Session Type Options:
- Continuing Education
- Symposium (90 or 165 minutes)
- Workshop (90 or 165 minutes)
- Roundtable
- Historical Highlights
- Informational
- Education-Career Development
- Regional Interest

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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 62nd Annual Meeting of the Society of Toxicology, at the Music City Center, Nashville, Tennessee, March 19–23, 2023.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 511.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 547.

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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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While all toxicology addresses the adverse effects that agents have on living organisms, clinical and medical toxicology play unique roles not often encountered in traditional toxicological practice. The seed in toxicology practice is to identify and understand the mechanisms by which substances cause effects, in order to determine the best treatment options for each patient. This course will introduce participants to the basics of the fields along with new and old tools that may be used by the clinical toxicologist.

Physiologically based kinetic (PBK) modeling predicts internal dose metrics by describing the critical physiological, physicochemical, and biochemical processes that determine the disposition of a chemical in an organism. Here, the general term kinetic is considered synonymous with pharmacokinetic, toxicokinetic, or biokinetic. Traditionally, in environmental toxicology, the calibration of parameters in a PBK model and the evaluation of its predictive capability rely heavily on comparing model simulations with in vivo blood or tissue concentration data obtained from laboratory animals. However, the availability of in vivo data is limited to extensively researched chemicals, which impedes the broader applications of PBK models by regulatory agencies. The recent paradigm shift toward using new approach methodologies (NAMs) to inform predictive approaches for chemical hazard and risk assessment necessitates the use of PBK models—developed and evaluated without data from live animals—to convert a bioactive concentration observed in cell-based toxicity assays to an equivalent human exposure level. In 2021, in order to keep pace with global efforts to develop and incorporate NAMs in chemical risk assessments, the Organisation for Economic Co-operation and Development (OECD) published a guidance document on characterizing, validating, and reporting Physiologically Based Kinetic Models without the use of animal data. This guidance document includes contextual information on the characterization and evaluation of PBK models in a regulatory context and highlights common in vitro and in silico approaches to parameterizing the models. The OECD guidance document provides a model reporting template and model evaluation checklist for evaluating the credibility of PBK models for their intended purposes. Adoption of the OECD PBK model guidance document is now encouraged when developing OECD Integrated Approaches to Testing and Assessment. This course offers training on key principles in the OECD guidance document. After a brief overview of the guidance, common, modern in vitro and in silico approaches for parameterizing a PBK model will be highlighted, as described in the second chapter of the guidance document. Next, various tools on how to evaluate the context, implementation, and scientific validity of a PBK model without using in vivo data will be presented, as recommended in the third chapter of the guidance document. At the end of the course, a hands-on exercise will give both novice and expert attendees an opportunity to apply their knowledge in evaluating example PBK packages. This course is designed for those involved in developing, applying, and promoting the acceptance of PBK models and those who seek to reduce/replace animal testing and incorporate more mechanistic investigations into the non-bioenergetic roles of mitochondria, these areas need to examine specific mechanisms of toxicity within mitochondria in order to mitigate potential toxicophores and help identify chemical series with high toxicity risk. Non-clinical species are vital to hazard identification and human risk assessment, the selected species should be relevant to human as far as primary pharmacology and metabolite profile. Additionally, it is imperative that robust exposure is demonstrated to ensure proper toxicological assessments, especially for non-clinical indications. Proper formulation selection is fundamental to address challenging properties of a compound and enable in vivo evaluation with desired exposure levels. The last speaker will discuss how the emergence of machine learning can enable off-target hypothesis generation for an unexpected toxicity observed in vivo. Toxicologists should promptly identify any causes of unexpected toxicities and propose models to screen out compounds with an unfavorable profile if it is off-target mediated. However, regardless of considerable time and financial investments, discovering the mechanism of toxicity may not be possible by empirical methods alone. Machine learning may help accelerate the generating of testable hypotheses. The course will conclude with an interactive session to focus on how toxicologists can achieve diversity in models of scientific expertise and ensure better decision-making early in the discovery stage. Overall, this course will inform toxicologists of new insights needed to utilize multidisciplinary tools to develop proactive safety paradigms that could reduce project delays and late-stage drug attrition. These concepts and approaches are generally applicable for predictive safety and investigative toxicology in any field, including academic research work.

Mitochondria are the central energy-producing organelle in the cells of most eukaryotes, though their roles within the cell expand far beyond bioenergetics. Recent work indicates that this dynamic organelle serves as a regulator of apoptosis, oxidative stress, calcium homeostasis, signal transduction, steroid hormone synthesis, and immunity among other pathways. As a result, mitochondria can alter cell and tissue function that leads to aging, neurodegenerative disease, cardiovascular disease, inflammatory disorders, and increased cancer severity. However, despite increasing investigations into the non-bioenergetic roles of mitochondria, these areas remain critically understudied and misunderstood. For example, mitochondrial iron uptake plays a critical role in iron homeostasis and related hepatotoxicity. Similarly, mitochondrial dysfunction is increasingly identified as a key factor in Gulf War Illness, suggesting newfound roles in other diseases. This Continuing Education course will explore the wide roles that mitochondria play in response to cellular stress and xenobiotics beyond bioenergetic alterations. It also will seek to challenge how researchers traditionally examine and include mitochondrial toxicity in their work. Speakers with expertise in mechanisms and assessment of mitochondrial toxicity will present an introduction to mitochondrial toxicity that will focus on the need to examine specific mechanisms of toxicity within mitochondria in order to understand the resultant adverse outcomes; this will include three case studies displaying the methods used and mechanisms of mitochondrial toxicity that extend beyond alterations to bioenergetics, including mitochondrial iron homeostasis and its role in acetalaminophen hepatotoxicity, antiviral medication-induced alterations to heart epigenetic and metabolic landscapes, and the role of mitochondria in the function of the innate immune system and inflammation. Next, we will progress from in-depth mitochondrial mechanisms to how this knowledge can be gained using mitochondria as biomarkers for renal exposures. Finally, in a world with an ever-expanding need for toxicological assessment of new drugs, toxins, and toxicants, we will explore how mitochondrial testing has been improved and conducted in large pharma and how large pharma is upscaling mitochondrial toxicity testing in order to meet increasing future demand. Following the session, attendees will have a clear understanding of the dynamic roles mitochondria play within the cell and at organizational levels, the challenges and current solutions analysis of mitochondrial endpoints presents, and a new perspective on the role of mitochondria in toxicological mechanisms. Further, they will be empowered to assess how these less-understood mitochondrial mechanisms may play key roles in their own work, ultimately expanding and improving toxicological evaluations.

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Traditionally, rats, mice, and rabbits have been used as preferred animal models (rodent and nonrodent) for nonclinical developmental, reproductive, and juvenile toxicity testing. Published regulatory guidelines recommend the selection of relevant animal models—based on individual project requirements—that may be influenced by systemic exposure and prior systemic toxicity data derived from general toxicology studies. Most compounds use traditional models that are quite effective and widely accepted by international health authorities. Species-specific dose-limiting toxicity, differences in metabolism across test species, and pharmacological relevance may require researchers to investigate nontraditional models such as guinea pigs, minipigs, and dogs. In addition, certain pharmacological classes (e.g., antibiotics in rabbits) or chemistries also may limit the use of traditional models. Further, during the COVID-19 pandemic, a severe shortage of nonhuman primates (particularly sexually mature females needed for ePNEP studies) shifted the focus toward nontraditional animal models. This course will walk participants through the various considerations for study designs as well as the pros and cons of more common nontraditional mammalian models in DART and juvenile toxicity testing. It also will touch upon the use of rabbits and their limitations in fertility and juvenile animal studies. The first presentation will describe how collaboration between animal model suppliers, industry, and academia has driven scientific development and expanded the use of the Göttingen minipig into DART and juvenile toxicology by highlighting specific projects. The second presentation will focus on the use of beagles in developmental and juvenile toxicity testing, including many critical aspects of study design, species differences, and terminology. The third presentation will explore the scientific considerations for the selection of alternatives to nonhuman primates in order to address embryo-fetal risk and provide examples of how transgenic, knockout and knockout animal models, and surrogate molecules can be used to support nonclinical hazard assessment for various biotherapeutics. The course will end with a summary of the current regulatory guidance on DART and juvenile animal toxicity testing, which includes discussion of the use of nontraditional species or models. Following this course, participants will be familiar with a variety of approaches to assessing embryo-fetal risk and juvenile toxicity in animal models not commonly used in DART as well as the current regulatory guidance supporting them.

Building on courses from previous years, this session seeks to provide members with a brief overview of systematic review methods and principles that will be followed by analysis of how these methods can be applied to or interpreted as information beyond traditional human health hazard endpoints (e.g., epidemiology and animal toxicity studies evaluating apical endpoints). Systematic review methods using unbiased, reproducible, and transparent approaches have been implemented in order to evaluate data streams. The application to a broader base of evidence is crucial to the practice of risk assessment, given that exposure characterization ecological receptors inform assessment conclusions. The first talk will provide a brief review of systematic review principles followed by an overview of the translation of principles and techniques of systematic review across diverse disciplines. A second talk will demonstrate how Ecotoxicity Database (ECOTOX) literature review and data curation protocols were designed to align with systematic review methods. Methods were developed to ensure that ecotoxicity data were extracted in sufficient detail in order to support independent evaluation, synthesis, and/or review of ECOTOX data documents ranging from scoring documents to regulatory decision-making. The focus of the third presentation will be the application of systematic review methods to environmental fate endpoints and how the systematic evaluation of these endpoints influences prioritization, assessment, and prediction of human health and ecological hazard and toxicity. The development of a fit-for-purpose evaluation framework to systematically assess data unique to environmental fate endpoints, including field/monitoring data and data estimated from environmental fate and transport models, will be described. The fourth presentation will focus on the application of systematic review methods to environmental exposure data—specifically the data required to estimate daily soil ingestion rates in humans. We also will discuss the application of systematic review principles, including the development of a population, exposure, comparator, and outcome statement specific to the soil ingestion of contaminants. The fifth speaker will consider challenges associated with using systematic review principles in the synthesis and integration of mechanistic data. Adjustments of systematic review methods needed to evaluate mechanistic data, including a definition of the objective/scope of the use of the data and selection/refinement of an appropriate critical appraisal tool for various types of mechanistic data (e.g., in silico, in vitro, in vivo, and epidemiological data), will be presented to demonstrate that these systematic review adjustments rely on knowledge of standard constructs and methods used in toxicology and risk assessment. The final speaker will consider evidence-based methods for assessing the correlation of in vitro data (from the US Environmental Protection Agency Toxicity Forecaster) and/ or in vivo data (from standard toxicity studies and human clinical trials) in order to predict human health hazard. A case study will be presented to show how data from these streams were evaluated systematically in order to predict the effects associated with two antidiabetic drugs in humans. As a whole, this course will demonstrate how systematic review methods that are developed to evaluate individual toxicity studies are being applied to other systems. The course will provide attendees with the working knowledge base required to assess diverse endpoints using systematic review methods. Furthermore, this course will appeal to attendees of diverse backgrounds and toxicological disciplines who are interested in studying and applying systematic review methods, including scientists developing and conducting studies related to ecotoxicity, environmental fate, exposure, mechanistic in vitro/in vivo toxicity testing, and others, along with risk assessment practitioners and regulators.

The rapid expansion of computational and in vitro methods for the analysis of chemical-biological interactions has evolved a wealth of open-source tools and resources that enable discovery and analysis. This supports increased use of these resources in order to aid chemical assessments and support the finding and generating of information for prioritization, study waivers, weight of evidence, and other regulatory applications. Many of these new tools are open access and web based, which enables broader access. This session will provide attendees with an understanding of open-source and often web-based tools and resources that can be applied in a context-specific manner for understanding chemical-biological interactions and informing chemical assessments. The course will open with an overview of regulatory testing strategies that incorporate the use of traditional methods in order to provide a background on how these tools and resources can be used. The next three presentations will provide an overview of resources that contain traditional and new approach methodology testing data, as well as tools that support the assessment needs. Case studies will be used as reference points for participants, who also can work through the course material at their leisure. These presentations will address the following questions: how can we find existing data for a given chemical or chemicals that are structurally similar, how can we determine whether existing data can be used to fill in gaps of information, and how can computational models be leveraged to generate predictions that increase the weight of evidence? The final presentation will highlight various new tools and opportunities to derive narratives of chemical-biological interactions. Together, these presentations are intended to inform participants on pertinent resources available and provide a practical guide for when and how to use them. The course objectives are to (1) introduce participants to the mechanisms in which nonanimal approaches can be used to support assessments; (2) highlight resources for access to data and tools that enable the following applications: waivers, read-across, defined approaches and integrated approaches to testing and assessments, and QSAR-based modeling; and (3) showcase new resources to complement narratives.
data, extension of qualitative weight of evidence considerations to quantification of key event relationships, and increased experience in application. This course builds on a training developed within the OECD program and the best practices of documentation and assessment, including updates to the AOP-Wiki in order to encourage the use of common ontologies and delineation of literature analysis strategies and factors that modulate quantitative relationships. Consideration of disease pathways associated with nonchemical stressors also contributes to the expansion of evolving knowledge. The course includes practical demonstration of the Wiki/knowledge base, tips for developers and assessors, an opportunity to use the AOP Wiki to find information within sample use cases, and examples of development and application. Each presentation will cover different aspects of AOP development and application and, using real examples, offer a comprehensive overall learning experience. The first presentation will update attendees on how the available guidance and tools for AOP development have evolved to reflect experience with increasing and expanding content and biological space. The second presentation will emphasize the importance of transparently and efficiently documenting evidence collection and evaluation, which includes sharing tools and effective practice to improve the effective and predictive AOPs. The third presentation will lead into a comprehensive demonstration and hands-on activity with the AOP Wiki, including changes to the publicly available interface, which will be of interest to prospective developers as well as those seeking to use currently available AOP information. The remainder of the course will feature a discussion of current and future AOP applications (including the development of IATAs) and a transition to next-generation risk assessment and decision-making using new approach methodologies (NAMs) featuring globally relevant application examples. The course will leave its participants familiar with the most recent developments in effective practices that support AOP development, assessment, and application. Attendees will also be acquainted with evolving supportive resources within the OECD program and use of the AOP Wiki.

Inhalation toxicology has always carried an air of mysticism when compared with other modes of administration. This presentation will provide the information necessary for better understanding of this perceived dark art and serve as a take-home introduction for those new to the inhalation toxicology field while providing regulatory guidance to those not so new to inhalation toxicology. It will consist of practical aspects of animal studies regarding the determination of dose and formulations. Further, this course will provide examples and interpretations on what is adverse and not adverse in reference to histopathological findings in the respiratory tract.

In vitro to in vivo extrapolation (IVIVE) efforts are critical for addressing low concordance of preclinical animal models for chemical-induced human toxicity and aligning with global efforts to reduce, refine, or replace animal testing. Notably, REACH in the EU, Toxicology in the 21st Century (Tox21) in the US, and other regulatory initiatives support the development of new approach methodologies (NAMs) and stratified approach fields of IVIVE. As such, a wave of recently developed, rationally designed in silico models, advanced in vitro systems, omics signatures (e.g., toxicogenomics, proteomics, epigenomics, metabolomics, and lipidomics), and physiologically based pharmacokinetic (PBPK) modeling approaches demonstrate utility for IVIVE across diverse organ systems. Despite these advancements, there is limited understanding of key concepts related to robust NAM development, validation, and implementation within a regulatory framework to progress IVIVE adoption. Based on gaps in IVIVE guidance, this Continuing Education course will explore successful IVIVE studies across research sectors and highlight important strategies and principles critical for regulatory consideration. Specifically, the course will focus on IVIVE strategy for prominent organ system toxicities, including inhalation toxicity, hepatotoxicity, developmental neurotoxicity, and cardiovascular toxicity. The course will begin with an overview of IVIVE concepts and utility across academic, government, and industrial toxicological sectors. The first speaker will highlight the value of IVIVE within the field of inhalation toxicity using IVIVE case studies that connect inhalation exposure to complex mixtures in vitro and tobacco-free nicotine product human risk assessment. Next, the second speaker will detail the use of toxicogenomics, PBPK modeling, and publicly available toxicological databases (e.g., TG-GATEs) to assess drug-induced liver injury IVIVE within a robust compound validation set and describe how these approaches can aid in de-risking hepatotoxicity across research sectors. The third speaker will focus on an integrated approach to testing and assessment case study for developmental neurotoxicity using a battery of NAMs in order to prioritize a class of compounds. This speaker will also explore how exposure data in humans can be used to interpret this information. The fourth speaker will provide a balanced viewpoint of the cardiovascular NAM landscape, including 2D and 3D cardiac and vascular toxicity model approaches, challenges, and strategies for IVIVE implementation. The final speaker will conclude the course with a discussion of regulatory considerations and pathways forward for the adoption of IVIVE approaches within adverse outcome pathway contexts and clinical packages. This course will focus on critical organ systems and provide guidance on pertinent assay parameters (e.g., cell types, model systems, and endpoints) and mechanisms of action shown to affect key toxicities that have the potential for assessment via IVIVE. Also, as experts in their respective fields, the speakers will impart knowledge regarding organ system pathophysiology and offer key insights into in silico, in vitro, and systems biology methodology that are essential for the successful development of IVIVE platforms. Together, the latest developments on assay guidance and strategies for IVIVE development can inform participants on the best approaches within the field and address key factors identified by investigators. Along with valuable insights into regulatory considerations and future IVIVE perspectives, this course offers key strategies for the development, implementation, and advancement of IVIVE practices across research sectors.
The learnings from this course are relevant for attendees from academic, industry, and government sectors who seek to explore or expand the use of computational models within their organizations.

Training in effective science communication is critical for scientists in all stages of their careers. However, as many of us advance beyond our graduate and postdoctoral training, we have fewer opportunities to convey our communication training to other scientists (both inside and outside our field). The purpose of this Continuing Education (CE) course is to introduce effective science communication, discuss how providing critical feedback helps make you a more effective communicator, and suggest mechanisms by which you can define yourself as a science communicator online. This innovative session will include real-time feedback via mobile devices (i.e., polling) in order to engage the audience. It also will include an interactive session in which participants are required to deliver a three-minute presentation in small groups (e.g., five people) using an oral format or a single slide so as to practice effective communication in a safe space. Attendees will receive constructive comments in real time and during the discussion session. The concluding question-and-answer session will allow for registrants to use the polling functionality to communicate their experiences from the interactive session.

This CE course will be complemented with a Wednesday presentation in the Tiny Tox Theater (ToxExpo, Hall C) in which participants and other interested attendees can share how they applied any learned or practiced communication skills during the meeting.

Out of necessity, chemical safety assessment is transitioning to more rapid and efficient methods for predicting toxicity, including the application of artificial intelligence and machine learning based approaches. Understanding regulatory needs and decision contexts is a critical precursor to building fit-for-purpose computational tools that will ultimately be adopted and implemented. The Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) has collated such information for many toxicological endpoints and methods, such as acute systemic and topical toxicities, ecological assessments, and in vitro to in vivo extrapolation. Once the context of use has been defined, rigorous data curation efforts are necessary to identify and establish training data for predictive model building; this data curation process can also be supported by cognitive algorithmic tools and software. Numerous methods and structural/physicochemical feature sets can be applied to build quantitative structure activity relationship (QSAR) models from large chemical toxicity training data sets, each with respective dis/advantages. A crowdsourcing approach has been applied to build consensus ensemble models that leverage the strengths and compensate for the weaknesses in any individual method and provide robust predictive performance and broad coverage of the chemical universe. Consensus QSAR models for endocrine disruption endpoints and acute systemic toxicity are being considered and evaluated. Specific case studies describing how these approaches are being used for various regulatory decision-making contexts such as chemical prioritization and waiving animal testing requirements will be discussed.

Hepatotoxicity is the injury imposed on the liver caused by exogenous chemicals and is the leading reason for drug attritions. Traditional screening for chemical hepatotoxicity relies on animal testing, which is costly, time-consuming, and has ethical concerns. High-throughput screening (HTS) protocols can test thousands to millions of chemicals using HTS assays, providing enormous amounts of information on relevant toxicity mechanisms in the past decades. This effort brings new challenges to computational toxicology studies in the big data era. Normally, traditional computational modeling approaches handle training sets with individual endpoints, which are not suitable to model a large amount of related HTS data. In this work, we developed a new machine learning approach to establish robust chemical adverse outcome pathway (AOP) models from dose dependent toxicity data obtained from HTS assays and used model outcomes to correlate to hepatotoxicity. Using these AOP models, the compounds of interest can be ranked by the outcome of each AOP model, and the mechanisms of highly ranked chemicals can be illustrated using the relevant assays. Furthermore, grouping chemicals based on chemical structure similarity revealed strong AOP model outcomes for chemical classes of hepatotoxicants, informing on their mechanisms of actions (MOA). The developed AOP models can be coupled with toxicokinetics results to directly predict chemical hepatotoxicity (Accuracy 71%, Recall 76%, Precision 85%). For the external validation purpose, we used the resulted models to identify several drugs that induce liver injury (DILI) and illustrate their toxicity mechanisms. Although presented as a case study, this new machine learning approach to automatically mine public toxicity data for predictive modeling purposes can be a universal computational toxicology strategy to apply to various chemical toxicity evaluations based on informative dose dependent mechanistic toxicity data.
We have developed a novel form of AI, based primarily on search engine algorithms, which is useful for the analysis of secondary pharmacology and mechanism of toxicity (MoT) analyses. A search engine AI approach has many advantages over traditional machine learning based approaches, including Transparency: The data supporting any target inference can be easily identified, traced back to its original source, and evaluated. This transparency contextualizes all findings and allows scientists to fully leverage their expertise when interpreting results. In clinical biology applications, the trajectories of all nodes are shown, as well as the precise reported activity against the putative target(s). Convincion: The most experimentally supported results are ranked first, regardless of how much data is searched or how many additional results are returned; Scale: Search engine algorithms can be tailored to work seamlessly and rapidly over multiple large, heterogenous, and highly complex data sets, identifying instances where multiple data sources converge on the same answer. For a given chemical series of interest, for example, our AI approach can identify if structurally related compounds converge on certain targets, toxicities, pathologies, adverse events, drug classes, protein families, protein domains, diseases, etc. Our AI is powered by a massive knowledge graph of over 700M nodes and edges covering all areas of biomedical research, from pharmacology to omics data to clinical trial information. Analyses can be initiated with chemical structures of interest, but also with gene lists from omics experiments, pathways, biomarkers, or metabolites from metabolomics profiles. Multiple approaches can be used together, such that a compound off-target or MoT can be pinpointed using a combination of chemical structure, transcriptional perturbation data, and metabolomics profiling. Here we present a case study demonstrating the use of our approach to discover putative off-targets of the anxiolytic drug carazolol. We will also present an example case study investigating the underlying mechanism of an unanticipated safety finding.

One of Genotoxic Toxicology’s functions is to identify compounds that have the potential to cause chromosomal damage. The standard regulatory method is the manual microscopic micronucleus assay. Micronucleus can manifest itself via various molecular mechanisms such as non-genotoxic and genotoxic insults to the DNA, which can be further classified as clastogen (chromosome fragments-containing micronuclei) or aneugen (full chromosome-containing micronuclei). Aneugens can be further subclassified as tubulin binders and aurora inhibitors. Follow-up assays are routinely needed to differentiate various mode of actions (MoAs) in regulatory settings. A new generation of software incorporated into a comprehensive in silico approach is used to evaluate the compound progression through development. Clastogens cannot progress unless indication justify versus aneugen effects are thresholded, with large enough safety margins, aneugen are considered non-genotoxic at therapeutic concentration and are safe to progress. In this study, we used 101 Genotoxicity Predictions for Rapid Compound Screening: A Case Study for Accurate Classification Using Machine Learning


Nonclinical safety assessment of new drug candidates typically includes embryo-fetal developmental toxicity studies, one component of which is an evaluation of fetal skeletal development by manual examination of Alizarin red-stained fetuses. However, the staining process generates hazardous waste and continuously demands storage of physical fetus specimen. In addition, evaluation of fetal skeletal morphology is subjective and, therefore, inherently prone to variability and/or biases. To overcome these challenges, micro-computed tomography (micro-CT) imaging has been introduced as an alternative approach, wherein unstained fetuses are scanned using a micro-CT scanner and the reconstructed 3D images are examined using a 3D visualization tool. At Merck, we have developed a protocol to acquire micro-CT images of animal fetuses at a high throughput. In addition, we have made attempts to automate the assessment on rabbit fetuses using automated image analysis techniques. Herein, we are providing an overview of our approach to segment and label each bone on the rabbit fetal skeleton using conventional image analysis techniques, as well as the latest attempts to improve the automated analysis using artificial intelligence (AI). Specifically, we conducted a study on the localization of vertebrae using a 3D regression convolutional neural network (CNN) model and a multi-task 3D CNN model for the segmentation and labeling of vertebrae. Through the propagation of our efforts, we are demonstrating the promise of using high-throughput micro-CT to enhance fetal skeletal assessment in nonclinical safety studies, while highlighting the areas warranting further investigations using AI.

Much research was recently put into the application of various AI approaches to digitalize drug toxicity in vivo data, including data extraction from various internal and external sources, text mining from regulatory toxicity study reports, followed by harmonization and transformation into SEND-like formats. This presentation will show how these digitalized in vivo data are applied in Predictive Toxicology. The first step to enable the full benefit of these digitalized toxicity data is to organize them into a structured database. This database can be then used for tailored searches around questions for example on specific toxicity findings, on-and off-targets or structurally similar drugs and these search results can be applied for read-across and risk assessment in drug research and development. The first use cases demonstrated how how search results for specific mode of action adverse effects like neutropenia or inflammatory findings of the gastrointestinal tract were used to trigger follow-up investigations, helped in elucidation of this potential mode of toxicity and to assess the relevance of these findings for the further development process as well as for human situation. As follow up, the organized data can then be used to generate training datasets for machine learning. Accordingly, we extracted most relevant toxicity findings like from histopathology, clinical chemistry, hematology examinations for main target organs, including liver, kidney, heart and developed individual in silico models for the prediction of these toxicity endpoints. So, the second case study will demonstrate how these in silico models helped to de-prioritize and structurally optimize lead series in early drug research, including lead structure candidates from the chemical thixo-pyrindinone class. These case studies show that applications of AI are playing an increasingly important role for in silico prediction and risk assessment of drug toxicity.

Wildfires are increasing in size and incidence across the world and are a significant source of air pollution in the form of particulate matter, toxic vapors, and noxious gases. Inundation of smoke plumes into affected communities poses a major challenge for air quality management and public health. In addition to biomass smoke, combustion of dwellings, businesses, automobiles, household appliances and synthetic and synthetic materials results in toxic emissions that could have profound and long-lasting adverse health and ecological outcomes. Notably, smoke from these fires, such as the 2018 “Camp fire” in California and the 2021 “Marshall fire” in Boulder, Colorado, may contain hydrogen cyanide, acidic aerosols, heavy metals, and a host of organic compounds that are known, or suspected to be, carcinogens, caustic agents, and/or irritants. The relative potency of these emissions (particularly in a mixture environment) is not well studied, and the Environmental Protection Agency’s Air Quality Index for criteria pollutants, which is used to communicate health hazards in near real time, does not necessarily capture the full range of potential air toxic exposures. This session will highlight the chemistry of smoke from structural fires, provide an overview of the exposures structural and wildland firefighters face, and describe efforts to assess the contribution of structural fires within wildland fire air samples. Later presentations will highlight what is known about the toxicology and carcinogenicity of these emissions and identify data gaps and approaches needed for risk assessment. This session aims to increase both member awareness and quantitative information of the health hazards associated with combustion of structures during and after wildfires. It also will discuss how toxicology can inform early decision-making for public health protection.
emissions released from different building products, under different fire scenarios. It will also discuss human tenability criteria for the concentrations of the individual toxic gas species generated by combustion.

1024 Exposure and Cancer Risks among Structural Firefighters
K. Fent. NIOSH, Cincinnati, OH. Sponsor: M. Gilmour

This talk will summarize recent studies that have evaluated cancer incidence and mortality in structural firefighters. The speaker will further discuss the predominant exposure pathways among firefighters, and how these pathways contribute to biomarkers of exposure, with an emphasis on known or probable carcinogens. He will wrap up with a discussion of the wildland-urban interface (WUI) and how this may impact firefighters’ exposures now and in the future.

1025 Investigating the Links between Chemical Exposures and Adverse Health Effects Associated with Camp Fire Smoke
Q. Zhang University of California Davis, Davis, CA. Sponsor: L. Van Winkle

We investigated the links between chemical exposures to wildfire smoke and adverse health outcomes through performing real-time measurements of ambient PM in Davis, CA during the Camp Fire period in November 2018. Specifically, we characterized the highly time-resolved temporal variations of PM concentration and composition using a high-resolution mass spectrometer (SP-AMS) and applied novel mass spectrometry analysis to estimate the total concentrations of phthalates in PM. Different sources of the PM were resolved as well. These results are combined with data on nonhuman primate pregnancies at various stages of development to investigate the adverse effects of wildfire smoke on pregnancy and fetus development.

1026 Mutagenicity and Toxicity of Combustion Products from Synthetic Materials and Comparisons to Biomass Emissions
Y. Kim University of North Carolina at Chapel Hill, Chapel Hill, NC.

This presentation will discuss differences in the chemical components of wildfire smoke depending on fuel types and burning temperatures and how such differences can promote similar or distinct toxicity outcomes. This will introduce how we develop a lab-scale combustion system to simulate various wildfire smoke emissions and present how our computational approach can be used to identify individual chemicals that drive specific toxic outcomes. This will also provide potential beneficial effects of particle filtration in reducing health impacts from acute exposure to wildfire smoke. This presentation will help to better understand health effects of smoke exposures from burning homes, structures, and man-made materials in the wildland urban interface (WUI) areas during wildfires. The views expressed do not necessarily represent the views or policies of the US EPA.

1027 Health Risk Assessment on Fire’s Front Line: Challenges for Characterization and Communication
A. Jarabek. US EPA, Research Triangle Park, NC.

The acute nature and chemical composition of exposures associated with structural fires pose a complex challenge to risk assessment approaches as well as to informing impacted populations of potential health effects. This presentation discusses specific components of current assessment approaches that require customization, including consideration of “C vs t” as determinants of toxicity; evaluation of chronic trajectories from acute measurements; specification of parameters for vulnerable populations; and mixtures methods with unknowns. Discussion of approaches for translation and communication of health risk, including visualization of data and public engagement, is also included. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.

1028 Mechanistic Insights on Gene x Environment Interactions in Autism
L. Smirnova. Johns Hopkins University, Baltimore, MD.

Autism spectrum disorders (ASD) constitute a global public health concern. In the US, incidences of ASD have increased from 1 in 5,000 children in 1975 to 1 in 44 children in 2020. Similarly, these increases have been documented in other countries around the world. Until recently, research on the etiology of neurodevelopmental disorders has focused largely on genetic causes. However, this research has clearly shown that single genetic anomalies account for only a small proportion of cases, and even in genetic syndromes highly associated with ASD, a significant percentage of carriers do not have ASD. Overall, genetic factors seem to account for 40-50% of ASD cases. There is now credible evidence that ASD is the result of complex interactions between genes and environmental factors. In contrast to genetic risk factors, which are currently irreversible, environmental factors are modifiable risk factors. Therefore, identifying factors that increase risk for ASD may provide valuable approaches for the primary prevention or mitigation of the severity of symptoms associated with these disorders. However, to date, the identities of environmental factors that influence ASD risk or severity and the mechanisms by which environmental factors interact with genetics to determine individual risk remain critical gaps. The phenotypic heterogeneity—the clinical hallmark of ASD—combined with the complex multigenic etiologies, significantly increase the difficulty of identifying specific environmental factors increasing risk. The complexity of ASD heritable factors also creates a range of sensitivities of the developing brain to the adverse effects of environmental factors, which has made it challenging to establish clear associations between exposure to environmental factors and diagnosis of specific disorders. To address this, it is critical to integrate approaches and findings from diverse fields, which is the goal of this session. Presenters with expertise in epidemiology, genetics, and mechanistic toxicology will present these diverse findings. The session will open with an overview of the broad spectrum of research activities (epidemiology, molecular, and bioinformatics-based studies) in the field of gene x environment (GxE) in ASD supported by the National Institute of Environmental Health Sciences. This will be followed by a discussion of how molecular epidemiology and systems biology approaches can be leveraged to study GxE interactions in ASD prevalence. The next two talks will illustrate how in vitro models are used to investigate molecular mechanisms underlying potential synergy between environmental chemicals and ASD-linked genetic susceptibilities. The session will conclude with a presentation demonstrating how animal models are used to validate in vitro findings of putative GxE. In summary, attendees will gain knowledge regarding current state-of-the-art, interdisciplinary approaches for studying mechanisms of GxE interactions that influence ASD risk and will learn about emerging data and remaining gaps.

1029 Challenges and Opportunities for Gene-Environment Interaction Studies Supported by the Extramural Division of NIEHS
K. McAllister. NIEHS, Research Triangle Park, NC. Sponsor: L. Smirnova

NIEHS has a strong interest in understanding the interaction of genetic and environmental risk factors for complex human diseases, and this Institute has a long history of exploring gene-environment interactions particularly relevant to many neurodevelopmental diseases. Genetic susceptibility is recognized as a significant component to the varying effects of toxicants on disease pathways. This underlying understanding of genetic susceptibility or resistance to environmental exposures is currently being examined in a variety of genetic epidemiology studies supported by NIEHS. NIEHS has also supported both solicited and unsolicited proposals related to other multi-faceted aspects of G x E research including the development of innovative statistical and bioinformatics methods, the use of sophisticated in vitro functional genomics approaches, and the use of novel population-based model organisms, such as the Collaborative Cross and Diversity Outbred mice. NIEHS also recognizes the importance of the ethical, legal, and social implications that may arise from individuals or communities being identified as at increased disease risk due to a combination of genetic and environmental factors and their interplay. The translational goal of this G x E research is to identify populations that are most sensitive to exposures to ultimately adopt prevention/intervention strategies to protect the most vulnerable subpopulations.

1030 Using Molecular Epidemiology to Better Understand the Interplay between Environment and Genes in the Causation of Autism Spectrum Disorder
A. Ponsoby. The Florey Institute for Neuroscience and Mental Health, Melbourne, Australia. Sponsor: L. Smirnova

Molecular epidemiology involves working at the interface of epidemiology and system biology. For environmental determinants of autism spectrum disorder, our work uses these approaches to investigate how putative harmful environmental agents, such as phthalate chemicals, may have a differential impact on outcomes depending on genetic vulnerability. Gene x environment interaction using pathway and/or network approaches can provide greater statistical power and information than examining the differing effect of phthalates on neurodevelopment by individual genetic SNP (single nucleotide polymorphisms) variants alone. The developing brain is highly sensitive to environmental disturbances, and adverse exposures can act through oxidative stress. Here, we first generated a genetic pathway function score for oxidative stress (gPFSox) based on the transcriptional activity levels of the oxidative stress response pathway in brain and other tissue types. Then, in the Barwon Infant Study (BIS), a population-based birth cohort (n = 1074), we demonstrated that a high gPFSox, indicating reduced ability to counter oxidative stress, is linked to higher autism spectrum disorder risk and higher parent-reported autistic traits at age 4 years. Past work in BIS has reported higher prenatal phthalate exposure at 36 weeks of gestation associated with offspring autism spectrum disorder. In this study, we examine combined effects and show a consistent pattern of increased neurodevelopmental problems for individuals with both a high gPFSox and high prenatal phthalate exposure across a range of outcomes, including high

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The significant increase of ASD in recent years cannot be explained solely by genetics but rather by gene x environment interactions. To reduce the enormous numbers of animals that would be required to study all the possible G X E interactions that could influence ASD, new approaches focusing on human genetic risk factors in human relevant organotypic models are needed. This will help to identify specific G x E interactions most likely contributing to the disease, which then can be validated in more complex in vivo models. A combination of three technologies (induced Pluripotent Stem Cells (iPSC), CRISPR/Cas9 gene editing and advanced 3D organoid cultures) were used to provide a proof of principle example of how G x E can be addressed in vitro. Mutation in the chromodomain helicase DNA binding protein 8 (CHD8) gene is one of the major genetic risk factors for ASD. A heterozygous CHD8 knockout iPSC line was generated using CRISPR/Cas9. Brain organoids were generated from CHD8 knockout and control iPSCs and were exposed to an organophosphate insecticide, chlorpyrifos (CPF). There is a significant body of epidemiological evidence suggesting an association between developmental exposure to CPF and altered neurodevelopment in children. A common ASD feature of imbalanced excitatory to inhibitory signaling (glutamate/GABA ratio) was confirmed in this in vitro model. CHD8 knockout brain organoids were more sensitive to the effects of CPF. Exposure to CPF reduced the level CHD8 protein in both control and mutant organoids, suggesting that the downstream target of mutation and exposure may be the same. Exposure led to reduced neurite outgrowth in both genotypes which was rescued by ticlopidine. Finally, we identified synergistic effects of mutation and exposure on the level of neurotransmitters and metabolites associated with ASD (including, one carbon, and trans-sulfuration, tryptophan and dopamine metabolism). These in vitro findings were then validated against existing human data on ASD metabolic biomarkers.

### 1034 Microplastics: Factors to Consider When Assessing Potential Environmental and Human Health Risks

**J. Norman.** American Chemistry Council Inc., Washington, DC.

The ability to detect, identify, and measure microplastics in the environment has increased greatly in recent years. With these increased efforts, new information needs and knowledge gaps have been identified. Microplastic research is in its beginning stages and presents toxicologists with a unique opportunity to design research programs that incorporate the latest methods and best practices. It is clear that interdisciplinary research, global collaborations, and communication among stakeholders are needed to enrich this emerging field of study. Given the heterogeneity of microplastic materials, studies that encompass exposure, hazard, and risk challenges must be designed. Developing a risk-based approach is ultimately necessary in order to accurately evaluate the potential impacts of microplastics on humans and the environment. The first speaker will highlight the research needs and challenges that any research community faces today. The second speaker will discuss the importance and relevance of pristine microplastic materials derived from consumer products and the implications of human ingestion. The third speaker will expand the discussion into secondary (i.e., weathered) microplastic materials, particle transformation mechanisms, and relevance to exposure assessments. The fourth speaker will share the unique challenges researchers consider when measuring airborne microplastic materials as an important first step to an exposure assessment. Finally, the last speaker will review new methodologies and additional considerations for combining information to be used in a holistic risk assessment. This session is designed to engage the attendees and launch a discussion for toxicologists interested in studying the effects of microplastics.

### 1032 Wnt-Specific Genetic Influences on Gene Regulation in a Population of Human Neural Progenitor Cells

**J. Stein.** University of North Carolina Chapel Hill, Chapel Hill, NC. Sponsor: L. Smirnova.

This presentation will discuss how cell culture-based genetic associations can be used to identify environmental chemicals that impinge upon stimulus-specific cellular and molecular mechanisms that influence risk for ASD and other neuropsychiatric disorders. Stimulation of the Wnt signaling pathway is known to affect neural proliferation and patterning and to modulate the risk for autism-associated mutations cluster in genes of the Wnt pathway. In addition, genetic associations of common brain variation associated with inter-individual differences in cortical surface area are clustered near Wnt relevant genes. To identify genetic variants that exert effects specifically during Wnt stimulation conditions, we added Wnt activators (Wnt3a, CT99021) to human neural progenitors from 84 different donors. We then measured gene expression via RNA-seq and chromatin accessibility via Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) in each of these donors. Wnt stimulation strongly increased chromatin accessibility at T-cell factor/lymphoid enhancer factor (TCF/LEF) binding sites. Context specific regulatory elements are significantly enriched in heritability of brain related traits. We detected hundreds of locations in the genome where genetic variation has differential effects in the context of Wnt signaling.

### 1033 Developmental Exposure to a Human-Relevant PCB Mixture Causes ASD-Relevant Phenotypes in Juvenile Mice Expressing Human Mutations That Modulate Calcium Signaling

**P. Lein.** University of California Davis, Davis, CA.

While ASD is thought to result from complex interactions between environmental and genetic risk factors, the identification of specific gene x environment interactions that influence ASD risk remains a critical data gap. This talk will present data from experimental animals studies designed to test the hypothesis that PCBs interact with human mutations that alter the fidelity of neuronal Ca2+ signaling to confer ASD risk. Mice that expressed a gain-of-function mutation (T4826I) in the wildtype (WT) littermates were exposed to the MARBLES PCB mixture at 0, 0.1, 1 and 6 mg/kg/day in the maternal diet throughout gestation and lactation. The MARBLES PCB mix simulates the relative proportions of the twelve most abundant PCB congeners found in the serum of pregnant women at increased risk for having a child with a neurodevelopmental disorder. Behaviors of relevance to ASD, e.g., ultrasonic vocalizations, spontaneous repetitive behavior and sociability, were tested in postnatal pups and weanlings, and dendritic arborization was quantified in the hippocampus and cortex of these animals, which were euthanized upon completion of behavioral testing. The findings from these studies suggest that (1) developmental PCB exposure causes ASD-relevant phenotypes that vary by sex and genotype; and (2) sex-specific responses to environmental factors may contribute to sex biases in the prevalence of ASD. Overall, these observations provide proof-of-principle evidence that PCBs interact with heritable human mutations to modulate neurodevelopmental outcomes of relevance to ASD.

### 1031 Role of the Autism Risk Gene, CHD8, in Chlorpyrifos-Induced Neurotoxicity in iPS-Derived Brain Organoid Model

**L. Smirnova.** Johns Hopkins University, Baltimore, MD.

The potential toxicity of a microplastic particle, so the use of representative test cation due to environmental weathering. The weathering of particles may change the potential toxicity of a microplastic particle, so the use of representative test material or when generating weathered microplastic test materials. Research -tic particles. Secondary microplastic particles occur when a plastic article breaks down and enters the environment. Secondary microplastic particles begin with similar characteristics as the original article but may undergo significant modification due to environmental weathering. The weathering of particles may change the potential toxicity of a microplastic particle, so the use of representative test material is critical. Context understanding the potential for microplastic toxicity. Efforts to develop standardized and fully characterized microplastic materials are underway to assist researchers distinguish adverse effects caused by the particle itself, from adsorbed chemicals or weathering, or a combination of the two contaminants. This presentation will provide attendees with an overview of the critical elements to consider from a quality assurance standpoint when selecting a standard microplastic material.
1037 Developing Standard Reference Materials and Determining Effects of Weathered Microplastic Particles

C. Savaes, Baylor University, Waco, TX.

Exposure to microplastics can occur in multiple environmental compartments, i.e., air, water, soil, and biota. However, one of the most prevalent sources is water. Across the globe, microplastic particles are detected in freshwater, wastewater, saltwater, and groundwater. And because of microplastics chemical composition and physical attributes, these particles readily sorb a multitude of other contaminants present in the surrounding matrices, i.e., organic matter, inorganic moieties, heavy metals, among others. The potential of sorbed substances on to the surface of microplastic particles combined with exposure to environmental conditions result in particle transformation producing “weathered” or “aged” microplastics. Laboratory studies designed to assess microplastic particle hazards depend on exposure concentrations and simulated real-world conditions and influence the physical, chemical, and toxicological properties. Therefore, there is a need to develop systematic analysis methods to enable read-across of relationships, and eventually correlations and generalizations, to inform public health. This talk will present a case study related to the exposures and induced toxic effects of weathered microplastic particles. The resultant data will provide a critical piece of information for future risk assessments.

1038 Measuring Aerosolized Microplastic Particles in Indoor and Outdoor Air

A. Elder, University of Rochester Medical Center, Rochester, NY.

The assessment of potential risk from exposure to aerosolized microplastics requires a greater understanding of their quantity, chemical identity, and size distribution. Aerodynamic diameter dictates whether the particle can be inhaled and where it deposits in the respiratory tract. The analysis of microplastic particle density, and its shape have an impact on aerodynamic behavior. Developing methods to distinguish aerosolized microplastics from other particles is the first step to understanding potential exposure and potential effects. This presentation will discuss the considerations and techniques developed to sample, isolate, and characterize microplastics in air. Future work will apply these techniques to outdoor air as a first step to understanding microplastic exposures.

1039 A Framework for Ecological and Human Health Risk Assessment of Microplastic Materials: Linking Characteristics to Toxicity

A. Koelmans, Wageningen University and Research, Wageningen, Netherlands. Sponsor: J. Norman

The assessment of microplastics in the environment represents a unique challenge to researchers. Microplastics in the environment are a complex mixture of polymer types, additives, shapes, sizes, and other defining characteristics. In order to describe potential risks associated with microplastics, researchers need to be able to distinguish between effects caused by microplastics from natural and other anthropogenic particles and chemicals. Microplastics also have the added challenge of potential effects being caused by the physical interaction with the microplastic particle (e.g., shape and size) or by interactions with the microplastic’s chemical composition. By assessing potential mode(s) of action for toxicity and their dose responses, probability density functions for particle characteristics and advanced statistical analyses can be used to determine the potential risks of any mixture of microplastics. This framework is applicable to both ecological and human health risk assessments. These unique sets of challenges will be discussed, and the latest risk assessment frameworks will be presented to the attendees.

1040 Biocompatibility Assessment of Absorbable/Degradable Materials Employed in Medical Devices: Challenges, Pitfalls, and Emerging Testing Methods

D. Diaz-Diestra, North American Science Associates LLC, Raleigh, NC.

The use of absorbable materials in medical devices has expanded over the last few decades. These materials are particularly attractive as they do not remain permanently in the body, do not require removal/revision surgeries, and may reduce or eliminate late-stage adverse responses. However, assessing the biocompatibility of these materials and/or risk they pose to patients can be quite challenging given their unique physical and chemical properties. The objective of this workshop is to discuss some of these challenges (e.g., solvent incompatibility during chemical characterization, high variability in leachable profiles, and limitation of preclinical models to predict long-term biological effects). The workshop will also consider ongoing efforts to develop and update methodologies for more comprehensive biocompatibility assessments in order to ensure patient safety and establish regulatory compliance. The first speaker will take a materials-based perspective and present an overview of absorbable materials development, degradation testing, and applications. The second speaker will discuss biocompatibility testing considerations in the regulatory approval process of absorbable-based medical devices. The third talk will review the challenges of biocompatibility evaluation of absorbable medical devices and efforts to improve our understanding of biological responses to these devices through degradation, which enables us to better predict long-term clinical outcomes. The next talk will provide an overview of the new changes introduced to ISO 10993-18:2020 and key scientific aspects of an extractable study, such as information gathering, selection of extraction conditions, sample extraction, sample processing, system selection and qualification, quantification, and identification. Additionally, questions on how to test novel materials for chemical characterization will be discussed. The final speaker will highlight new approach methods for risk assessing extractable compounds from biodegradable medical devices. Also discussed will be best practices and potential pitfalls in applying and interpreting in silico predictions for mutagenicity and Cramer classification. This speaker will also present case studies demonstrating the application of in silico methods to connect structurally discrete polymer degradants to the device materials of construction and possibly to support a chemical category read-across approach.
### 1043 Challenges with Biological Evaluation of Absorbable Medical Devices

S. Skoog, US FDA/CDHR, Silver Spring, MD.

Bioabsorbable materials are widely used in medical devices as they provide significant advantages as non-permanent implants, providing mechanical support and function for an optimized duration to permit healing and/or tissue regeneration. These devices are of significant interest to patients as they do not remain permanently in the body, do not require removal/revision surgeries, and may reduce/eliminate late-stage adverse responses. Absorbable materials have also been utilized in combination products to provide controlled release of drugs. The use of bioabsorbable materials within medical products has dramatically expanded over the past few years, including introduction of novel materials and investigations for new, high-risk device applications; however, assessing the biocompatibility of these materials and/or risk they pose to patients is quite complex since these materials are constantly evolving in the body. Currently, biocompatibility evaluation is conducted on devices prior to degradation, which underestimates the complexity of bioabsorbable materials and does not account for the device degradation products or material property changes over time. Furthermore, standard, extraction-based methods may not provide a physiologically-relevant test sample. Therefore, evaluation of safety and effectiveness of devices containing absorbable materials relies heavily on animal studies which may need to be carried out through complete degradation of the device; however, these studies are time-consuming and expensive and may be insufficient to predict clinical performance due to the use of healthy animal models, interspecies differences, and limited time points. Additionally, current standards for evaluation of absorbable medical devices provide limited useful feedback for biocompatibility assessment. This presentation will review the challenges with biocompatibility evaluation of absorbable medical devices and efforts to improve our understanding of biological responses to these devices throughout degradation to better predict long-term clinical outcomes.

### 1044 New Approach Methods for Toxicological Risk Assessment of Extractable Compounds from Biodegradable/Bioresorbable Medical Devices

J. Cohen, Gradient, Boston, MA.

Biocompatible materials are seeing increased use in medical device applications due to their desirable biocompatibility, mechanical properties, and tunable degradability. However, chemical characterization studies on these materials identify large numbers of unique and complex extractable compounds that lack toxicological data packages. This presentation will provide an overview of predictive toxicology tools currently available for evaluating risks to human health, along with a discussion of potential pitfalls and best practices. First, ICH M7 and ISO 21726, describe computational toxicology methods for predicting mutagenic potential and assigning appropriate thresholds of toxicological concern. In silico programs for predicting mutagenicity or Cramer class must not be used as a black box; one must consider applicability domain and confidence when reconciling conflicting predictions. Comparing Cramer classification predictions to those based on expert judgment for a test set of medical device extractables across several in silico platforms highlights potential pitfalls. showed relatively poor agreement across the five modules: Toxtree version 3.10 (“Cramer rules,” “Cramer rules with extensions,” and “revised Cramer rules”) and the Organisation for Economic Co-operation and Development (OECD) Quantitative-Structure-Activity Relationship (QSAR) Toolbox version 4.4.1 (“Cramer” and “Cramer (Extended).” Some modules showed improved performance for specific chemical classes (e.g. aromatics vs aliphatics). Lastly, extractables profiles of biodegradable devices are often largely comprised of polymer degradants that also lack toxicity data. Connecting degradants to the materials of construction may support justifying a category read across approach. Such an approach would require documenting similarities (and differences) across category members in terms of chemical structure, reactive functional groups, physicochemical properties, predicted toxicity, mode of action, and toxicokinetic and metabolic profiles. In silico tools for predicting mode of action, structure activity relationships, toxicokinetic and metabolic profiles can aid in filling data gaps to support assessing groups of polymer degradants as a chemical class. A case study of hypothetical polyurethane polymer degradants will be presented.
In recent years there has been a rapidly expanding number of approved therapeutic products which target the liver. These new products have led to improved understanding of pathomechanisms and pharmacological pathways. In parallel, substantial progress has been made to systematically monitor patients in clinical studies, as well as evaluate and manage hepatotoxicity associated with new pharmaceuticals and biological agents. In particular, the observation that drug-induced acute hepatic cellular injury with jaundice will progress to acute liver failure (ALF) in ~10-50% of patients as well as reflects an increased liability of a study drug to cause idiosyncratic life-threatening DILI within in a larger treatment population (Hys’ Law) is core principles embodied in the 2009 FDA guidance on the premarketing clinical evaluation of DILI. Findings of hepatotoxicity during drug development and sometimes in early post-marketing settings typically inform product labeling and recommendations for risk management. Nonetheless, there are critical important challenges and gaps that remain. First, depending on both the causative agents and susceptibility characteristics of individual patients, DILI can be triggered by different mechanisms and is linked to a variety of clinicopathological phenotypes that can be clinically severe. Second, biological therapies that target complex pathways. In parallel, substantial progress has been made to systematically evaluate the toxicity of chemicals currently on the market as well as those chemicals have been evaluated for potential risks to human health and safety.

Drug induced liver injury (DILI) is an uncommon but potentially lethal adverse drug reaction. Despite consist progress in DILI research and advances in our understanding of its pathogenesis, DILI remains a challenging problem for drug-developers, clinicians, and regulatory agencies. DILI is a major cause of acute death in >50% of cases in most Western countries (>50% of the cases). Clinically, DILI may mimic almost any known type of liver disease, which often leads to challenges in diagnosis and management. In the absence of universal diagnostic biomarkers, the diagnosis of DILI still requires a comprehensive assessment (non-clinical and clinical), careful review of the clinical presentation, and ruling out of other possible causes. Various instruments and scales for causality assessment currently have limited utility during drug development. It is increasingly accepted that DILI events can be categorized into three groups: (1) Intrinsic (predictable, linked to toxic exposure levels of a drug); (2) Idiosyncratic (rare and unexpected, linked to a yet poorly understood genetic or other predisposing factor); and (3) NIHD (linked to an unwanted biological action of a drug in an individual patient). Each of these categories, there are several well-recognized phenotypes, which are defined based on clinical and pathological criteria. Acute hepatocellular injury has been observed and studied most often, but it is increasingly recognized that other forms of DILI such as drug-induced (DI)-cholostatic liver injury, DI-steatohepatitis, DI-autoimmune hepatitis, and immune-mediated liver injury caused by checkpoint inhibitors (ILIC), can also be serious and even life-threatening. It is well established that DILI may present differently in patients with pre-existing liver disease and can have a worse outcome. Given the increasing number of clinical trials enroll- ing patients and the increased public awareness of chemical/biological effects. Secondary assays may comprise of more complex in vitro systems containing liver cells in a more physiologic state e.g. 3D tissue structures, co-cultures and liver-on-a-chip, enabling assessment of consequences of chronic drug exposure and subtle signaling. Furthermore, complex test systems may be deployed on a case-by-case basis in which a specific biological variable (for example, inflammation, drug metabolism, etc.) is assessed, such as NAFLD, inflammation or genetic variables) is introduced in a manner that can be used for both hazard identifica- tion and risk assessment related to idiosyncratic DILI or DILI where the patient phenotype may alter sensitivity to drugs. It is essential that the baseline physio- logical phenotype of the in vitro model is considered, in particular the predictions, before undertaking toxicological investigations, to ensure that the most appropriate methods are used to determine the potential DILI liability of a new drug. Integrating data from the in vitro toolbox with exposure, signals from early in vivo toxicity studies (clinical chemistry, histopathology) and mathematical modelling is crucial to gain further insights into the ultimate likelihood of DILI in humans and thereby suggesting potential clinical development strategy.
lead to major differences in biological activity. This is one of the reasons the same analogs are sometimes used in read-across for different endpoints based on a similarity index calculated using a general fingerprint. Thus, the goals of this session are to (1) address the misconceptions about chemical similarity by redefining it based on use, context, and how it differs from the perspectives of medicinal chemists, model developers, and regulators; (2) demystify the machine-learning approaches to black boxes and tools relating to structure to activity by identifying the most influential structural features that define similarity for a specific outcome; and (3) identify the best practices regarding how and when to apply machine-learning and class-based approaches depending on the goal and context of use. The first speaker will give an introductory presentation defining the different relevant concepts that play a role in the design and use of the similarity and class-based approaches. The second speaker will provide an overview of the different tools used to encode chemical information. The following talk will address the concept of similarity and the fundamentals of supervised and unsupervised approaches. The fourth speaker will discuss the endpoint-specific similarity measures in relation to the selection of the most adequate analogs. The fifth speaker will highlight the best practices for applying different approaches to example studies. The last speaker will present on the application of similarity measures for supervised and unsupervised approaches.

**1053 Applications of Class-Based Approaches in Risk Assessment and Toxicological Research**

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

This presentation will set the stage for the following speakers by introducing the concept of similarity and its use in the different predictive and investigative approaches commonly applied in toxicological research and risk assessment. This talk will also give an overview of the lessons learned and best practices from different studies of interest to the Division of National Toxicology program at NIEHS involving the use of clustering and classification approaches and its applications to investigate adverse effects of chemicals on human health and the environment. Examples of class-based methods and related cheminformatic tools as part of New Approach Methodologies (NAMs) and their applications to facilitate toxicology research and provide information for risk assessment, will be provided based on case studies about classes of chemicals of high interest such as organo-halogenated flame retardants (OFRs), polyaromatic hydrocarbons (PAHs) and polyfluoroalkyl substances (PFAS).

**1054 Structure Similarity Based on Chemical Descriptors, Fingerprints, and Structural Alerts**


Due to the challenge of assessing the risk of the huge number of chemicals contained in consumer products and in the environment, computational science has been applied with increasing success to various aspects of toxicology. One hypothesis of computational toxicology is that chemicals with similar structures have similar toxicity and cause toxicity in similar mechanisms. Machine learning and deep learning, including unsupervised and supervised learning, have been widely applied in computational toxicology. In general, machine learning and deep learning derive relationships between toxicity testing results and chemicals based on structure similarity measurements between chemicals. Therefore, how to describe chemicals in chemical structure similarity calculation is vital for the success of applying machine learning and deep learning in computational toxicology. Various methods have been developed for describing chemical structures to facilitate machine learning and deep learning in computational toxicology. This presentation will review some most frequently used structural description methods, including structural alerts, molecular descriptors, and fingerprints, discuss their roles in machine learning and deep learning for safety evaluation and risk assessment of chemicals, and showcase applications of structure similarity measurements based on those structural description methods and calculation software packages, and offer a prospect of structural descriptions to enhance applications of machine learning and deep learning in computational toxicology.

**1055 The Multifaceted Challenges of Chemical Similarity**

D. Fourches, Durham, NC. Sponsor: K. Mansouri.

The notion of chemical similarity is pivotal to the fields of cheminformatics and in silico molecular modeling. In the context of risk assessment of environmental compounds, the similarity between pairs of compounds and the pairwise similarity distribution within a given molecular series are routinely computed. The fundamental assumption is that chemicals above a significant threshold of pairwise similarity can be considered biologically similar. However, this notion is filled with various methods to calculate chemical similarity using 1D, 2D, and 3D structural characteristics of a chemical as well as with counterexamples of activity cliffs (i.e., compounds with high similarity but opposite bioactivity). In this presentation, we will (i) review the top similarity metrics to better highlight their relevance for small organic molecules; (ii) discuss several examples of activity cliffs identified by those similarity metrics; and importantly (iii) broaden the concept of contextualized chemical similarity (CCS) with the example of protein degraders (PROTAC-GMT-PROTeolysis-Targeting Chimera).

**1056 Endpoint-Specific Similarity Measures for Read-Across and Weight-of-Evidence**


Read-across is a method to perform data gap filling to address (eco)toxicological properties for target compounds by using available information on related analogues. A key aspect in read-across is therefore the choice of the source chemicals, which can be based on different similarity metrics. We will present some attempts to better identify suitable candidate source chemicals according to the best practices for applying different approaches to example studies. The last speaker will present on the application of similarity measures for supervised and unsupervised approaches.
for this transformative vision to be realized. Well supported by current knowledge, the first principle is that new computational tools (for detecting alteration in gene expression that result from endogenous or exogenous influences on the test organism. The second principle is that alterations in gene expression are indicators of adverse or adaptive biological responses to stressors in an organism. Principle three is that transcriptomics can be employed in order to establish a benchmark dose response curve that will inform from short-term in vivo dose toxicology. Dose-response curve which a concerted molecular change is not expected. Finally, principle four states that the use of a transcriptomic point of departure (set at the concerted molecular change dose level) will support human health protective risk assessment. If all four principles are substantiated, this vision is expected to transform aspects of the industrial chemical and agrochemical risk assessment process that are based on establishing safe exposure levels for mammals across numerous toxicological contexts. This results in a significant reduction in animal use and provides equal or greater protection of human health. In this Roundtable, we plan to discuss these principles and elicit feedback from experts in the field regarding the feasibility, limitations, and barriers they foresee, particularly for regulatory acceptance.

**1060 Evaluating and Rethinking the Use of Dogs in Agrochemical Evaluation and Registration**
L. Murphy, Corteva Agriscience, Indianapolis, IN.

The goal of this session is to assemble various perspectives on the need and use of the dog as a required species for agrochemical human safety evaluation. This session will examine the problem statement that the 90-day dog study required for agrochemical registration may not always be needed to adequately assess hazard identification and human safety and risk. A historical perspective will be presented regarding the rationale for use of the dog in chemical testing and the changes related to dog study requirements by regulatory agencies. Two different approaches to retrospective analyses of active-substance registration in the US and Europe, respectively, will demonstrate the limited value of a 90-day dog study in determining human health risks from exposures to agrochemicals. Two case studies from herbicide and fungicide active ingredients reevaluated by the Agencia Nacional de Vigilância Sanitária (Anvisa) will consider how rat toxicity study data and 90-day dog studies can be used to determine species sensitivity for thyroid effects. A case example will be presented to demonstrate the use of in silico and in vitro tools and available in vivo data to support waiving a 90-day dog study. Following these presentations, a panel discussion will allow panel members from regulatory agencies, industry, and nongovernmental organizations to highlight key challenges and opportunities to determine when a 90-day dog study is unnecessary. Attendees will be encouraged to share their perspectives on the criticality of 90-day dog studies in agrochemical risk assessment and regulatory decisions. Topics for open discussion will include research ideas to investigate if human-relevant safety assessment decisions can be obtained from approaches other than the use of dogs, types of data and approaches that can inform the selection of human-relevant species, proposed criteria for waiving 90-day dog studies, and potential case studies to address the questions raised in this abstract.

**1061 OK, Google, “How Do I Science?” Roles and Responsibilities of Developers and Users in Computational Toxicology**
L. Burgoo, Raptor Pharm & Tax Ltd., Apex, NC.

As interest in computational approaches to understanding chemical-biological interactions soars so does the call for making the data and tools more accessible. This argument for democratization of data and methods—the idea that everyone should have access to the data and tools with which to understand adverse effects and outcomes—is not without good cause. Increasing the adoption of computational approaches has the potential to create more ethical and relevant chemical assessments in a cost-effective manner. Democratization will help drive tool and algorithm development and may one day lead to a more efficient and safer drug development process. Web-based interfaces combining data and user-friendly tools offer a low barrier of entry that can enable individuals with no training to run complex analyses that only a few years ago required a high degree of training. The benefits are apparent: more people will have access to more data, and more minds will be able to see, interact with, and ultimately draw insights from the data. What could possibly go wrong? This session aims to discuss the opportunities open computational toxicology present for increasing engagement and better science along with the opportunities for misuse and abuse. Do data generators and developers have an obligation to ensure the responsible use of these resources? What about the responsibilities of users of both the tools and the resulting analyses? Ultimately, what are the ethical, legal, and social implications of data democratization? And on the flip side, what are the implications of gatekeeping access to these resources? This session will begin with an overview by the Co-Chair, followed by a brief description of each panelist’s position(s). This will lead into a moderated discussion between the panel and audience during which we will discuss these topics with the aim of increasing the collective awareness and identifying opportunities and challenges to purposefully move forward into the computational era of toxicology.
significant deficiencies especially in their ability to assess toxicity. In particular, toxicities such as CRS were not predicted based upon these models due to the requirement for interactions among immune and non-immune cells that are not present in standard immunodeficient mouse strains such as the frequently used NOD-SCID-gC-/- strain. Further humanization with engraftment of human hemopoietic cells can generate some CRS-like toxicities, but these additional modifications vastly increase model costs and require careful planning to achieve reproducibility. Beyond the challenges of modeling immune dysregulation phenomenon such as CRS, most therapeutic targets of human T cell-based immunotherapies are human specific and the therapeutic targets are not therefore generally present in normal mouse tissues making preclinical assessment of on-target, off-tumor toxicity largely unattainable. This presentation will review the role of preclinical animal models in developing T cell therapies such as CAR T cells and the current approaches to assessing toxicity risk of these complex cellular therapies.

Chimeric antigen receptor T (CAR T)-cell therapy has emerged as a powerful new therapeutic option for patients with hematologic malignancies. Although several CAR T clinical trials have shown encouraging results, broad use of CAR T therapies is limited by various reasons, including life-threatening toxicities such as cytokine release syndrome (CRS) and neurotoxicity, individual variability, and high cost. Several mouse models, with or without immunocompromised NSG™ variants, have been reported as in vivo tools to assess the toxicity and efficacy of CAR T therapy. Naive NSG™ mice bearing B cell precursor leukemia cell line Nalm-6 showed significant human cytokine induction after being treated with CAR T cells. Another study used CD34-humanized NSG™-SGM3 mice bearing ALL-CM leukemia cell line, and showed that CAR T-induced toxicity, including CRS and fever, is tumor burden-dependent. While both humanized mouse platforms will be discussed, we recently have developed a novel PBMC-humanized mouse model. This model is more physiologically relevant, and simultaneously assesses the efficacy, safety, and downstream toxicity of CD19 CAR T-cell therapy. Various NSG™ variants including those that show enhanced sensitivity to CRS due to the expansion of additional human immune cell populations were used. Mice were humanized using PBMCs and treated with autologous or allogeneic CAR T cells, and efficacy, safety, and downstream toxicity were assessed. PBMC humanized mice treated with autologous CD19 CAR T showed significant CAR T expansion and efficacy. Both T-cell and myeloid cell-associated cytokines were induced in these humanized mouse models after CAR T cell treatment, and downstream toxicity could be observed. We further addressed the efficacy and safety of autologous CAR T treatment in PBMC humanized mice bearing human B-cell lymphoma Raji tumor cells. To potentially utilize the platform for universal "off-the-shelf" CAR T therapeutics, we modeled the platform by treating PBMC humanized mice with allogeneic CAR T. The allogeneic CAR T treatment showed minimal CAR T expansion, and efficacy and safety profile varied depending on the PBMC donor. In summary, we have developed a novel PBMC-humanized mouse model to test the efficacy, safety, and downstream toxicity of both autologous and allogeneic CAR T-cell therapy.

Botanical supplements and herbal medicines are used globally and are growing in popularity for both disease treatment and prevention in some regions, especially because of the COVID-19 pandemic. Botanicals encompass plant-derived products, fungi, and algae and are naturally complex with varying chemical compositions because of factors such as differences in growing conditions, extraction processes, and changes to the finished product. Ensuring the safety of these products is an important public health priority, yet most regulatory structures do not require toxicity testing and rely on information related to the history of safe use. Conventional in vivo and in vitro toxicity testing methods that are often used for safety assessments of chemicals and pharmaceuticals are considered inadequate for use on single, discrete chemicals rather than the complex mixtures that represent most botanical substances. As a result, toxicity data for many botanical mixtures are lacking, and few new approach methodologies (NAMs) have been evaluated for their suitability with these complex mixtures. This session will provide an overview of the current regulatory landscape for botanical products, discuss gaps and recent efforts and study data to improve or design new methodologies, and highlight successes and challenges associated with study design, method development, and data interpretation of these approaches. The session will begin with a brief outline of botanical dietary supplements, highlighting the importance of evaluating toxicity for these complex mixtures because of their potential for dose, route, and duration of exposure/application. Conventional in vitro and in vivo methods giving preference to those not requiring mammalian animals. Typically, both humanized mouse models and humanized models have been used to study the potential risks associated with these mixtures. Similarities and differences between humanized mice and humanized models will be discussed. The session will then be followed by an overview of the current regulatory landscape. The next three talks will highlight specific progress and assay results, including novel data generation, across various NAMs that can be used to evaluate diverse endpoints of toxicological importance. These include developmental neurotoxicity batteries, in vitro enteroxicity and hepatotoxicity assays, and toxicogenomics approaches to evaluate systemic toxicity.

Safety concerns have been raised due to the occurrence of certain natural plant constituents in botanicals. The pure constituents and, in some instances, also parts of the plant or extracts thereof may exert various types of toxicity depending on the dose, route and duration of exposure/application. The compounds of concern may either originate from the plant used or from a contamination with other plants or weeds. Hazard considerations mostly based on animal or in vitro tests may include genotoxic or carcinogenic properties, system and/or organ toxicity etc. New and Alternative Methods (NAMs) are more and more widely used in order to restrict animal experiments to a minimum. NAMs include all types of in silico and in vitro methods giving preference to those not requiring mammalian animals. Typically, these methods comprise in vitro genotoxicity testing, toxicity testing in tissue-specific cell culture, in vitro absorption and metabolism studies etc. Furthermore, in silico searches for structural alerts including read-across of related structures are applied. In addition to hazard identification, combined cell culture-based toxicokinetic and toxicodynamic testing strategies may allow modeling of effective target concentrations of a chemical and/or its metabolite(s) upon a defined (human) exposure. The testing should be applied to selected constituents (to be quantified by targeted chemical analysis) of a plant species (or family) known from the literature. Furthermore, non-targeted analysis and testing of whole extracts may provide additional information. Since natural plant constituents often occur as large numbers of derivatives (constituents), establishing relative toxic potency factors may be warranted to allow for a science-based risk assessment. For this purpose, NAMs provide an excellent tool, even allowing structure-related predictions of potencies. Examples will be demonstrated for the family of pyrrolizidine alkaloids, for phototoxic furanocoumarins and for the diverse group of alkylbenzenes.
### 1071 Dietary Supplements: Regulatory Overview and the Evaluation of Safety

R. Yeager. US FDA, College Park, MD.

The Dietary Supplement Health and Education Act (DSHEA) of 1994 transformed the FDA's authority over dietary supplements. In the past 28 years, the dietary supplement market has grown significantly from approximately a $4 billion industry comprised of about 4,000 unique products to an industry worth almost $50 billion, with as many as 80,000 different products available to consumers. Making informed decisions about dietary supplements can have a significant and positive impact on Americans’ health and consumers need to have access to safe, high-quality, and properly labeled products. The FDA works to achieve the right balance between preserving consumers’ access to lawful supplements, while still upholding our solemn obligation to protect the public from unlawful and, possibly, unsafe products. As the FDA regulates the dietary supplement marketplace, it’s critical that we work closely with our partners in industry, academia, and government to achieve our unified goal of protecting public health and safety. As the dietary supplement industry develops new products and ingredients, advances new delivery systems, and innovates in other ways, the FDA must do more to leverage its existing resources and authorities to evaluate these products. The Botanical Safety Consortium gathers the leaders from industry, academia, and government to promote scientific advances in evaluating the safety of botanical ingredients and mixtures in dietary supplements.

### 1072 Development of Neurotoxicity Battery for Botanicals at the US Division of Translational Toxicology

C. McPherson. NIEHS, Research Triangle Park, NC.

Researchers at the Division of the National Toxicology Program along with governmental, industry, and academic partners have been engaged in efforts to develop and evaluate a battery of developmental neurotoxicity assays to screen chemicals for potential to disrupt the complex processes necessary for normal neurodevelopment. This battery includes 2D and 3D in vitro assays to measure neuronal cell migration, neurite growth, and neural network formation and function, as well as the zebrafish assay to measure neurobehavior. To extend the application of these assays from single chemicals to complex mixtures, we recently evaluated a series of botanical extracts with evidence of potential neurotoxicity based on the literature. The botanicals include aconite, guarana, yohimbine, bupverupum, and wormwood. Multiple samples for each botanical ingredient were evaluated in the battery. Initial results indicate that some of the samples elicited changes in locomotor function in zebrafish (both increasing and decreasing activity). However, consistent results across related samples were not observed indicating that variability in samples could complicate interpretation of results. Further work will explore uptake of key constituents to allow for more direct comparison of the results from different formulations of the tested botanical ingredients.

### 1073 In Vitro Experimental Systems for the Evaluation of Human Enterotoxicity, Hepatotoxicity, and Herb-Drug Interaction Potential of Botanicals

A. Li. Discovery Life Sciences, Columbia, MD.

Some herbal supplements are now known to be associated with hepatotoxicity, enterotoxicity, and pharmacokinetic herb-drug interactions in the human population. Two metabolically competent in vitro experimental systems, namely, cryopreserved human intestinal mucosa (CHIM) and cryopreserved human hepatocytes (CHH), have been used or proposed to be used in the evaluation of the hepatotoxic, enterotoxic, and drug-interaction potential of botanicals. Both experimental systems are competent in the complete human enteric (CHIM) and hepatic (CHH) drug metabolism pathways, including phase 1 oxidation and phase 2 conjugation. Drug-drug interactions are focused on CYP3A4 inhibition and induction, while toxicity is evaluated using cellular ATP contents and MTT metabolism as endpoints. In addition, a novel experimental system, the metabolism-dependent cytotoxicity assay (MDCA) utilizing HEK293 cells, a human renal cell line devoid of xenobiotic metabolism as the target cells for cytotoxicity, with permeabilized cryopreserved human hepatocytes (MetMax human hepatocytes) as an exogenous metabolic system, and pathway of metabolism defined by co-factor supplementation, is applied in the evaluation of the potential formation of cytotoxic reactive metabolites from the botanicals.

### 1074 Incorporation of In Vitro Techniques for Botanical Safety Assessment

J. Naicoff. Procter & Gamble, Cincinnati, OH.

One of the common data gaps in the assessment of botanicals is related to the determination of their systemic toxicity potential. Comprehensive transcriptional profiling allows the determination of the effects of a given botanical on gene expression in an organism or cell type, elicited by virtually any mechanism, and with that the identification of the specific cellular mechanisms and pathways modified by exposure to this complex mixture, which could indicate potential toxicity. The comprehensive transcriptional response of MCF7, HepG2, A549 and ICell cardiomyocytes was evaluated (Tempo-Seq) after exposure to vehicle-control or to milk thistle, ginseng, Ashwagandha, blue cohosh or Usnea lichen extracts at 3 non-cytotoxic concentrations for each botanical (at a fixed concentration for each botanical, at each dose of exposure for each cell type evaluated), and used to determine which the specific cellular pathways were modified by the exposure, using pathway enrichment analysis. Connectivity mapping (CMap) was also used to identify chemicals with similar transcript profile to the one elicited by each botanical. This information was used to identify chemicals with similar biological activities and to perform potential toxicity for each botanical. For example, the transcriptional profile of Ashwagandha identified in A549 and HepG2 treated cells had a high degree of similarity to the one elicited by withaferin A, a constituent found in Ashwagandha. Further, top connections include pharmacologically active chemicals targeting specific cellular pathways including: CIP2A, c-MYC (celastrol), SHT1A (thujone) A1A and A2A receptors (phenoxybenzamidine) among other biochemical pathways. Pathway enrichment analysis indicated that the main upregulated pathways by Ashwagandha are MTOCR1, p53, TNFA signaling via NFkB and others, while the main downregulated pathways are cell cycle control, E2F, G2M checkpoint and myc targets, among others. In all, this study demonstrates the utility of transcriptional profiling together with CMap analysis for identification of MOAs associated with botanical exposure via a functional read across approach.

### 1075 Placenta as a Target of Environmental Exposures: From Bench to Society

R. Strakovsky. Michigan State University, East Lansing, MI.

Pregnancy is considered among the most vulnerable life stages, for both the mother and the child. Despite being a critical bridge between the maternal exposure and fetal development, the placenta is a severely understudied organ. Led by Dr. Almudena Veiga-Lopez and Dr. Rita Strakovsky, this session features a diversity of early career, mid-career, and well-established investigators who come from federal and academic institutions and have expertise in epidemiology, animal models, and clinical and molecular epigenetics. These investigators will highlight the importance of the placenta as a target of chemical exposures, with certain chemicals (organophosphates and pyrethroids) pointing to the placenta as a chemical reservoir since they accumulate in the placenta and fetal compartment to a higher degree than that of the maternal serum. The session will feature new and exciting research related to the impacts of various environmental chemicals which cross the placental barrier with potential implications for placental disorders, such as preeclampsia and miscarriage.

### 1076 Phthalate Exposure and Longitudinal Ultrasound Measures of Placental Function in the Human Placenta Project Cohort

K. Ferguson. NIEHS, Research Triangle Park, NC.

Dr. Ferguson’s research group will present data from a human study containing prenatal phthalate and phthalate-replacement exposure measurements and repeated measures of placental function. Phthalates are endocrine disrupting chemicals which cross the placental barrier with potential implications for pregnancy and fetal development. Several prior cross-sectional epidemiologic studies have shown associations of phthalates with placental health outcomes, but these studies have been limited to measures of placental weight at delivery, crude biomarkers of placental function, or cross-sectional umbilical or uterine artery blood flow assessments in utero. Nevertheless, molecular epidemiology studies indicate that prenatal phthalate exposure is associated with changes in epigenetic and protein expression markers in the placenta. These findings may confirm gross changes to placental development and function in vivo. In this presentation, Dr. Ferguson will present on the association between phthalate metabolite levels and placental outcomes including microvascularization, calcifications, and...
Placental Glucose Transfer after Gestational Parturicte Matter Exposure

P. Stapleton, Rutgers Ernest Mario School of Pharmacy, Piscataway, NJ.

Exposure to air pollution, and specifically aerosolize particulate matter, during pregnancy has been associated with reduced fetal growth in epidemiological and laboratory studies. Limited fetal growth has been identified as a developmental risk factor for many pregnancy, neonatal, and lifetime conditions including perinatal mortality and metabolic diseases; however, the functions of the placenta within this model has been woefully overlooked. We recently identified that nanosized/ultrafine metallic or plastic particles have the propensity to migrate from the mother’s lung and deposit within the placenta, thereby providing local and direct interaction(s) between the placentals trophoblasts and the xenobiotic particle. Glucose is the primary fuel source for fetal growth, development, and metabolism; therefore, the particle must pass through the maternal interface, through the placenta, to the fetus for utilization. Therefore, we hypothesized that glucose passage through the placenta to the fetus may be impaired after maternal inhalation of aerosolized titanium dioxide nanoparticles, thereby limiting fetal nutrient resources and subsequent growth. Initial results of these studies indicate a sex-dependent effect in fetal blood glucose after maternal gestational exposure. In this presentation, we will discuss our recent studies focused on placental glucose metabolism and glucose transport to the fetal compartment. Our findings indicate that alterations to placental health and function due to gestational environmental exposure may not only influence perinatal health, but also promote the developmental origins of metabolic disease.

Environmental Modulator of Placental Tryptophan Catabolism and Influence on Maternal-Fetal Immune Tolerance

M. Susiario, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Humans are ubiquitously exposed to endocrine disruptors including bisphenol A (BPA). Wheningested orally, the biologically active parent BPA compound undergoes rapid first pass metabolism predominantly to BPA glucuronide. Detectable levels of unconjugated BPA, however, have been found within tissues of the maternal fetal interface suggesting an inefficient deconjugation process that could leave humans at risk of adverse health effects. Exposure to the endocrine disruptor bisphenol A (BPA) is linked to human miscarriages and pregnancy complications, however the underlying mechanisms are still poorly understood. In contrast, the potential reproductive toxicity of the analogs including tetrabromobisphenol A (TBBPA) is relatively understudied. Pregnancy loss can be caused by aberrant maternal fetal immune tolerance. One proposed mechanism is disruption in indoleamine 2,3-deoxygenase 1 (IDO1)-mediated tryptophan catabolism that impairs expansion of regulatory T cells (Tregs) and creates excessive pro-inflammatory responses against the semiallogeneic fetus. Our laboratory is interested in elucidating whether environmental exposure during pregnancy alters maternal immune environment in mice. We investigate the effects of exposure to 10 mg/kg body weight (bw)/day BPA and 500 microgram/kg bw/day TBBPA during pregnancy. The exposure paradigm results in maternal serum BPA levels of 2 ng/mL that is within the range of internal BPA exposure in humans. The exposure level of TBBPA is below its oral reference dose of 600 microgram/kg bw/day for reproductive toxicity in rodents. Exposure to BPA and TBBPA results in higher rates of pregnancy loss in allogeneic pregnancies. These effects were associated with lower number of maternal CD4+ T cells and Tregs. Interestingly, BPA and TBBPA-induced pregnancy loss were linked to lower protein expression of the IDO1 in trophoblast giant cells. Our studies suggest that placental tryptophan catabolism plays a role in pregnancy maintenance and that maternal exposure to BPA and TBBPA adversely influence pregnancy success in mice through mechanisms that reduce Tregs and impair IDO1-dependent maternal fetal immune tolerance.

Legacy and Replacement Per- and Polyfluoroalkyl Substances (PFAS) Target the Placenta

S. Fenton, NIEHS/NTP, Research Triangle Park NC.

Legacy, well characterized per- and polyfluoroalkyl substances (PFAS) are being replaced with a variety of PFAS congeners that are data poor; specifically, with little known about their effects on the placenta or developing fetus. In epidemiologic studies, perfluorooctanoic acid (PFOA), a bioaccumulative PFAS, is reported to increase risk for pre-eclampsia. The latter of which is also reported in mice. PFOA’s replacement, commonly called HFPPO-DA or GenX, significantly decreased mouse pup weight at culling, increased placental weight, decreased the fetal/placental weight ratio, and caused excessive gestational maternal weight gain at 2 mg/kg/d. Similar effects were evident in mice at 5 mg/kg GenX (half-life >550 days), even though the half-life of GenX is <1d. The PFAS induced unique patterns of adverse lesions of the placenta at term. However, gestational exposures to 1 mg/kg/d PFOA or GenX led to nearly identical latent cardiometabolic and liver outcomes that varied by sex. Affected biological pathways related to the latent health outcomes and/or fetal growth have been identified in male and female placentas from these studies. To characterize placental effects in PFOA more broadly, we varied family members tested in a multiplexed high throughput screen for proliferation, cytotoxicity, and mitochondrial membrane potential changes across a 10-point linear concentration curve ranging from 50-500 µM in JEG-3 human placental trophoblasts. Concentration-response model estimates (EC50) were calculated for at least one endpoint in roughly 80% of evaluated PFAS. Six of those PFAS were compared from 1-300 µM for effects on cellular migration and expression of 46 critical genes involved in placental growth, differentiation, and pregnancy maintenance. We observed gene expression changes in 300 µM GenX-exposed JEG-3 cells that are consistent with its persistent inhibition of trophoblast migration at <200 µM, such as reduced IGF2 and ERBB, and increased CTNNB1 expression. Uptregulation of ABCG2 expression in JEG-3 cells after 24 h exposure to GenX (3 or 300 µM) or PFOA (100 µM), and significant increases in expression of estradiol-related or -modifiable genes (i.e., ESRR, GPR1, VEGFA,HS1D1) by 100 µM PFOA or 300 µM GenX suggest important modes of action for future study.

Single Cell and Cell Type-Specific Analyses in Human Placental Tissues: Implications for Chemical Exposures and Adverse Birth Outcomes

K. Bakulski, University of Michigan, Ann Arbor, MI.

The placenta mediates adverse pregnancy outcomes and is perfused in chemical toxicants exposures circulating in maternal blood. Environmental toxicology and epidemiology studies demonstrate maternal exposures to chemicals are associated with gene expression and DNA methylation differences in bulk placental tissues. The placenta is a complex structure with specialized cells, and placentacell type heterogeneity limits mechanistic interpretation of bulk placental gene expression and epigenetic measures. Using single cell and sorted cell methods, we generated human term placental cell type specific gene expression profiles for 19 fetal and 8 maternal cell types and cell type specific DNA methylation profiles for 6 fetal cell types. We applied these reference profiles as tools to deconvolute cell type proportions in independent datasets and tested for differences in cell abundance. We demonstrated that overabundance of extrathrophoblast cell types mediated 35% of the association between preeclampsia and FL1 gene over expression in the placenta, underscoring the role of placental extravillous trophoblasts in opening maternal spiral arteries, a key pathway in preeclampsia pathology. We newly apply these cellular deconvolution methods to placental toxicology studies of common chemical exposures with documented placental adverse effects. We applied these methods to placental tissues across the course of gestation as well as within specific periods (e.g., trimesters). Importantly, unlike in prior studies, exposure measures are based on spot urine phthalate and phthalate alternative metabolite measurements from 8 time points across pregnancy, which allows for better estimates of maternal exposure across the course of gestation as well within specific periods (e.g., trimesters). Furthermore, urinary metabolite measurements are the gold standard for phthalate exposure assessment and prefer over placental measures since contamination of tissue and blood samples occurs readily in the laboratory. Additionally, the novel outcome measures were captured at 8 time points during gestation by ultrasound, which may provide information on phthalate-induced placental dysfunction at a sub-clinical level.

Placental Transcriptomic Alterations Underlie Neurodevelopmental Effects of Pesticide Exposure

C. Marist. Emory University, Atlanta, GA. Sponsor: B. Strakovsky.

In both human and animal studies, organophosphate and pyrethroid pesticide exposures during the gestational period have been linked to offspring neurobehavorial deficits. Despite policies aimed at reducing use, these chemicals remain widely utilized around the globe. Organophosphates, such as chlorpyrifos, act through inhibition of acetylcholinesterase and through impacts on serotonin. In contrast, pyrethroid pesticides, such as deltamethrin, act by inhibiting voltage-gated sodium and chloride channels, through GABAergic signaling, and through impacts on the dopamine system. Both pesticides can cross and accumulate in the placenta, with concentrations in placenta generally higher than observed in maternal samples and lower than fetal brain and liver. This is of interest, as a number of neuronally active pathways, such as dopamine and serotonin production and response are active within the placenta. Exposure of human trophoblast cells to chlorpyrifos impacted gene involvement in vasculatization and placental development, while direct effects of pyrethroids on the placenta are not comprehensively characterized. We have examined in utero exposure to common pyrethroids and organophosphate pesticides using a hybrid study in a rodent model and human observational cohort of farm-working pregnant women to investigate the functional role of placenta in linking exposure and postnatal neurodevelopment. In a C57BL/6J mouse developmental exposure model using whole transcriptome sequencing of both offspring...
Flame retardants are high-production volume chemicals used worldwide within several product categories, including electronic equipment, furniture, building materials, and vehicle parts. They constitute a wide group of chemicals with diverse physicochemical properties that are combined to achieve maximum efficacy to delay combustion. However, many flame retardants can migrate from end-use products into the ambient environment over time, increasing the potential for human exposure and subsequent health effects. While newer flame retardants are formulated and introduced into commerce, legacy flame retardants continue to be present in end-use products, specifically within older furniture and cars. Chronic leaching of flame retardants in these two settings can potentially cause further exposures and health risks. This is of specific concern within minority populations and lower-income households, many of whom are likely to purchase used furniture/cars or continue to use older products which leads to potential health disparities in many areas. Thus, we need a better understanding of the exposure patterns and health effects of flame retardants with the goal of designing safer formulations and undertaking behavioral interventions to reduce exposures. The objective of this globally relevant Symposium is to assemble leading investigators who are actively conducting research on flame retardant exposure and health effects so that a multidisciplinary discussion on topics related to flame retardant exposure, toxicology, risk assessment, epidemiology, and sustainable chemistry may ensue. Following a brief introduction on flame retardant regulatory policies, the first two speakers will discuss personal flame retardant exposures from diverse probable sources (i.e., indoor, food, and vehicular microenvironment), exposure disparities in historically underrepresented and low-income populations in two distinct geographical locations (California and New York), and potential health effects and behavioral interventions necessary to reduce exposure. The third speaker will review the impact of adult flame retardant exposures on neuronal excitability, behavior, and energy homeostasis in a mouse model. The fourth speaker will focus on using zebrafish as an in vivo screening model for flame retardants hazard assessments, biomarker identification, and prioritization for testing. Finally, the fifth speaker will consider the use of toxicological data to advance the safer and sustainable design of flame retardants. The session will end with a panel discussion. Overall, the session will interest researchers and stakeholders with a focus on flame retardant use, exposure, and safety. It also will provide a well-rounded discussion on the current state of research and flame retardant exposures and exposure disparities, health risks, molecular targets, and risk assessment strategies that guide safer and sustainable chemistry in the future.

**1084 Brominated Flame Retardants as a Persistent Health Threat: Exploring Health Impacts in Vulnerable Human Populations**

A. Kupcu, Columbia University, New York, NY.

Polybrominated diphenyl ethers (PBDEs) are a class of legacy brominated flame retardants that were produced as a mixture to satisfy fire-safety regulations. Although PBDEs were voluntarily phased out in 2004, exposure continues due to infrequent replacement of PBDE-containing products and a long environmental half-life. These sources are particularly relevant for low-income and minority populations who are more likely to purchase used furniture or infrequently replace old products. Furthermore, it is anticipated that PBDEs from consumer products in landfills will migrate into the food chain, resulting in new exposure sources. PBDEs are endocrine disrupting compounds with documented adverse impacts on child neurodevelopment in both humans and animal models. However, early life PBDE exposure may also impact child growth and adiposity. Perinatal PBDE exposure in rats demonstrate that PBDEs increase body weight, altering adipokines, carbohydrates, lipids, and steroid metabolism. However, to date, findings in human populations have been mixed. We examined the associations of prenatal PBDE exposures with child birth outcomes, growth, and adiposity in a population of Dominican and African American mother-child pairs from New York City. We find that higher BDE-153 was associated with lower birth weight z-score and longer gestation. Moreover, higher BDE-199 was associated with lower birth length z-score. However, neither cord levels of individual PBDEs nor the total PBDE mixture were associated with overall BMI z-scores in later childhood, suggesting that these early indicators do not lead to more growth, nor growth, but this presentation will explore the evidence for the impacts of prenatal exposures to PBDEs on child health, focusing on understudied health outcomes in understudied populations.

**1085 The Influence of Adult Exposures to Organophosphate Flame Retardants on Energy Homeostasis, Behaviors, and Hypothalamic Neural Excitability**

T. Repeke, Rutgers, The State University of New Jersey, New Brunswick, NJ.

Organophosphate flame retardants (OPFRs) are used in household and workplace products and are considered endocrine disrupting compounds. We have reported that exposure to an OPFR mixture causes sex-dependent disruptions of energy homeostasis through alterations in ingestive behavior and activity in adult mice potentially via estrogen receptors (ERs). Because feeding behavior and energy expenditure are coordinated by the hypothalamus, we hypothesized that OPFR disruption of energy homeostasis occurs through modulation of the melanocortin circuitry within the arcuate nucleus. We exposed male and female transgenic mice expressing green fluorescent protein in either NPY/AgRP or POMC neurons to a common mixture of OPFRs for 4 weeks. We examined neuronal excitability and hormone sensitivity using whole-cell patch clamp electrophysiology. OPFR exposure depolarized the NPY/AgRP resting membrane and dampened the M-current within the same neurons from female mice. These neurons were also more sensitive to ghrelin, which more potently reduced the M-current in OPFR-exposed females. POMC neurons from female mice exposed to OPFRs exhibited elevated baseline excitability and experienced greater excitatory synaptic input. In a follow-up study, adult male and female mice were treated with the same mixture of OPFRs for 7 weeks on a low-fat diet (10% kcal fat) or a high fat diet (45% kcal fat). Consistent with our previous observations, OPFRs altered weight gain in males while females remained unaffected. OPFRs also disrupted metabolism and behavior. During the night, males exhibited elevated activity and oxygen consumption, while in females these parameters were decreased. OPFRs disrupted feeding behavior and abolished diurnal water intake patterns in females, while increasing nighttime fluid consumption in males. Despite no effect on glucose homeostasis, OPFRs altered circulating insulin and leptin in females and ghrelin in males. Together, these data support a sex-selective effect to increase neuronal output from the melanocortin circuitry governing feeding behavior and energy expenditure; thus, influencing the effects of diet-induced obesity by altering activity, ingestive behavior, and metabolism. Broadly, this work suggests that flame retardants should be assessed for impacts on the hypothalamic neurocircuitry that controls metabolism from adult and perinatal exposures.

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Aging is associated with cellular changes in the brain which drive functional deficits in both cognition and motor movement associated with common age-related neurodegenerative diseases such as Alzheimer’s and Parkinson’s. Many studies to date have focused on how genetic differences drive individual susceptibility to disease presentation. However, it has become increasingly apparent in recent years that an equally important contributor to the age-assembler of the age-associated cognitive impairments is environmental factors including exposures to, for example, pesticides and medicines. These can affect important cellular processes needed to maintain cellular or tissue integrity resulting in: (1) reduced ability to remove damaged proteins and other cellular components through a process called autophagy and (2) age-related increases in a cell fate known as senescence which can result in widespread tissue damage. This presentation will concentrate on the tails side of the toxic aging coin, assessing the role of environmental toxins as they relate to development of these age-related changes known to contribute to age-related neurodegeneration.

Validation is an indispensable step to facilitate regulatory acceptance. Still, it is on implementation of animal-intensive studies. Recent progress in the development of fit-for-purpose and biologically relevant NAMs enable adoption of alternative methods for new molecule development, and regulatory registration purposes. NAMs that have been validated and adopted as OECD test guidelines (OECD TGs) are currently being applied to replace traditional in vivo testing for global registration of new active ingredients and formulations. For many complex toxicological endpoints, a battery of promising NAMs, not yet adopted as OECD TGs, could be applied to understand mode of action, assess key mechanistic events in the development of toxicity, biological relevance, and for better interpretation of data via weight of evidence assessment, while establishing protective and robust point of departure for human health risk assessment. In this presentation, direct and indirect mechanisms of thyroid toxicity are used as proof-of-principle, to demonstrate the applicability of various promising NAMs, including in silico and in vitro assays, zebra fish models, and omics approaches for toxicity profiling of plant protection products at various stages of molecule development, for stage-gate advancement decisions and for global registration and re-registration purposes. In addition, the significance of compiling well-characterized positive and negative reference chemicals, and experiences and key learnings on implementing multi-organ microfluidic systems for thyroid models will be discussed.

Validation is an indispensable step to facilitate regulatory acceptance. Still, it is currently being identified by many stakeholders as the main reason for the lack of sufficient advancement in the implementation of New Approach Methodologies (NAMs) in a regulatory context. An evolution of early practices is needed to embrace emerging technologies, the increased complexity of the information measured (e.g., ‘omics), and the need for data integration to address complex endpoints. For example, it may not always be necessary to conduct inter-laboratory ring trials to assess the reproducibility of a NAM. Ring trials are unnecessarily lengthy and expensive, and their added value is probably minimal as they are usually more a reflection of laboratory quality or expertise rather than NAM reliability. Instead of ring trials, it may be more valuable to focus on well-designed training and transfer studies to show that the NAM can be repeated in a naive laboratory. Moreover, post-validation proficiency testing adds confidence to the capacity of a laboratory to use the test method. On the other hand, as more validation studies are sponsored and managed by method developers, ensuring data integrity, transparency and independent review of the study becomes paramount. This talk provides an overview of the challenges and opportunities for adapting the validation practices to keep pace with scientific progress whilst ensuring scientific confidence and the protection of human health and the environment. Examples of current validation studies applying new practices will be presented and discussed.
The OECD is a forum to discuss criteria for development and implementation of toxicity testing methods. The suitability of these methods is assessed by principles in OECD Guidance Document (GD) 34 on Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment, often referred to as the "validation handbook." Like most OECD documents, GD 34 is intended to be flexible, modular, and broadly applicable to the needs of Member Countries, and thus, the approaches for demonstrating validity are amenable to a variety of national legislations. Considerable experience was gained developing (in vivo) test methods before validation principles were published. Similarly, it is expected that the principles for building confidence and validating NAMs may be refined with experience developing and implementing these methods. The OECD Integrated Approaches to Testing and Assessment (IATA) Case Studies Project provides examples of NAMs designed to address a variety of regulatory scenarios. Though not all approaches are amenable to all regulatory needs or legislative mandates, the critical review and discussion of such applications has helped to define steps to build confidence in such innovative methods. The new paradigm for assessing confidence in NAMs presented earlier in this session was adapted from the principles for developing the OECD Test Guideline on Defined Approaches for Skin Sensitisation, which initially started as a series of IATA Case Studies. This talk will discuss how a new paradigm for assessing relevance of NAMs continues to evolve with the experience gained through case studies. A parallel process of evaluating NAMs that are not (yet) proposed as Test Guidelines, and thus do not meet the level of validation required for the mutual acceptance of data (MAD), provides users with a high degree of scientific confidence based on a thorough description of the methods, harmonised data reporting standards, a description of the strengths and uncertainties, and transparent expert review.

The scientific confidence framework is made up of several components: establishing fitness of purpose of the new approach method (NAM); evaluating the human biological relevance; conducting technical characterization of the NAM; describing needs for data integrity and transparency; and the need for independent review. This presentation will provide real world, regulatory examples for applying the scientific confidence framework to develop robust conclusions of regulatory readiness and regulatory acceptability. Needs for data integrity and transparency; and the need for independent review.

Leadership development is becoming an increasingly important topic in academia, industry, and governmental organizations. We recognize that science does not take place in a vacuum. Scientists cannot do the work alone, and that skills such as communication, teamwork, and leadership are essential to bolstering innovation and discovery in their field. In modern science, it is impossible to keep up with the incredible speed of scientific knowledge alone. Teams, collaborations, or even consortia are required to make the scientific breakthroughs for which we all strive. Teams can come from people who come from the same institution and share the same background or mixed groups forming interdisciplinary collaborations. Collaborations can be formed voluntarily or are forced by funding agencies. Teams are structured within organizations in order to enhance the workflow. However, the teams are formed and whatever their consistency, their success relies on good scientific leadership. As we climb the professional ladder, we rely ever more on leadership skills. Yet, leadership development has never been a primary concern in scientific training. During (post-)graduate training, trainees learn how to be good researchers, but there is little focus on equipping them to be leaders. Development of leadership skills is considered an individual endeavor, and scientific leadership is seen as a role people acquire rather than an intentionally guided process. Nevertheless, leadership affects the whole team, not just the leader. Being a good scientific leader requires key skills. But what are these scientific leadership skills, and how do you acquire them? And do skills needed in academia also apply to government or industry work? How can scientific leaders create a positive and inspiring working environment? In sum, what key requirements and challenges enable effective leadership in science? The goal of this session is to answer these questions through presentations by established scientific leaders across distinct organizations and diverse backgrounds. While all session speakers began in academia and now work in a toxicology-related field, they have been appointed to a wide field of leadership positions in academia, government, industry, and science. Each speaker will reflect on their journey through the scientific leadership field and how they became a scientific leader. They will present what their scientific leadership role entails and address what they see as key skills and key challenges in their current roles. Additionally, they will give tips and advice on how to acquire the necessary leadership skills. The individual talks will be followed by a panel discussion in which we can focus on the differences and similarities between the different fields of leadership. The extended discussion period will allow time for answering audience queries.
The blood-brain barrier (BBB), which is comprised of the brain microvascular endothelial cells (BMVECs) that line brain capillaries, is a highly restrictive and selective interface that controls molecular transport between the bloodstream and central nervous system. To regulate import and export of diverse biomolecules, the BBB possesses a diverse cohort of membrane transporters with a wide array of substrate specificities. In general, it is extremely challenging to predict how well any given compound will be transported across the BBB. Further, studies of BBB permeability in vivo are laborious and low-throughput, and it is well accepted that BBB permeability in mice is not necessarily representative of that in humans (for example, due to relative differences in transporter expression and compound affinity for these transporters). For these reasons, interest in human in vitro BBB models has grown considerably in the last several decades. Although sources of human material were previously restricted to primary tissue isolates (which are not widely accessible) and immortalized cell lines (which do not exhibit robust BBB function), the advent of human induced pluripotent stem cell (iPSC) technology can now provide an unlimited source of material for constructing BBB models. In this presentation, I will describe my group’s development of protocols to differentiate iPSCs into BMVEC-like cells and assembly of these cells with other neurovascular progenies into microphysiological BBB models. This talk will focus on the development of new strategies involving CRISPR screening and molecular engineering of small molecule biosensors to better understand molecular transport, which has parallels for studying BBB penetration of potential toxicants.

Human blood-brain barrier (BBB) models derived from induced pluripotent stem cells (iPSCs) have become an important tool for discovery and preclinical evaluation of central nervous system (CNS) targeting drugs and gene-based therapies. Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary form of gene-modified cell-based immunotherapy with potential for targeting solid tumors, as well as glioblastomas. Crossing the BBB is an important step in the systemic application of CAR-T therapy for the treatment of glioblastomas and other CNS malignancies. In addition, even CAR-T therapies targeting non-CNS antigens such as the well-known CD19-CAR-T therapies, are known to trigger CNS side-effects including brain swelling due to BBB disruption. In this study, we used iPSC-derived brain endothelial-like cell (iBEC) transwell co-culture model to assess BBB extravasation of CAR-T-based immunotherapies targeting U87MG human glioblastoma (GBM) cells overexpressing the tumor-specific mutated protein EGFRvIII (U87vIII). Two types of anti-EGFRvIII targeting CAR-T cells with varying tonic signaling profiles (CAR-F263 and CAR-F269) and control Mock T cells were applied on the luminal side of BBB model in vitro. CAR-F263 and CAR-F269 T cells triggered a decrease in transendothelial electrical resistance (TEER) and an increase in permeability. CAR-T extravasation and U87vIII cytotoxicity were assessed from the abluminal compartment using flow cytometry and IncuCyte real-time viability imaging, respectively. A significant decrease in U87vIII cell viability was observed over 48 hrs, with the most robust cytotoxicity response observed for the constitutively activated CAR-F263. CAR-F269 T cells showed a similar cytotoxic profile but were both approximately 4-fold less efficient at killing the U87vIII cells compared to CAR-F263, despite similar transmigration rates. Visualization of CAR-T cell extravasation across the BBB was further confirmed using iBEC-on-CHIP models. The described BBB assay was able to discriminate cytotoxic efficacies of the different EGFRvIII-CARs and to provide a measure of potential alterations to BBB integrity. Collectively, we illustrate how iPSC-derived BBB models provide a measure of potential alterations to BBB integrity. Collectively, we illustrate how iPSC-derived BBB models provide a measure of potential alterations to BBB integrity. Collectively, we illustrate how iPSC-derived BBB models provide a measure of potential alterations to BBB integrity. Collectively, we illustrate how iPSC-derived BBB models provide a measure of potential alterations to BBB integrity.

The National Heart, Lung, and Blood Institute (NHLBI) in collaboration with the Department of Defense Joint Program Committee-6/Combat Casualty Care Research Program (JPC-6/CCCRP) has recently launched the Trans-Agency Blood-Brain Interface Program (BBI) to create next-generation human BBB models. In this session, we will discuss the current state of the Trans-Agency Blood-Brain Interface Program (BBI) and the underlying neurovascular mechanisms are largely unknown and under-researched. To address this challenge, a new program was launched to support innovative projects with a substantial focus on the neurovascular-blood unit in normal and pathological states. Additionally, this initiative fosters collaborations between hematologists/vascular experts, neuroscientists, and tissue chip developers to create enhanced/transmodified platforms that more closely model the human brain-blood barrier (BBB). The blood component has been a critical research gap in BBB research and an improved, human-like BBB prototype/model would serve as an invaluable resource to the scientific community. Research addressing vascular, hemostatic, hematopoietic, and/or immune cell interaction across the BBB is of particular interest. This initiative is focused on developing a new generation of in vitro human BBB model systems of the human BBB, redefining the neurovascular unit and stimulating the development of a new field of science to complement research currently based on animal models. This program aims to infuse the field with hematology, vascular, and circadian expertise to advance translational research by bringing the blood back to the BBB and facilitating the development of a more complete neurovascular blood vessel model with direct relevance to humans.

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The overall integration of NAM data into risk assessment is one of the most challenging aspects of NGRA. This session will also examine the current status of IVIVE for NAM integration.

**1107 The Next-Generation Risk Assessment Approach Developed by Cosmetic Europe and the Role of PBK Modeling**

A. Najar, Beiersdorf, Hamburg, Germany; Sponsor: J. Wambaugh

Next Generation Risk Assessment (NGRA) of a chemical is a framework that integrates non-animal/new approach methodologies (NAMs) to ensure human safety of cosmetics ingredients without generating animal data. NGRA requires the extrapolation of in vitro points of departure (PoDs) to equivalent external exposures relevant to cosmetic exposure scenarios and following the Seurat-1 workflow published by Berggren et al. (2017). PBK modeling provides a means for translating in vitro concentration-effect response relationships to in vivo dose-response relationships in humans. The presented case study uses PBK modeling for reverse-dosimetry of in vitro PoD on the estrogenic pathway for genistein and daidzein and considering relevant cosmetic exposure scenarios. The genistein PBK model was validated using in vivo kinetic data, which confirmed the ability to reproduce the observed kinetic parameters. Genistein was used as a read-across analogue to gain confidence in the developed PBK models for the target compound, daidzein. The current case study demonstrates the applicability and crucial role of PBK modelling in NGRA, as well as provides strategies for building valid PBK models without animal data using the read-across concept proposed by the OECD. Challenges to improve the predictive performance of the developed models will be addressed by subsequent presentations with respect to in vitro distribution (Proença, Kramer, and Wambaugh) and PBK modeling (Gardner and Escher).

**1108 A Strategy to Optimize In Vitro Disposition Modeling for Next-Generation Risk Assessment**

N. Kramer1 and S. Proença2; 1Wageningen University and Research, Wageningen, Netherlands; and 2Universiteit Utrecht, Utrecht, Netherlands.

In vitro concentration-effect relationships are traditionally based on nominal concentrations, but this dose metric accurately reflects the concentration of the chemical causing toxicity in cells in vitro. To the extent to which a chemical partitions into cells will depend on physicochemical properties and binding affinities to assay constituents such as plastic, serum protein, and cell lipids. As part of the ONTOX project, a gap analysis was performed to systematically evaluate and extend the chemical and assay applicability domain of in vitro disposition models for in vitro assays assessing molecular perturbations related to steatosis, cholestasis, developmental neurotoxicity and tubular necrosis. A literature review was performed to compare the chemical and assay applicability domains of in vitro disposition models published in literature. Using artificial intelligence, a database has been constructed listing chemicals associated with the aforementioned ontologies and their physicochemical and kinetic properties. The database is used to analyze the chemical space of these chemicals and assess the extent to which they fit within the chemical and assay applicability domains of in vitro disposition models.

**1109 Challenges of In Vitro Disposition Modeling: First Insights from the Tox21 Project**

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

Next Generation Risk Assessment (NGRA) hinges on quantitative determination of surrogate points of departure (PoDs) using in vitro-in vivo extrapolation (IVIVE). Understanding in vitro disposition is critical for IVIVE since the free, effective concentration might be a hundredth or a hundred times the nominal test concentration. While mathematical models exist for predicting in vitro disposition from physico-chemical properties, the data for evaluating these predictions represent limited chemical structure diversity. The chemical library of the U.S. Federal Tox21 screening program contains thousands of diverse chemicals. The Tox21 library has already been screened in concentration-response mode for diverse bioactivities using high-throughput in vitro assays. In some cases of PoDs – based on nominal in vitro tested concentration – have been identified. This presentation will describe how Tox21 has been collecting new data characterizing in vitro disposition of sentinel chemicals to assess any differences between nominal and free concentration. These data permit evaluation of a variety of mathematical models for in vitro disposition across a wider range of physico-chemical properties, including key chemical classes found in commerce and the environment. Accurate prediction of in vitro disposition will enhance the predictive power of quantitative NGRA.

**1110 Incorporating Metabolite Information into PBK Models for In Vitro to In Vivo Extrapolations**

I. Gardner; Certara UK Limited, Sheffield, United Kingdom.

As physiologically based kinetic (PBK) models simulate the absorption, distribution, metabolism, and excretion of chemicals through the body, they are well suited to extrapolate effect concentrations of chemicals from in vitro toxicity assays to human bioequivalent doses, an approach referred to as IVIVE. IVIVE-PBK models have been used extensively to describe the plasma and tissue concentration profiles of drugs and chemicals. Within the Riskhunt3r project (www.riskhunt3r.eu) different strategies have been investigated to produce IVIVE-PBK models for some exemplary compounds. This includes using only in silico estimates of model inputs compared to in vitro model inputs and a middle out approach whereby in vitro data and some human pharmacokinetic data were used to inform the PBK model input parameters. A second important objective of the work in this project was to investigate approaches to include key metabolites into the PBK model. Some representative examples of parent and metabolite IVIVE-PBK models will be presented.

**1111 The Development of In Vitro Models of Barrier Organs for Parameterizing Next-Generation PBK Models**

S. Escher; Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover, Germany; Sponsor: J. Wambaugh

Consumers and workers are exposed to chemical substances via different routes of exposure. In order to accurately determine the risk for chronic exposure to chemicals, mathematical PBK models are needed. The Tox21 project is using PBK models to understand the extent to which a chemical partitions into cells. The extent to which a chemical partitions into cells will depend on physicochemical properties and binding affinities to assay constituents such as plastic, serum protein and cell lipids. As part of the OnTox project, a gap analysis was performed to systematically evaluate and extend the chemical and assay applicability domain of in vitro disposition models for in vitro assays assessing molecular perturbations related to steatosis, cholestasis, developmental neurotoxicity and tubular necrosis. A literature review was performed to compare the chemical and assay applicability domains of in vitro disposition models published in literature. Using artificial intelligence, a database has been constructed listing chemicals associated with the aforementioned ontologies and their physicochemical and kinetic properties. The database is used to analyze the chemical space of these chemicals and assess the extent to which they fit within the chemical and assay applicability domains of in vitro disposition models.

**1112 A Current Perspective on Environmental Chemicals and Their Role in Initiating and Exacerbating Cardiovascular Disease**

D. Carlin; NIEHS, Research Triangle Park, NC.

Pollution accounts for more than nine million premature deaths worldwide—out of which two-thirds of the deaths are attributed to exposure to environmental toxicants (e.g., volatile organic compounds [VOCs], metals/metalloids, environmentally persistent free radicals, particulate matter, and polycyclic aromatic hydrocarbons). These toxicants are abundant in water, automobile exhaust, wildfires, combustible products, and household chemicals. Copious amounts of these chemicals also are present at Superfund and other hazardous waste sites. These toxicants often target the heart and lungs. Studies over the last three decades have established a causal link between particulate matter exposure and cardiovascular disease. Data from recent studies also suggest that environmental chemical exposures increase the risk for stroke, hypertension, and type 2 diabetes. More specifically, VOCs (e.g., acrolein and 1,3-butadiene), metals (e.g., cadmium and lead), and metalloids (e.g., arsenic) also increase the risk for vascular inflammation and atherosclerosis, which are the underlying causes of most cardiovascular disease. However, it is unclear how pollutants increase the incidence and severity of cardiovascular disease. Even less is known about how such exposures relate etiologically and mechanistically to disease development. This Symposium will assemble experts from diverse research programs (e.g., epidemiology, clinicians, mechanistic) in order to discuss the state-of-the-science pertaining to underlying complex biological mechanisms/pathways associated with chemical exposure and cardiovascular disease. In addition, this session will cover other areas requiring further attention, including a look into the effect of nonchemical stressors (e.g., socio-economic, physical, psychological, lifestyle, and poor nutrition), novel mechanistic pathways and assays used to determine cardiovascular toxicity effects, and target areas for potential preventive and therapeutic strategies.

**1113 Harmony between the Lung and the Heart: Codependent Responses to Pollution**

V. Antony; University of Alabama at Birmingham, Birmingham, AL.

Cardiopulmonary disease secondary to environmental insults is well recognized and documented. It is recognized that mortality resulting from an exposure to PM and other particulate matter during the population of Medicare age is directly proportional to PM 2.5 exposure even below the EPA accepted levels, irrespective of smoking history. The underlying causes are linked to both pulmonary and cardiac disease. While cardiac disease and strokes remain the primary cause of mortality in the US, Chronic Obstructive Pulmonary Disease (COPD) is the third...
largest cause of death world-wide and is a significant co-morbid condition in both myocardial infarctions and strokes which are caused by tissue hypoxia in the relevant organ system. Exposure to pollutants causes exacerbation of airway diseases such as COPD and asthma leading to hypoxemia which, in turn, can lead to increased risk of myocardial hypoxia as well as an increased risk for strokes. We have recently shown that increases in procoagulant molecules by exposure to air pollutants appear to be a significant driver of the thrombotic potential of DEP exposure and a hypercoagulable state also predisposes humans to myocardial infarctions and stroke. Pollutants not only cause exacerbation of underlying lung disease but also may be the primary cause of lung disease. Cadmium (Cd), a HM in cigarette smoke (which may contain 2-3 micrograms of Cd) is a common pollutant from coal fired power plants and coke furnaces and has an in vivo half-life of 25 years. Cd causes COPD as well as exacerbation to a procoagulant state with higher levels of fibrinogen/fibrin in the blood and lungs. Pulmonary artery hypertension (PAH), which can occur secondary to lung diseases mentioned above, leads to right atrial enlargement and heart failure and can also be caused by direct exposure to certain environmental pollutants such as monocrotaline. In summary, the heart and the lung are directly and indirectly in their responses to pollutants. These links are also physiological, where an insult to one organ is reciprocated in the other. Understanding these links will help us better develop strategies and therapeutic modalities to address both.

Find up-to-date information at www.toxology.org.

1114 Air-Blood Interface as a Critical Mediator of the Cardiovascular Toxicology of Particulate Matter and Potentially Other Inhalation Exposures

T. Dupas, Louisiana State University, Baton Rouge, LA.

Inhalation of particulate matter air pollution has been linked to increased risk of death due to cardiovascular disease. Epidemiologic linkages between inhalation exposures and cardiovascular disease are well-known, but mechanisms for this pathogenesis are not fully understood. Early reports correlated inhaled exposure with both pulmonary and systemic inflammation, the latter of which is known to promote vascular inflammation and by extension, vascular diseases such as atherosclerosis. However, recent studies utilizing particles of smaller sizes and doses suggest that PM-induced vascular dysfunction can occur absent cellular inflammation or circulating cytokines. The air-blood interface in the lung, i.e., the alveolar-capillary barrier, is a critical location where gas exchange occurs and where blood vessels and pulmonary cells interact. The cells that comprise the air-blood interface may thus be a source of mediators released systemically to impact vascular function downstream of the lung. Our laboratory has shown that endothelial (ET-1)–1 to the plasma for PM exposure and PM-induced vascular dysfunction. The lung is a rich source of ET-1 production, and in rodent models, pulmonary ET-1 release has been shown correlated with the severity of heart failure. However, systemically released ET-1 is only one possible mediator of vascular dysfunction secondary to inhalation exposures. Other potential mediators include microRNAs and metabolites of biomolecules. For example, pulmonary oxidative stress leads to the production of lipid and protein oxidation products that may be transported to impact cell signaling downstream of the lung. This symposium will review recent literature and potential systemic mediators that may link inhalation exposures with the initiation or exacerbation of vascular disease distal to pulmonary vascular beds.

1115 Air Particulate Matter, Gut Dysbiosis, and Cardiometabolic Toxicity

J. Araujo, University of California Los Angeles, Los Angeles, CA.

A large body of evidence supports the causal link between particulate matter (PM) exposure with atherosclerosis and various metabolic endpoints including insulin resistance, diabetes, and fatty liver disease, suggesting an intersection between metabolic and cardiometabolic toxicology. However, the precise pathogenic mechanisms remain unknown. We are investigating whether exposure to PM results in changes in gut microbiota composition, and if these changes could causally mediate the development of cardiometabolic health effects using various animal models and exposure paradigms. In particular, we have assessed the effects of exposure to diesel exhaust on lipid metabolism in mice through assessment of plasma lipids and lipoproteins, oxidized fatty acids 9-HODE and 13-HODE, ApoE KO mice exposed to inhaled diesel exhaust (DE, 6 hours/day, 5 days/week for 16 weeks) exhibited elevated plasma cholesterol and triglyceride levels, increased hepatic triglyceride content and higher hepatic levels of 9-HODE and 13-HODE, as compared to ET-1–1 to the plasma for PM exposure and PM-induced vascular dysfunction. ApoE KO mice exposed to inhaled diesel exhaust (DE, 6 hours/day, 5 days/week for 16 weeks) exhibited elevated plasma cholesterol and triglyceride levels, increased hepatic triglyceride content and higher hepatic levels of 9-HODE and 13-HODE, as compared to control untreated mice. Cd treatment with Cd (10 mg/kg/day) for 8 weeks exhibited statistically significant differences in the abundance of bacterial taxa, as well as alpha and beta diversity indices of the DE-exposed gut microbiome vs. FA (p<0.05). There were significant positive associations between these cecum bacterial species and plasma and hepatic lipids. Furthermore, DE-exposed mice had significantly decreased mRNA and protein levels of hepatic 12-lipoxygenase (12-LO). Interestingly, DE-exposed mice showed markedly reduced fecal acetate levels that significantly associated with lipids and cecal microbiome. A direct effect of short chain fatty acids on hepatocytes was observed after treatment of HepG2 cells with whole DE particles (DEP) together with acetate, which significantly inhibited 12-LO mRNA expression as compared to DEP alone. Overall, these data suggests that DE could have induced changes in the hepatic content of oxidized lipids via induction of gut dysbiosis and decreased intestinal levels of acetate, resulting in decreased protection against lipid oxidation. Ongoing work with other experimental exposure models is being conducted to determine if PM-induced gut dysbiosis is also associated with the induction of other cardiometabolic health effects.

1116 Metallomics and Cardiovascular Health: Opportunities for Prevention and Intervention


Arsenic, cadmium and lead are established risk factors for cardiovascular disease of atherosclerotic origin. For other metals, the evidence is growing. Numerous disadvantaged communities around the US, including Native American communities, are disproportionately exposed to metals through natural and anthropological sources. Evidence supports that arsenic, cadmium and other metals are related to increased left ventricular wall thickness and left ventricular hypertrophy in young adults, as assessed using transthoracic echocardiography at baseline and follow-up, as well as with clinical outcomes (coronary heart disease, strong, heart failure) in older adults. Given these epidemiological findings, which are supported by experimental and mechanistic research, interventions are needed to protect these communities from the vascular effects of metal exposures. For short half-life metals, such as arsenic and uranium, removing them from drinking water, as implemented by the Strong Heart Water Study, can improve vascular health. For metals with long half-lives, such as cadmium and lead, new strategies are needed. Evidence from the South African Lung Haemodynamics and Tuberculosis (SALT) has shown that divalent metal chelation can prevent cardiovascular disease, providing a new powerful strategy for cardiovascular health. Metal chelation could become a new pharmacotherapy to reduce metal and cardiovascular disease burden. Toxicological research can further investigate mechanisms for the cardiovascular benefits of metal chelation.

1117 The Disheartening Role of Volatile Organic Compounds in Cardiopulmonary Disease

S. Srivastava. University of Louisville, Louisville, KY.

Volatile organic compounds (VOCs) are a class of toxicants abundant in indoor and outdoor air. Major sources of VOCs include petroleum products, industrial solvents, tobacco smoke, wildfire, and a variety of household products. VOCs such as trichloroethylene, benzene, acrolein, xylene and butadiene are also major chemicals of concern at most Superfund sites because exposure to these VOCs could have a variety of adverse health effects including type 2 diabetes (T2D) and stroke. Emerging data also suggest that benzene exposure could be a risk factor for atrial enlargement and heart failure. Our pre-clinical and population-based studies suggest that chronic exposure to VOCs could increase blood pressure, inflict vascular injury, accelerate atherosclerosis, and promote cardiac dysfunction. Most of these unfavorable health effects are promoted by VOC-induced endothelial toxicity. Mechanistic studies indicate that exposure to VOCs such as benzene and acrolein augment the transcription of stress response genes, promote endoplasmic reticulum (ER) stress, and induce unfolded protein response (UPR) in endothelial cells. Our data support the hypothesis that VOC exposure can increase endothelial dysfunction, promote cardiac dysfunction, and affect cardiovascular outcomes. We will discuss how pharmaceutical and genetic manipulation of these pathways regulate VOC-induced endothelial functions, atherogenesis, and heart failure.

1118 Continuing toward Best Practices in Organizing, Assessing, and Applying Mechanistic Data in Hazard Characterization and Risk Assessment

B. Wikoff, ToxStrategies Inc., Asheville, NC.

A well-attended and highly interactive workshop during the 2022 SOT Annual Meeting assembled scientists knowledgeable in the consideration and application of key characteristics and mechanistic pathway constructs, namely mode of action (MOA) and adverse outcome pathway (AOP). Presentations and lively discussion highlighted misunderstandings regarding appropriate application of these complementary constructs that are used to organize and assess mechanistic data. Audience participation led to helpful suggestions and a collective commitment to also consider principles of good practice for their application in hazard characterization and risk assessment based on increasing understanding of their interface and overlap. This Workshop Session seeks to carry this momentum into the 2023 meeting with the objective of advancing discussion to inform the development of best practices for organizing, assessing, and applying mechanistic information in pathways based on key events and key event relationship across levels of biological organization in order to link exposure to specific adverse effects. As will be addressed in the first presentions, key events represent nodes of convergence of mechanistic pathways that are measurable and informative for predictive inference and regulatory application, including the use of new approach methodologies. AOPs and MOAs serve, then, as convenient
organizational and integrating constructs for mechanistic information from a range of sources, including computational predictive models and in vitro and in vivo regulatory toxicological studies, and epidemiological investigations. This pathway-based integration, in turn, provides important information regarding plausibility, human relevance, and consistency with anticipated patterns of dose-response. More recently introduced key characteristics facilitate systematic identification and organization of mechanistic evidence into categories of activity, rather than being characterized by levels of biological organization, they are based on empirical associations between chemical and biological properties and human disease outcome (e.g., cancer, reproductive effects). In some cases, this has led to the perception that they are checkbox markers of an effect, which was a key discussion point raised by audience members at the 2022 session. As part of the session, a 65-minute discussion will foster engagement of session participants in a continued evaluation aimed at improving common understanding of the nature of the interface between and among these constructs. It also serves as a platform for sharing experiences with application and recommending components of best practice. Next steps in considering best practices in organizing, assessing, and applying mechanistic information evaluation are integrated with cancer evidence from exposure and mechanism knowledge into categories of activity. The results of mechanistic data to inform predictive inference in regulatory application. The development of new approach methodologies (NAMs) that provide mechanistic insight into pathophysiology creates an unprecedented opportunity to generate human-specific test methods for regulatory toxicology—a significant unmet need in pulmonary toxicology due to unsolvable species differences between humans and animals. This framework has been adopted broadly by both regulatory agencies and stakeholders as a means to improve the consideration of the adequacy of support of mechanistic hypotheses for observed effects. The Organization for Economic Cooperation and Development (OECD) framework and program for the development of Adverse Outcome Pathway (AOPs) introduced more recently (2012) builds on this experience, to advance the application of mechanistic data to inform predictive inference in regulatory application. Descriptions of chemical agonistic toxicodynamic pathways of key events at different levels of biological organization are formalized in a public knowledge base to support chemical-specific regulatory applications such as MOA analysis and more mechanistically based integrated approaches to testing and assessment (IATA). Experience in the OECD program engaging both the human health and ecological communities and interface with the regulatory community has evolved greater common understanding and resulting advancement of the development and application of mechanistic data. Perspective on the appropriate reliance/contribution of mechanistic data in hazard characterization based on this progress is provided. Collective learnings from engaged communities based on this experience is also presented, with a view to considering the implications for best practice of learnings for better integration of mechanistic constructs such as AOP, MOA, and key characteristics, adopted by the International Agency for Research on Cancer in hazard identification for carcinogens.

**1119 Evolution and Status: Application of Mechanistic Data in Hazard Characterization**

M. Whelan. European Commission Joint Research Centre, Ispra, Italy. 

Since the late 1990’s, the World Health Organization (WHO) International Program on Chemical Safety (IPSC) has developed and evolved the Mode of Action (MoA) framework, to consider the incorporation of mechanistic data in the assessment of both cancer and non-cancer effects and their human relevance based principally on data from toxicological studies in animals. This framework has been adopted broadly by regulatory agencies with much experience gained in the consideration of the adequacy of support of mechanistic hypotheses for observed effects. The Organization for Economic Cooperation and Development (OECD) framework and program for the development of Adverse Outcome Pathway (AOPs) introduced more recently (2012) builds on this experience, to advance the application of mechanistic data to inform predictive inference in regulatory application. Descriptions of chemical agonistic toxicodynamic pathways of key events at different levels of biological organization are formalized in a public knowledge base to support chemical-specific regulatory applications such as MOA analysis and more mechanistically based integrated approaches to testing and assessment (IATA).

**1120 Mechanistic Information Evaluation in the Report on Carcinogens: From Key Characteristics of Carcinogens and Mechanistic Questions to How Deep to Dive and Study Evaluation-to-Evidence Synthesis**

A. Wang. NIEHS, Research Triangle Park, NC. 

In cancer hazard evaluation for potential listing in the Report on Carcinogens (RoC), we have used key characteristics of carcinogens (KCCs) to help search and organize diverse mechanistic information in a robust systematic evaluation. Studies met the inclusion criteria, sometimes in tens of thousands in an assessment, are labeled/tagged with relevant KCC(s) and other features (e.g., study types and substances) by human reviewers assisted by artificial intelligence. With this overview of available information, we can ask specific mechanistic questions (which may be related to one or multiple KCC(s), a specific hypothesis, or read cross) and evaluate the information by questions. To balance practicality and the desire of in-depth evaluation of all included studies, we established a process to determine “how deep to dive” for studies related to each mechanistic question and whether to analyze “raw” data from repositories (e.g., omic data). For example, if a mechanistic question is controversial or the evaluation will rely depend on a few more informative studies for one mechanistic question, these studies will be evaluated for risk of bias and study sensitivity, while other studies may be narratively evaluated. We evaluate the strength of evidence for each mechanistic question and synthesize the evidence across mechanistic questions to conclude to all mechanistic questions and develop evidence for recommendation. In mechanistic information evaluation are integrated with cancer evidence from exposure humans and animals for recommendation of a listing status in RoC. We continue to improve the approach and tools for cancer mechanistic information evaluation and welcome your input.

**1121 Proposal for Moving toward Best Practice**

D. Wikoff, and B. Meek. 1ToxStrategies Inc., Asheville, NC; and 2University of Ottawa, Ottawa, ON, Canada.

The last presentation in this session will involve the proposal of possible options for moving towards best practices in utilizing NAMs and organizing, assessing, and applying mechanistic data in hazard characterization and risk assessment, building upon discussions in the evolution of the session proposal. An initial proposal for discussion will consider next steps based on bridging of apparent misunderstandings identified in the 2022 workshop and receipt of continuing input, structured in a series of interactive sessions at related scientific meetings. It will also take into account increasing knowledge of the interrelationships of mechanistic constructs such as KCs and pathway-based approaches and increasing experience in their broad application. Initial proposals will build on transparent, evidence-based methods and evolving best practice in the integration and application of pathway-based approaches. It is anticipated that a stepwise approach for best practices to draw on motivation from the strengths of existing constructs can be used as a concrete starting place for discussion. 1Key characteristics to help design search strategies and organization of existing mechanistic information, and priorities for additional screening by NAMs and/or development of IATAs. Determination of how individual studies or activity related to a single key characteristic might be used to inform decision-making, depending on the nature and reliability of the assay(s) and purpose of the assessment. 2Best practices in use of key characteristic concepts in consideration of biologically plausible pathways.

**1122 Developing Human-Relevant New Approach Methodologies to Measure Key Events in Pulmonary Adverse Outcomes: Focus on Chronic Endpoints**

H. Behring. Institute for In Vitro Sciences Inc., Gaithersburg, MD.

The development of new approach methodologies (NAMs) that provide mechanistic insight into pathophysiology creates an unprecedented opportunity to generate human-specific test methods for regulatory toxicology—a significant unmet need in pulmonary toxicology due to unsolvable species differences between humans and test animals. To generate clinically meaningful mechanistic understanding that is fit to protect human health, a clear understanding of how key events upstream of pulmonary adverse outcome pathways (AOPs) in humans can be measured and/or predicted in vitro and in silico is needed. This workshop will leverage the knowledge derived from human clinical assessments as drivers for establishing quantitative metrics for key event detection and evaluation using NAMs. The Chair will open the session with a brief welcome and review of the Workshop’s focus: highlighting opportunities for ongoing development of human-relevant NAMs for pulmonary AOPs resulting from inflammatory key events. The first speaker will outline the quantifiable clinical manifestations of pulmonary fibrosis. Current findings will be reviewed from both a clinical perspective and a NAMs application standpoint. Available approaches used to detect key events outlined in current AOPs will also be discussed. Next steps for characterizing the quantitative relationships between pulmonary AOPs and upstream inflammatory key events will be the primary focus of the discussion.

**1123 Pathologic Processes of Idiopathic Pulmonary Fibrosis Provide Guidance for Key Event Detection in New Approach Methodologies**


Idiopathic pulmonary fibrosis (IPF) is a prototype of fibrotic lung diseases that exhibits the histologic pattern known as usual interstitial pneumonia. The fibrotic lung diseases are a group of conditions that result in fibrosis, or scar tissue, in the lung tissue that include chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and other key events leading to sensitization and fibrosis. The fifth speaker will discuss the use of precision-cut lung slices for measurement of inflammation and other key events leading to sensitization and fibrosis. The fifth speaker will describe an innovative in vitro model system that mimics human airway physiology and accurate control of breathing flow conditions. The final portion of the workshop will feature an interactive roundtable that will include a moderated discussion on the status of current NAMs in order to address regulatory needs for chronic exposures, including exposure-threshold benchmarks based on clinical manifestations. This Workshop will provide an overview of clinical pulmonary pathophysiology and describe several NAMs in development that will be used to inform clinical risk assessment. Several highly relevant inflammatory disease aetiologies will be reviewed from both a clinical perspective and a NAMs application standpoint.

Available approaches used to detect key events outlined in current AOPs will also be discussed. Next steps for characterizing the quantitative relationships between pulmonary AOPs and upstream inflammatory key events will be the primary focus of the discussion.
lung can be diverse with overlapping features, characterized by varying degrees of inflammation and fibrosis. Although animal models for lung fibrosis have been developed, they do not accurately recapitulate and predict the response of human lung tissue to various insults. However, with numerous molecules involved in the Key Events of fibrosis (which include alveolar epithelial cell injury, fibroproliferation and matrix remodeling) having been identified as potential biomarkers, appropriate new approach methodologies capable of reproducing these molecular responses may provide insights to IPF disease progression. Certain genes such as TOLLIP, MUC5B, associated with a differential response to treatment and with the development and/or the progression of IPF look promising, and may be informative for the development of NAMs. Additionally, the bacterial signature in IPF lungs, as shown from microbiome analyses, as well as mitochondrial DNA seem to have promising roles as biomarkers. Of the current pulmonary test systems available, human precision-cut lung slices (PCLS) offer native architecture and contain all the cell types at the time of slicing, including those associated with KE following pro-fibrotic pathway initiation. With recently developed human PCLS cryopreservation techniques and demonstrated long term culture, the KE associated with the IPF adverse outcome can be explored using human lung tissue. The development of such New Approach Methodologies can significantly enhance our understanding of the condition, leading to mechanistic discoveries to inform patient therapy as well as test methods to predict the fibrotic potential of chemicals and other stressors.

Human precision-cut lung slices (PCLS) offer a human-relevant ex vivo test system with the capability of producing a human-like response. Reproducing these molecular responses may provide insights to IPF disease progression. Certain genes such as TOLLIP, MUC5B, associated with a differential response to treatment and with the development and/or the progression of IPF look promising, and may be informative for the development of NAMs. Additionally, the bacterial signature in IPF lungs, as shown from microbiome analyses, as well as mitochondrial DNA seem to have promising roles as biomarkers. Of the current pulmonary test systems available, human precision-cut lung slices (PCLS) offer native architecture and contain all the cell types at the time of slicing, including those associated with KE following pro-fibrotic pathway initiation. With recently developed human PCLS cryopreservation techniques and demonstrated long term culture, the KE associated with the IPF adverse outcome can be explored using human lung tissue. The development of such New Approach Methodologies can significantly enhance our understanding of the condition, leading to mechanistic discoveries to inform patient therapy as well as test methods to predict the fibrotic potential of chemicals and other stressors.

**1124 Pathophysiology of Respiratory Sensitization: Use of Clinical Hallmarks Combined with Adverse Outcome Pathway to Inform the Development of New Approach Methodologies to Identify Chemical Respiratory Allergens**


Respiratory sensitisation upon exposure to low molecular weight (LMW) chemicals is a significant occupational health problem in several industries and is also of concern due to the use of consumer products. Under the umbrella of occupational asthma, there are two broad classes of mechanisms that can result in hypersensitivity of the airways, often presenting with similar symptoms of cough, wheeze, shortness of breath and/or chest tightness. As it is difficult to tease out immune-mediated respiratory sensitisation from non-sensitizers, chemicals classified as false negatives by other new approach methodologies or NAMs data can be combined with clinical hallmarks to align them to the KEs in the AOP. In addition, key considerations in the form of a scoring matrix, to bear in mind while using the clinical data, along with the importance of considering history and routes of exposure to the LMW chemical are outlined. Finally, using the AOP as guidance, we summarise how in vitro new approach methodologies or NAMs can be developed, they do not accurately recapitulate and predict the response of human lung tissue to various insults. However, with numerous molecules involved in the Key Events of fibrosis (which include alveolar epithelial cell injury, fibroproliferation and matrix remodeling) having been identified as potential biomarkers, appropriate new approach methodologies capable of reproducing these molecular responses may provide insights to IPF disease progression. Certain genes such as TOLLIP, MUC5B, associated with a differential response to treatment and with the development and/or the progression of IPF look promising, and may be informative for the development of NAMs. Additionally, the bacterial signature in IPF lungs, as shown from microbiome analyses, as well as mitochondrial DNA seem to have promising roles as biomarkers. Of the current pulmonary test systems available, human precision-cut lung slices (PCLS) offer native architecture and contain all the cell types at the time of slicing, including those associated with KE following pro-fibrotic pathway initiation. With recently developed human PCLS cryopreservation techniques and demonstrated long term culture, the KE associated with the IPF adverse outcome can be explored using human lung tissue. The development of such New Approach Methodologies can significantly enhance our understanding of the condition, leading to mechanistic discoveries to inform patient therapy as well as test methods to predict the fibrotic potential of chemicals and other stressors.

**1125 An In Vitro Alveolar Co-culture Model to Discriminate Respiratory Irritants and Sensitizers**


Reactive electrophiles are a significant inhalation hazard in consumer products and occupational settings. Such chemicals can lead to irritation of the respiratory tract as well as allergic pathologies. For the latter, still no validated in vivo or in vitro methods exists for a precise classification of respiratory sensitizers. To address this unmet need, and to provide a platform for investigating the human-specific mechanisms of pulmonary irritation and sensitization, we have developed an in vitro model that mimics the alveolar-capillary barrier. This 3D alveolar in vitro test system designed is built on the microporous membrane of cell culture inserts, using human cell lines: alveolar type II pneumocytes-A549, endothelial-EA.hy926 and monocytic leukaemia-THP-1 cells. The test system was exposed at the air-liquid interface (ALI) to known low-molecular weight chemical respiratory sensitizers and non-sensitizing pulmonary irritants, and the expression of CD54, CD86 and TSLP cell surface markers on THP-1 cells was measured by flow cytometry. The obtained data show that the test system can discriminate respiratory sensitizers from non-sensitizers. Additionally, tested pro-haptens were correctly identified as respiratory sensitizers, chemicals classified as false negatives by other new approach methodologies for respiratory sensitization evaluation. This work contributes to the understanding of respiratory adverse outcomes and supports the need for further testing of chemicals able to elicit an allergic response.

**1126 Human Precision-Cut Lung Slices to Study Key Events Associated with Respiratory AOPs**

V. Patel. Institute for In Vitro Sciences Inc., Gaithersburg, MD.

Human precision-cut lung slices (PCLS) are a reliable ex vivo test system that provides control over key parameters of smoking behavior including puff time, the pathophysiology of differentiated human mucociliated bronchiolar epithelium generated from ECs in real-time while mimicking clinically relevant breathing and vaping topography exactly as happens in humans. They observed that the addition of even small quantities of VEA was sufficient to alter size distribution and significantly enhance total particles inhaled from ECs. Moreover, they demonstrated the utility of the biomimetic robot for studying influence of nicotine and breathing profiles on respirator and restrictive lung disorders. This new non-living platform offers potential to serve as a novel preclinical scientific research, decision-support tool when insight into toxicological impact of modifications in ENDS is desired. Dr. Benam will conclude the lecture by discussing challenges in the field to be tackled and some ideas for future directions.

**1127 Quantitative Analysis of Particle Disposition Using Bioinspired Engineered Systems**

K. Benam. University of Pittsburgh, Pittsburgh, PA.

Accurately modelling the effects of particle distribution in the human airway is critical to understanding the toxicity of exposure to chemicals and mixtures in particulate matter. In this talk, Dr. Benam will present on application of tissue microengineering Organ-on-a-Chip technology to create a ‘Human Lung Small Airway-on-a-Chip’ which supports full differentiation of pseudostratified mucociliated epithelial cells from normal or diseased donors underlined by a functionally matured microvascular endothelium. Dr. Benam will next describe a state-of-the-art in vitro model system that permits analysis of the effects of whole cigarette smoke delivered under physiologically relevant flow conditions that mimic breathing on the pathophysiology of differentiated human mucociliated bronchiolar epithelium cultured in a microfluidic ‘Airway-on-a-Chip’. The automated smoking instrument provides control over key parameters of smoking behavior including puff time, inter-puff interval and number of puffs per cigarette, which have been shown to be critical contributors to smoke-induced injury in humans. In addition, Dr. Benam will present human Vaping Mimetic Real-Time Particle Analyzer (HUMITIPA). Vitamin E acetate (VEA) has been strongly linked to outbreak of EC or vaping product use-associated lung injury (EVALI). How VEA leads to such an unexpected morbidity and mortality is currently unknown. To understand whether VEA impacts the disposition profile of inhaled particles, Dr. Benam and his team created a biologically inspired robotic system that quantitatively analyzes submicron and microparticles generated from ECs in real-time while mimicking clinically relevant breathing and vaping topography exactly as happens in humans. They observed that the addition of even small quantities of VEA was sufficient to alter size distribution and significantly enhance total particles inhaled from ECs. Moreover, they demonstrated the utility of the biomimetic robot for studying influence of nicotine and breathing profiles on respirator and restrictive lung disorders. This new non-living platform offers potential to serve as a novel preclinical scientific research, decision-support tool when insight into toxicological impact of modifications in ENDS is desired. Dr. Benam will conclude the lecture by discussing challenges in the field to be tackled and some ideas for future directions.

**1128 How to Be an Active Ally and Upstander for Promoting an Inclusive Environment in Toxicology**


Unconscious bias and microaggressions impact the workplace, interactions with colleagues, and productivity. Left unexamined, these biases can affect decision-making processes and how toxicologists conduct research or work effectively at their jobs. This Workshop will invite participants to engage in a respectful environment where they can learn about the concept and impacts of implicit biases, how to identify and recognize microaggressions and other hostile behaviors, and certain tools to support colleagues in difficult situations. Several examples will be shared on how unconscious bias can appear in our work and everyday decisions and negatively impact marginalized groups in STEM. Most importantly, the Workshop will describe concrete, specific strategies and provide resources individuals can use to minimize the effects of implicit bias and become an active ally in promoting a positive workplace climate. The session will consist of an interactive presentation and career stages sharing their experiences and highlighting areas for ally engagement. Overall, the session will provide resources and strategies to empower participants to recognize unconscious bias and microaggressions and become active supporters in promoting a positive workplace climate.
The interactive training will be provided by Dr. Meredith Hastings, who is a Professor in the Department of Earth, Environmental, and Planetary Sciences at Brown University and serves as Deputy Director of the Institute at Brown for Environment and Society, an interdisciplinary research center focused on understanding interactions between natural, human and social systems. The training will be composed of (1) lecture/presentation introducing concepts (and define terms) of microaggression and biases in the workplace, and (2) interactive discussion of scenarios and instances for bystander intervention and allyship in cases of biases, such as racism, homophobia, and gender discrimination. Ultimately, this training/workshop will equip attendees with skills to recognize ways in which exclusionary behaviors manifest in different research settings and how they are experienced by people of different gender, racial, ethnic and other identities and to implement direct and indirect behaviors to intervene in the situation of microaggression and biases. Below is the detailed breakdown of Dr. Meredith’s training. In part 1, Dr. Hastings will introduce the concept of implicit bias and microaggressions. The participants will learn about (1) the science behind implicit bias, including how it was discovered and how we measure it; (2) different types of microaggressions and who experiences them. During this part of the session, the participants will also be encouraged to complete a short self-identity exercise, which is to be shared and discussed only voluntarily.

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It is necessary to demonstrate that a NAM has achieved a requisite degree of scientific confidence for one or more specific applications before it can be used to support product stewardship or regulatory decisions. The term ‘fit for purpose validation’ (FFPV) emerged in such discussions, and while various components of such a process have been discussed, a uniform approach for FFPV has yet to be adopted. Accordingly, we have proposed a scientifically rigorous, yet flexible framework, that can be widely applied to evaluate best, if not all, types of NAMs.

This NAM Scientific Confidence Framework (SCF), centered on explicitly answering the scientific method, consists of 7 components: 1) problem formulation and hypothesis (an explicit proposition that the NAM can be used to provide actionable information for a specific decision context) 2) the biological relevance & plausibility of the NAM; 3) assay performance (documentation of sensitivity, specificity, reliability, & domain of applicability of the NAM); 4) documentation of the performance of inference (prediction) models based on the NAM response – outcome response relationship; 5) dissemination of the data, inference models, etc. to support independent replication; 6) a narrative rationale making the case that there is/is not sufficient scientific confidence in the NAM to support the specific application for the chemistry domain of interest, and 7) verification through independent scientific peer review. We will illustrate the application of this NAM SCF to the evaluation of the key characteristics of carcinogens, to TTCs, and points of departure from ToxCast data, and an AOP.

**1136 Achieving Successful Reproducibility for Next-Generation Toxicological Methods**

A. Karmaz, Inotiv, Research Triangle Park, NC.

There is an abundance of new approach methodologies (NAMs)—particularly computational models—being developed to support chemical assessment for screening and prioritization. The advantage of NAMs in toxicity testing is that they are cost- and time-effective, minimize the use of animals, and generate an abundance of data. However, integration of these methods for use in chemical assessments persists, as stakeholders require some form of evaluation, verification, or validation for these methods. The approaches to NAM evaluation include reproducibility and performance assessment benchmarked against previous in vivo experiments where the expected performance can vary or even be unreasonable. Thus, it is important to address how to set standards for evaluating NAM robustness both qualitatively and quantitatively and to ensure that data, approaches, and results are effectively shared and transparent. Whether assessing statistical summary metrics or performance of reference chemicals, defining expectations and harmonizing how model assessment is conducted will help us move toward NAM adoption. Presentations in this session will tackle not only how to set expectations but also how to evaluate performance. They will also examine certain approaches to building confidence in models and best practices for publishing these data, including the role of the FAIR data principles. The session will begin with a brief introductory presentation by one of the session Co-Chairs to highlight any perceived lack of reproducibility, how this applies to NAMs, what challenges exist for publishing and evaluating these approaches, and how the research community can better support their development through data sharing and FAIR data principles. The second speaker will address approaches to conducting variability assessment for evaluating reproducibility and the importance of characterizing the in vivo study being used as reference. The third speaker will present a study designed in vivo in rat for which they selected a chemical inventory for which they defined a set of standards for evaluating NAM performance in predicting organ-level effects in repeat dose studies of adult animals by investigating the reproducibility, variability, and differences in organ-level findings in repeat dose studies, using organ lowest effect level (LELs) for liver, kidney, stomach, spleen, thyroid, and adrenal from the Toxicity Reference database. The concordance of organ-level findings in replicate studies, defined by chemical only, and chemical and species, and chemical and study type, and the variance in treatment-related organ lowest effect level (LEL) values, were estimated. Differences between chronic (CHR) and subchronic (SUB) organ-level effects were evaluated. Finally, in vitro estimates of organ-level effects were quantified. Total concordance (% chemicals with agreement across studies), depending on organ and replicate definition, was 39 - 88%. Multilinear regression modeling, using study descriptors as covariates, was used to estimate total variance, mean square error (MSE), and root residual mean square error (RMSE) in organ LELs. The MSE values (estimates of unexplained variance) suggest study descriptors accounted for 52-69% of total variance in organ LELs. RMSE ranged 0.4 - 0.6 log10 (mg/kg)/day. Odds ratios indicated it is unlikely to observe organ effects in a CHR study if the chemical was negative in a SUB study. Paired randomization testing indicated that mean CHR organ LELs were less than mean SUB organ LELs for most tissues, with mean CHR-SUB differences of 0.1 to 0.5 log10 (mg/kg)/day. Finally, in vitro bioactivity data from liver and kidney models were used to estimate administered equivalent doses (AEDs) via in vitro to in vivo extrapolation. Paired randomization testing indicated the observed mean difference between LEL and AED values may approach 0.5 log10 (mg/kg)/day for liver and kidney. Overall, variability in repeat dose organ LELs suggests a good NAM might predict organ LELs within ± 1 log10 (mg/kg)/day with accuracy approaching 70%, and existing NAMs may predict liver-related LELs within estimates of variability in replicate in vivo studies. This abstract does not necessarily reflect US EPA policy.

**1137 How Do We Achieve Reproducibility in 21st-Century Toxicology?**

R. Nault, Michigan State University, East Lansing, MI.

New approach methodologies (NAMs) are developed by diverse stakeholders from academia, industry, and government in a concerted effort to reduce costs and accelerate human health risk assessment. As NAMs continue to proliferate without clear expectations about data sharing and publication, or standard for evaluating their performance, there is a risk of creating a reproducibility crisis and impeding progress towards adoption. Essential to evaluating NAMs, whether they are in vitro assays or computational models, is the transparency of the models and analytical methods including metadata and code. Efforts to promote improved data sharing and management such as the Findable, Accessible, Interoperable, Reusable (FAIR) principles can serve to facilitate reproducibility and reuse of NAMs, but there is a need for the establishment of community-based expectations regarding the reporting and dissemination of NAMs as well as metrics for benchmarking performance. Ultimately, through implementation of robust community-based standards and FAIR data sharing principles it is expected that we can facilitate the benchmarking and evaluation of NAMs accelerating their adoption for human health risk assessment.

**1138 Expectations for New Approach Methods Performance in Predicting Organ-Level Effects in Repeat-Dose Animal Studies**

K. Paul-Friedman, US EPA, Research Triangle Park, NC.

Building scientific confidence in new approach methodologies (NAMs) may include a comparison to in vivo results. This work suggests benchmark expectations for NAM performance in predicting organ-level effects in repeat dose studies of adult animals by investigating the reproducibility, variability, and differences in organ-level findings in repeat dose studies, using organ lowest effect level (LELs) for liver, kidney, stomach, spleen, thyroid, and adrenal from the Toxicity Reference database. The concordance of organ-level findings in replicate studies, defined by chemical only, and chemical and species, and chemical and study type, and the variance in treatment-related organ lowest effect level (LEL) values, were estimated. Differences between chronic (CHR) and subchronic (SUB) organ-level effects were evaluated. Finally, in vitro estimates of organ-level effects were quantified. Total concordance (% chemicals with agreement across studies), depending on organ and replicate definition, was 39 - 88%. Multilinear regression modeling, using study descriptors as covariates, was used to estimate total variance, mean square error (MSE), and root residual mean square error (RMSE) in organ LELs. The MSE values (estimates of unexplained variance) suggest study descriptors accounted for 52-69% of total variance in organ LELs. RMSE ranged 0.4 - 0.6 log10 (mg/kg)/day. Odds ratios indicated it is unlikely to observe organ effects in a CHR study if the chemical was negative in a SUB study. Paired randomization testing indicated that mean CHR organ LELs were less than mean SUB organ LELs for most tissues, with mean CHR-SUB differences of 0.1 to 0.5 log10 (mg/kg)/day. Finally, in vitro bioactivity data from liver and kidney models were used to estimate administered equivalent doses (AEDs) via in vitro to in vivo extrapolation. Paired randomization testing indicated the observed mean difference between LEL and AED values may approach 0.5 log10 (mg/kg)/day for liver and kidney. Overall, variability in repeat dose organ LELs suggests a good NAM might predict organ LELs within ± 1 log10 (mg/kg)/day with accuracy approaching 70%, and existing NAMs may predict liver-related LELs within estimates of variability in replicate in vivo studies. This abstract does not necessarily reflect US EPA policy.

**1139 Leveraging In Vivo Variability to Assess Performance of Predictive Models and the Role of Reanalysis to Build Confidence via Consensus**

A. Karmaz, Inotiv, Research Triangle Park, NC.

Regulatory agencies rely upon rodent in vivo acute oral toxicity data to determine hazard categorization and appropriate precautionary labeling as well as to perform quantitative risk assessments. We developed a reliable, robust reference data set to characterize the reproducibility and inherent variability in the in vivo acute oral toxicity test method, which then was used to contextualize results and set expectations regarding NAM performance. Briefly, a large acute oral toxicity data inventory for 11,992 chemicals was compiled and made available to 35 participating international research groups who submitted a total of 139 predictive models. The same inventory was subset to identify chemicals with at least two independent in vivo studies resulting in an observed variability quantified by a margin of uncertainty of ±0.24 log10 (mg/kg) associated with discrete in vivo rat acute oral LD50 values. The predictive models submitted by consortium members were evaluated within their respective model applicability domains using external validation and the in vivo defined margin of uncertainty. Predictions were then combined into a collective set of model predictions and analyzed. This “re-analysis” approach wherein independent groups applied their own methods, and independently derived results were assessed for consensus serves to leverage the collective strengths of each distinct model and help build confidence in the consensus prediction. Compared to the reproducibility of the in vivo study, as measured by conditional probabilities, the collective models-based consensus showed high accuracy confirming robust performance.

**1140 How Will Transparency Make Our Work Ethical and Reproducible?**

L. Burgoo, Raptor Pharm & Tox Ltd., Apex, NC.

Scientific inference is ultimately predicated on our ability to make inferences about the population response based on studies of the sample response. Ultimately, the quality of inferences is based on the quality of the study design. There can be many reasons why we cannot reproduce a study, most commonly due to lack of sufficient information to actually perform the study again. When we do have sufficient information to perform the study, reproductions typically fail due to sampling bias.
i.e., the samples used do not accurately represent the population), which can manifest as unacceptably high variability between studies. From an applied ethics perspective, the inability of samples to accurately reproduce the population will lead to inappropriate inference, and inappropriate inference is always harmful. Thus, we have an ethical imperative to identify these instances before study reproduction efforts are underway, and in fact, we have an imperative to avoid sampling bias, and other study biases, before the study is even published - this is the goal of scientific integrity. This talk will illustrate how a few small steps will go a long way to help with transparency and scientific integrity. Examples will be provided such as moving towards a culture of protocol pre-registration - where a study protocol is pre-registered for all to see, and this includes the analysis plan. Furthermore, ways that authors can make all of the data analyzed in the study (including data that were not analyzed) available will be discussed. Showing resources for how we can share the code and parameters used to analyze the data, including versions of software, such as Docker images to share the analysis environment and all of these (meta) data. There are steps that can be taken immediately, leveraging existing tools, that will enable others to reproduce the work and the analyses.

**W 1141 Challenges and Future Directions in NAM Applications to Mixtures Risk Assessment**

D. Hines, RTI International, Research Triangle Park, NC.

Characterizing toxicity and risk for complex chemical mixtures poses unique challenges. Mixtures of interest for risk assessment can include combined exposure to environmental contaminants (e.g., Superfund sites) or commercial products with unknown active ingredients that may be unknown but are present at varying concentrations have different kinetics and may affect multiple mechanistic pathways leading to one or more biological outcomes. Furthermore, mixture components may contribute to chemical interactions that can result in synergy or antagonism. New approach Methodologies (NAMs) have emerged as nonanimal alternatives to traditional toxicology test methods but have yet to be widely implemented in regulatory risk assessment. These NAMs include in vitro assays that can generate one or more mechanistic outputs to characterize toxicity, as well as computational and statistical approaches that can be used to integrate, evaluate, and interpret in vitro results. This Workshop identifies the current usefulness and limitations of NAMs related to informing regulatory risk assessment of mixtures. It also discusses emerging methodologies used to address these challenges in chemical spaces, which range from household products to pharmaceuticals. This session will focus specifically on progressing toward the application of NAMs in regulatory settings for mixtures in the US and other countries. The session will begin with a brief introductory presentation by the session Chair to provide an outline of the Workshop and highlight the importance of extending NAMs to mixtures regulatory applications. The second speaker will provide a regulatory perspective on the needs and potential uses for NAMs that are relevant to mixtures. This presentation will focus on what questions NAMs should address so that they are useful in regulatory settings. The third speaker will discuss the advantages and challenges of NAMs compared with traditional regulatory risk assessment approaches, focusing specifically on mixtures applications. This presentation will highlight certain capabilities of current NAM approaches to filling knowledge gaps and facilitating mixtures analysis as well as identifying their limitations. The fourth speaker will provide an example application of NAMs analysis of mixtures by discussing cell-based toxicogenomic studies conducted on natural product mixtures. The floor will then be open for discussion to facilitate conversation focused on addressing current limitations and progressing NAMs toward regulatory application in risk assessment. The discussion will include the questions (1) how can emerging NAMs help to address these limitations and inform mixtures risk assessment approaches, (2) how can NAMs provide advantages over traditional regulatory risk assessment approaches, (3) can NAMs be used to inform on undefined mixtures, such as those from natural products or ingredient breakdown, and (4) what NAM resources are currently available to help managers assess mixtures?

**W 1142 A US FDA/CDER Perspective on Nonclinical Testing Strategies: Classical Toxicology Approaches and New Approach Methodologies**


Nonclinical testing of human pharmaceuticals is conducted to assess the safety of compounds to be studied in human clinical trials and for marketing of new drugs. Although there is no exact number and type of nonclinical studies required for safety assessments, all are important components of the testing effort. The traditional approach is outlined in various FDA and ICH guidance documents and involves a combination of in vitro assays and whole animal testing methods. Recent advances in science have led to the emergence of numerous new approach methodologies (NAMs) for nonclinical testing that are currently being used in various aspects of drug development. These nonclinical nonclinical testing methodologies can predict clinical outcomes, although improvements in these methods that can increase predictivity of clinical outcomes are encouraged and needed. Although most of FDA/CDER’s experience with NAMs has been with individual chemicals, issues with mixtures can occur. For example, we recognize the need to assess human skin sensitization of mixtures. However, it is important that validation of a NAM for mixtures should include testing of mixtures to ensure that the NAM works with mixtures as well as with individual components. In addition, the type of information needed to evaluate whether the NAM is adequate to support making a regulatory decision for the safety of a mixture will be discussed. This presentation discusses FDA/CDER’s view on the challenges and opportunities of using NAMs in drug development especially as they relate to mixtures. It addresses the need to understand the appropriate ways of extending NAMs to mixtures. Examples where NAMs are currently being used in nonclinical safety assessments, and where they may supplement and/or enhance current testing methods, are also discussed.

**W 1143 Advantages and Challenges of New Approach Methodologies in Regulatory Risk Assessment of Chemical Mixtures**


This presentation summarizes current challenges, the potential use of novel scientific methodologies, and ways forward in the risk assessment and risk management of mixtures. Generally, there is consensus on methodologies to address mixtures; however, there are still several data and methodological gaps to be addressed. New approach methodologies (NAMs) can support filling knowledge gaps on the toxicity and mode(s) of action of individual chemicals. It is practically impossible to test all possible mixtures and single substances experimentally. Therefore, smart strategies are needed to assess the potential hazards using new tools that rely less on in vivo testing and incorporate instead alternative experimental and computational tools. Their main strengths lie in their integrated use and putative contribution of different aspects of the hazard from combined exposure to multiple chemicals. But in order to benefit from these tools in the hazard assessment of mixtures, more guidance on their use is needed to facilitate a more widespread application. In the presentation, the potential for using NAMs to support mixture risk assessment will be discussed, such as in whole-mixture as well as component-based approaches and use for grouping substances. Possible uses will be illustrated using examples based on human biomonitoring data.

**W 1144 Case Studies Using New Approach Methods for Evaluation of Complex Mixtures**

D. Daston, Proctor & Gamble, Cincinnati, OH.

Botanical dietary supplements, personal care products, and household goods (e.g., cleaning products) are examples of mixtures that represent everyday exposures. When the components of mixtures are well characterized the assessment of safety of a consumer product is relatively straightforward, with NAMs being particularly useful in elucidating modes of action that might be expected to interact. Evaluating the safety of uncharacterized complex mixtures such as botanical extracts is more difficult due to challenges in both chemical analysis and testing. This necessitates the application of predictive approaches that include both computational and in vitro evaluation. Case studies describing application of NAMs to complex mixtures will be presented and include botanical extracts evaluated in high-throughput receptor binding screening and transcriptomics, coupled with analytical chemistry to identify major components of the extracts, as well as exposure considerations. Key considerations include benchmarking with known active and inactive compounds and distinguishing adaptive responses from adversity in an in vitro context.

**W 1145 Crucial Role of Physiologically Based Pharmacokinetic (PBPK) Modeling in Human Health Risk Assessment in Different Sectors**

A. Schepky, Beiersdorf, Hamburg, Germany.

The evaluation of the public health impact of thousands of chemicals in commerce and the environment might be accelerated using valid nonanimal, new alternatives methodologies NAMs. Next-Generation Risk Assessment (NGRA) of a chemical is a framework that integrates NAMs in order to assure human safety in the absence of animal testing. The key principle of the NGRA is to extrapolate the in vitro point of departure to external equivalent exposure regimes associated with product use (i.e., in vitro to in vivo extrapolation [IVIVE]). Physiologically based pharmacokinetic (PBPK) models integrate the knowledge on the absorption, distribution, metabolism, and excretion (ADME) of a chemical in order to describe its fate in the human body, which provides a means for this extrapolation. Application of PBPK models facilitate extrapolations across studies, species, routes, and over various dosing scenarios. PBPK modeling is fundamental to the development of biologically based dose-response models used to make quantitative predictions and address uncertainty and variability related to kinetics and dynamics of a chemical. This session will address the concepts, model structures/software, and implementation of PBPK models in chemical risk assessment. The first presentation will briefly introduce generic PBPK and NGRA to the audience. The next presentation will illustrate PBPK in chemical risk assessment using ultraviolet light filters (UV absorbers) as an example. The third presentation will compare and contrast the issues with pharmaceuticals with those of other chemicals in commerce regarding reporting templates and transparency and reproducibility. The final presentation
will discuss PBPK modeling for humans, test species, and farm animals. Beginning with examples of food and feed risk assessment, this presentation also will discuss anticipated developments, implementations of generic PBPK models into the European Food Safety Authority’s Tkplate, and harmonizations of sensitivity analysis. This brief Workshop will conclude with a panel discussion in which the presenters will be asked to address (1) when deterministic versus probabilistic PBPK models are required for chemical risk assessment, (2) best practice model evaluation criteria, and (3) how to increase confidence and regulatory acceptance of PBPK models. Time will be allotted for additional questions from the Workshop attendees; audience questions to the panel will be encouraged and welcome.

PBPK Modeling for Chemical-Specific Risk Assessment: Homosalate after Sunscreen Application Case Study


The evaluation of the safety of chemicals for humans necessitates using non-animal/new alternatives methodologies. In the current study, a physiologically-based kinetic (PBK) model was developed for UV filters, e.g. homosalate, to support the human health safety evaluation without generating animal data. The developed PBK models were implemented for to route-to-route and inter-species extrapolation. This would facilitate the replacement of default assessment factors with more specific, chemical-derived factors for the Margin of Safety calculation. Legacy in vivo data were employed to develop the PBK model. In addition, The PBK model was developed using measured human in vitro ADME data. The derived PBK model was subject to sensitivity analysis to identify influential chemical-specific parameters followed by considering the calculation of the confidence interval (CI) of Omnirex and AUC. Model performance was evaluated by comparing predicted and observed values from a US-FDA clinical trial (Identifer: NCT03582215, https://clinicaltrials.gov/). Final estimation of the internal dose metrics was obtained in a virtual population and considering input parameter uncertainty. The developed PBK model estimated reasonably well the internal exposure of homosalate according to different exposure scenarios with a medium to high level of confidence. PBK models for other UV filters are under development. In the absence of in vivo data, such human PBK models will be the heart of future completely non-animal-based chemical risk assessment. Reference Najjar, A., et al. (2021) Use of Physiologically-Based Kinetics Modelling to Reliably Predict Internal Concentrations of the UV Filter, Homosalate, After Repeated Oral and Topical Application. Frontiers in Pharmacology 12, 802514. DOI: 10.3389/fphar.2021.802514.

Enabling Risk-Based Decisions with Pharmacaco- and Toxicokinetic Models while Addressing Transparency and Reproducibility

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

Pharmacokinetics/Toxicokinetics enables quantitative characterization of chemical risk by allowing insight into the dose-response relationship. However, some aspects of kinetics are chemical-specific and require parameters and other features that must be derived from data and/or structure-based predictions. Models and their parameters are typically vetted through the scientific peer-review process which, while important, has often been shown in many cases to be insufficient for risk evaluators (Mclanahan et al., 2012). This presentation will compare and contrast the issues for pharmaceuticals and chemicals in commerce. We will then investigate both 1) model reporting templates and 2) “generic” models with consistent form and parameterization as alternatives that enable transparency and reproducibility to better inform regulatory decision making. Traditional approaches to developing PBK models were focused on single chemicals, with chemical-specific consideration of key physiology - these models are resource-intensive and often dependent upon data derived from animals. More recently, generic PBK models with standardized physiologies that depend on in vitro measurements have emerged as a higher throughput alternative to traditional PBK.

Generic PBPK Modeling for Humans, Test Species, and Farm Animals: Their Role in Food and Feed Risk Assessment and Further Developments

J. Dorne. European Food Safety Authority, Parma, Italy. Sponsor: A. Schepky.

Generic human physiologically-based (PBK) models have been developed at the European Food Safety Authority (EFSA) for a number of test species (mouse, dog, rabbit), and several farm animals (cattle, pig, sheep, chicken). These models have been published, made open access, assessed, and evaluated using relevant case studies using historical data. In addition, the models were implemented as tools following the principles laid out in the OECD guidance to support their implementation in the food and feed safety arena. Applications include forward and reverse dosimetry to predict kinetic parameters and concentrations in target organs from exposure (external dose) or recalculated exposure from biomonitoring studies (internal dose) respectively. This presentation aims to describe the development of such models, illustrate their validation, and use through case studies in the first place. Ongoing developments will also be discussed with particular attention to recent data collection on physiological parameters and metabolic and transporter interactions with human health. Additionally, new scenarios will be presented in the context of other PBK applications. A. Najjar, A. Schepky.

Placental Biology, Toxicology, and In Vitro Modeling for Predictive Developmental Toxicology

J. Rogers. ToxStrategies Inc., Raleigh, NC.

The placenta serves critical functions for pregnancy maintenance and in utero development in mammals, and its structure and function can be compromised by diverse chemical toxicants, e.g., maternal conditions. Placental insufficiency is associated with fetal growth restriction (FGR) and other adverse effects on pregnancy in humans and animals. It is often attributable to perturbation of the unique cell populations in the placenta. New approach methods (NAMS) for development toxicity assessment without animals will need to predict FGR and other pregnancy complications, but they are typically evaluated against known structural teratogens and non-teratogens. The human placenta, which is not represented in current cellular or non-placental animal NAMS (e.g., stem cells, zebrafish, C elegans) for developmental toxicity, can be modeled in vitro to assess development of the placenta, placental metabolism, and the placenta’s transport of nutrients and waste products. In vitro placental models could improve prediction of FGR and other placenta-mediated effects in humans. Placental responses to toxic exposures and conditions, including transcriptomic, proteomic, and epigenomic analyses and signatures related to effects on prenatal development and their relationship to long-term effects on offspring, will be explored. Some aspects of placenta cell physiology are sexually dimorphic, and the importance of this finding will be discussed. A variety of in vitro methods for toxicity assessment will be presented, representing different cell types and different stages of placenta. Findings for environmental contaminants, including per- and polyfluoroalkyl substances, phthalates, and polycyclic aromatic hydrocarbons, will be discussed. This Workshop will highlight the placenta as a unique and critical target for developmental toxicity assessment, which hopefully will spur discussion of models of placental toxicology as NAMS are developed for predictive developmental toxicology.
the underlying mechanisms remain unresolved, recent investigations in rodent and human cell lines indicate that PFBA and other PFAS may cause placental toxicity. Placental trophoblasts serve critical roles in placation, and impaired trophoblast function is linked to common pregnancy complications. In culture, primary cell/tissue trophoblast models display representative functional and molecular characteristics of their in vivo counterparts. Due to these properties, primary cell/tissue trophoblast models have been employed as a proxy to investigate chemicals for human placental toxicity. As an example, we highlight our efforts in using primary human villous cytotrophoblasts (CTBs) to profile the impacts of environmental chemicals such as PFOS on cell function and the transcriptome and proteome. We demonstrate PFOS to induce significant cytotoxicity in a concentration- and time-dependent manner. Additionally, at subcytotoxic concentrations, PFOS alters key regulatory and biological pathways responding to hormones and response. Importantly, PFOS alters key molecules and biological pathways regulating hormone response, lipid metabolism, and innate immunity, implicating key signaling pathways (e.g., PPAR) in PFOS response. Using a modeling approach, we predict specific proteins and related pathways (e.g., innate immune response) to be altered at concentrations (<1μM) relevant to human exposure. In summary, we provide supporting evidence related pathways (e.g., innate immune response) to be altered at concentrations (<1μM) relevant to human exposure. In summary, we provide supporting evidence related pathways to be altered at concentrations (<1μM) relevant to human exposure. In summary, we provide supporting evidence that PFOS induces human placental toxicity in vitro and affects biological pathways important for placental function and disease.

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1152 Placental 'Omics Data: New Insights into Chemical Toxicity and the Developmental Origins of Health and Disease


Phthalates are ubiquitous endocrine disrupting chemicals that alter placental growth and function, and potential exposure to phthalates has been linked to a variety of infant and childhood health outcomes through the Developmental Origins of Health and Disease (DOHAD) paradigm. Transcriptomic profiling can reveal underlying functional changes in both placental cell lines and human cohort studies related to prenatal exposure to phthalates, as well as other environmental exposures. These approaches offer new insights into some of the mechanisms that may exert the most substantial disruptions to the placenta, which can inform chemical monitoring. Understanding these transcriptional changes can also inform molecular mechanisms of chemical perturbations, which can provide insight into mechanisms of toxicity. In the latest cohort study of its kind (N=760), we have identified genes and noncoding RNAs whose placental expression is associated with urinary concentrations of 16 different phthalate metabolites in the 2nd and 3rd trimesters. The highest number of associations were with a secondary metabolite of diisononyl phthalate, and we also observed associations between Di (2-ethylhexyl) phthalate and its metabolites and placental gene expression that were significant only in male samples. In parallel, we have performed comparative in vitro work in both primary placental cell lines and extravillous trophoblast cell lines, which revealed that Monol (2-ethylhexyl) phthalate induces transcriptional changes that are related to concentration, fetal sex, and trophoblast cell type. We identified a higher number of transcriptomic changes in extravillous trophoblasts than primary cells, and changes to the placental transcriptome is primary cell from male participants, which included genes that were also significant in our cohort study. The genes with differential expression had enriched binding sites for a number of transcription factors, including nuclear hormone receptors PPARG, PPAR, AR and ESR1, which is in alignment with known mechanisms of toxicity. Our next steps involve the integration of chromatin mapping to investigate the upstream regulators of these gene expression changes. Taken together, this work highlights the potential that transcriptomic approaches can provide in establishing mechanisms of toxicity.

Climate change is leading to a steady overall rise in average temperatures across the globe. Even more concerning is the increase in extreme weather events (e.g., extreme heat events, drought, tornados) that are more likely to result in more serious catastrophes and disproportionate health impacts. Extreme heat events, which are expected to increase in frequency and severity, are characterized in the US by high temperature (> 95°F) combined with high humidity over multiple consecutive days. According to the US Centers for Disease Control and Prevention, these events contribute to the death of more than 600 people per year, which may be an underestimate of the actual number because many of the recorded fatalities are assigned to comorbidities. It also is projected that climate change will cause more areas to experience lengthier and more frequent, intense extreme heat events. Furthermore, human activities concentrate heat exposures, such as in urban heat islands. The broader ramifications of extreme heat events are not fully characterized at the geographic scales relevant for decision-making, including morbidity (e.g., acute and chronic illnesses, adverse pregnancy outcomes, and psychosocial disorders), mortality, and lower productivity of outdoor workers. Contributing factors include disparities because of socioeconomic and other factors, along with financial costs. The overall burden of these extreme heat events will likely persist for several decades after reductions in greenhouse gas emissions. This session will highlight the latest information on the effects of extreme heat events on human populations, including cardiopulmonary and mental health impacts. The presentations will focus on a few critically important topics related to extreme heat events, including the driving factors behind regional variations and sources of vulnerability. Findings will show that responses to this crisis must include developing climate-resilient health systems and adaptation policies that seek to reduce risk among the most vulnerable while simultaneously considering other stressors. Attendees also will hear about assessment of risk related to rising ambient temperature and vulnerable populations. In addition, the effect of extreme heat on mental health and behavior will be explored. Finally, we will assess the risks of compounding heat-related risks with wildfire smoke and other environmental exposures. This session and the abstracts therein do not represent official US Environmental Protection Agency policy.

1154 Acute Cardiopulmonary and Neurobehavioral Effects of Extreme Heat: Heightened Risk from Living Conditions and Subsequent Environmental Exposures

M. Flaiming. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Climate change is expected to continue increasing the average daily ambient temperature, as well as introducing more frequent and intense heat waves across the globe in the coming decades. Concurrently, urban environments are becoming increasingly populated worldwide, where people are exposed to higher ambient temperatures through a phenomenon known as the Urban Heat Island (UHI) effect. These conditions are a major concern for vulnerable populations, such as those with pre-existing conditions (e.g., cardiovascular disease). The World Health Organization (WHO) has characterized cardiovascular disease (CVD) as the leading cause of death in 2021 and there is epidemiological evidence to suggest that a warming climate will contribute to an increase in cardiovascular mortality. Thus, further examination of these factors (extreme heat events (EHE) and underlying CVD) and their interactions is crucial in addressing the growing public health concern. More importantly, epidemiological data need to be supported with biological plausibility in order to adequately characterize the risk, identify vulnerable populations, and propose mitigation and treatment strategies. Using in vivo rodent models, we evaluated the effects of EHE and long-term exposure to high ambient temperatures on cardiovascular, pulmonary, and behavioral responses using repeated high-frequency echocardiography, whole-body plethysmography, and Morris water maze and novel object recognition, respectively. Results from these studies show that increased heat contributes to altered cardiopulmonary function and increased arrhythmogenesis and is further impacted by living conditions (depleted versus enriched), which appears to play a role in disease progression and expression of genes important in environmental sensing (e.g., transient receptor potential channel expression). Furthermore, these factors alter the responses to subsequent environmental exposures, especially other extreme events like wildfire smoke. These conditions represent a set of daily stressors that implicate disruption and imbalance in the homeostatic regulation (e.g., autonomic nervous system) of the cardiovascular and pulmonary systems, alter body resiliency, and increase the likelihood of deleterious health outcomes from subsequent environmental exposures.
The impact of extreme heat (EH) on physical health outcomes has been well documented. However, impacts of elevated temperature on specific mental health outcomes remain poorly understood. Climate epidemiology can leverage existing large healthcare claims datasets to assess the relationship between extreme weather and adverse mental health outcomes. The objective of our initial study was to quantify the association between ambient heat and mental health-related emergency department (ED) visits in the contiguous US among adults, overall, and among potentially sensitive subgroups. The outcome of interest was ED visits with a primary or secondary discharge psychiatric diagnosis during the warm seasons among adults with commercial or Medicare health insurance living in 2,775 US counties. County-specific daily maximum ambient temperature, on a continuous scale, were used to estimate Parameter-elevation Relationships on Independent Slopes (PRISM) model. We assessed the daily incidence rate of cause-specific mental health diagnoses, as well as a composite endpoint of any mental health diagnosis, assessed using primary and secondary discharge diagnosis codes. We identified data from ~3.5 million ED visits among ~2.2 million unique individuals (~45% male). Days of extreme heat were associated with an incidence rate ratio (IRR) of 1.08 (95% confidence interval [CI]: 1.07, 1.09) of ED visits for any mental health condition. Associations were also pronounced for ED visits due to specific illness, including anxiety and stress disorders, mood disorders, substance use disorders, schizophrenia, childhood onset behavioral disorders, and self-harm. Associations were more pronounced among men and in the Northeast, Midwest, and Northwest climate zones. Days of extreme heat were associated with higher rates of mental health-related ED visits. Results may be informative for clinicians providing mental health services during EH and may also aid in preparing for expected increases in health service needs when times of extreme heat are anticipated. Importantly, this work focuses on a dataset of health-related data that is among the most valuable in our database. Further work should aim to characterize subclinical mental health outcomes associated with EH, as well as the mental health effects of exposures in low-income and underinsured/uninsured populations.

The NCATS BioPlanet is a comprehensive pathway resource that incorporates the universe of nearly 1,700 human pathways sourced from publicly available, manually curated sources, which have been subjected to thorough redundancy and consistency cross-evaluation. The BioPlanet web browser enables integrated visualization of pathway information, connections between gene targets, pathways, diseases, toxicity endpoints, and available assays that measure the bioactivity of pathway targets. The platform provides in addition visualization of pathways on a 3-dimensional globe, in which the distance between any two pathways is proportional to their degree of gene component overlap. This presentation will demonstrate the BioPlanet as a biological big data visualization tool and its application in the selection and design of in vitro assays for the clinical response measurement to strengthen hypothesis generation.

Large-scale data curation efforts have introduced complex networks of disparate toxicological information that can be difficult to understand and communicate. Data visualization is a form of communication that can be used to build an understanding of the types of information within these large, complex databases and how that information is connected. Effectively communicating through data visualization allows the audience to understand how the data support a conclusion or to form new hypotheses altogether. Poorly made graphics can lead to misinterpretation and hinder science communication by distorting the information being presented. This session will introduce attendees to the computational tools used to visualize and contextualize complex toxicological data. The focus will be on building a data story through data visualizations within the framework of the US Environmental Protection Agency CompTox Chemicals Dashboard. The session will focus on the use of data visualization—specifically, the OrbiTox tool—to understand and predict connectivity between multidomain data. This talk will discuss the importance of data visualization for effectively communicating complex data and emphasize existing tools that facilitate visualization of large, complex data.

The use of animals in pharmaceutical and chemical safety testing is a privilege that should be respected with the implementation of the 3Rs (replacement, reduction, and refinement). The current shortages in routine nonrodent species, such as nonhuman primates, acknowledged by the US Food and Drug Administration in a guidance published in February 2022 created an urgency to reduce the use of primates. Where in vitro assays are not yet mature to replace the whole nonrodent species in the Wake of Primate and Dog Shortages. K. Sokolowski. Denali Therapeutics, South San Francisco, CA.

The NCATS BioPlanet is an integrated platform for exploring the universe of cellular signaling pathways for toxicology, systems biology, and chemical genomics. R. Huang. NIH/NCATS, Rockville, MD.

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are how these disadvantages compare to other species and if these disadvantages preclude the use of rabbits in general toxicology studies. While several factors affect the decision for species selection, including pharmacological relevance, pharmacokinetics and ADME considerations, tolerability, the extent of available background data, practical considerations around the route of administration, and precedent with certain drug classes, there are no perfect models. In this Workshop, we aim to bring together stakeholders from veterinary medicine, industry, contract research organizations, and government to explore concerns for using the rabbit for general toxicity testing and identify data gaps to alleviate concerns with the use of rabbits. Ideally, this effort will propel companies to include rabbit in their species selectivity screens and use them when identified as a relevant species, which could build the historical control database for rabbits. Ultimately, diversity of nonrodent species available for general toxicology studies will provide flexibility across industry that can alleviate shortages of nonrodent species.

W 1162 Introduction
K. Sokolowski, Denali Therapeutics, South San Francisco, CA.

The introduction will provide background on the use of rabbits and declare the issues to be addressed by the workshop. Selection of a sensitive toxicity species is important to identify relevant and translatable toxicities to humans. A review of reported concordance rates across species demonstrates the utility of rabbits as a sensitive species in toxicology studies. Three case studies of recently approved drug products using both rabbit and nonhuman primate in the general toxicology studies will be presented for comparison of translatable toxicities. This talk will highlight the specific rationale for use of rabbits in toxicology studies and differentiate the intrinsic scientific limitations of the rabbit as a nonrodent species from the technical, commercial, and regulatory limitations.

W 1163 General Overview of Rabbit Husbandry and Veterinary Care
P. Tumer. Charles River Laboratories, Wilmington, MA. Sponsor: M. Fortin

Identified concerns for the use of rabbits in discovery and preclinical safety studies is the rabbit’s peculiar nature and perceived fragility as a research model. Rabbits are in the order Lagomorpha, which puts them on a branch of the phylogenetic tree next to rodents, but closer to primates than dogs or minipigs. Unusual among common research animal species, rabbits are herbivores and hind-gut fermenters adapted for high fiber diets and colonies should be managed accordingly. A prey species, rabbits are sensitive to certain stressors (e.g., handling that can make heart rate and blood pressure increase) and the signs of stress can be subtle (e.g., reduced movement around their enclosure rather than outward signs of distress). However, proper management techniques such as acclimation and adequate staff training to recognize signs of distress can be employed to make the rabbit a successful species for collecting relevant and translatable data for safety. This talk will set the stage by providing a veterinary perspective on working with rabbits in examining novel therapeutics, biomaterials, and medical devices as well as tips for successfully integrating rabbits into research programs.

W 1164 What Else Can Rabbits Be Used For in Pharmaceutical Development Other Than Embryo-Fetal Development Studies and Vaccine Development?
E. Lewis. Charles River Laboratories, Horsham, PA.

Specialized Contract Research Organizations (CRO) are equipped to use rabbits in pharmaceutical development and routinely use this species in embryo-fetal development (EFD) studies, vaccine development, medical device and intraocular general toxicity studies. However, there are other uses for the rabbit that are not routinely employed, such as juvenile animal studies and toxicology studies administering test articles through common and uncommon routes (e.g., oral, intravenous bolus and infusion, intramuscular, intraarticular, etc.) In a recent poll, selection of the dog and NHP were based on historical control data in those species, dog or NHP used as the precedent species for a particular drug class, and dog or NHP were routine species used by the company without further studies to identify other relevant species, such as rabbit (Prior et al., 2020). In comparison, the rabbit lacks the historical control data of the dog and NHP; however, there is sufficient historical control data in rabbit regarding the traditional general toxicology endpoints (e.g., background histological lesions, hematology, clinical chemistry, clinical behavior, and ophthalmology). Capability also exists for the use of rabbits in cardiovascular and respiratory safety pharmacology studies; however, there are limitations on the historical control data. As the rabbit is an altricial species, their use in juvenile animal studies rivals that of the NHP. This talk will describe the extent of historical control data on the rabbit, identify the needs of industry to build the historical control data in particular areas, and promote the possibilities of the use of this species for pharmaceutical development.

W 1165 Regulatory Perspectives on Use of Rabbit as an Alternative Nonrodent Species for Drug Development
P. Wang, US FDA, Silver Spring, MD.

In general, the safety assessment of pharmaceuticals involves a toxicologic framework that should include two species, a rodent and a nonrodent, as this framework has been shown to increase the chances of identifying human-relevant toxicities. The choice of which rodent and nonrodent species to use, and the justification for why the species represents an appropriate system for assessing human safety of the pharmaceutical rests with the pharmaceutical developer. In the past, most small molecule developers typically relied upon the dog as the nonrodent species; however, for reasons that are not entirely clear, there has been a shift in recent years towards the use of the nonhuman primate (NHP). Biotherapeutic protein developers also often rely upon the NHP for safety assessments, as the NHP is often the only pharmacologically relevant species. The COVID-19 pandemic has caused a marked disruption in the NHP supply. There have also been indications that dog supplies are constrained in some regions. This has created a climate in which pharmaceutical developers are faced either with the prospect of substantially delaying their development program, or of identifying alternative nonrodent species. This talk will discuss some of the considerations of using the rabbit for assessing general toxicity endpoints, as well as FDA experience with the rabbit as a nonrodent species.

W 1166 Expanding Our Knowledge of Neurological Disease Etiologies: Current Research on the Neural Exposome
D. Jett, NIH/NINDS, Bethesda, MD.

The complex etiologies of many neurological disorders, which include both heritable and nonheritable factors, present formidable challenges for researchers. This Symposium will present and discuss the neural exposome concept as a means of addressing multifactorial etiologies of disease. Drawing from the broader context of the exposome, which describes the totality of internal and external exposures across the lifespan that affect human health, the neural exposome are those exposures that have an impact on neurological diseases and disorders and overall nervous system health. These include environmental exposures to toxicants, social activity and stress, diet and nutrition, and the microbiome. Speakers David A. Jett, PhD, and Yuxia Cui, PhD, will provide an introduction to the exposome and available tools and resources at the National Institutes of Health that are used for exploring the neural exposome. The session also will feature illustrative examples of neural exposome research related to Parkinson’s disease, Alzheimer’s disease and related dementias, and amyotrophic lateral sclerosis as presented by Beate Ritz, MD, PhD, Gary W. Miller, PhD, and Eva Feldman, MD, respectively. Collectively, the speakers will highlight the progress, unmet needs, and potential of neural exposome research to advance development of more effective prevention and intervention strategies.

W 1167 Introduction to the Neural Exposome
D. Jett. NIH/NINDS, Bethesda, MD.

Human genetics has provided unprecedented insight into the etiologies of inherited neurological diseases and disorders as well as the role of the more common genetic mutation of each disease. Even though what we have learned from the genome is extremely valuable, the majority of health risk factors cannot be explained by genetics alone. The neural exposome incorporates the interactions between the genome and all nonheritable factors such as environmental exposure to toxic chemicals, social activity and stress, diet, and the microbiome that affect gene expression across the lifespan. It has become widely recognized that a new frontier of biomedical research is needed to complement the genomic information, and advances in exposome science to provide a great opportunity for the scientific community to make progress in uncovering the causes of a variety of human health conditions. The neural exposome is especially complex because the nervous system is continually changing as a function of experience and exposures across the lifespan. For instance, neurodegenerative diseases such as Alzheimer’s and Parkinson’s, or those with incomplete penetrance such as C9orf72 amyotrophic lateral sclerosis (ALS) and LRRK2 Parkinson’s disease, must be influenced by acute or chronic environmental exposures. Studies on the impact of the neural exposome are emerging, and there are many examples of how specific exposures affect the nervous system. There are also new insights into the mechanistic underpinnings of how the neural exposome modifies nervous system health that describe the synergy among the different kinds of neural exposomic factors such as environmental exposures and how they influence gene expression.
Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by clinical symptoms and structural changes in brain. Although genetic factors have been identified, the genetic contributors explain only a small portion of disease causation and progression. Our laboratory has taken a multi-pronged exposomic approach to examine environmental factors in AD and cognitive function. We are applying our exposomic analysis to three human studies: the Washington Heights and Inwood Community Aging Project (WHICAP), the Estudio Familiar de Influencia Genética en Alzheimer (EFICA), and the Reference Ability Neural Network (RANN) Study. In conjunction with and parallel to these human studies, we are conducting gene x environment follow up studies in C. elegans. For example, there were four metabolites (tyrosine, carnitine, cystine and N,N-dimethylyaniline N oxide) that were associated with increased p-tau expression in C. elegans and also elevated in cerebral spinal fluid in humans with AD. We use liquid chromatography and gas chromatography-based high-resolution mass spectrometry in these human studies to analyze exogenous chemicals and endogenous metabolites in plasma and cerebrospinal fluid. Dr. Miller will report initial findings from these studies that demonstrate relationships between environmental exposures and a range of phenotypic outcomes in this presentation.

### 1169 Leveraging the Exposome for ALS Prevention


Amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disease that lacks effective treatments. The identification of novel, modifiable risk factors are urgently needed for this fatal disease as most ALS patients die within two to four years after diagnosis. We developed a new multipollutant environmental risk score (ERS) based on concentrations of persistent organic pollutants and pesticides in blood from ALS cases compared to age-matched controls. We observed a seven-fold increase in ALS risk in individuals with a high ERS and a concomitant significant decrease in survival. Using both publicly available datasets and our own Michigan ALS cohort, we recently created a robust ALS polygenic risk score (PGS). Using both publicly available datasets and our own Michigan ALS cohort, we also recently created a robust ALS polygenic risk score (PGS). A PGS with 275 single nucleotide polymorphisms had the best model fit. A standard deviation increase in ALS PGS was associated with 1.25 (95% CI: 1.02, 1.53) higher odds of ALS with an area under the curve (AUC) of 0.654, which improved ALS risk prediction (p-value=1x10^-6) over a model without the ALS PGS. Using an exposomic approach combining ERS with PGS allows for the identification of at-risk individuals who may benefit from personalized interventions to either slow disease progression or, in select populations, promote ALS prevention.

### 1170 Exposomic Studies of Pesticides and Parkinson’s Disease in Rural Central California


Pesticides are an important component of agriculture worldwide and the types of chemicals in use have grown tremendously. Among environmental agents, pesticides also have been most consistently shown to increase the risk of Parkinson’s disease (PD). Thus, it is paramount to assess how different pesticides affect PD risk singularly or in combination. California pesticide use reporting captured more than 600 active ingredients applied as pesticides in agriculture over half a century. This pesticide use report information allowed for detailed and long-term exposure assessment in 1600 participants of two PD studies together with the collection of extensive residential and occupational data and bio-samples. In this presentation, we will discuss how we employed pesticide use report-based exposure data to gain insights into the pesticide exposome and its interactions with multiple ‘omics’ layers in humans that affect PD risk including identifying epigenetics (methylations) and metabolic signatures, and more recently, the human gut microbiome. For example, we will show that chronic low-level OP exposure is associated with differential DNA methylation in blood with the nicotinic acetylcholine receptor signaling pathway being most overrepresented. We will also present results for metabolic pathways associated with organophosphate, organochlorine and pyrethroid exposures related to mitochondrial energy metabolism, fatty acid, lipid, and amino acid metabolism suggesting that exposures result in oxidative stress, inflammatory reactions, and mitochondrial dysfunction. These human real-world study results have and continue to inspire additional lab-based experiments to address mechanisms through which pesticides contribute to PD onset and progression.

### 1171 The NIEHS Exposure Science and the Exposome Program: Advancing Tools and Technologies for Studying the Exposome

V. Cui. NIEHS, Research Triangle Park, NC.

A fundamental challenge of incorporating the exposome in precision health research is how to model environmental exposures. The National Institute of Environmental Health Sciences (NIEHS) has been at the forefront of accelerating scientific and technological advancements to characterize the exposome. This presentation will provide an overview of the research supported by the Exposure Science and the Exposome Program at NIEHS to develop a suite of complementary tools and methods for comprehensive assessment of the exposome. In particular, the presentation will focus on the Human Health Exposure Analysis Resource (HHEAR, https://hhearprogram.org/), an infrastructure and resource to support eligible NIH-funded researchers who want to add on or expand environmental exposure analysis in their study. HHEAR seeks to provide the research community access to centralized, high-quality, exposure-assessment services including state-of-the-art laboratory analysis of biological and environmental samples across the life span, statistical analysis, and expert consultation on study design, laboratory analysis, and data analysis and interpretation. The HHEAR Data Center (https://hhear-datalcenter.msm.edu/) provides a repository for exposure data generated by HEAR labs and previously collected epidemiologic data associated with an HHEAR study. More than half of the HHEAR studies are now publicly accessible with harmonizable environmental health data for secondary and pooled analysis.

### 1172 Human Cells as Nonanimal Alternative Approaches for Immunotoxicity Testing

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

Immunotoxicology, including the discipline of immunotoxicology, is a field of science that is at a crossroads between in vitro approaches to assess in vivo versus the need for in vivo models. Approval of the ban for toxicity testing of cosmetic constituents using in vivo models by the EU in March 2013 marked a turning point for the necessity to consider and develop alternative methods. This initiative led to the development and approval of the first in silico and in vitro alternatives to animal testing for immunotoxicity testing that are now accepted for regulatory purposes in many countries worldwide. The pressure to reduce animal use in toxicity testing is not limited to cosmetics, and enormous scientific efforts have been expended to develop nonanimal alternative approaches. The immune system by design is a highly complex and integrated system that presents challenges for the development of in vitro approaches. Challenge accepted: the field of immunotoxicology has worked arduously in pursuit of appropriate and informative in vitro models. Utilization of human cells for these models provides direct translatability to human health and risk assessment. The focus of this session is to highlight some of the innovations that are leading to breakthroughs in the incorporation of in vitro alternatives for hazard identification and risk assessment. Following a brief introduction, our first speaker will present the concept of wholistic in vitro immunotoxicity testing using human whole blood to interrogate innate, humoral, and cell-mediated immunity. The second speaker will discuss the species differences that underscore the need for human models to evaluate antibody production in immunotoxicity testing and provide evidence of a regulatory node within the immunoglobulin heavy chain gene that may predict sensitivity to xenobiotics that alter antibody production. The third speaker will take us back to the developing immune system and provide evidence of an in vitro model using human CD34+ immune progenitor cells to inform developmental immunotoxicity. The fourth speaker will discuss the application of in vitro techniques using human cells, specifically the HuLA assay for immunosafety, in pharmaceutical safety assessment. The fifth speaker will provide insight into regulatory needs surrounding the applicability of nonanimal alternatives in safety toxicology and human health risk assessment.

### 1173 Building a Comprehensive In Vitro Toolbox for Immunotoxicology Safety Assessment Using Human Whole Blood

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

The immune system is charged with not only maintaining overall health and well-being but also plays a critical role in maintenance of homeostasis in most other organ systems of the body. Therefore, the immune system is highly complex and dynamic and built to exist in an environment of continually shifting challenges from intrinsic and extrinsic forces. Given the pinnacle role for the immune system in homeostasis and health, the field of Immunotoxicology has been charged with protecting the immune system from toxicity. Traditional approaches for immunotoxicity safety assessment have relied heavily on use of animals to study the effects of xenobiotics on complex multi-cellular functions. However, there is immense pressure to adopt the 3Rs principle of Replacement, Reduction, and Refinement of animal use in toxicity testing. Considering the ever-expanding ban on animal testing for cosmetic ingredients, the field of Immunotoxicology has done an exemplary job of developing and validating non-animal alternative testing methods for skin sensitization. Successful recapitulation of the complex process of skin sensitization using
in silico and in vitro technologies provides promise for adopting NAMs for other complex immune functions. It is critical for a comprehensive safety assessment to investigate the impact of xenobiotics on innate, humoral, and cell-mediated immunity that demand complex interactions of many immune cell types. The goal of this talk is to present evidence for development of in vitro techniques capable of assessing immunotoxicity to all major arms of the immune system. Human whole blood has been used to develop antigen-driven functional immune responses including Natural Killer activity, T-cell activation, antibody production, and cytokine profiling. Together with comprehensive immunophenotyping for identification of cell populations and cytotoxicity, this human whole blood model provides promise as a comprehensive toolbox for in vitro hazard identification for xenobiotics affecting the immune system. Proof of performance will be illustrated using known immunosuppressive compounds including polyclonal aromatic compounds.

1174 A Regulatory Node within the Immunoglobulin Heavy Chain Gene May Predict Sensitivity to Xenobiotics That Alter Antibody Production

C. Sulentic. Wright State University, Dayton, OH.

The immunoglobulin heavy chain (IgH) gene is responsible for the expression of all Ig classes/isotypes, i.e. IgM, IgD, IgG, IgA, and IgE. These isotypes have different effector functions in mediating immunity. Therefore, environmental and/or genetic-induced alterations in processes that control IgH expression will significantly affect human health. Toxicants that bind the aryl hydrocarbon receptor (AhR) are well-established inhibitors of IgH expression and antibody levels. However, in the context of human IgH expression and antibody production, the role of the AhR is not well defined. This is further complicated by the fact that our understanding of basic immune function has been largely based on rodent models. There are significant differences in the IgH gene between rodents and humans that will likely translate to functional differences. Given the importance of antibodies in defense and disease, this represents a significant knowledge gap in assessing human B-cell function and potential sensitivity to xenobiotics. This presentation will discuss the importance of using human B cell models to assess antibody production in immunotoxicity testing and propose a regulatory node within the IgH as an environmental sensor and predictor of immunotoxicity, using AhR ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin as a case study.

1175 Human Cord Blood-Derived CD34+ Hematopoietic Stem Cells as an In Vitro Model for Investigating Developmental Immunotoxicity

N. Kaminski. Michigan State University, East Lansing, MI.

The large array of different cell types that constitute the immune system arise from CD34+ hematopoietic stem cells (HSCs). Sibling cell fate decisions made in the development of these various immune cell lineages is facilitated by the transition of HSCs to multipotent progenitors, followed by the appearance of common myeloid or common lymphoid progenitors ultimately resulting in cell lineage specification. Historically, xenobiotics that interfere with immune cell development were identified using rodent models, primarily mice. However, it is also well established that although there are many similarities between the mouse and human immune system, critical differences also exist. The objective of this presentation is to discuss a novel in vitro model system using human cord blood derived CD34+ HSCs that recapitulates critical events allowing for the cellular development and lineage commitment for most of the major immune cell types, as confirmed by scRNAseq and flow cytometry. Moreover, an aryl hydrocarbon receptor (AhR) ligand will be used as a case study to demonstrate how perturbations of the events involved in lineage commitment can markedly and selectively skew cell type specification. With the current emphasis on establishment of new approach methodologies for toxicity evaluations, this model system focused on developmental immunotoxicology provides numerous advantages over the traditional models, particularly being the utilization of human primary HSCs, which eliminates the need for extrapolation across animal species.

1176 HuLa Assay in Pharmaceutical Design and Safety Assessment: Can it Be Used for Prediction of Immunosuppression and Immunostimulation?

M. Collinge. Pfizer Inc., Groton, CT.

Assessment of the immunomodulatory effects of xenobiotics is important both in the context of pharmaceuticals and environmental chemicals. Holistic approaches that incorporate multiple immune cell types and functions are particularly powerful as an initial screen and, depending on the outcome, can be followed up with additional cell type specific and mechanistic assays. The in vitro T-dependent antibody assay (TDAR) has long been utilized for this purpose, requires antigen presentation as well as B and T cell function, and is highly predictive of immunotoxicity, particularly when applied in combination with other assays. An in vitro cell based assay that uses human cells that can be used in a similar manner to the in vivo TDAR would be desirable. To that end, the human lymphocyte activation (HuLA) assay was developed. The development of the assay, its qualification and evaluation by multiple pharmaceutical companies will be described, along with the ways the assay can be applied. While largely used for immunosuppression, its potential use for immunostimulation will be discussed.

1177 What Does the US FDA/CDER Need to Utilize In Vitro Immunotoxicity Data for Human Health Risk Assessment?

L. Myers. US FDA, Silver Spring, MD.

The FDA is committed to advancing the 3 Rs (replacement, reduction, refinement) in drug development. In vitro toxicological assessments have helped advance the 3Rs at the Agency and are increasingly being submitted to help answer many nonclinical safety questions. One of the major hurdles facing regulatory use of data generated using novel in vitro assays is whether the new assay will be as robust as the traditional models (sometimes referred to as the “gold standard”) for answering the safety question for that endpoint. In immunological safety assessment, many regulatory agencies have or are actively working towards accepting safety data produced using several in vitro assays to address hypersensitivity, immune stimulation / cytokine release, immunomodulation, as well as other endpoints. This talk will discuss the current approaches using in vitro data to address immune safety during drug development, with a focus on human cells, human cell lines, or humanized cells. This talk will also discuss the opportunities for advancement for in vitro assessment of immune safety / immunotoxicology as well as discussing concepts to consider when developing novel assays for human risk assessment.

1178 Metals Matter: The Potential Effects of Metal-Mediated Cell Death in the Pathogenesis of Cardiopulmonary Diseases

L. Cai. University of Louisville School of Medicine, Louisville, KY.

Metals are present everywhere on the earth. In biology, essential metals maintain important functions for the cell, including division, growth, and differentiation, ultimately contributing to organ function maintenance; however, nonessential metals can be introduced to our body undesirably by several routes. These nonessential metals in the body cause pathologies, such as metabolic abnormalities and cardiovascular diseases (CVDs) because of their cytotoxic and/or genotoxic toxicities. In fact, metal toxicities can be caused not only by nonessential metals but also by essential metals at inappropriate levels. These cases include iron- and copper-mediated cell death, ferroptosis and cuproptosis, respectively. However, how these metal-mediated cell deaths, such as pulmonary pathogenesis and diabetic CVDs, affect our health, how other metals affect ferroptosis and cuproptosis, and whether these metal-mediated cell deaths cause CVD remain unknown. This Symposium will begin with a brief introduction, whereupon the first speaker will address the importance of mitochondrial and how the damage and dysfunction occurs because of several metals leads to various cellular dysfunctions and cytotoxicity. The second speaker will focus on exposure to cadmium and related pulmonary toxicities, including various kinds of cell death, which may be mediated by cadmium’s contribution to essential metal dyshomeostasis. The third speaker will introduce the role of ferroptosis in the development of diabetic cardiomyopathy. The fourth speaker will address copper dyshomeostasis and health effects, while the last speaker will introduce the new concept of cuproptosis and underlying mechanisms with the potential roles in the pathogenesis of diseases. This Symposium, therefore, will assemble clinicians, translational toxicologists, and bench investigators to discuss the health impacts of essential metal dyshomeostasis-mediated cell death, including various health risks, and how essential metal dyshomeostasis is caused by chronic exposure to nonessential metals in many conditions, such as occupational, air pollution, and environmental contamination. To conclude this Symposium, we will demonstrate how metals are important for our life and health.

1179 Mitochondria as a Target of Metals

J. Meyer. Duke University, Durham, NC.

Mitochondrial uptake of essential metals is carefully regulated, often because mitochondria play critical roles in metabolic processes. For example, mitochondria play critical roles in heme and iron-sulfur cluster synthesis. Some non-essential metals accumulate in mitochondria by a number of mechanisms including mimicry of essential metal transport. There is also strong evidence for mitochondrial toxicities of many non-essential metals, including arsenic, mercury, lead, and cadmium; however, all of these also have non-mitochondrial targets. I will provide a brief review of mitochondrial impacts of non-essential metals and interactions with essential metals, including work by our group and others on the effects of lead and cadmium on mitochondrial functions. I will discuss how we work to identify mechanisms by which arsenic targets mitochondria, as well as our finding that human disease variants in mitochondrial homeostasis genes result in altered sensitivity to arsenic exposure in the nematode Caenorhabditis elegans. Next, I will present work in which we tested the hypothesis that mitochondria are a target of

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Cadmium-Induced Lung Toxicity

V. Antony. University of Alabama at Birmingham, Birmingham, AL.

Cardiopulmonary disease secondary to environmental insults is well recognized and accepted. It is recognized that all-cause mortality of the population of Medicare age is directly proportional to PM 2.5 exposure even below the EPA accepted levels, irrespective of smoking history. The underlying causes are linked to both pulmonary and cardiac disease. While cardiac disease and stroke remain the primary cause of mortality in the US, Chronic Obstructive Pulmonary Disease (COPD) is the third largest cause of death worldwide and is a significant co-morbid condition in both myocardial infarctions and strokes, which are caused by tissue hypoxia in the relevant organ system. Exposure to pollutants causes exacerbation of airways diseases, such as COPD and asthma, leading to hypoxemia that in turn, can lead to increased risk of myocardial hypoxia as well as an increased risk for stroke. Pollutants not only cause exacerbation of underlying lung disease but also may be the primary cause of lung disease. Cadmium (Cd), a metal in cigarette smoke (e.g., cigarette contains 2-3 micrograms of Cd), is also a common pollutant from coal fired power plants and coke furnaces and has an in vivo half-life of 25 years. Cadmium causes COPD, as well as exacerbation of other metal toxicity, and leads to a procoagulant state with higher levels of fibrinogen/fibrin in the blood and lung. Cadmium exposure initiates autophagy through ferroptosis leading to cell death of lung tissues exposed to it. This leads to both airway and lung parenchymal injury with aberrant responses. A better understanding of these mechanisms will help us develop therapeutic modalities to prevent lung disease following exposure to lung pollutants.

Essential Role of Ferroptosis in the Development of Diabetic Cardiomyopathy

L. Cai. University of Louisville School of Medicine, Louisville, KY.

Ferroptosis was described as a form of cell death induced by the small molecule erastin, which inhibits the import of cystine, leading to glutathione (GSH) depletion and inactivation of the phospholipid peroxidase glutathione peroxidase 4 (GPX4). As an iron-dependent form of regulated cell death, ferroptosis occurs through the lethal accumulation of lipid-based reactive oxygen species when GSH-dependent lipid peroxide repair systems are compromised and is also tightly related to amino acid, glutathione, lipid, and iron metabolisms. We have investigated the essential role of ferroptosis in the pathogenesis of diabetic cardiomyopathy (DCM) in mice with type 2 diabetes and a new ex vivo DCM model. Advanced glycation end-products (AGEs), an important pathogenic factor of DCM, were able to induce ferroptosis in engineered cardiac tissues (ECTs), evidenced by increased levels of Phgp2 and lipid peroxides and decreased ferritin and SLC7A11 levels. Typical morphological changes of ferroptosis in cardiomyocytes were observed using transmission electron microscopy. Inhibition of ferroptosis with ferrostatin-1 and deferoxamine prevented AGE-induced ECT remodeling and dysfunction. Ferroptosis was also evidenced in the heart of type 2 diabetic mice. Inhibition of ferroptosis by liproxstatin-1 prevented the development of diastolic dysfunction at 3 months after the onset of diabetes. The protective effect of sulfaphenazole, NRF2 activator, on ferroptosis was found AMP-activated protein kinase (AMPK)-dependent. Therefore, we concluded that ferroptosis plays an essential role in the pathogenesis of DCM, sulfaphenazole prevents ferroptosis and associated pathogenesis via AMPK-mediated NRF2 activation. This suggests a feasible therapeutic approach with sulfaphenazole to clinically prevent ferroptosis and DCM.

Metabolic Effects of Copper Misbalance


Copper is an essential enzyme cofactor and a signaling molecule required for proper differentiation and functional maturation of most cells. In this presentation, we will discuss how copper misbalance affects the metabolic status of tissues and alters the communications between different cell types within tissues. In particular, we present evidence that in the liver excess copper activates oxidative-stress response factor NRF2, which leads to upregulation of sulfotransferases, misbalance of oxysterols and sulfated steroids, inhibition of nuclear receptors and increased inflammatory response that can be reversed by liver nuclear receptor agonists. The development of liver pathology, especially inflammation and fibrosis, depends on copper accumulation in both parenchymal and non-parenchymal liver cells and is affected by copper misbalance in the intestine. The relevance of this altered metabolic signaling to human disorders of copper misbalance such as Wilson disease and obesity will be highlighted.

Copper Induces Cell Death by Targeting Lipoylated and Fe-S Cluster Proteins


Copper is an essential co-factor for all organisms, and yet it becomes toxic if concentrations exceed a threshold maintained by evolutionarily conserved homeostatic mechanisms. The precise mechanisms through which excess copper induces cell death are, however, largely unknown. Our work now establishes that structurally diverse copper ionophores induce a new form of regulated cell death that is distinct from known cell death mechanisms such as apoptosis and ferroptosis. This copper-dependent cell death mechanism is largely dependent on mitochondrial respiration. Increased mitochondria respiration promotes copper-dependent cell death whereas blocking cellular respiration attenuates this process. Moreover, this copper-dependent death occurs via direct binding of copper to lipoylated components of the tricarboxylic acid (TCA) cycle in a process that is highly dependent on the function of the ferredoxin 1 (FDX1) enzyme. This results in lipoylated protein aggregation and global iron-sulfur cluster protein loss leading to proteotoxic stress and ultimately cell death. Taken together, our findings suggest that two evolutionarily ancient cellular mechanisms (lipoylation and Fe-S clusters) are crucial targets of copper induced cytotoxicity.

The Future of Fire Safety: Exploring the Intersection of Wildfires and Human Health

C. Wright. Chemical Insights Research Institute, Marietta, GA.

Wildfires are an emerging public health threat capable of hindering the economic and social infrastructure on which we daily rely. Climate change will sharpen this threat; therefore, larger conversations among the scientific community are sorely needed to address this unprecedented global issue. Understanding the complexity of wildfires and their impact on human health is key to mitigating loss of life and quality of life as well as reducing environmental consequences, including the reduction of indoor and outdoor air quality. In this session, our panelists will identify challenges in data collection and potential knowledge gaps that impede our ability to fully quantify the human and economic costs of wildfires. Our panel of experts will examine the chemical processes that occur during urban wildfires, discuss the complexity of wildfire emissions, and examine what is known about human exposure and adverse health outcomes. Specifically, our presenters will provide new evidence on biomarkers of exposure, such as brominated flame retardants and per- and polyfluoroalkyl substances and their link to epigenetic alterations found in exposed vulnerable populations, including firefighters. While it is widely known that firefighters have higher cancer incidence rates than the general public, our presenters will reveal recent metabolomic data that may shed light on cancer initiation pathways in first responders. The efficacy of indoor air pollution mitigation strategies on cardiometabolic health in wildfire-affected individuals will also be explored and evaluated. These findings may aid in the development of therapeutic and intervention mitigation strategies to protect exposed first responders and communities during wildfires.

Wildfires at the Wildland: Urban Interface and Their Health Impact on First Responders and Communities

M. Black. Chemical Insights Research Institute, Marietta, GA. Sponsor: C. Wright.

In this presentation, we will provide the current state of the science involved in wildland urban interface (WUI) fires. The chemistry and health impacts of WUI fires are poorly understood, but WUI fires can lead to higher human exposures than remote wildland fires because of their proximity to communities. Current knowledge gaps in our understanding of complex fuel sources, their combustion products and contribution to wildfire smoke, and impacts on first responders and community will be examined and discussed. Exposure pathways, including air, water and soil, will be emphasized to enhance the audiences understanding of potential human health risks of wildfire exposures.

Evaluation of Biomarkers of Exposure in Southern California Firefighters Responding to Wildland-Urban Interface Fire Incidents

M. Calkins. NIOSH, Cincinnati, OH. Sponsor: C. Wright.

WUI firefighters may experience structural firefighting exposures without wearing the PPE routinely used by municipal firefighters during a structural response (e.g. self-contained breathing apparatus (SCBA), turnout gear) and without the ability to follow recommended decontamination practices for structural fire response. In this presentation, the results of biomarkers of exposure measured in firefighters from southern California enrolled in the Fire Fighter Cancer Cohort Study (FFCCS)—a national collaborative research study with NIOSH and
the universities of Arizona and Miami. Discussion will include comparisons to the general U.S. population, pre- and post-fire response, and characteristics of the fire incidents.

1187 Epigenetic Biomarkers of Toxicity in California Firefighters Working in the Wildland-Urban Interface
J. Goodrich. University of Michigan, Ann Arbor, MI.

This presentation will focus on DNA methylation and microRNA (miRNA) expression as early indicators of toxicity and exposure in wildland-urban interface (WUI) firefighters. Data derived from baseline and post-exposure evaluations of blood samples (n=100) obtained from the Fire Fighter Cancer Cohort study will be discussed including DNA methylation across 750,000 loci via the Infinium EPIC array along with the relative abundance of 800 miRNAs using a linear mixed model approach. The relationship between differential methylation and miRNA expression across time and the implications on firefighter health will be presented. This presentation aims to establish epigenetic modifications as relevant biomarkers of exposure and disease susceptibility in vulnerable populations during wildfire events.

1188 Assessment of Adverse Pregnancy Outcomes among US Female Firefighters

Several firefighter occupational exposures have been previously linked with adverse reproductive outcomes among non-firefighters. We used cross-sectional survey data to investigate the burden and occupational factors associated with miscarriage, preterm birth, and infertility among a cohort of US women firefighters. Within a subset of these women, we used dried blood spot samples to compare anti-Mullerian hormone levels to non-firefighter controls. The focus of this presentation will be a summary of our analyses, highlighting differences in risk between volunteers versus career firefighters, wildland/wildland-urban-interface (WUI) firefighters versus structural firefighters, and potentially, cumulative occupational exposures.

1189 Modulation of PM2.5-Mediated Cardiometabolic Indicators in Wildfire-Exposed Individuals through Residential Air Filtration
J. Zhang. Duke University, Durham, NC. Sponsor: C. Wright.

This presentation will describe the design and protocol of a new study to examine the impact of a 6-month residential HEPA filtration intervention on cardiometabolic outcomes during the presence or absence of wildfire events. Participants will be a cohort of ethnically diverse individuals at risk for type 2 diabetes (overweight or obese older adults) residing in the Los Angeles area where air pollution levels are among the highest in the US and is associated with wildfires. We will present preliminary data on wildfire exposure biomarkers, metabolic dysfunction biomarkers, and participants’ demographic, housing, and community characteristics. In this crossover trial, participants will be block-randomized to start either the HEPA filtration or the sham (no HEPA) filtration, each lasting for 6 months. With a 6-month washout period between HEPA and sham filtration, each participant will be followed for 18 months, during which different wildfires will be monitored and recorded.

1190 Cancers, Chemicals, and the Microbiome
J. Toyoda. Mote Marine Laboratory & Aquarium, Sarasota, FL.

The human body is host to as many microbial cells as human cells, and the gut microbiome is estimated to contain 50- to 100-fold more genes than the host. The vast genetic functions of the gut microbiome provide critical services, such as toxicant metabolism, synthesis of nutrients and bioavailable metabolites, and regulation of adaptive immune response. Gut-associated communities impact human health, and increasing evidence indicates dysbiosis, or microbiome perturbation, contributes to a variety of human diseases, including cancer. Despite strong links between dysbiosis and cancer outcomes, mechanisms by which toxicants and microbes interact to influence carcinogenesis remain unclear. The goal of this session is to educate SOT members on the importance of the gut microbiome to cancer onset, development, and therapy by highlighting (1) microbiome interactions with chemical carcinogens, (2) microbiome-mediated mechanisms of cancer development, and (3) how microbiome targeting can improve cancer outcomes. Speakers will share emerging findings in microbiome-carcinogen interactions, including the roles of the gut microbiome in the biotransformation of arsenic and metabolic activation of triclosan. Speakers will also discuss microbiome-mediated cancer outcomes in colon and liver via enzymatic, immune-related, and barrier-associated mechanisms. The Workshop also will explore the potential of the microbiome as a druggable target to reduce chemical toxicity, alter disease progression, and enhance cancer therapy through advantageous modification of the gut microbiome using prebiotics and enzyme inhibitors. Session attendees will better understand the roles microbes play in toxicant metabolism, cell-signaling pathways, gut barrier function, and tumor immunity. Further, they will gain familiarity with a variety of mechanisms by which the microbiome alters cancer outcomes using an array of molecular approaches.

1191 Use of Multi-omics to Decipher Signaling Molecules of Xenobiotic-Gut Microbiome-Host Interactions

The gut microbiome is a key modulator of human health and there is a growing number of studies that evaluate the systemic, regulatory, and metabolic role of the gut microbiome. This work demonstrates that xenobiotics interact with the gut microbiome providing a new angle to evaluate chemical toxicity. Modulation of the gut microbiome is also regarded as a promising approach to treat cancer or improve cancer treatment outcomes. However, how carcinogens, microbiome and host interact at the molecular level remains a significant gap. This not only impedes mechanistic understanding how altered gut microbiome causes or contributes to cancer, but also prevents microbiome modulation to reduce adverse outcomes from toxicant exposure. Toward this goal, with multi-omics and system biology approaches, we demonstrated significant changes in signaling molecules between conventionally raised and germ-free mice and have shown that exposure to carcinogens such as arsenic, benzopyrene, and formaldehyde induced different toxicological responses in mice with different gut microbiomes. We also demonstrated that carcinogen-altered gut microbiome causatively leads to disease in animals. Using arsenic as an example, we have shown how the gut microbiome cross-talks with the host through microbiome-regulated signaling molecules, including bile acids. Ongoing research shows that the gut microbiome plays a key role in affecting the profiles of mutagenic DNA adducts arising from exposure to benzopyrene, a potent human carcinogen. We have also demonstrated that modulating signaling molecules that reflect gut microbiome-host interactions can effectively prevent or treat diseases, including inflammatory bowel disease. Similarly, the administration of microbiome-related metabolites, identified from omics profiling, caused long-term radioprotection, mitigation of hematopoietic and gastrointestinal syndromes, and a reduction in proinflammatory responses during ionizing radiation. Taken together, deciphering microbiome-mediated chemical signaling and involved host signaling pathways provides important research avenues to understand and molecular mechanisms underlying carcinogenesis. Gut-host interactions, develop suitable biomarkers of gut microbiome toxicity, and discover druggable microbiome targets to improve cancer treatments outcomes or prevent cancer onset.

1192 Microbial β-Glucuronidase Enzymes Induce Colitis and Tumorigenesis by Reactivation of a Common Antimicrobial Additive in Gastrointestinal Tract
J. Zhang. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Emerging research supports that triclosan, an antimicrobial agent found in thousands of consumer products, exacerbates colitis and colitis-associated colorectal tumorigenesis in animal models. While the intestinal toxicities of triclosan require the presence of the gut microbiota, the molecular mechanisms involved have not been defined. Here, we show that intestinal commensal microbes mediate metabolic activation of triclosan in the colon and drive its gut toxicity. Using a range of in vitro, ex vivo, and in vivo approaches through biochemical analysis, metabolite analysis, crystal structure analysis, and sequencing analysis, we identify specific microbial β-glucuronidase (GUS) enzymes involved and pinpoint molecular motifs required to metabolically activate triclosan in the gut. Finally, we show that the targeted inhibition of bacterial GUS enzymes abolishes the colitis-promoting effects of triclosan, supporting an essential role of specific microbial proteins in triclosan toxicity. In addition to the reactivation of xenobiotics, our recent findings show that microbial GUS enzymes can also act as a promising therapeutic target to address colitis and related problems, and to alter the dysbiotic composition of gut microbiota. Together, our results define a mechanism by which intestinal microbes contribute to the metabolic activation and gut toxicity of triclosan. They further highlight the importance of considering the contributions of the gut microbiome in evaluating the toxic and carcinogenic potential of environmental chemicals.

1193 The Microbiome Controls Anti-tumor Immune Responses in the Liver

The microbiome includes commensal bacteria and other microorganisms, and their encoded genes and functions, that colonize the epithelial surfaces of our body. Under healthy conditions, the host and its microbiome exist in symbiotic as a metaorganism by providing a nutrient-rich microenvironment in return for aid in digestion and metabolism. In addition, they have been shown to have local and systemic effects on cancer onset, progression and therapy response. Primary sclerosing cholangitis (PSC) and inflammatory bowel disease are risk factors for cholangiocarcinoma. In addition, dysbiosis has recently been shown

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in patients with PSC with and without inflammatory bowel disease. We therefore investigated the effect of gut microbiome perturbations in a murine model of cholangiocarcinoma. In two different murine models of PSC, we found a decrease in gut barrier function allowing gut-derived bacteria and lipopolysaccharide to appear in the liver where they induced CXCL1 expression in hepatocytes through a TLR4-dependent mechanism ultimately leading to an accumulation of CXORZ+ polymorphonuclear neutrophilic cells (PMN-MDSCs) which are widely considered to be a critical negative regulator of immune responses. PMN-MDSCs suppressed anti-tumor immune responses in the liver and promoted growth of murine cholangiocarcinoma. This study clearly demonstrated that the gut microbiome controls hepatocytes to form an immunosuppressive environment and promote cholangiocarcinoma growth.

**Modulating the Gut Microbiome to Improve Cancer Immunotherapy Efficacy**
R. Pidgeon, McGill University, Montréal, QC, Canada.

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancers. However, long-lasting tumor control occurs in a minority of patients. The composition and function of the gut microbiome is a key immune mediator of ICI activity. Therefore, microbiome manipulation is a promising therapeutic approach to improve ICI efficacy. Gut microbiome composition and function can be manipulated in two major ways: supplementation of exogenous bacteria (via probiotics or fecal microbiome transplants) or modulation of endogenous microbes (via dietary prebiotics or xenobiotics). We hypothesized that dietary prebiotics could reshape the endogenous gut microbiome to improve ICI efficacy. We found that the pomegranate-rich camu-camu berry can improve ICI efficacy in syngeneic MCA-205 sarcoma or E0771 breast cancer-bearing mice. This anti-tumor effect correlated with increased intratumoral CD8+ T cell populations and was microbiome-dependent as microbiome depletion inhibited camu-camu activity. However, a drawback of working with animal- or whole-fruit is variability in prebiotic composition. Therefore, we sought to isolate the active dietary prebiotic in camu-camu. Using reversed-phase chromatography, camu-camu fractions were isolated and iteratively screened in tumor-bearing mice for anti-tumor activity. We identified the pomepeol castalagin as the microbiome-dependent bioactive compound in camu-camu. 16S rRNA sequencing revealed that, like camu-camu, castalagin gavage in both mouse models increased the relative abundance of immunogenic taxa (notably Ruminococcus and Akkermansia muciniphila). In the gut, castalagin is metabolized to ellagic acid and various urolithins, depending on microbiome composition. We observed that gut microbiomes of patients that responded to ICIs were significantly more likely to metabolize castalagin compared to non-responders. Metagenomic sequencing revealed that metabolizers had significantly different microbiomes compared to non-metabolizers. In addition, RNA-seq analysis on bacterial isolates identified putative genes in the castalagin metabolic pathway. Taken together, the pomepol castalagin acts as a prebiotic and improves ICI efficacy through gut microbiome modulation. Further work is needed to fully elucidate the mechanism by which castalagin impacts the microbiota composition and how it is metabolized by gut bacteria.

**Moving Stem Cell-Derived New Approach Methods toward Regulatory Acceptance**
L. Pang, US FDA/NCTR, Jefferson, AR.

New approach methods (NAMs) offer the potential to accelerate molecular evaluation in pharmaceutical drug development and chemical hazard assessment data acquisition. They also translate effects to protect human health and the environment. Over the past decade, numerous stem cell-derived NAMs have been developed; but their adoption and implementation into regulatory applications remain limited. To build confidence and increase the impact of NAMs, it is important to understand the technologies of being used the relevance, sensitivity, specificity and toxicity of endpoints featured in the in vitro models; the decision context to establish protective and/or predictive points of departure; the variability and concordance to traditional animal studies; and the interindividual variability that may affect the responses of different subpopulations. This workshop’s speakers will provide examples on how stem cell-derived NAMs are of use for hazard identification/risk assessment of environmental chemicals, drug screening, and toxicity detection/prediction. These novel perspectives provide insights on the suitability of using stem cell-derived NAMs to derive protective thresholds of health hazards, application of donor-derived stem cells to address issues of interindividual variability in gene-environment interactions, toxicodynamic assessment of chemical hazard, the predictive value of stem cell-derived neural spheroids to preclinical species, and the promise of using a diverse panel of stem cell-derived cardiomyocytes in translational drug-induced cardiotoxicity. The efforts in assay development in supporting potential regulatory uses will be highlighted. The hurdles encountered on the qualification/verification pathways, the needs of different stakeholders, and strategies to promote the communication and interactions of stakeholders will be discussed in the last 30 minutes of the session. There have been active discussions of the potential regulatory use of NAMs among the Critical Path Institute Predictive Safety Testing Consortium, the IQ Microphysiological Systems Affiliate, and the utility, benefits, limitations, and challenges in applying new technologies to inform internal and regulatory decision-making. This workshop provides unique insights and marks an important investment toward the eventual regulatory acceptance of stem cell-derived NAMs, as it requires the general consensus of stakeholders on performance criteria of the methods and the translatability, qualification, and verification of the model.

**Computational Morphodynamics: Advanced Modeling of Human Stem Cell-Derived Data**
T. Knudsen, US EPA, Research Triangle Park, NC.

Previous screening of the ToxCast chemical library in a pluripotent H9 human stem cell (hPSC) assay predicted developmental toxicity with a balanced accuracy 78-84% for well-curated reference compounds [Zurlinden et al. 2020]. Since the molecular mechanisms of most closely resembles the 'epiblast', an early embryo by gastrulation, in silico models built that self-organize emergent phenotypes and positional information of the epiblast can offer dynamic knowledge representation beyond performance classifiers to test the veracity of presumed mechanisms. A new and fully executable computer model of the human epiblast was built to translate hPSC-derived bioactivity data into advanced developmental trajectories through in silico self-organization of endomesodermal domains. Determination of progenitor cell fate is dependent upon positional information and temporal colinearity of an autonomous HOX clock, which can be altered through perturbation of a signaling network (e.g., FGF, BMP, NODAL, ATRA). This unique model of gastrulation combines ToxCast chemical bioactivity data with the molecular logic of signaling networks to mechanistically predict early developmental hazard through analysis of resulting mesodermal topography. Such models mechanistically drive biomolecular lesion(s) into higher levels of biological organization and can support regulatory implementation by putting hPSC-derived chemical effects data into motion (toxicodynamics). This abstract does not necessarily reflect Agency policy.
Drug-induced neurotoxicity is a leading cause of safety-related attrition for therapeutics in clinical trials, often driven by poor predictive capability of preclinical in vitro and in vivo models of neurotoxicity. Here we compare the predictive value of preclinical species and IPS-derived neural spheroids for a set of 84 clinical stage pharmaceuticals with varying degrees of seizurogenic and neurodegenerative liability. By measuring and integrating 7 parameters of neuronal health and function, a neurotoxicity score is calculated using a logistic regression model. This integrated iPSC Neural neurotoxicity score results in a 93.33% specificity and a sensitivity of 53.49%, demonstrating a very low false positive rate for the prediction of seizures, convulsions and neurodegeneration. In contrast, nonclinical species showed a higher sensitivity (75%) and much lower specificity (30.4%). Importantly, we find that this model greatly improved the inter-individual variability in the detection of neurotoxicity in discovery-stage drugs, catching >85% of small molecules that caused neurotoxicity in vivo. Finally, we integrate these in vitro and in vivo observations with a summary of other recently published iPSC-derived models of neurotoxicity to make a case for the robust utility of these NAM neurotoxicity models in drug discovery.

The cardiotoxicity induced by many oncology drugs significantly limits their clinical use and hampers the benefit of the lifesaving anticancer treatments. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a robust and human-relevant platform for cardiac safety assessment and have been reported to recapitulate the clinical inter-individual variation in susceptibility to doxorubicin (DOX)-induced cardiotoxicity (DIC) (Burnidge et al. 2016). Herein, we tested the feasibility of using hiPSC-CMs derived from a diverse set of donors to examine their variability in DIC. Similar to observations in clinical practice, the panel of hiPSC-CMs exhibited variable cytotoxic responses and the overall DIC effect was dose-dependent. Transcriptomic analysis of hiPSC-CMs upon DOX exposure largely replicated the known DIC mechanisms. Moreover, we assessed the sensitivity of the panel of hiPSC-CMs to DOX based on in vitro cytotoxicity and found that the results may reflect in vivo DIC risk, as the transcriptomic changes of DOX-sensitive and DOX-resistant cell lines were consistent with those generated from patients who showed different clinical susceptibility to DIC. In addition, the inter-individual variability of DIC is larger than the intra-individual variability. Taken together, our study suggests that a panel of hiPSC-CMs can be used to assess the inter-individual variability of oncology drug-induced cardiotoxicity and may enable patient stratification prior to the initiation of anticancer treatments. The model may also aid in internal decision making in the R&D pipeline of oncology drug candidates.

This presentation will highlight the purpose of peer review, the role it plays in scholarly publishing, and the unexpected benefits it can offer both reviewers and authors. We will summarize the peer review process from submission to publication. Although the editorial process can differ between journals, we will outline the general path that submissions follow—beginning with initial editor review, to inviting reviewers, to assessing peer review comments—and how journal editors typically reach decisions. We will then discuss the revision process, with insight into how editors and reviewers should evaluate revised manuscripts and factors that are considered when making a final decision. We will consider the roles of different editor types related to the peer review process, including Editors-in-Chief, Science Editors, Deputy Editors, Associate Editors, and Managing Editors, and encourage participants to describe any other roles within their journal or organization that are integral to peer review. We will discuss various models of peer review, both established and emerging, including open, single- and double-anonymous, and post-publication peer review, then examine the strengths and weaknesses of each. We will end this session with an overview of peer review ethics and considerations before accepting an invitation to review and when it may be more appropriate to decline an invitation (e.g., conflicts of interest, confidentiality, objectivity, timeliness).

Peer reviewing skills can inform the authorship process. Scientists from all stages of their careers are encouraged to participate and share their questions, concerns, experiences, and advice about reviewing manuscripts.

This presentation will offer practical tips that lead to increased opportunities to conduct peer reviews for a variety of scholarly journals as well as best practices for reviewing papers in experimental toxicology and the environmental health sciences. We will start with practical advice about performing initial evaluations of manuscripts, consulting author and reviewer guidelines for more information, and when to reach out to an editor with questions or concerns. The key elements of reviewer evaluations and written reviewer comments will be discussed along with the type of information to include in private comments to editors versus open comments to the author. Differences will be discussed in approaches to evaluating common scholarly article types (e.g., full-length research articles, brief reports, review articles) and additional considerations that are involved in re-reviewing revised manuscripts. Throughout this presentation, we will highlight techniques to convey comments politely and constructively. We will end by describing best practices for authors to respond to reviewer and editor comments—including when and how it may be appropriate to push back on requests.

This session, led by two early career scientists, will feature a live review of a short manuscript, allowing time for ample audience discussion and questions. We will begin with a brief overview of emerging peer review models that rely on crowdsourcing and ways to engage as both a reviewer and an author. This will include descriptions of resources that may be of interest to SOT members, including BioRxiv and preprint servers specific to certain regions (e.g., AfricArXiv) that may be underrepresented in traditional models of scientific review and/or publishing. We will spend the remainder of the session on a live review of a short, nontechnical manuscript, allowing ample time for audience discussion and questions. This portion of the workshop will feature opportunities for both small and large group discussions to enhance learning and to share experiences and tips. We will discuss the major sections of a manuscript (e.g., objectives, conclusions) and the key factors to consider when reviewing each section. Attendees will gain hands-on experience in crafting reviewer comments that are focused and constructive.

This presentation will provide our experience, as early career scientists, in reviewing manuscripts and receiving reviews on our own manuscripts, and how those experiences helped shape our skills as both writers and reviewers. We will share personal experiences and examples about the mentorship and training we received on the peer review process as well as our first solo reviews. We will also discuss how to interact with editors throughout the review process, differences in journals and
The goal of human health risk assessment is to characterize the potential harmful effects of a chemical exposure within human populations and the doses at which these effects occur. A key aim is to protect broad populations, which includes sensitive subpopulations. Traditionally, uncertainty around human variability in dose response is addressed by applying a default uncertainty factor of 10. While great investment has been made in precision medicine approaches, toxicity testing paradigms for chemical assessment do not inform quantitative assessment of population-level variability and susceptibility arising from myriad factors, such as genetics, life stage, concurrent stressors, and comorbidities. In order to improve the chemical safety assessment methods in this regard, population-based models are needed to obtain estimates of interindividual variability. Considerable progress has been made in the development and application of new approach methodologies (NAMs) that test high numbers of chemicals in a cost-efficient and timely manner. In addition, NAMs have the potential to experimentally incorporate human population variability and susceptibility factors and, ultimately, to allow risk assessors to define more significant uncertainty factors. This session will feature speakers from academia, government, and contract research organizations. These experts will discuss approaches using novel NAMs to address population variability and susceptibility. The introduction will address the challenges of interindividual variability and susceptibility in human hazard and risk assessment, describe the current 10-fold uncertainty factor, and highlight the opportunities of NAMs to experimentally quantify the population variability. Speakers will present on the use of mouse diversity outbred-derived progenitor cells and high content imaging in order to address genetic population variability in neurotoxicity. They will also review recent efforts in population in vitro human models in the context of risk assessment and describe key challenges and opportunities in their application. Moreover, speakers will describe the use of human data and NAMs to cover human variability in toxicokinetics and/or toxicodynamics using simulation techniques in the context of deriving occupational exposure limits. Recent progress at the European Food Safety Authority to integrate human variability with toxicokinetics in chemical risk assessment—with a particular focus on phase I and phase II metabolism and transporters—will be discussed. Presentations will be followed by an interactive panel discussion covering how NAMs can be applied to inform risk assessment in population variability and susceptibility. What challenges and uncertainties do NAMs pose? To what other human population variability and susceptibility challenges can NAMs be applied in the future? How can such information be implemented in risk assessment practices?

1207 Diversity Outbred Mice That Mimic Human Heterozygosity and Are Highly Genetically Diverse
A. Harrill, US EPA, Research Triangle Park, NC.
Diversity Outbred (DO) mice that mimic human heterozygosity and are highly genetically diverse provide an ideal resource to interrogate toxicity responses unique to sensitive sub-populations. A Tox21 Consortium cross-agency partnership utilized DO neural progenitor cells derived from genetically unique outbred mice, coupled with the US EPA’s established high throughput phenotypic profiling (HTPPP) assay to interrogate inter-individual variability in developmental neurotoxicity susceptibility. DO cell lines were exposed to a chemical test set in 8-point dose response and stained with six fluorescent probes to visualize multiple organelles, including nucleus, nucleoli, endoplasmic reticulum, Golgi, cytoskeleton, plasma membrane, and mitochondria. 1,300 phenotypic measures were quantified from confocal imaging and well-level data were normalized to each cell line’s DMSO control, with phenotypic profiles assessed at concentrations at the cytotoxicity LOEL and below. Phenotypic response across lines was quantified using global Mahalanobis distance and a benchmark concentration (BMC) associated with the threshold for phenotypic effects was determined for each cell line. These BMC data were then analyzed using a Bayesian framework to calculate toxicodynamic variability factors for each chemical tested, which were then compared to the default uncertainty factor for inter-individual variability (3.16) to determine whether data-driven uncertainty factors may be more protective of sensitive subpopulations. These initial data represent an important first step toward a high-content screening strategy that may inform population variability in dose and phenotypic response. This abstract does not necessarily reflect US EPA policy.

1208 Population-Based In Vitro Models to Address Interindividual Variability in Risk Assessment
W. Chiu, Texas A&M University, College Station, TX.
Addressing inter-individual differences in potential hazardous effects of chemicals remains a longstanding challenge in human health risk assessment that is typically addressed heuristically through use of 10-fold default “uncertainty” or “safety” factors. Although it has long been recognized that chemical-specific data would be preferable to replace these “defaults,” only recently have they emerged experimental model systems and organisms with the potential to experimentally quantify the population variability for specific chemicals. Progress is most evident in the use of population of human cell models, the most common of which are human lymphoblastoid cell lines (LCLs) and induced pluripotent stem cell (iPSC)-derived organotypic models. For instance, we have shown that LCLs can be used to characterize population variability in vitro hazard and concentration-response over a hundred chemicals, as well as mixtures and complex substances. Human iPSC-derived progenitor models are most advanced for cardiomyocytes, and we have similarly used this model to quantify population variability in both functional as well as viability phenotypes across over a hundred drugs and environmental chemicals. Moreover, we have shown in vitro-to-in vivo extrapolation approaches can be developed that result in predictions consistent with in vivo toxicity data. Overall, we find that the extent of toxicodynamic variability depends strongly on both chemical and endpoint, with greater variability for functional versus viability endpoints. We conclude that population-based models are now beginning to realize their potential to address long-standing data gaps in inter-individual variability.

1209 Empirical Data Describing Interindividual Variability: Comparison with Intrasppecific Assessment Factors Used for Deriving Health-Based Guidance Values
K. Schneider, FoBiG, Freiburg, Germany. Sponsor: H. Hoogendurk
Consideration of inter-individual variability in susceptibility ("intraspecies extrapolation") is an important step in deriving health-based guidance values. However, empirical data describing this variability are scarce. In a research project for the German Federal Institute for Occupational Safety and Health (BAuA) we evaluated and compiled human in vivo data from published toxicokinetic studies to describe variability in toxicokinetic parameters such as the area under curve (AUC). From the data we derived distributions for covering variability in 95% and 99% of the target population. These distributions describe the uncertainty in this extrapolation step resulting from substance-to-substance variability. In a similar way we searched for human in vivo data describing variability due to toxicodynamic reasons. Due to several shortcomings of such data, for describing variability in toxicodynamics, we referred to a study using in vitro data. The authors investigated in vitro cytotoxicity of many substances in more than 1000 cell lines and documented variability as the ratio between the medians and 5th or 1st percentiles of the EC50 (effective concentration 10%) determined for each substance-cell line combination. From these data again we derived two distributions covering variability in 95% and 99% of the population, resp. We combined the distributions for toxicokinetic and -dynamic variability by probabilistic methods (Monte-Carlo analysis) and compared the resulting extrapolation for intraspecies extrapolation with current assessment factors for deriving occupational exposure limits and similar values. These factors in use show a broad range from >1 to 10. Accordingly, we observed large differences in the probability, with which these factors are able to cover uncertainties of this extrapolation step. Conclusions from these observations are discussed in the larger context of harmonising methods for deriving health-based guidance values. The percentage of the target population that is covered and the weighting with which uncertainties are addressed define the protection level provided for by the guidance values. Probabilistic approaches allow for a transparent comparison of protection levels achieved by different methodologies.

1210 Recent Developments at the European Food Safety Authority to Integrate Human Variability in Toxicokinetics in Chemical Risk Assessment
J. Dome, European Food Safety Authority, Parma, Italy. Sponsor: H. Hoogendurk
Over the last decade, the European Food Safety Authority has been involved in a number of collaborative research projects with European Institutions to support the implementation of New Approach Methodologies (NAMs) in human risk assessment of chemicals. One particular aspect that still requires further investigations is the integration of human variability in toxicokinetics to support the use of quantitative in vitro in vivo extrapolations models for compounds of relevance to the food and feed safety area. In order to address this topic, bayesian meta-analyses of pharmacokinetic data from the pharmaceutical database reflecting acute exposure (Cmax) and chronic exposure (AUC, Clearance) to generate pathway-related variability distributions for QIVIVE modelling and derive pathway-related uncertainty factors. Results from such meta-analyses are presented for isoform-specific phase I enzymes (CYP2D6, CYP2C9, CYP2C19, CYP3A4, Paraoxonase-1; carboxyl-esterases etc), phase II enzymes (UDP-glucuronidyl-transferases and
Targeted protein degraders are rapidly evolving classes of drugs with great promise to address difficult drug targets in oncologic and non-oncologic disease. However, there is limited public discussion or scientific consensus on the application of rigorous, consistent, and effective methods for assessment of their clinical safety. Molecular glues, heterobifunctional degraders, and other targeted protein degraders use endogenous protein degradation processes to target protein degradation processes to molecularly specific and diverse therapeutic protein targets with therapeutic potential. The goal of this Workshop is to discuss the preclinical and translational safety assessment of heterobifunctional degraders (often called proteolysis targeting chimeras or PROTACs) and molecular glues, focusing on the key challenges of early de-risking, species selection, and clinical translation. The first speaker will provide an introduction to the topic, including a description of the different approaches to targeted protein degradation, illustrated by recent data on functional genomic profiling of molecular glues and PROTACs. The second speaker will address early de-risking strategies, focusing specifically on the use of a novel proteomic platform to identify off-target proteins associated with therapeutic modalities that induce protein degradation and gene silencing. The third speaker will discuss strategies for assessing the safety of targeted protein degraders in drug discovery, including the discussion of in vitro assays to inform in vivo studies. The fourth speaker will address the challenge of selecting pharmacologically relevant species to address the toxicity of targeted protein degraders by using cereblon (a commonly utilized E3 ligase) as a case study. The final speaker will present a regulatory perspective on the challenges and opportunities of protein degradation therapies. The roundtable discussion will focus on key points raised during the Workshop, with a specific emphasis on how approaches used for targeted protein degraders may differ from those used for traditional small molecule development. Overall, this Workshop will be of great interest to the many biotech and pharmaceutical companies that are engaged in this area and to the regulators involved in supporting these programs. The Workshop also will be of great relevance to academics researching protein degradation mechanisms and their translation to therapeutics.
of the assays and methodologies to assess the pharmacological and toxicologic profile of the targeted protein degrader to establish an effective nonclinical assessment strategy to ensure clinical safety. These may be additional assessments (e.g., in vivo animal studies, in vitro assays, in silico analyses) to what is routinely conducted under traditional small molecule development programs, in order to identify and mitigate human risk. While targeted protease degradation therapies have the potential to be a powerful class of therapeutic products, many regulatory challenges remain to ultimately balance safety and risk identification with the potential for clinical benefit.

**1217 21st-Century Agrochemical Evaluation: Discussing a New Vision**

G. Hilton,
PETA International Science Consortium Ltd., Stuttgart, Germany.

A rapidly growing human population, in parallel with increasing pressures due to a changing world and climate, is increasing the demand on the agrochemical industry and heightening the need for safe and effective crop protection products. Although the existing agrochemical safety evaluation paradigm is well established based on classical toxicology methods, it is unlikely to meet the emerging challenges of a developing and ever-expanding sustainable agriculture. The science underpinning chemical testing methods is advancing at a remarkable pace, and the ability to implement modern tools in assessing safe uses and risks of crop protection chemicals has dramatically increased. New approach methods (NAMs) can help generate robust data for integrated human and ecological safety assessment while decreasing animal use. It has become critical to consider how to incorporate these advancements, leverage existing knowledge, and prioritize the needs for testing in both human and ecological settings from the perspective of environmental risk assessment for agrochemicals. This session will provide a platform to discuss the development of fit-for-purpose safety evaluation for agrochemicals and assess applicability of these approaches to meet changing global and local needs for regulatory decisions. The discussion will help identify existing barriers to progress in the current safety assessment paradigm and the opportunities to overcome these barriers, as well as the potential for developing science to improve the risk assessment of agrochemicals. The first presentation will focus on challenges faced by the agrochemical industry in its attempts to proactively promote regulatory uptake of nonstandard safety assessment approaches, from scientific challenges in the One Health space to stakeholder engagement and perceived conflicts of interest. The second presentation will provide an overview of a multi-sectoral, multidisciplinary project that was recently launched by RESI in order to transform the evaluation of agrochemical safety evaluation. The third presentation will discuss the impact of the classification system under the Globally Harmonized System and a way forward that could enable incorporation of NAMs for that purpose. The fourth presentation will highlight opportunities and challenges on how NAMs could address current regulatory requirements and future chemical testing needs. Before opening the dialog to all attendees, a panel will discuss the following questions: (1) what are the barriers to achieving a 21st-century approach to risk assessment, (2) what actions and incentives are needed to facilitate a new paradigm of agrochemical safety assessment that provides the opportunity to fulfill data needs with new approaches, (3) how do we build trust in a 21st-century approach to risk assessment, (4) who are the key actors in implementing change, and how do we foster collaboration?, (5) what type of expertise should we leverage to ensure success, and (6) how should changes be implemented?

**1218 Evidence-Based Methods in Toxicology: Progress in the Past Decade and Collaborative Approaches for the Future**

H. Schaefer,
FDA, Silver Spring, MD.

The adoption of evidence-based methodologies in the field of toxicology and environmental health has experienced rapid growth in the past decade. This demonstrates the advantages that systematic review processes (e.g., transparency, reproducibility, rigor) bring to an assessment process but also highlights the challenges that face in applying evidence-based medical techniques to toxicology. Success in advancing methodologies has been achieved via collaborative efforts to share experiences and develop, test, and refine tools and approaches, as well as provide educational opportunities. This session aims to summarize and provide case examples of the advancements and challenges in evidence-based methods in toxicology. It also will serve as a forum for participants to learn about the direction of evidence-based methods, collaboration opportunities, and educational opportunities. This session will open with a brief introductory talk to characterize the scope of advances made in evidence-based toxicology over the past decade. A series of presentations will then highlight application of these methods to investigations of safety of food, drugs, and chemicals. A concluding talk will summarize future directions and challenges the toxicology community faces and its vision for the future. An open comment period will allow for identification and discussion of ongoing collaboration efforts open to the field.

**1219 A Day in the Life of an Industry Toxicologist**

M. Huang,
Premier Consulting, Cincinnati, OH.

Often, toxicologists are segmented into academia, government, and industry. However, the nature of industry work can vary widely. This session aims to highlight the different industry sectors in which toxicologists work and capture the sectors’ similarities and transferable skills. Participants, whether a current trainee or a career toxicologist, interested in exploring a new sector will gain a better understanding of what toxicology looks like in industry through real-life examples. Participants will hear from working professionals who use toxicology in a variety of for-profit sectors. Speakers will represent companies, consulting groups, and contract research organizations from the pharmaceutical, medical device, consumer product, food, occupational health, and risk communication spaces.

Each speaker will discuss their responsibilities and tasks, what a typical workday entails, and important skills and knowledge needed for success. They will also present a case study highlighting the kinds of problems they encounter in their work and how they are addressed (or are still being addressed) them. Following speaker presentations, a panel discussion with the speakers will explore common themes and cite skills across the different sectors that individuals looking to work in industry can focus on developing. The panel also will discuss practical advice for the job search and interview processes. Following the panel discussion, participants will have a chance to ask questions during the allotted Q&A and networking time.

**1220 Epitranscriptomic Mechanism of Metal Toxicity and Carcinogenesis**

C. Yang,
Case Western Reserve University, Cleveland, OH.

Analogous to DNA methylation and nuclear core histone posttranslational modifications, RNA molecules are chemically modified. The recognition of crucial biological functions of RNA modifications led to the birth of the term epitranscriptomics, which includes all forms of naturally occurring chemical modifications in all types of RNA molecules. Like DNA and histone protein modifications, RNA modifications also are dynamically regulated by three groups of proteins: (1) “writers” that deposit modifications on RNA molecules, (2) “erasers” that remove modifications from RNA molecules, and (3) “readers” that recognize and bind the modified RNA molecules to mediate their functional outcomes. Among more than 100 forms of chemical modifications in various RNA molecules, the N6-methyladenosine (m6A) modification is known as the most prevalent internal modification in eukaryotic messenger RNAs (mRNAs). RNA modifications regulate RNA splicing, stability, structure, and translation, which lead to changes in gene expression. Heavy metals are common environmental and occupational pollutants and important etiologic factors for cancer and many other diseases, and their underlying mechanism is not well understood. The epitranscriptomic effects of metal exposure is an exciting and emerging field in toxicology. The goals of this Symposium are to introduce current epitranscriptome research, present detection technologies for epitranscriptomic modifications, and discuss the role of epitranscriptomic dysregulation in metal toxicity and carcinogenesis. To achieve these goals, this session will convene a panel of outstanding epitranscriptome and metal researchers consisting of early career, mid-career, and well-established investigators. First, Dr. Thomas J. Begley, a pioneer and leading expert in epitranscriptome research, will present an overview on current epitranscriptome research and epitranscriptomic mark detection technologies, followed by exciting research on tRNA modifications under various toxicant stress conditions. Next, three experts (Drs. Allison Kupcso, Zhishan Wang, and Yu-Ying He) in metals toxicology will present their cutting-edge cell culture, animal model, and human studies to show how epitranscriptomic dysregulation is critically involved in metal-induced type 2 diabetes mellitus, cancer susceptibility, and carcinogenesis, respectively. Since few compelling epitranscriptomic studies have been performed in the field of metal toxicology, this Symposium will feature only recent research on the epitranscriptomic effects of arsenic and chromium. These topics will be of interest not just to metals researchers but also to those studying the mechanisms of action of other toxicants. This session will introduce the concept of epitranscriptomics to those unfamiliar with the subject and those more focused on epigenetics. It also will attract an expanded SOT audience of metallurgists and other toxicology researchers.

**1221 The tRNA Epitranscriptome Has Emerged as a Dynamic Regulator of Gene Expression That Plays Key Roles in Cancer Etiology**

T. Begley,

The epitranscriptome – the dozens of enzyme-catalyzed chemical modifications found on all types of RNA – is a key regulator of gene expression. Detecting epitranscriptomic marks is challenging, and an overview of next-generation sequencing technologies and mass-spectrometry based methods to analyze RNA modifica-

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1222 The Epitranscriptome as a Novel Mechanism of Arsenic-Induced Diabetes: Evidence from Human Populations

Arsenic, a naturally occurring metalloid, is associated with numerous adverse health effects, including increased risk of Type 2 Diabetes Mellitus (T2DM) – a top 10 leading cause of death in the US. However, the mechanisms behind these effects remain unknown. We propose that the RNA modification, N6-methyladenosine (m6A), the epitranscriptomic modification that regulates mRNA stability, is involved in the impacts of As on T2DM. Research in model organisms suggests that As exposure alters the m6A epitranscriptome and that m6A plays a key role in T2DM, yet these impacts have yet to be assessed in humans. We investigated m6A modifications in blood leukocytes from a cohort of older individuals from the Normative Aging Study (NAS) with toenail As measured prior to exposure (n=19). We next examined m6A reader, writer, and eraser mRNA expression and profiled m6A using methylated RNA immunoprecipitation sequencing in blood. Arsenic concentrations were positively correlated with mRNA levels of m6A readers and negatively correlated with m6A erasers. These correlations were also observed with fasting plasma glucose in participants with T2DM. Furthermore, As was associated with m6A levels in five mRNA transcripts involved in T2DM and inflammation in humans. This preliminary evidence suggests a role for the epitranscriptome in As-induced T2DM. Future studies will explore the role of m6A in a population of American Indians that suffer disproportionately from T2DM and have a high burden of As exposure. This work has the potential to delineate novel pathways of As-related T2DM, identify interventions, and inform recommendations for arsenic levels in water of vulnerable communities.

1223 RNA m6A Writer METTL3 Promotes Chronic Hexavalent Chromium Exposure-Induced Cancer Stem Cell-Like Property and Tumorigenesis
Z. Wang. Case Western Reserve University, Cleveland, OH.

Hexavalent chromium [Cr(VI)] is a well-known and common environmental and occupational carcinogen, however, the mechanism of Cr(Vi) carcinogenesis has not been well understood. Similar to DNA and histone proteins, RNA transcripts are also subjected to a variety of chemical modifications. The term epitranscriptome refers to the collection of all forms of RNA modifications. Among more than 100 types of RNA modifications, the N6-methyladenosine (m6A) modification is now recognized as the most common internal modification in eukaryotic messenger RNAs (mRNAs). Although the roles of DNA and histone protein modifications in Cr(Vi) carcinogenesis have been actively explored, whether RNA modifications are critically involved in Cr(Vi) carcinogenesis have not been determined. To study the epitranscriptomic effect of chronic Cr(Vi) exposure, we first performed the m6A writer expression in parenteral non-transformed human bronchial epithelial cells significantly reduced their RNA m6A levels and the transformed phenotypes, as evidenced by their decreased cancer stem cell (CSC)-like property and tumorigenesis. Moreover, stably knocking down METTL3 expression in parenteral non-transformed human bronchial epithelial cells significantly impaired the capability of chronic Cr(Vi) exposure to induce cell transformation and CSC-like property. These findings reveal an important role of epitranscriptomic dysregulation in Cr(Vi) carcinogenesis.

1224 m6A mRNA Methylation Regulates Arsenic-Induced Tumorigenesis
Y. He. University of Chicago, Chicago, IL.

Analogous to DNA and histone modifications, RNA molecules are chemically modified at an increasing number of sites in the field called epitranscriptomics. More than 100 chemical RNA modifications have been identified. Among these modifications, N6-methyladenosine (m6A) RNA methylation is the most abundant internal modification in messenger RNA (mRNA) and non-coding RNA in eukaryotic cells, which regulates RNA metabolism, including RNA decay, translation and nuclear processing. However, the regulatory and functional role of m6A RNA methylation in arsenic-induced tumorigenesis remains unclear. Here we demonstrated that the m6A mRNA demethylase FTO promotes arsenic-induced tumorigenesis. FTO as an m6A RNA demethylase is degraded by selective autophagy, which is impaired by low-level arsenic exposure to promote tumorigenesis. We found that in arsenic-associated human skin lesions, FTO is up-regulated, while m6A RNA methylation is down-regulated. In keratinocytes, chronic relevant low-level arsenic exposure up-regulated FTO, down-regulated m6A RNA methylation, and induced malignant transformation and tumorigenesis. FTO deletion inhibited arsenic-induced tumorigenesis. Moreover, in mice, epidemis-specific FTO deletion prevented skin tumorogenesis induced by arsenic and UVB irradiation. Targeting FTO genetically or pharmacologically inhibits the tumorigenesis of arsenic-transformed tumor cells. Using m6A seq analysis followed by regulatory and functional analyses, we identified NEDD4L as the m6A-modified gene targeted by FTO. Finally, arsenic stabilizes FTO protein through inhibiting p62-mediated selective autophagy. FTO up-regulation can in turn inhibit autophagy, leading to a positive feedback loop to maintain FTO accumulation. Our study reveals FTO-mediated dysregulation of mRNA m6A methylation as an epitranscriptomic mechanism to promote arsenic tumorigenicity. Our results may open up new opportunities for developing new preventive and therapeutic strategies to reduce skin cancer burden.

1225 Opening Our Eyes: Ocular Gene Therapies from Theory to Practice
K. Schafer. Greenfield Pathology Services Inc., Greenfield, IN.

The approval of the first ocular gene therapy in December 2017 marked a shift in the way we think about the treatment of ocular diseases, providing hope for patients with previously untreatable inherited retinal dystrophies. Its success has produced a relative explosion of interest in the field of ocular gene therapies. These therapeutic strategies, once considered solely as reparative gene therapies, have emerged as a potential method for transfecting cells in the eye by serving as endogenous therapeutic protein biofactories, which would result in the successful treatment of more prevalent diseases. As this is a new and rapidly evolving field, the goal of the session is to introduce the ocular gene therapy landscape, share current nonclinical study designs and development strategies implemented for ocular gene therapies, consider current knowledge gaps and future directions. We will discuss common barriers to development, nonclinical safety findings, assessment of risk-benefit, and the translatability of nonclinical findings to patients. Experts will share experiences, case examples, and data from recent years. At the end of the session, there will be an extensive panel discussion to engage the audience on the presented topics. Attendees of this session will leave with an end-to-end overview of points to consider in the development of ocular gene therapies.

1226 Nonclinical Considerations in the Development of Ocular Gene Therapies
J. Brassard. Brassard Toxicologic Pathology Consulting, Tustin, CA.

Gene therapy is part of a promising class of therapeutics with a broad range of potential indications, not only for genetic disease but also on hematologic, neurodegenerative, oncologic, and ocular diseases for which there are no viable therapeutic alternatives. This presentation will cover nonclinical considerations in the development of ocular gene therapies. After a brief review of the basics for intraocular drug development and for systemic gene therapies in general, the presentation will focus on the development of gene therapies for the ocular disease based on the lessons learned from successful product development of systemic AAV gene therapies and recent experiences in developing ocular gene therapies.

1227 Preclinical Development Strategies for Ocular Gene Therapies

The preclinical development strategy for AAV therapeutics must consider many factors for successful translation to the clinic and regulatory approval. First, a good understanding of the disease, and the mechanism of action and the cellular location of the disease-causing protein are necessary to design effective therapies. The choice of AAV capsid is dictated by the specific cell type to be targeted, for example, photoreceptors, retinal ganglion cells, or retinal pigment epithelium cells.
This knowledge will also guide the choice of promoters and enhancers used during the vector design. Another important consideration is the identification of mathematical models that accurately replicate the disease, which will facilitate proof-of-concept and pharmacology studies. If good animal models do not exist, creative in vitro models can be used to support the development of the vector. For Phase 1/2 clinical trials, robust safety studies are required to be conducted according to Good Laboratory Practices (GLP). In addition to toxicology assessments, these studies often include an analysis of the biodistribution of vectors over time and immune responses to the vector capsid and transgenic protein. One underappreciated aspect of preclinical development is the need for robust assays to evaluate efficacy and safety, and the time required to qualify and/or validate these assays. In summary, a preclinical development strategy that includes informed vector design, thoughtful planning of pharmacology and safety studies and early development of critical assays will enable translation of AAV therapeutics for ocular diseases to the clinic and eventual drug approval.

### Regulatory Aspects and Opportunities in the Development of Ocular Gene Therapies

R. Allen. US FDA, Silver Spring, MD. Sponsor: K. Schaefer

The development of investigational gene therapy products for the treatment of retinal disease has steadily increased over recent years. While the potential benefits of gene therapies have been shown, it is also important that the risks associated with these products are appropriately assessed prior to administration into the target clinical population. This presentation will provide an overview of CBER’s current regulatory expectations for the preclinical development of gene therapy products intended to treat retinal disorders.

### Clinical Aspects of Ocular Gene Therapy Development: Maximizing Benefit/Risk


Ocular AAV gene therapies show promise for treating both inherited and acquired retinal disorders through gene replacement or editing, vectorized therapeutic delivery and optogenetics approaches. Gene replacement strategies to replace nonfunctional genes with normal copies in inherited retinal diseases, biofactory approaches to generate soluble proteins such as anti-VEGF biologic agents for age-related macular degeneration and emerging gene-editing tools to modify gene expression at the gene level. Intravitreal delivery of AAV gene therapies is required to selectively deliver the therapeutic gene into the nuclei of target cells. The choice of AAV vector and route of administration are key determinants of the efficacy and safety of these delivery platforms and will vary according to the therapeutic and target tissue. For example, intravitreal AAV2 and AAV7m8 can target inner retinal cells, while AAV2, AAV5, AAV7, AAV8 and AAV9 can transduce photoreceptors and RPE cells when given subretinally. AAV has been shown to activate innate pattern recognition receptors such as TLR-2 and TLR-9 resulting in the release of inflammatory cytokines and type I interferons. The vector can also induce capsid-specific and transgene-specific T cell responses and neutralizing anti-AAV antibodies which may limit the therapeutic effect. However, the target organ of retinal gene therapy, the eye, is relatively immune-privileged. Most intravitreal inflammation following gene therapy delivery has been mild and self-limiting and not accompanied by a loss of efficacy. In terms of route of delivery, subretinal injection avoids the inner limiting membrane (ILM) barrier and utilizes an immune privileged space. Although there is risk of surgical complication, effective transduction of outer retina can be achieved with therapeutic success. Intravitreal gene therapy delivery is associated with dose-dependent uveitis that can result in adverse outcomes. Intravitreal injection provides widespread inner retinal transduction which is more pronounced at points where the ILM is thinner. A well-informed clinical development plan should be supported by a clear protocol and risk-based proactive medical monitoring. Standardized assessment of and management of the peri- and post-operative uveitis is required to maximize patient safety and robustly inform the clinical plan.

### A Physiologically Based Kinetic (PBK) Model for Thyroid Hormone Homeostasis and the Effect of PXR/CAR Inducers in Rat and Human

B. Riffle. BASF Corporation, Research Triangle Park, NC.

Thyroid hormone (TH) homeostasis regulated via the hypothalamic pituitary thyroid axis through a coordinated feedback system which alters synthesis of thyroxine (T4) and triiodothyronine (T3), metabolism, clearance and elimination. Thyroid hormone homeostasis is critical many biological functions including neurodevelopment. In rodents chemical inducers of the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) reduce T4 & T3 concentrations by liver glucuronidation and increased biliary secretion and fecal TH elimination. This chemical induced disruption of rodent thyroid hormone homeostasis is of unknown relevance to human thyroid hormone homeostasis. A PBK model was developed to evaluate species differences in TH storage, clearance and to predict effects of PXR/CAR activators on hormone homeostasis in humans. Rat models were parameterized with TH data and validated against endogenous hormone data using existing literature. Human T4 & T3 disposition was predicted from allometrically scaled rat model and validated against radiolabeled data in plasma, liver and excreta and endogenous human data in euthyroid individuals. Produced data on effects of phenobarbital (PB) in rats on T4 glucuronidation were used to predict the percent decrease in total T4 in PB-treated rats at 100 mg/kg/day for 5 days. Preliminary predictions of increased fecal clearance in humans are consistent with limited data available for patients taking PB for therapeutic purposes. The successful extrapolation of the rat
Adverse outcome pathways (AOPs) connect a molecular initiating event with a larger-scale biological effect at the cellular, organism or population level and provide a theoretical framework for extrapolating toxicity testing data from in vitro assays. Quantitative mathematical/computational models (qAOPs) associated with qualitative AOPs are needed to provide predictions of health effects that may arise from an environmental toxicant exposure in order to regulate these putative toxicants and protect public health. While it may seem straightforward to develop a qAOP from a published qualitative AOP or one available in the AOPIwiki (aoipwiki.org), a considerable amount of model development effort is required unless quantitative models already exist for some or all of the key event relationships in an AOP. In addition, interspecies differences need to be accounted for when using animal data to predict human health effects. This presentation will provide an overview of best practices to enhance the conversion of AOPs to qAOPs with a specific focus on additional data needed for interspecies extrapolation. Examples will be drawn from existing qAOPs (e.g., for aromatase inhibition within the hypothalamus-pituitary-gonadal axis), and a qAOP for acetylcholinesterase inhibition leading to neurodegeneration.

Toxicology 2.0 must reflect real-world-based exposure designs (in silico, cellular, organoids, models, organisms, longitudinal epidemiological studies). Population-scale measurements based on biobanks and ecobanks that inform on the distribution of 1000s of chemical and non-chemical stressors in relevant populations (general population, relevant subgroups, disease cohorts) are needed. Study designs and computational approaches must be aligned to provide interpretable and actionable results. Dr. Miller will present findings from multiple cohorts to demonstrate the feasibility of the approach. Ethical issues, policy implications, community engagement, and citizen participation must keep pace with the technology. A critical implementation step is to scale-up mass spectrometry technology for high-quality inexpensive assessment of up to 5000 chemicals that can be tagged to environmental and chemical screening, in vitro/in silico clinical trials and identifying intrinsic and extrinsic susceptibilities; (2) Precision Health aims for individual, personalized preventive interventions, and pharmaceutical and non-pharmaceutical therapies; and (3) Targeted Public Health Interventions & Environmental Regulations have to address population & spatial-temporal variability in genome and/or epigenome as well as past and present exposome. The remaining speakers in this session will delve into the areas of Exposure, Technology, and Evidence integration in more depth, as well as provide overarching perspectives as to the path forward.
Interactions of cells with each other, their extracellular matrix scaffold, and gradients of soluble factors in perfusion devices; such features have been adapted to several different tissue types that have been tested with prototypical compounds. However, the group identified several areas that will need to be addressed moving forward to enable more predictive and population-based chemical testing. Specifically, the biological capabilities of MPS will need to include a) reliable and genetically-diverse cell sourcing, b) improved protocols for differentiating patient-derived stem cells into adult cell phenotypes across essential tissues, c) integrative and non-invasive biomarkers, d) integration of dynamic physiology and pathophysiology outcomes, e) population heterogeneity and susceptibility through life-course(s), and f) biological surrogates for non-chemical stressors. Technological capabilities of MPS will be further developed include a variety of models of increasing architectural complexity (monolayer/suspension cultures, organoids and organoids (multi-organoid) systems) for different stages of drug/chemical development, representation of healthy and diseased populations by a personalized multiscale of possible futures, platform standardization, increase in throughput, validation against in vivo outcomes, incorporation of biosensors with near-real time outputs, and automated fabrication. Furthermore, the above biological and technological capabilities will need to be added cost-effectively for screening a large numbers of chemicals. Case studies will be presented to illustrate the capabilities of existing commercially available MPS and enhanced capabilities of prototype MPS. Ultimately, the ability to generate patient-specific and population-relevant MPS on-demand for screening will usher in a paradigm shift within toxicology.

1240 The Central Role of Computational Toxicology in Toxicology 2.0
N. Kleinstreuer. NIEHS/NIECTAM, Research Triangle Park, NC.
The field of computational toxicology has grown rapidly over the last decade, with the maturation of cognitive algorithmic tools and software to mine, process, and model data to provide reliable and robust predictions of chemical property, activity, and toxicology. Progressing and integrating computational capabilities will be of the utmost importance to provide the enabling structure upon which the next scientific revolution in toxicology is based. Augmented intelligence approaches use big data and computational tools to apply machine learning, natural language processing, mathematical modeling, and data analytics to support and enhance human intellect. Current and future applications of augmented intelligence to predictive toxicology will be discussed, with examples ranging from automating data curation and annotation, to predictive modeling, to big data-derived hypothesis generation and testing, to evidence interpretation, to establishing scientific confidence and implementing new approaches. Data generation, curation, and sharing are central to the shift, significantly driving the opportunities. Technological and biological capabilities highlighted as priority development needs include comparable, compatible, integrable multi-omic databases, quantitative in vitro to in vivo extrapolation and the development in silico “digital twins” of in vitro and in vivo systems. The continuous adaptation of augmented intelligence growth (e.g., explainable A.I.) will facilitate successful implementation of next-generation toxicological assessments.

1241 Evidence Integration for Regulatory Use of Toxicology 2.0
Integrating evidence from multiple sources evidence streams (epidemiological, animal toxicology, in vitro, in silico, non-chemical stressors, etc.) plays a key role and is integral to the process of translating evidence into knowledge that can inform decision-making. The emergence of “big data” along with “big compute” requires Toxicology 2.0 to develop novel evidence integration approaches, combining multiple evidence streams across diverse sources of structured and unstructured information. At the workshop, the group developed a vision as was developed to conduct complex rapid/real-time evidence integration by combining advance-ments made in data mining, applications of artificial intelligence (e.g., natural language processing), with the transparency and rigor tenants of systematic review. To implement this vision, workshop participants the workshop identified a need for collaborative, open platform(s) to transparently collect, process, share, and interpret data, information, and knowledge on chemical and non-chemical stressors. Creating these platforms is foundational for rapid and real-time evidence integration and will empower all steps to support the protection of human health and the environment. A number of needs were identified to create this platform, in particular software development to create dynamic and accessible interfaces, defining standards and key data elements to facilitate analysis of meta-data and automated annotation, and consideration for quality control.

1242 The Implementation of Tox-21 and Toxicology 2.0
T. Hartung. Johns Hopkins University, Baltimore, MD.
To date, exposure considerations typically follow the identification of a hazard. Future Tox-21c 2.0 must be driven by the identification of negligible exposure (e.g., thresholds of toxicological concern) to deprioritize risk assessments and guided by the identification of relevant exposures through exposomics. The adaptation to technical progress, especially MPS and A.I., requires harmonization of reporting and quality assurance. The key challenge lies in integration of these different evidence streams. Evidence-based Medicine (EBM) can serve as a role model with systematic reviews, defined data search strategies, inclusion and exclusion criteria, quality scoring, risk-of-bias analysis, metaanalysis, etc. While this is mostly applicable to existing data and studies, a new challenge is the prospective application for the composition of test strategies (Integrated Test Strategies – ITS, Integrated Approached to Testing and Assessment – IATA, and Defined Approaches - DA). A key role for Probabilistic Risk Assessment was identified. Major challenges are the validation of such new approaches and training, communication, and outreach.

1243 Grappling with the Grim Reaper: Epigenetics of Aging
S. Phatak. Nutrilite, Buena Park, CA.
Aging-related diseases are responsible for 90% of reported deaths. Current global life expectancy is roughly 73 years of age for women and 69 years of age for men, and living past age 100 is rare for humans. The term “blue zone” is used to describe populations with extraordinary longevity and an increased proportion of centenarians. According to the United Nations interactive data set, 433,653 centenarians were living in 2015, which accounts for only 0.06% of the world population. Worth noting is that quality of life also is favorable in these long-living communities, which share a similarity in that health span more closely matches life span. Unraveling the great mystery of aging begins by understanding how epigenetic reprogramming contributes to disease and aging. Recent data related to epigenetic aging in COVID-19 patients also will be explored. This Workshop will begin with an overview of the topic and provide relevant background information to guide participants along a quest for the fountain of youth. The epigenetic clock theory of aging aims to explain our inevitable decline in biological function, and a wealth of knowledge has been gained through the study of different model organisms, from immortal species to human twins. Features of the epigenomic landscape shift over time, with work indicating that aberrant DNA methylation may serve as a predictive feature of life span. Exposures to common environmental stressors, including biocides, tobacco, metals, dietary micronutrients, and medications, will be explored. By remodeling the epigenome, these lifestyle factors have been shown to induce disease, including cancer, metabolic syndrome, and cognitive decline, all of which are characteristic of aging. Multi-omics approaches in preclinical and clinical models will be discussed. Furthermore, indirect or parental exposures may have the capacity to accelerate aging in offspring and have the potential for long-term impact. Epigenetic aging, therefore, also should be a notable factor in the progress of aging. Specifically, our focus will shift toward identifying life- and health-extending intervention strategies that solve this complex aging puzzle. The talks will be presented by a diverse panel of speakers arranged by career sector, career stage, region, and background, and the session will conclude with a joint Q&A panel to facilitate discussion.

1244 Single Nucleotide Resolution Analysis Reveals Pervasive, Long-Lasting DNA Methylation Changes by Developmental Exposure to a Mitochondrial Toxicant
O. Lozoya. JOVIA Q! Solutions, Durham, NC.
Mitochondrion-driven alterations of the epigenome have been reported, but whether they are relevant at the organismal level remains unknown. The viable yellow agouti mouse (Aym) is a powerful epigenetic biosensor model that reports on the DNA methylation status of the Aym locus, which is established prior to the three-germ-layer separation, through the coat color of the animals. Here we show that maternal exposure to rotenone, a potent mitochondrial complex I inhibitor, not only changes the DNA methylation status of the Aym locus, which is established prior to the three-germ-layer separation, but also broadly affects the liver DNA methylation levels (e.g., explainable A.I.). We show that maternal exposure to rotenone is a potent mitochondrial complex I inhibitor, not only changes the DNA methylation status of the Aym locus, which is established prior to the three-germ-layer separation, but also broadly affects the liver DNA methylation levels (e.g., explainable A.I.). It is also a potent mitochondrial complex I inhibitor, not only changes the DNA methylation status of the Aym locus, which is established prior to the three-germ-layer separation, but also broadly affects the liver DNA methylation levels (e.g., explainable A.I.). It is also a potent mitochondrial complex I inhibitor, not only changes the DNA methylation status of the Aym locus, which is established prior to the three-germ-layer separation, but also broadly affects the liver DNA methylation levels (e.g., explainable A.I.). 

1245 Telomeres in Toxicology: Occupational Health and Aging
M. Shob. CDC/ATSDR, Atlanta, GA. Sponsor: S. Phatak.
The ends of chromosomes shorten at each round of cell division, and this process is thought to be affected by occupational exposures. Occupational hazards may alter telomere length homeostasis resulting in DNA damage, chromosome aberration, mutations, epigenetic alterations, and inflammation. Therefore, for the protection of genetic material, nature has provided a unique nucleoprotein structure known as a telomere. Telomeres provide protection by averting an inappropriate activation of the DNA damage response (DDR) at chromosomal ends and preventing recognition of single and double strand DNA (ssDNA and dsDNA) breaks or chromosomal end-to-end fusion. Telomeres and their interacting six shelterin complex proteins in
coordination act as inhibitors of DNA damage machinery by blocking DDR activa-
tion at chromosomes, thereby preventing the occurrence of genome instability, perturbed cell cycle, cellular senescence, aging and apoptosis. However, inappro-
priate DNA repair may result in the inadequate distribution of genetic material
during cell division, resulting in the eventual development of tumorigenesis and
other pathologies. This article reviews the current literature on the association of
changes in telomere length and its interacting proteins with different occupational
exposures and the potential application of telomere length or changes in the regulat-
ory proteins as potential biomarkers for exposure and health response, including
recent findings and future perspectives. The findings and conclusions in this report
are those of the authors and do not necessarily represent the official position of
the Centers for Disease Control and Prevention, the Agency for Toxic Substances
and Disease Registry, and the National Institute for Environmental Health. The use of
product names in this presentation does not constitute an endorsement of any
manufacturer’s product.

1246  Cell Composition and Epigenetics of Environmental Exposures
Related to Diseases of Aging
K. Bakulski. University of Michigan, Ann Arbor, MI.

Epigenetic modifications are labile to environmental influences and epigenetic
dysregulation is a hallmark of multiple aging diseases. Environmental epigeneti-
cuties routinely measure DNA methylation in readily available tissues. In a
United States nationally representative longitudinal study of older adults, we
show blood-based accelerated DNA methylation aging is associated with chronic
diseases, such as diabetes, hypertension, and presenile dementia. We further
demonstrate that accelerated DNA methylation aging is associated with complex
environmental exposures, such as neighborhood segregation, among older adults.
Tissues and cell types exhibit specific epigenetic patterning and heterogeneity
between samples complicates epigenetic studies. Environmental exposures can
influence cell composition within tissues. In a United States nationally representa-
tive cross-sectional survey we show how environmental chemical biomarker levels
are highly associated with immune cell composition in an age-specific manner.
Tissue-level epigenetic measures represent a convolution of epigenetic signals
from individual cell types. Failure to account for cell-type heterogeneity in epige-
etic studies of aging limits identification of biological mechanisms and biases study
results. Properly addressing cell-type heterogeneity limits sources of potential bias,
avoids misinterpretation of study results, and allows investigators to distinguish
shifts in cell-type proportions from direct changes to cellular epigenetic program-
ming, both of which provide insights into environmental disease etiology and aid
development of novel methods for prevention and treatment. We will discuss the
relationships between exposures, aging diseases, tissue-level DNA methylation,
and cell-type heterogeneity in study design and analysis and paths forward for new
aging toxicology and environmental epidemiology studies among older adults.

1247  Epigenetic Editing and the Environment: A Role for Transcription
Factors as Mediators of the Relationship between Exposure, the
Epigenome, and Outcomes
E. Martin. NIEHS, Research Triangle Park, NC.

DNA methylation is the best studied epigenetic mark in human populations. It has
been found to be associated with age, disease, lifestyle choices and exposures.
However, what is not well understood is how these factors change the epigenome.
One potential mediator between exposure and environment is the action of
transcription factors. It is well known that transcription factors can drive differ-
ences in DNA methylation, histone modification and chromatin opening. It has
also been shown that transcription factors interact with toxicants either directly by
binding or indirectly through activation of cellular signaling pathways. Furthermore,
it has been shown that transcription factors drive epigenetic changes throughout
the aging process and that some of these transcription factors are impacted by
environmental exposures. This talk will explore how activation of transcription
factors by endogenous and non-endogenous ligands alters the epigenome in unique
ways and the implications for these epigenetic changes on aging. Overall, the data
suggests that exogenous ligands, specifically phthalates and progestin-based birth
control, do not activate the target transcription factor, progesterone receptor, in the
same way as the endogenous ligand, progesterone, resulting in unique patterns of
epigenetic change. This suggests that toxicant-induced transcription factor activa-
tion is mediated by other factors in the cell such as other transcription factors and
epigenetic editing enzymes.

1248  Environmental Influences on Human Epigenetic Aging
and Variability
A. Cardenas, and A. Bozack. Stanford University, Palo Alto, CA. Sponsor: S. Phatak
Exposure to chemicals, such as human carcinogens, influence health and disease
and disrupt biological aging processes. Data from epidemiological studies show
that occupational benzene, trichloroethylen (TCE) and smoking accelerate various
epigenetic aging clocks in adults. In children, prenatal and early-life exposures have
been found to be associated with epigenetic aging. For example, in Chile we report
that adults exposed to high levels of Arsenic in their drinking water had greater
epigenetic age acceleration while another study of Latino children in California
showed that maternal preconception adverse childhood experiences are associ-
ated with epigenetic age acceleration. We have tested other pharmacological
interventions that include low dose aspirin and zinc supplement. Additionally, we
consider the effects of social isolation within a simulated space travel experiment in
the Mars-500 mission, a high-fidelity 520-day ground simula-
tion experiment, testing multiple epigenetic aging biomarkers. Finally, we showed
that prenatal exposures accelerate epigenetic clocks estimated at birth. Overall,
there is evidence that both chemical and psychosocial exposures accelerate
epigenetic aging through the life course. However, various epigenetic clocks
have differential sensitivity to exposures. There is also evidence suggesting that
the early life period is susceptible to aging disruptions which might suggest that
the biological aging process starts at conception and might be programmed at
development. Prevention efforts for age related diseases should consider targeting
both psychosocial and chemical exposures.

1249  New Approach Methodologies in the Pharmaceutical Sciences: Novel
Strategies Challenging the Traditional Testing Paradigm
to Increase Regulatory Confidence

Drug discovery and development is based on a historical foundation of animal
testing data, with confidence built over decades; however, recently identified
shortcomings necessitate new approaches to the standard testing paradigm.
Though new approach methodology (NAM) is a relatively recent term for nonanimal
testing methods for chemical hazard identification and risk assessment, similar
in vitro/in silico methods have long been used in the pharmaceutical sciences to
inform and augment and inform of in vivo testing. Availability of new
in vitro models, such as complex in vitro models (CIVM) (e.g., organoids, spheroids,
3D tissues) and microphysiological systems (MPS) (e.g., bioprinted tissues,
organ-on-a-chip) have provided opportunities to integrate in vitro-in vivo testing
and potentially improve human risk assessment. However, the readiness of CIVM/MPS
with respect to reproducibility, applicability of readouts for specific contexts of use,
and suitability for regulatory decision-making has yet to be broadly realized. Though
several CIVM/MPS representing discreet organ systems have been developed, their
utilization as a collective or as linked systems to provide whole-body assessment
for pharmaceuticals is limited. Thus, the readiness of a single or even multiple CIVM/
MPS to completely replace animal studies in the traditional pharmaceutical testing
paradigm remains questionable. The arrival of new therapeutic modalities (e.g., cell
therapies, biologics) for which the traditional in vivo testing paradigm is not entirely
suitable have provided an opportunity to pressure test the utility of CIVM/MPS and
other NAMs for pharmaceutical risk assessment. The session goal is to highlight
successes and challenges in utilizing NAMs—particularly CIVM/MPS—in pharma-
ceutical testing along with recommendations for progressing the field. Introduced
first will be the International Consortium for Innovation & Quality in Pharmaceutical
Development (ICIQ) and NAMs, particularly CIVM/MPS affiliate; specifically emphasized will be the collective efforts of its pharmaceutical scientists to advance the field of CIVM/MPS with respect to increasing confidence in reproducibility and regulatory decision-making capabi-

ties. The next presentations will provide examples of CIVM/MPS use to make lead
candidate selections and to assess the efficacy, pharmacokinetics, and potential
toxicity across several therapeutic modalities. Lastly, a regulatory perspective on
the opportunities and challenges in the field will be provided. The session will close
with a panel discussion. Panelists will be challenged to identify (1) opportunities for
CIVM/MPS integration throughout the drug life cycle and (2) in vivo animal models that
exhibit clinical translation challenges CIVM/MPS developers can target for future
model development. Session attendees will be invited to provide perspectives for
advancing the field with the goal of capturing consensus on short- and long-term
expectations for NAMs regarding drug discovery and development.

1250  Introduction: The Collective Efforts of Pharmaceutical
Scientists in Utilizing CIVM/MPS to Augment Pharmaceutical
Testing Approaches
P. Hardwick. Bristol-Myers Squibb Company, San Diego, CA.

Recognizing the potential for complex in vitro models (CIVM) and microphysiologi-
cal systems (MPS) to transform the way in which scientists approach the testing of
pharmaceutical candidates, the ECVAM CIVM and MPS was officially formed as a
subgroup of the International Consortium for Quality & Innovation in Pharmaceutical
Development (IQ) in 2018. Since its formation, the primary goal of the IQ MPS
Affiliate has been to identify challenges and opportunities and work to facilitate
the implementation of CIVM and MPS in drug development. This presentation will
introduce the concept of CIVM for drug discovery and development and provide a
presentation of the workshop and discuss the collective activities of pharmaceutical scientists
to increase confidence in this burgeoning field of pharmaceutical testing tools
for pharmacology, ADMET (absorption, distribution, metabolism, excretion),
and toxicology applications. Recent efforts to provide recommendations for minimally
expected functional capabilities of CIVM and MPS on an organ system level will be discussed. Additionally, efforts to facilitate dialogue amongst pharmaceutical scientists and global regulators to evolve the use of CIVM and MPS for regulatory decision making will also be introduced.

Over the last couple of decades, we have been witnessing the systemic shift in the current trends of drug development paradigms to more complex modalities targeting complex pharmacology emerging that are quite human specific. In these cases, a translatable in vivo model may not be available. Accordingly, there is a need to increase the complexity and thereby the predictability of the current in vitro models that are fully humanised and can recapitulate the species-specific biology more accurately. Initially in cell therapy development, efficacy studies were limited to simple 2D well plate-based assays, quickly followed by in vivo models. While that approach has shown some success with haematological targets, moving to solid tumours with range of target expression levels requires a more sophisticated approach to ensure the right efficacy for the therapy. Although the 2D well plate-based simpler assays are still the workhorse of in vitro assessment, recent human-specific complex targets benefit strongly from a combination approach where a series of simple assays are complemented with high complexity, more physiologically relevant models to enable decision making. The value of MPS in cell therapies is even more evident when looking for increased efficacy of combination therapy approaches, which again are already independently reliant on highly complex human-specific immune reactions that do not translate well to clinic from the traditional simple in vitro or in vivo models. So, a more integrated approach combining multiple different in vitro models with varying complexity integrated with in silico and in vitro models is a more promising approach for clinical success. For the safety assessment of new cell therapies, a similar approach is valid as well for the same reasons as in assessment of efficacy. Low confidence of the clinical translatable ability of the current, traditional preclinical models for cell therapy safety assessment leads to greater caution in therapeutic target selection and the selectivity of targets that are expected to inherently have less safety liabilities. This conservative approach may be limiting the development path of potentially highly successful therapies. The anticipation is that with increased access to more sophisticated preclinical safety assessment tools, previously untreatable cancers may be more accessible.

MP5 aim to recapitulate the architecture, cell-cell interactions, and tissue-microenvironment that is representative of the very complex biology beyond standard two-dimensional culture. Thus human MPS provide an opportunity to improve preclinical-to-clinical translation. Oncology combination therapies have the potential for huge clinical benefit but identifying tolerable regimens can be extremely challenging due to overlapping bone marrow (BM) toxicity. While this can be managed by adjusting combination-doses and drug-sequencing/schedules, this can be time-consuming, often with sub-optimal outcomes. To guide this we have developed a human BM MPS that maintains stem cells with concurrent differentiation into erythroid, myeloid and megakaryocyte cells. AZD5153, AstraZeneca’s highly potent BET/BRD4 inhibitor and venetoclax (approved BCL2 selective inhibitor) both carry haematological side effects as monotherapies, which may be ameliorated in combination. We observed co-dosing alone, concurrently, or sequentially in the MPS. Concurrent treatment exacerbated haematological toxicity compared to monotherapies (p<0.001), which was mitigated by sequential dosing (p<0.001). Antisense oligonucleotides (ASOs) are increasingly being developed as a therapeutic modality, however preclinical safety evaluation of ASOs lacks predictivity due to the similarity of in vitro models and a lack of in vivo cross-species reactivity. We have developed a polarised, dual channel human kidney proximal tubule-on-a-chip that recapitulates transporter-mediated drug toxicity. Following exposure (48h) to ASOs SPC5001 (PCSK9; clinically nephrotoxic) or 556089 (MALAT1; non-toxic), ASO uptake (target-downregulation) and SPC5001-induced cytotoxicity (5µM; lactate dehydrogenase release) were observed. Moreover, SPC5001 continuous exposure (20 days) resulted in time-dependent release of kidney-injury biomarkers kidney injury molecule 1, neutrophil gelatinase-associated lipocalin and clusterin. We have demonstrated the potential of these MPS to enhance the human-relevance of pre-clinical safety assessment, paviing the way for refinement/replacement of in vivo studies. Recent data is indicating that we may be able to improve safety and development in the pharmaceutical industry, including a lack of standardisation, high cost and time requirement, and low sample volumes or cell numbers for downstream analyses.

Nonclinical studies assessing the safety of pharmaceutical drugs during development have long supported the marketing of increasing numbers of highly effective and safe therapeutics. However, as science evolves to identify more complex molecular targets involved in the mechanistic drug safety and efficacy, the need increases for better, faster, more accurate and predictive testing methodologies in drug development. During drug discovery, candidate drug selection is often based on high throughput in vitro assays that screen for wanted mechanisms of action while screening out unwanted toxicities. However, during drug development, drug manufacturers predominantly conduct the nonclinical safety studies in various animal models. This is because the strengths and weaknesses of animal models are relatively well understood and there is arguably less perceived uncertainty associated with allowing regulatory agencies to review such in vivo data. Nevertheless, as per the FD&C Act and the Code of Federal Regulations (21 CFR), FDA continues to provide flexibility to sponsors, to use in vitro and in vivo studies to support the safety of drugs under development. Consequently, there has been increasing interest in the use of new alternative methods (NAM) in order to achieve the goals of the 3Rs (refine, reduce and replace). While studies using NAMs constitute a very small number of those studies submitted in IND and NDA packages, such NAMs tend to focus on pharmacology endpoints rather than toxicology endpoints. Therefore, there is now an opportunity to expand the use of NAMs from their application in drug discovery, and to integrate these methodologies into drug development. A more mechanistic understanding of drug toxicities might contribute to a decrease in drug toxicities that are observed during late phase clinical development or during post marketing. The adoption of NAMs into the drug approval process can only happen when there is confidence on both the part of drug manufacturers as well as the drug regulators. Building confidence in new methodologies is therefore key to their adoption and widespread use. The presentation will provide perspectives on reaching consensus between stakeholders on key features of new alternative methods and how these methods can support the marketing of safe and effective drugs.

One of the last frontiers in biomedical research involves the repair of damaged tissues and organs, including the central nervous system. The prospective use of stem cells to mend injured organs is very exciting but requires both potency
and safety assessment strategies. During this Workshop, cell-based approaches to treating disease and organ failure will be delineated, with an emphasis on the safety criteria necessary for the use of stem cell approaches to tissue repair. At this time, there are a few licensed cellular products derived from functionally mature and differentiated cells, and aside from cord blood-based products, there are currently no approved products based on multipotent and pluripotent cells in the US market. This is partly due to the complexity and heterogeneity of the cellular products, which pose several manufacturing and regulatory challenges. In addition, cells are typically exposed to various conditions and exogenous factors during the manufacturing process, which makes the cells functionally different from their original states. This emphasizes the need for new methods and quality attributes to reliably predict the biological functions of manufactured cellular products. Industry-related scientists from therapy discovery/evaluation groups, academic researchers, and regulatory scientists will benefit from the in-depth information provided in this session. The closing panel discussion will emphasize the importance of developing a consensus definition of cell-based therapies and examine various approaches to identifying potential toxicity and therapeutic efficacy. ‘Kwee, Brian; J., and Kyung E. Sung. University of Tennessee Health Science Center, Memphis, TN. Sponsor: US FDA/CBER, Silver Spring, MD. K. Sung.

1259 International Perspectives on Safety Assessment Approaches for Cell-Based Therapies
R. Roberts. Aponix, Alderley Edge, United Kingdom.
The Health and Environmental Sciences Institute (HESI) Cell Therapy - TRacking Circulation and Safety (CT-TRACS) program is an international, public-private, multidisciplinary collaborative group that aims to facilitate the translation of cell therapy products (CTPs) to the clinic by driving the development of tools, methods and scientific knowledge required to evaluate the safety of therapeutic cells. Through knowledge exchange between stakeholders from all sectors, the committee is addressing key safety concerns that are especially challenging for CTPs: (1) biodistribution, to determine the fate of cells once administered in the patient and developing online tools/resources that will be freely available to the broader cell therapy community (Helfer et al. 2021; Pereira Mouriés et al., 2022); (2) tumorigenesis, to assess the potential risk of these products to form tumors in the patients (Sato et al. 2019). Several multi-site studies are currently ongoing to evaluate tailored and fit-for-purpose methods that address tumorigenicity concerns for human Pluripotent Stem Cells derived CTPs (Henry et al. 2022; Watanabe et al. submitted), CAR-T derived therapies, as well as for detection of potential off-target editing in CTPs edited with technologies like CRISPR. The objectives from these efforts are to develop robust methods, internationally harmonized, for predicting tumorigenicity, and to aid researchers, developers and regulators to assess the safety of CTPs with more confidence, contributing to faster/earlier decision-making.

1258 Two Strategies to Improve and Refine Stem Cell-Based Therapies
C. Morshed. KITE Research Institute of the University Health Network, Toronto, ON, Canada. Sponsor: W. Slikker.

Neural stem cells (NSCs) have garnered significant scientific and commercial interest in the field of regenerative medicine. Strategies aimed at exploiting the fundamental biology of the cells (self-renewal and capacity to generate cells of the neural lineage) holds promise for developing cell-based therapies to treat neurodegenerative disease and neurotrauma. Two distinct approaches have been explored as a means to harness the potential of NSC pools: (1) Cell transplantation and (2) endogenous NSC activation using novel biologic and tools. Both have shown success in the treatment of neurological disorders. I will discuss our current understanding and application of NSC based regenerative strategies, highlighting the limitations and promises that have been exposed from pre-clinical models of stroke utilizing these stem cell-based approaches.
aim to go beyond measuring cellular toxicity using traditional measures using a strategy called image-based profiling. This approach extracts hundreds of features of cells (or other biological samples, such as tissues or whole organisms) from images using advanced computational approaches such as deep learning. Just like transcriptional profiling, the similarities and differences in the patterns of extracted features reveal connections among diseases, compounds, and genes. We are therefore able to identify, at a single-cell level, how diseases, drugs, and genes affect cells, which can uncover small molecules’ mechanisms of action or toxicity, discover gene functions, predict assay outcomes, discover disease-associated phenotypes, identify the functional impact of disease-associated alleles, and find novel therapeutic candidates. Public data is becoming available at a scale that may allow unprecedented computational models to predict toxicity assay outcomes for a given treatment including small molecules. With these data and variants of the assay like LipocyteProfiler and CardioProfiler, we intend to implement drug discovery-accelerating applications at scale.

**1263 High-Throughput Phenotypic Profiling with Cell Painting Assay and Applications in Next-Generation Risk Assessment**

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

New Approach Methods (NAMs) are any technology, methodology or approach that provides information on chemical hazard without the use of animal models. NAMs data are increasingly being considered for use in risk assessment in lieu of or in combination with data from traditional animal-based toxicity studies. Researchers at the US EPA have proposed a tiered NAMs-based hazard evaluation strategy with an integrated strategy for accurately predicting toxicity using high-throughput phenotypic profiling (HTPP) to generate bioactivity data for hundreds of chemicals in a variety of human-derived cell models. This presentation will discuss the use of one such assay, high-throughput phenotypic profiling (HTPP) with Cell Painting, for: 1) determining molecular points-of-departure (mPODs) that correspond to threshold concentrations where cellular biology is perturbed and 2) generating high-resolution phenotypic profiles that characterize the biological activity(s) of the test chemicals. The presentation will then discuss potential applications for each of these data analysis outputs in chemical risk assessment using a dataset comprising phenotypic profiles for hundreds of chemicals from the ToxCast library screened in concentration-response mode in three different cell types (U2 OS, HBECS-KT, TeloHAGE). The first application converts mPODs (in units of micromolar) to administered equivalent doses (AEDs) using *in vitro* to *in vivo* extrapolation (IVIVE) and reverse dosimetry. Bioactivity exposure ratio (BER) analysis then compares the AEDs to exposure predictions to identify and flag chemicals where human exposure estimates overlap with bioactive doses. The combinatorial use of HTPP data from multiple human-derived cell lines will be explored in the context of this application. The second application focuses on mechanistic prediction using bioactivity profile similarity. Target chemical profiles are compared to a collection of profiles from annotated reference chemicals with known molecular targets and mechanistic predictions are confirmed using orthogonal assays. In addition, bioactivity profile similarity combined with chemical structure similarity can be used for chemical-biological read across (CBRA) and prediction of *in vivo* toxicity. Participants in this session will gain an understanding of how HTT methods may be applied to inform NAMs-based next generation risk assessment. *This abstract does not reflect US EPA policy.*

**1264 The Application of Cell Painting for Predictive Human Health Applications in Agrochemical Screening**

J. LaRocca, Corteva Agriscience, Indianapolis, IN.

During the plant protection product (PPP) discovery process, the identification of potential toxicological effects is crucial to the design of sustainable environmental and human health molecules. Studies submitted in PPP registration dossiers are typically low throughput and are designed to involve complex systems such as whole mammals for human health this typically involves a comprehensive testing packing largely focused on rodent testing ranging from acute to chronic exposures. Fulfilling the 3R (Refinement, Reduction, and Replacement) principles and supporting animal welfare regulation (e.g., Directive 2010/63/EU), new approach methodologies (NAMs) such as Cell Painting are leveraged for early-stage discovery HTP screening to aid in molecule design. Here we describe the application of Cell Painting to human HepaRG cells. Human HepaRG cells were chosen because liver is a common target organ for PPPs and these cells have increased metabolic capacity compared to other common liver cell lines. The HepaRG Cell Painting assay was validated with established positive controls, such as berberine chloride, and further compared to a high throughput (HTP) assay using a proprietary Human In Vitro (HIVIT) panel. In vitro to in vivo extrapolation was conducted on a subset of chemicals to assess predictive accuracy for short-term in vivo points of departure. This work will highlight the use of Cell Painting, but also discuss some of the limitations with in vitro models. The application of these assays to the discovery toxicology paradigm will be discussed, as they represent fast and efficient alternatives to in vivo rodent testing for high-throughput screening approaches.

**1265 Cell Painting for Screening of Pharmaceutical Compounds: Current Applications, Advantages, and Challenges Associated with Application to Drug Discovery and Development**

D. Dalmas Wilk, GlaxoSmithKline plc, Collegeville, PA.

Cell Painting (CP) is a morphology based high content imaging (HCI) platform that measures phenotypic changes in individual cells across multi-cellular compartments/organelles as a function of time and/or exposure to chemicals and/or novel therapeutic agents. This new approach methodology (NAM) can be used alone or integrated with other tools for assessment of pharmacology, drug discovery and development (D&D), and toxicology. The utilization of CP can decrease the need for in vivo animal data as well as aid in the identification of drug candidates with a superior safety profile. In addition, utility in the development of customized mechanistic assays to evaluate target safety are also being considered to identify phenotypic profiles and risk factors for frequently occurring organ toxicities without sufficient screening tools. However, the application of CP to toxicology is still in its infancy. Given the large amount of multifaceted data there are, there are many special considerations that need to be considered and harmonized before practical implementation in regulatory decision making. This talk will highlight the current state of CP in D&D as a means for analysis of organ toxicity. Key challenges associated with experimental design including selection of cell types and need for time/concentration dependent analyses will be discussed. Given the large amount of multifaceted data generated using CP, key challenges associated with data analyses and the application to gain mechanistic understanding and prediction of toxicity at a systems toxicity level will be highlighted. Additional challenges related to interpretation of results as it relates to *in vitro* to *in vivo* translation given that phenotyping is performed both in vitro and in vivo will be discussed. Cell-based assays will also be discussed. Despite challenges that need harmonization, application of CP holds promise in both reducing animal use during D&D as well as aid in designing compounds with a superior safety profile.

**1266 Exploring Cell Morphological Profile Information for the De-risking of Small Molecules**


The practical implementation of (new approach methodologies) NAMs as the basis of regulatory decisions for phytopharmaceutical products in the next 10 to 15 years will require global cooperation between the different stakeholders from academia, regulatory bodies, and industry. The characterization of compound-induced in vitro biomarker responses can provide both desired activity and adversity in cell-based assays will also be discussed. Despite challenges that need harmonization, application of CP holds promise in both reducing animal use during D&D as well as aid in designing compounds with a superior safety profile.

**1267 Refining Inhalation Risk Assessments by Integrating New Approach Methodologies**

W. Kloen, ToxStrategies Inc., Asheville, NC.

Evaluating risk from the inhalation route has always presented a challenge. This is because of the underlying species differences in the biology of the respiratory tract and the complexities of material transport and tissue dosimetry of inhaled substances. Further complicating the assessment are significant anatomical differences between the respiratory tracts of traditional test species and humans. This talk will highlight the current state of NAMs with scalability potential, rich biological information content and, when combined with ADMET properties, could provide predictive *in vivo* adverse outcomes. At Bayer, we are exploring the information contained in Cell Painting images for the small molecule safety evaluation and human health risk assessment for pesticides. Morphological profiles were analyzed in the frame of *in vitro* point of departure (POD) determination, of acute toxicity prediction, and for the prediction of other *in vitro* assay results. U2OS cells are currently being used as a first approach, but we believe that a specific set of cell lines needs to be defined, to reflect a maximum of derived toxicological endpoints. Toxicokinetic models, derived from the US EPA R “HTTK” package and from a Bayer in house model (hybrid model combining deep learning and PBPK models) will also be discussed in their applications for reverse dosimetry to extrapolate *in vivo* dose levels from *in vitro* POD. Finally, the analysis of causal associations between morphological profiles and toxicological outcomes, would allow us to define unsafe morphological profiles, that could then be used to develop the concept of safety-by-design using AI guided *de novo* chemical design using generative models such as Autoencoders.
chemical being evaluated, inhalation toxicity could not be thoroughly assessed via the inhalation route in animal models. In their analysis, the scientific advisory panel generally agreed that the proposed NAMs presented opportunities for refining the assessment but called attention to several areas for improvement. A few major critiques were (1) the underlying model system appropriate for understanding whole lung toxicity, (2) were the endpoints subtle enough to understand repeated exposures, (3) were the acute exposures sufficient for long-term extrapolation, and (4) were dosimetry assumptions/modeling approaches appropriate? Since that evaluation, many advances have been made that encourage the use of alternative approaches within inhalation risk assessment and toxicity. This Symposium will include five presenters. The first speaker will provide a historical context of inhalation risk assessment, the inherent challenges of animal models, and the evolution toward the use of human experimental platforms. The second speaker will highlight advances in exposure assessment, focusing on target tissue dosimetry in discrete regions of animal and human respiratory tract under realistic exposure conditions. Finally, the last two speakers will present case studies that feature these approaches and discuss how data from these techniques can be incorporated into and compared against existing animal data. Overall, this session will discuss the current state of inhalation risk assessment and highlight relevant NAMs that can be used to understand and refine inhalation risk.

### 1268 Introduction to Inhalation Risk Assessment and Initial NAM Considerations

M. Perron, US EPA, Washington, DC; Sponsor: W. Klaren

The Environmental Protection Agency’s (EPA) Office of Pesticide Programs (OPP) conducts human health risk assessments to evaluate the potential health effects of pesticides in residential and occupational settings from multiple routes of exposure. For evaluating effects via the inhalation route, pesticide registrants conduct subchronic inhalation toxicity studies using experimental animals according to EPA or OECD test guideline requirements. However, anatomical and physiological differences between experimental animals and humans can impact the airflow and deposition of inhaled substances, which can subsequently influence the extrapolation of animal study results to humans. Furthermore, there are particular challenges with testing respiratory irritants that generate concerns for animal welfare. In recent years, efforts to develop new approach methods (NAMs) to refine inhalation risk assessment using in vitro test systems with human tissues in conjunction with human dosimetry modeling have provided opportunities to overcome several of these challenges. This presentation will discuss advances of applying a NAM to refine inhalation risk assessment for a contact irritant pesticide, Chlorothalonil, and the process taken by the EPA to implement the approach.

### 1269 Nasal, Bronchial, Small-Airways, and Alveolar 3D Model Validation for Inhalation Hazard Identification

S. Constant, Epithelia, Geneva, Switzerland.

The main function of the human airway epithelium is to generate sterile atmosphere for the alveolar region where the gas exchange occurs. As the first line of defense against airborne pathogens, the airway epithelium acts as a key barrier through mucociliary clearance and host defense mechanisms. Airway epithelium is also an important immunoregulator through production of key messengers and physical interactions with immune cells. Upon activation, respiratory epithelial cells react by producing pro-inflammatory cytokines, chemokines and metalloproteinases for the recruitment and activation of immune cells as well as initiation of adaptive immunity. Interest in the use of 3D reconstituted human in vitro nasal, bronchial, small-airways and alveolar tissues is increasing in recent years for the study of respiratory diseases such as Asthma, Chronic Obstructive Pulmonary Disease (COPD), Bacterial and viral infections. Several applications of in vitro reconstituted Acute Lung Injury (ALI) models based on primary human cells in inhalation toxicity assessment will be discussed: (i) acute, long-term and sub-chronic testing strategies including static or dynamic exposures; (ii) inflammatory effect assessment; (iii) trans-epithelial permeability of xenobiotics; (iv) recent advances in the detection of respiratory sensitizers and irritants and (v) evaluation of ciliotoxic effect. These advanced models can serve an important role in hazard identification and concentration-response assessment that can modify an overall risk assessment for inhaled substances.

### 1270 Critical Role of Inhalation Dosimetry When Incorporating New Approach Methodologies

R. Cortlev, Greek Creek Toxicokinetics Consulting LLC, Boise, ID.

This presentation will focus upon important characteristics of inhalation dosimetry models and how they can be used to achieve exposure alignment across in vivo animal and in vitro human experimental platforms with realistic human exposure scenarios that are critical for toxicological risk assessments. In recent years, mechanistic inhalation dosimetry models that are based upon key physical, chemical, and biological processes governing material transport and interaction with airway tissues in animals and humans have fallen into three main categories: Computational fluid dynamics (CFD), physiologically based pharmacokinetic (PBPK), or multiple path particle dosimetry (MPPD) models, either alone or in combination. Each approach has its own strengths and weaknesses, and the choice depends upon the application. Case studies with reactive vapours and aerosolized pesticides and pigments will be presented where these in silico approaches were successfully used to either establish limitations and concerns for new acute animal inhalation toxicity testing, or to develop relevant human equivalent exposures corresponding to prior animal in vivo studies or new in vitro in toxicity studies with human airway cells grown at air-liquid interface in lieu of additional animal testing. As more examples of dosimetry modelling in NAMs are utilized by regulatory agencies in the coming years, the development of future models will become more refined and streamlined, especially if steps are taken to integrate dosimetry modelling with real-world exposure characterizations and new developments in in vitro human cell or tissue toxicity testing.

### 1271 Development of a Next-Generation Risk Assessment Framework Informed by Adverse Outcome Pathways

M. Dent, Unilever, Bedford, United Kingdom.

A next generation risk assessment (NGRA) approach for the safety assessment of polymers in personal and homecare products has been developed underpinned by the knowledge of Adverse Outcome Pathways (AOP) relevant for these materials. To date, we present one comprehensive AOP framework of including Polyhexamethylene phosphate (PHMG) in hypothetical disinfectant products. PHMG was tested across multiple NAMs, mapped against the AOPs for lung fibrosis (173), acute inhalation toxicity (302) and declines in lung function (148). Cells were treated daily for up to 12 consecutive days with nebulised PHMG in dose-response manner in the MuCell®-HF assays and EpiAlveolar™ system using a cloud chamber from Vitrocell®. In the MuCell®-HF experiments, TEER was measured daily, and the following biomarkers were measured at day 1, 7, and 11: LDH, cilia beating frequency, mucin secretion, and cytokines. In the EpiAlveolar™ system, cytokines and oxidative stress biomarkers were measured at the same timepoints. PHMG was also tested in the cell-free lung surfactant function inhibition assay. Concentration-dependent effects were modelled to derived points of departure. Exposure to PHMG led to a time and dose dependent toxicity response in both cell models. The EpiAlveolar™ cell system was more sensitive to the exposure to PHMG which led to loss of TEER accompanied by increased release of cytokine markers at early timepoints. At a later timepoints, there was significant cytotoxicity in the alveolar cells associated with PoDs lower than the predicted exposure. These results might reflect the in vivo situation in humans where PHMG led to acute interstitial pneumonia with diffuse alveolar damage after these exposures.

### 1272 Inhalation Risk Assessment Case Study from Using Three Structurally Dissimilar Pesticides

T. Osmint. Science Strategies, LLC, Charlottesville, VA.

Project Trio - A case study using three pesticides (Pyrethrins, Piperonyl Butoxide, MGK-264). This work was inspired by the EPA’s Office of Pesticide Program’s consideration of a New Approach Methodology (NAM) for the risk assessment of chemicals causing point of contact effects. It is especially applicable for assessing effects in the rat, a species that unlike humans, are obligate nose breathers. Several applications of including Polyhexamethylene phosphate (PHMG) in hypothetical disinfectant products. PHMG was tested across multiple NAMs, mapped against the AOPs for lung fibrosis (173), acute inhalation toxicity (302) and declines in lung function (148). Cells were treated daily for up to 12 consecutive days with nebulised PHMG in dose-response manner in the MuCell®-HF assays and EpiAlveolar™ system using a cloud chamber from Vitrocell®. In the MuCell®-HF experiments, TEER was measured daily, and the following biomarkers were measured at day 1, 7, and 11: LDH, cilia beating frequency, mucin secretion, and cytokines. In the EpiAlveolar™ system, cytokines and oxidative stress biomarkers were measured at the same timepoints. PHMG was also tested in the cell-free lung surfactant function inhibition assay. Concentration-dependent effects were modelled to derived points of departure. Exposure to PHMG led to a time and dose dependent toxicity response in both cell models. The EpiAlveolar™ cell system was more sensitive to the exposure to PHMG which led to loss of TEER accompanied by increased release of cytokine markers at early timepoints. At a later timepoints, there was significant cytotoxicity in the alveolar cells associated with PoDs lower than the predicted exposure. These results might reflect the in vivo situation in humans where PHMG led to acute interstitial pneumonia with diffuse alveolar damage after these exposures.
1273 What's All the Buzz about Tungsten?  
A. Bolt, University of New Mexico, Albuquerque, NM.

Tungsten is an emerging environmental toxicant. Because of its many desirable properties, including strength and flexibility, tungsten is frequently incorporated in a broad spectrum of goods used in everyday life, electronics, power tools, munitions, and implanted medical devices. As a result of this surge in the use of tungsten, more and more epidemiological studies have identified increased human exposure to high levels of tungsten in susceptible subpopulations in environmental, medical, occupational, and military settings. Multiple adverse health outcomes, including increased incidence and/or mortality of cancer, cardiovascular disease, stroke, chronic kidney disease, diabetes, and pulmonary disease, have been associated with human exposure. However, dissecting the individual contribution of tungsten in disease etiology can be challenging due to exposure to complex toxicant mixtures. Great strides have been made experimentally in our understanding of how tungsten is driving health outcomes using in vitro and in vivo models. Researchers have been able to identify toxicity in target organs and cell types as well as molecular mechanisms of action. Even so, the research performed to this point has barely scratched the surface, and major research gaps remain. Interestingly, recent data also have suggested tungsten is an essential micronutrient in the human gut microbiome, which adds yet another layer of complexity to our understanding of the role of tungsten in human health. This Symposium will feature a range of perspectives, from human epidemiological studies to molecular mechanisms driving disease pathogenesis, with the purpose of providing exciting, interactive presentations that highlight emerging research being conducted to investigate the impact of tungsten exposure on human health. Presenters will review current epidemiological studies providing evidence of tungsten exposures, health outcome data, and how co-exposure with the essential metal molybdenum can modify health effects using data from multiple human cohorts throughout the US. Presenters will also discuss key in vivo and in vitro studies that will provide important data focused on organ-specific toxicity, identification of molecular targets, and the potential beneficial effects of tungsten exposure and the implications for human health. Overall, this session will provide a unique platform for discussion on the current state of the tungsten toxicity field and where we go from here. Topics discussed will include multiple disciplines, disease outcomes, and perspectives on the effects of tungsten exposure on human health. This Symposium will stimulate discussion with leading experts in the tungsten research field, which will provide opportunities to identify gaps in our knowledge base and also stimulate collaborations to fill those research gaps.

1274 Environmental Tungsten Exposure and Metabolic and Renal Outcomes in a Rural Community  
K. James. University of Colorado Anschutz Medical Campus, Denver, CO.

Chronic Kidney Disease (CKD) and diabetes are debilitating and costly diseases with a national prevalence of 12% and 14% respectively. In the San Luis Valley (SLV), Colorado, a comparatively high prevalence of CKD (18%) and diabetes (18%) exist among a relatively young (<50 years old) population. The SLV is a mineral-rich region with elevated levels of heavy metals in the air, dust, and drinking water including tungsten. Recent research has identified a positive association between tungsten and CKD and diabetes while accounting for exposure to other heavy metals including arsenic, cadmium, and lead. This research utilizes historically collected biological markers of metal exposure and subclinical markers of kidney injury and diabetes as well as genetic material, from the San Luis Valley Diabetes Study to explore whether genetics play a role in the susceptibility to tungsten and the development of CKD and diabetes while accounting for exposure to multiple metals as measured in urine. This novel longitudinal study will further elucidate the renal and metabolic toxicity of tungsten based on subclinical markers of disease.

1275 Tungsten Exposure and Cardiovascular Health Effects in Diverse US Populations: Evidence from the Strong Heart Study and MESA  

Tungsten (W) interferes with molybdenum (Mo) binding sites and has been associated with prevalent cardiovascular disease (CVD). We evaluated if (1) W exposure is prospectively associated with incident CVD and (2) the association between urinary W levels and incident CVD is modified by urinary Mo levels. The association between W and CVD incidence and mortality was positive although non-significant at lower urinary Mo levels and significant and inverse at higher urinary Mo levels. Although prior cross-sectional epidemiologic studies in the general US population found positive associations between urinary tungsten and prevalent cardiovascular disease, our prospective analysis in the Strong Heart Study indicates this association may be modified by molybdenum exposure.

1276 The Role of the Bone Niche in Tungsten-Enhanced Breast Cancer Metastasis  
A. Bolt, University of New Mexico, Albuquerque, NM.

Multiple epidemiological and in vivo studies indicate that exposure to tungsten is associated with increased incidence and/or mortality of cancer and enhanced tumorigenesis and metastasis. However, limited experimental data exist investigating how tungsten exposure contributes to the carcinogenesis process. Due to a cohort of breast cancer patients accidentally exposed to tungsten during intraop- erative orthopedic surgery, our lab was investigating how tungsten exposure affects breast cancer progression and risk of metastasis. The bone is a site of long-term storage and toxicity following drinking water exposure to tungsten. The bone is also a known site of breast cancer metastasis and recurrence, which is very difficult to treat and has a high mortality rate. The bone is also a vital source of immune cells populations that suppress the anti-tumor immune response to drive tumor progression and metastasis. Using an aggressive orthotopic breast cancer mouse model we show that chronic oral exposure to tungsten results in tungsten deposition and enhanced breast cancer metastasis to the bone niche. Enhanced bone metastasis is associated with dramatic changes in the bone niche including osteol- ogy, signaling pathways and molecular defects associated with tungsten exposure. Multiple different members of the bone niche are associated with tungsten exposure. As a result, we were able to identify toxicity in target organs and cell types as well as molecular mechanisms of action. Even so, the research performed to this point has barely scratched the surface, and major research gaps remain. Interestingly, recent data also have suggested tungsten is an essential micronutrient in the human gut microbiome, which adds yet another layer of complexity to our understanding of the role of tungsten in human health. This Symposium will feature a range of perspectives, from human epidemiological studies to molecular mechanisms driving disease pathogenesis, with the purpose of providing exciting, interactive presentations that highlight emerging research being conducted to investigate the impact of tungsten exposure on human health. Presenters will review current epidemiological studies providing evidence of tungsten exposures, health outcome data, and how co-exposure with the essential metal molybdenum can modify health effects using data from multiple human cohorts throughout the US. Presenters will also discuss key in vivo and in vitro studies that will provide important data focused on organ-specific toxicity, identification of molecular targets, and the potential beneficial effects of tungsten exposure and the implications for human health. Overall, this session will provide a unique platform for discussion on the current state of the tungsten toxicity field and where we go from here. Topics discussed will include multiple disciplines, disease outcomes, and perspectives on the effects of tungsten exposure on human health. This Symposium will stimulate discussion with leading experts in the tungsten research field, which will provide opportunities to identify gaps in our knowledge base and also stimulate collaborations to fill those research gaps.

1277 Modulators of Tungsten-Induced Toxicity Identified by Whole Genome-CRISPR Screen  
K. Mann. McGill University, Montreal, QC, Canada.

Tungsten is increasingly used in manufacturing items including construction equipment, ammunition, jewelry, and medical devices. Due to increased usage, there is also increased exposure occupationally and to end-users. Despite this, there is very little known regarding the toxicities associated with tungsten exposure. We have shown that tungsten exposure alters B lymphocyte development, skews adipoocyte/bone differentiation from mesenchymal stem cells, and accumulates in the bone, likely as phospho-tungstase. While we have described some disruptions in signaling cascades associated with B cell differentiation, we know very little about the molecular events associated with tungsten exposure. To this end, we utilized an in vitro whole genome CRISPR screen to define those genes that provide protection against and vulnerability to sodium tungstate or phosphotungstate in the NALM6 preB lymphocyte cell line. We have identified a putative mechanism for tungsten's entry into the cell, which correlates with some of the bone/connective tissue defects associated with tungsten exposure. Multiple different members of the bone niche cascade downstream of amino acid sensing that leads to mTOR activation were identified in the CRISPR screen, suggesting a role for these molecules in potential metabolic changes associated with tungsten. These results show the utility of performing whole genome screens to identify molecular targets and/or signaling pathways and provide insight into tungsten-induced toxicity.

1278 Tungsten: An Overlooked Micronutrient for the Human Gut Microbiome  
G. Schut. University of Georgia, Athens, GA. Sponsor: A. Bolt.

Our understanding of how microbes impact human health has been revolutionized over the past decade, driven by the availability of vast amounts of metagenome data. The majority of the microbiome diversity is found in the gastrointestinal tract, where it aids in digestion and impacts overall human health. A striking revelation from the gut microbiome genome data is the presence of transporters and enzymes involved in the use of tungsten, an element previously shown to be required for some thermophilic microbes living primarily in volcanic vents but not considered to be involved in human health. Genes encoding enzymes of the so-called tungsten orange insulase (WOI) family are present in the genomes of several members of the dominant bacterial orders in the human gut microbiome, such as Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria and Synergistetes, along with genes encoding a tungstate-specific transporter known as Tup. A subset of these WORs have been shown to be involved in the detoxification of food- and microbe-derived tungsten, converting it to tungstic acid and releasing tungsten into the gut lumen. While we have described some disruptions in signaling cascades associated with tungsten exposure. Multiple different members of the bone niche cascade downstream of amino acid sensing that leads to mTOR activation were identified in the CRISPR screen, suggesting a role for these molecules in potential metabolic changes associated with tungsten. These results show the utility of performing whole genome screens to identify molecular targets and/or signaling pathways and provide insight into tungsten-induced toxicity.
The rodent cancer bioassay is a key element in the current standard approach for assessing human carcinogenic potential for agrochemicals as well as other industry sectors. The length of time, costs, resources, and numbers of animals needed to perform the bioassay have led to the development of alternative approaches to progress the modernization of carcinogenicity assessment away from lifetime rodent cancer bioassays. Decades of research have described the limitations of the rodent cancer bioassay for identifying human carcinogenic hazard and risk. These limitations have led to several international initiatives to establish approaches that modernize carcinogenicity assessment. For example, alternative approaches that include weight of evidence-based assessment of in vitro, in silico, and short-term in vivo tests have the potential to substantially reduce animal use while still protecting public health and better determining human carcinogenic hazard and risk. Frameworks to evaluate alternative approaches for carcinogenicity assessment of agrochemicals were developed by the Rethinking Chronic Toxicity and Carcinogenicity Assessment for Agrochemicals Project. These frameworks are being addressed through the European Partnership for Alternative Approaches to Animal Testing. This context produced a recognition of the need to map international efforts in order to develop and adopt new approach methodologies for carcinogenicity assessment. These initiatives generally recognize that more mechanistic approaches provide more granularity, increase testing efficiency, and may be more biologically relevant for evaluating both hazard and risk of chemical carcinogenicity to humans. The application of a mechanistic understanding to support new approaches to carcinogenicity assessment will be valuable for regulatory decision-making. The proposed session will outline the landscape of ongoing activities related to modernizing carcinogenicity assessment, present case studies for how this would work, provide regulatory perspective and feedback on what is possible and the challenges that must be addressed, and offer a perspective regarding the feasibility of achieving a globally harmonized approach to carcinogenicity assessment without long-term bioassays. This session includes international perspectives from government, industry, and nongovernmental organizations to explore a modern paradigm for science-based carcinogenicity assessment. It is intended to stimulate discussion with Workshop attendees in order to gain feedback and help progress the roadmap to modernized agrochemical carcinogenicity assessment.

To aid progression towards a modern paradigm of chronic safety assessment, several organizations collaborating in multi-stakeholder initiatives are developing WoE-based approaches and frameworks to support carcinogenicity assessment without long-term bioassays. These initiatives include collaboration under: The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), European Partnership for Alternative Approaches to Animal Testing (EPAA), Organization for Economic Cooperation and Development (OECD) Integrated Approaches to Testing and Assessment (IA(T)A) for non-genotoxic chemical carcinogens, and ReThinking chronic toxicity and carcinogenicity assessment for agrochemicals project (ReCAAP). While these initiatives are unique in their own actions and approaches, they are aligned to achieve the common goal of establishing a WoE-based carcinogenicity assessment that could fulfill regulatory needs for chronic safety assessment – without the rodent cancer bioassays. Given the breadth of activity, this presentation serves to scope the landscape of related projects to avoid redundant efforts, identify ongoing challenges, and identify opportunities to converge – including collaborations to develop case studies that will help build confidence in both regulators and the regulated industry, provide a reference point for international agencies identify of discrepancies in global approaches, and to provide a forum for defining a path towards implementation and acceptance of a WoE-based agrochemical carcinogenicity assessment.

Find up-to-date information at [www.toxicology.org/2023](http://www.toxicology.org/2023) | #2023SOT | #ToxExpo
Cannabis and cannabis-related products, including cannabidiol (CBD) resin-type
extracts from *Cannabis sativa*, high-CBD varieties of hemp, and cannabinoi
based extracts and products, are now available in all 50 states and Washington, DC. The presence of
pesticides, toxic elements, solvents, and mycotoxins in these commodities poses a potential health risk and has resulted in multiple recalls by manufacturers. Due to the Schedule I status of cannabis, the regulation of contaminants in cannabis and cannabis-based products is largely absent at the federal level. Individual states are setting action levels that may vary by up to four orders of magnitude for some common contaminants, such as the organophosphate insecticide chlorpyrifos and the fungicide myclobutanil. On the other hand, the illegal cannabis market continues to thrive. Products that fail state action levels may end up on the black market. Toxic effects such as the pesticide azoxystrobin are sometimes detected in high quantities. As the US cannabis market transitions from its illegal past, government regulations and cannabis testing industries remain in their infancy. The goal of this workshop is to engage the community with the latest findings on cannabis-borne contaminants and their risk to human health as well as the ethical, legal, and societal issues surrounding cannabis contamination and safety. This workshop includes five presentations by speakers from the public, industrial, academic, and journalistic sectors. It will explore (1) the common and emerging contaminants in cannabis, including pesticides, toxic elements, solvents, microbes, and mycotoxins; (2) the potential health hazards of cannabis contamination to recreational and medical users; and (3) the difficulties faced by regulators, cannabis manufacturers, laboratories, and other stakeholders in tackling this public health challenge. It will end with a 20-minute cross-disciplinary discussion of governmental, industrial, scientific, and ethical issues related to this public health challenge and possible solutions to these issues.
In 2021, The Arizona Republic investigated pesticide contamination in marijuana sold through licensed dispensaries in the state. The newspaper found products being sold exclusively to medical patients with as much as 10.4 ppm (or 26 times over the state limit of 0.4 ppm) for imidacloprid, a common pesticide in the industry. The cultivator had obtained passing certificates of analysis for the products that were provided to the retailer, a requirement for products to be sold in Arizona. The newspaper’s investigation found that state regulators had received a tip about contaminated products, and investigated the laboratory that provided the passing analyses. The state found that the laboratory did not falsify its results, which means the laboratory got clean samples while contaminated products went to the market.

The investigation also found that the cultivator had sent different samples of its products to different laboratories, an apparent violation of state rules. Following the newspaper’s report of the contamination, state lawmakers proposed changes to the state testing rules that would have addressed the shortcomings, which included a state-run “secret shopper” program to test for contamination. The bill also would have required the licensed laboratories in the state to submit all failing certificates of analysis to the state. While the bill did not pass, the legislative process highlighted the difficulty of navigating special interests, law enforcement, and public accountability to address a public health issue in a once-illegal cannabis market. This investigation sparks a broader discussion of ethical and legal issues on cannabis safety in the U.S.

Public health is increasingly affected by environmental issues—such as exposure to emerging contaminants—which account for a significant fraction of the toxicological burden. These exposures may be obvious, as with disasters such as chemical spills, or they may be less apparent, as with health conditions with unknown origins, evident only as changes in exposure or health trends when those communities would be most exposed or most susceptible if exposed. Timely access to high-quality, actionable information can protect public health, enable public health authorities to effectively respond to environmental health emergencies, and coordinate activities across the state and various stakeholders. Research studies analyzing the geographic distribution of air pollution and other types of environmental contamination documented the persistence of environmental health disparities between communities. Due to the shortage of published data, only limited research has been published on the geospatial distribution of environmental exposure disparities that may have been in the environment for some time but for which concerns have been raised more recently and health data are lacking. These exposures are considered by environmental health professionals to be among the most difficult to address due to unpredictable nature. The response to issues of concern require capabilities, capacity, and communication with pertinent organizations to enable rapid generation of translationally relevant data for public health decision-making. High-quality, reliable data are necessary to assess which contaminants have had hazardous potential so that steps can be taken to limit exposure and risks to the public. Engagement with the affected communities, particularly when those communities would be most exposed or most susceptible if exposed, are necessary so that action(s) such as intervention, remediation, and litigation will be well informed. This session aims to engage participants to discuss their emerging contaminants and issues of concern in public health, effective communication tools, and the lack of data on environmental exposure disparities that can be unique in terms of geography, income status, race/ethnicity, underlying health status, or other variables that cause disproportionate exposures associated with the emerging contaminants and issues of concern. The discussion will revolve around proactive identification of emerging contaminants and issues of concern by using iterative horizon-scanning, scoping, or mapping activities that (1) utilize existing methods or identify research projects with methodological needs; (2) evaluate ongoing projects and approaches to emerging contaminants and issues of concern, such as drinking water contaminant; (3) develop a potential system of cross-agency or multidisciplinary sharing of information, methodology, and capabilities that can be used to protect human health. This framework has been developed to evaluate and prioritize ECIC projects within the overall DNTP portfolio, as one of the strategic areas of focus for responsive research. Key strategies within this framework will identify public health emergencies and emerging issues, develop rapid mobilization of scientific resources, and identify research gaps to enhance response time, and facilitate fit-for-purpose prioritized responses that draw upon various DNTP resources (literature-based reviews, and health-based and mechanistic studies) to address emerging public health issues, without significant effect on the progress of other ongoing research activities. Some examples of ECIC projects in our portfolio include crumb rubber exposures, chronic kidney disease of unknown origin, cord blood exposure in diverse communities, and developmental studies on boron in drinking water. Activities include steps to establish communications with other organizations (e.g., government agencies, private entities, and environmental advocacy groups) to identify emerging contaminants and knowledge gaps leading to potential collaborations. Public health is increasingly affected by environmental issues—such as exposure to emerging contaminants, for which there is insufficient information available for understanding key aspects of risk to human health. Public health emergencies and emerging issues arise unexpectedly, yet regularly, and decision-makers often need rapid, high-quality, and actionable data to protect public health. Research within the Division of the National Toxicology Program (DNTP) at NIEHS has addressed both emergencies (e.g., Elk River chemical spill) and emerging environmental health topics (e.g., algal blooms) over the last several decades. However, rapid mobilization of scientific resources in response to such situations can be challenging. A framework has been developed to evaluate and prioritize ECIC projects within the overall DNTP portfolio, as one of the strategic areas of focus for responsive research. Key strategies within this framework will (1) prioritize public health emergencies and emerging issues, (2) develop rapid mobilization networks, characterize new scientific capabilities, and identify research gaps to enhance response time, and (3) facilitate fit-for-purpose prioritized responses that draw upon various DNTP resources (literature-based reviews, and health-based and mechanistic studies) to address emerging public health issues, without significant effect on the progress of other ongoing research activities. Some examples of ECIC projects in our portfolio include crumb rubber exposures, chronic kidney disease of unknown origin, cord blood exposure in diverse communities, and developmental studies on boron in drinking water. Activities include steps to establish communications with other organizations (e.g., government agencies, private entities, and environmental advocacy groups) to identify emerging contaminants/issues and knowledge gaps leading to potential collaborations. Continued discussions at the state, national, and global levels will enhance the use of limited resources by avoiding duplication of efforts, increase productivity, and identify and engage communities and groups advocating for scientific solutions to address human health concerns. Ultimately, our goal is to strengthen the science base around ECIC, enhance our ability to effectively respond to environmental health emergencies, and coordinate activities with other federal programs such as the legislatively mandated NERCI and the PFAS inter-agency workgroup.

A kidney disease of unknown cause is affecting agricultural workers in hotspots throughout the world. Termsd CKDu, this kidney disease has emerged as the leading cause of death among middle-aged men living in Meso-American countries (Nicaragua, El Salvador, and Guatemala), Sri Lanka, and a few communities in India. In this presentation, I will present the history of other regional nephropathies, the clinical and geographic description of the current CKDu epidemic, and the leading hypotheses about the potential causes for this form of kidney disease, including potentially heat stress, new infections, drinking water contamination and
agrochemical exposures. I will highlight preliminary data from other regions, includ-
ing the US, which demonstrate a high incidence of end-stage kidney disease in agricultural regions (Rio Grande Valley, Texas and Central Valley, California), as well as higher risk for incident end-stage kidney disease in the Agricultural Health Study participants with a prior hospitalization for pesticide exposure. We will also review data demonstrating a higher prevalence of CKD in other occupations (e.g., brick masons and farming). Studies evaluating CKD in diverse populations are hampered due to the nature of the disease (with long expected lag between exposure and disease), and the resource poor environment of the highest prevalence regions. Agricultural workers in the described hotspots are a marginalized population exposed to a range of understudied environmental and agrochemical exposures. Since filtered or secreted organic and inorganic compounds concentrate in the kidneys, even low-level toxin exposure can damage the tubules and cause progres-
sive kidney disease. CKDx demands new methods and multi-disciplinary teams to investigate a potentially new threat to the agricultural worker’s health.

**2000** Effect of Food-Grade Titanium Dioxide on DNA Methylation in Human Cells

Food-grade titanium dioxide (TiO₂) containing a nanoscale particle fraction is widely used as an additive in many foodstuffs, including cheeses and sauces, skimmed milk, ice cream, and confectionery products, e.g., as coating on sweets and chewing gum. However, the significant toxicity of food-grade TiO₂ nanomaterial is currently recognized, which suggests considerable risk to human health. The present study aims to further investigate the underlying mechanisms of food-grade TiO₂ toxicity by examining the alterations in cytosine DNA methylation induced by exposure to this nanomaterial in vitro. Human colorectal (Caco-2) and hepatic (HepG2) cells were treated with food-grade TiO₂ at sub-cytotoxic doses for 24 and 72 hours. Treatment with food-grade TiO₂ resulted in global and gene-specific DNA methylation alterations. Global DNA methylation decreased in each of the cell lines, while in these cells, four genes indicative of cellular stress response and toxicity (DNAJC15, GDF15, INSIG1, and PTGS2) exhibited altered DNA methylation. Additionally, treatment with food-grade TiO₂ altered the expression of chroma-
tin-modifying genes involved in establishing and maintaining DNA methylation 
patterns (DNMT1, DNMT3A, DNMT3B, MBD2, and UHRF) in a cell-type and
time-dependent manner. The results of this study demonstrate that sub-cytotoxic concentrations of food-grade TiO₂ induced treatment-related DNA methylation changes, supporting potential involvement of this epigenetic mechanism in the toxicity of this nanomaterial. Hence, for complete assessment of potential risk from food-grade TiO₂ exposure, epigenetic studies are critical.

**2002** Racial Disparities in Breast Cancer: Escalating the Effects of Chemicals with Exposure Disparities in Normal Breast Cells from Diverse Donors with High-Throughput Transcriptomics
N. Zhao, A. Tapanvi, T. Thong, and J. Colacino. University of Michigan School of Public Health, Ann Arbor, MI.

Despite well-documented disparities in breast cancer survival stratified by social determinants of health, genetics, diet, and race, the impacts of chemical exposures remain unclear. Biomonitoring data from the US National Health and Nutrition Examination Survey (NHANES) reveals disproportionate levels of toxicants in black women compared to white women, including pesticides, PFAS compounds, and heavy metals. Mounting evidence points to these chemicals and their analogs in the ability to alter mammary gland morphogenesis and induce phenotypic plasticity at these human-relevant doses. Thus, there is an urgent need to investi-
gate the impacts of environmental toxicants on breast cancer-associated biology. To address this gap, we perform high throughput transcriptomic (HTTR) profiling using the PlexWell whole transcriptome assay to characterize interindividual differ-
cences in response to chemicals with documented exposure disparities. Previously, we established conditionally reprogrammed (CR) cultures using cryopreserved punch biopsy samples of healthy, nulliparous women collected by the Susan G. Komen Normal Tissue Bank. Here, we test 6 CR cell lines (3 African American and 3 European American) treated with the following chemicals at a range of doses (control, 0.1, 1, and 10 µm): 1) Water as the vehicle control (sodium arsenite, cadmium chloride, copper chloride, lead acetate); 2) DMSO as the vehicle control (DDE, BPA, BPS, PFNA). Each contrast (sample x treatment x dose) relative to its control was performed in triplicate for a total of 432 exposure conditions. Raw count data was processed to produce differentially expressed gene (DEG) datasets using limma for analysis. Gene set enrichment analysis was performed using the MisgDB Hallmark gene sets. A false discovery rate threshold of 5% was applied for multiple comparisons. Unbiased clustering of global gene expression revealed widely varying differences between individuals, supporting the hypothesis that chemicals induce different transcriptional changes in cells from different individ-
uals. UpSet plots, heatmaps, and scatterplot visualizations also demonstrated unique distinctions in the number, directionality, and significance of DEGs for each toxicant, where all toxicants had at least two individuals who uniquely contribute as the largest sets of DEGs in both directions. Feature rank correlations of log2-fold changes between individuals for heavy metals, such as Cadmium Chloride at 10µm (range r = 0.176 to 0.676), observed stark contrasts against organic chemicals like BPS at 10µm (range r = −0.024 to 0.062), further suggesting that molecular mechanisms mediating toxicity are chemical and cell-line specific. DEGs across multiple individuals were commonly implicated in dysregulated cancer pathway signatures such as targets for MYC, E2F, G2M checkpoints, and epithelial-mes-
enchymal transitions. Overall, these results provide new insights into chemical exposures and highlight the utility of precision models to characterize the heterogeneity in breast cancer biology and susceptibility. Future work is needed to develop targeted prevention therapies and reduce health inequities that underlie vulnerable populations.

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Chemical toxicants frequently display strong tissue specificity that may result from different factors including exposure level and duration, (de)toxication capacity, cell type composition and proliferation status. Therefore, mapping the difference in transcriptomic responses towards chemical-induced stress between different tissue types is top essential to obtain improved understanding. This will reveal preserved networks across cell types but also will uncover cell specific responses which should be taken into account during chemical safety screening using specific cell types. Here, cell types derived from distinct organ types (human neuronal LUMHES, liver HepG2 and primary human hepatocytes (PHHs), kidney RPTEC and lung ALL-PBEC cells) were exposed to CDDO-Me, tunicamycin, TNFα and cisplatin to evaluate concentration dependent activation and temporal dynamics of specific stress response pathways, namely oxidative stress, unfolded protein, inflammatory and DNA damage response, respectively. First, cells were exposed in a wide concentration range for 24 h followed by transcriptome analysis of a targeted gene set of ~3500 genes using the TempO-seq technology. Thereafter, cells were exposed for 1, 2, 4, 6, 8, 12, 16 and 24 h at a single concentration to follow activation dynamics using ComB0-seq analysis for whole transcriptome mapping. In general, large differences were observed in the nature and the extent of transcriptional responses between the cell models. For all four compounds the majority of the transcriptional changes in the same genes and directions was observed between two liver cell models HepG2 and PHH. Moreover, evaluation of temporal dynamics of stress response activation could discriminate between networks responding similarly over time as well as networks showing distinct activation patterns across cell models. To conclude, both cell model dependent and preserved networks could be identified that allow for improved understanding when using these models during chemical safety testing. These findings caution against generalization and over-interpretation of findings obtained with a single model and stress the need to apply a battery of different test systems when evaluating safety of novel chemicals. Supported by the RISK-HUNT3R project funded by the European Union under the Horizon 2020 programme (grant agreement 654537), EU-ToxRisk project (grant agreement 681002), IMI MIP-DIL project (grant agreement 115356) and Division of the National Toxicology Program at NIEHS, NIH, USA (ZIA ES103318-03).

2004 Comparing RNA-seq and TempO-seq mRNA Data Sets: A Case Study

US EPA, Research Triangle Park, NC.

There are multiple technological platforms available for quantifying mRNA levels to use for transcriptomics studies. With the increase in TempO-seq generated mRNA data, it is important to determine whether TempO-seq and RNA-seq generated data are comparable and/or can be combined. For this analysis, two different TempO-seq data sets (Cell Atlas and Toxic) were compared for each target using principal component analysis (PCA) for four overlapping cell types (Daudi, HepG2, MCF-7, and U-2 OS) and the PCA showed that the data sets could be combined. The TempO-seq data were then compared to RNA-seq data for 12 cell types (A549, Daudi, HBECC-3-KT, HepG2, HME-1, HUVEC, MCF-7, RPE-1, RPTEC, TIME, U-2 OS, and T47-D) using PCA. For the four overlapping cell types within both Cell Atlas and TempO-seq data sets, the average of all replicates per cell type was used for this comparison to RNA-seq. Statistical testing using PCA on TempO-seq versus RNA-seq mRNA expression data for the 19,119 overlapping genes showed that there was a clear platform divergence pattern within the first principal component (PC1) for all cell types evaluated. This meant that these TempO-seq and RNA-seq data should not be combined without further steps. Normalizing the data by calculating the relative log expression (RLE) compared to the average expression level across cell types in each platform removed the platform divergence observed when using short-term, molecular-based assays for the characterization of toxicity and preservation of chronic-animal-based studies. This abstract does not necessarily reflect US EPA policy.
Copper (Cu) is an essential trace element, which is consumed through drinking water, grains and vegetables. In fact, Cu is increasingly being introduced into the environment, due to industrial and agricultural processes. As a cofactor for various enzymes Cu is vital for numerous biological redox processes, but in excess can be harmful or even toxic. A tightly regulated homeostasis is crucial, since Cu dysregulation can lead to neurological diseases such as Menkes and Wilson disease. Therefore, we investigated the toxicity of Cu oversupply and Cu dyshomeostasis in the context of neurodegeneration. We used the multicellular model organism C. elegans, since the nematode constitutes a distinguished in vivo model conserving various orthologous genes, and taking into account Cu homeostasis. C. elegans strains use include wildtype worms, as well as loss-of-function mutants of the copper metallochaperone protein (atox-1Δ) and of the copper storage protein ceruloplasmin (cpaΔ).

Additionaly, the species agnostic platform facilitates transcriptomics-based cross-species extrapolation by making the data more comparable across species. The platform uses the Seq2Fun algorithm to map RNA-seq reads from eukaryotic species to an ortholog database comprised of protein sequences from >600 eukaryotic species (EcoOmicsDB) with a translated search. The availability of these tools presents a unique opportunity to examine the EcoToxChip RNA Seq dataset for cross species comparisons. Accordingly, the objectives were to A) demonstrate the utility of EcoOmicsAnalyst for comparative transcriptomic analysis across multiple datasets, and present a single repository of principal component analyses across species; B) validate the species agnostic platform of relevance to ecotoxicology, EcoOmicsAnalyst and Seq2Fun platform, was developed to facilitate RNA Seq analysis of non-model species lacking a reference transcriptome. The platform uses the Seq2Fun algorithm to map RNA-seq reads from eukaryotic species to an ortholog database comprised of protein sequences from >600 eukaryotic species (EcoOmicsDB) with a translated search. The availability of these tools presents a unique opportunity to examine the EcoToxChip RNA Seq dataset for cross species comparisons. Accordingly, the objectives were to A) demonstrate the utility of EcoOmicsAnalyst for comparative transcriptomic analysis across multiple datasets, and present a single repository of principal component analyses across species; B) validate the species agnostic platform of relevance to ecotoxicology, EcoOmicsAnalyst and Seq2Fun platform, was developed to facilitate RNA Seq analysis of non-model species lacking a reference transcriptome.
**2011** BTBD9, a Restless Legs Syndrome–Associated Gene, Regulates Manganese-Induced Toxicity

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Manganese (Mn) is an essential mineral, but excess exposure can cause dopaminergic neurotoxicity. Restless legs syndrome (RLS) is a common neurological disorder, but the etiology and pathology remain largely unknown. The purpose of this study was to identify the role of manganese in regulation of a RLS genetic risk factor—BTBD9—and characterize the function of BTBD9 in Mn-induced oxidative stress and dopaminergic neuronal dysfunction. We found that human subjects with high blood Mn levels were associated with decreased BTBD9 mRNA levels, compared with subjects with low blood Mn levels. In AS49 cells, Mn exposure decreased BTBD9 protein levels. In Caenorhabditis elegans, loss of hpo-9 (BTBD9 homolog) resulted in more susceptibility to Mn-induced oxidative stress and mitochondrial dysfunction, as well as decreased dopamine levels and alterations of dopaminergic neuronal morphology and behavior. Overexpression of hpo-9 in mutant animals restored these defects, and the protection was eliminated by mutation of the forkhead box O (FOXO). In addition, expression of hpo-9 upregulated FOXO protein levels and decreased dopamine and protein kinase B levels. These results suggest that elevated Mn exposure might be an environmental risk factor for RLS. Furthermore, BTBD9 functions to alleviate Mn-induced oxidative stress and neurotoxicity via regulation of insulin/insulin-like growth factor signaling pathway.

**2012** The Heavy Metal Lead (Pb) Alters the Neuron/Glia Balance in the Developing Human Brain by Shifting Progenitor Cell Fate and Maturation Trajectories


Prenatal toxicant exposures interfere with nervous system development and can lead to persistent changes in the brain. Modeling exposures in primary fetal tissue and human induced pluripotent stem cell-derived 3D organoids, we show that early-life exposure to the heavy metal lead (Pb) alters the generation and maturation of different cell types—neurons and glia—in the developing human brain.

Previous studies have largely focused on neuronal toxicity, but here we focus on radial glia, the primary progenitor cells in the developing brain, and astrocytes, specialized glial cells that have important roles in neurodevelopment. Astrocytes protect the brain from toxins by participating in the blood brain barrier, regulating inflammation, and providing redox and metabolic support to other cells. We demonstrate that both fetal and organoid-derived cells rapidly take up Pb and establish a Pb exposure paradigm that achieves biologically relevant tissue levels of Pb. After 1 week of Pb exposure, organoids upregulated gene expression of metallothioneins, which are involved in metal homeostasis and buffering. There was also an increase in NRF2-mediated antioxidant response element (ARE) transcription as well as increased expression of heme oxygenase-1 (HMOX1). Astrocytes upregulated the expression of glutathione S-transferase (GST) and heme oxygenase-1 (HO-1), which are involved in detoxification and antioxidant mechanisms.

**2013** Early-Life MeHg Exposure of Human iPSC-Derived Cortical Cells Persistently Alters the Homeostatic Neuronal State

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The developing brain is exceptionally vulnerable to toxic insults. Early-life exposure to methylmercury (MeHg) results in long-lasting functional effects in the cortical structures of the developing brain and especially affects glutamatergic (GLUergic) neurons. To understand the mechanisms behind altered GLUergic function and the persistency of effects, this study utilized a human induced pluripotent stem cell (hiPSC)-neuroded cortical model, testing the hypothesis that developmental MeHg exposure results in persistently altered homeostatic neuronal state visible in functional and genetic outcomes. hiPSC-derived cultures were continuously exposed from day 4-10 of development (stage with neuroepithelium and radial glia stage) or 4-10 + 14-20 (stage with RG, plus intermediate precursors and early nonspecified progenitors). RG+IPC stage (0.1 µM exposure to MeHg) was performed and significant upregulation of phosphorylated eIF2α (p-eIF2α) in mature cultures was found following developmental exposure to 1 µM (p<0.001), and trended toward upregulation of p-eIF2α at the 0.1 µM exposure. To assess whether these changes were associated with altered function, mature cells were cultured on micro-electrode arrays (MEAs) and spontaneous network activity was assessed for 40 days (D110-D150). Assessment of spontaneous neuronal network activity and (network) bursting behavior indicated that the prior RG and RG+IPC exposures significantly altered neural activity patterns. RG exposure to 0.1 µM induced network spiking and bursting, whereas exposure to 1 µM decreased spiking and bursting activity. RG+IPC exposure (both concentrations) decreases spontaneous neuronal network activity and bursting. The data establish that early-life MeHg exposure results in altered transcriptional and functional state in mature hiPSC-derived cortical cultures. More importantly, these novel data indicate that effects are persistent and proceed to manifest even more than 100 days after cessation of exposure. This research was supported by NIH R01 ES073731 (ABB/MA).

**2014** Role of Arrestin Domain-Containing Protein 1 (ARRDC1)-Mediated Microvesicles (ARMMs) in Cadmium-Induced Neurotoxicity

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Exposures to heavy metals are often associated with neurodevelopmental toxicity. Intercellular communication is often perturbed by metal exposures but the precise mechanism and role of such perturbation in metal-induced neurotoxicity remain poorly understood. Neural cells can secrete membrane-encapsulated vesicles into the extracellular milieu. These extracellular vesicles (EVs) contain functional signaling molecules mediating intercellular communication. The present study aimed to investigate the effects of heavy metal cadmium (Cd) on intercellular communication by evaluating the role of arrestin domain-containing protein 1 (ARRDC1)-mediated microvesicles (ARMMs). We utilized a lentiviral CRISPR/Cas9 system to knockout ARRD1 to repress ARMMs release in immortalized human neural progenitor ReNcell CX cell line and compared the responses to Cd between scramble control and knockout cell lines. Cytotoxicity endpoint (LDH) and oxidative stress results showed that ARRD1-knockout cells were more susceptible to Cd; the addition of extra ARMMs reversed the increased susceptibility to Cd, indicating that ARMMs play a protective role that can alleviate cytotoxicity induced by Cd. Mechanistic evidence including the expression of genes related to antioxidative responses was consistent with the toxicity results and proteomics data showed that the biosynthesis of amino acids and metabolic pathways were disrupted. The release of EVs was significantly increased in a dose-dependent manner, demonstrating that cellular communication was stimulated. Proteomics data showed that Cd altered the proteins in EVs that were from proteasome and involved neurodegenerative diseases such as Parkinson’s and Huntington’s disease. Furthermore, the potential of ReNcell differentiation to neuronal cells was compared between scramble control and knockout cells, and the effects of Cd exposure were studied. ARRD1-knockout cells showed weaker neuronal cell markers for mature neurons and astrocytes after differentiation, indicating ARMMs are involved in this process. Cd significantly inhibited the differentiation for both scramble and knockout cells.
Astrocytes are the first line of defense against xenobiotics crossing into the brain. As primarily glycolytic cells, mitochondrial metabolism in astrocytes is not required for their survival. However, in this work, we demonstrated that astrocytic mitochondrial metabolism is essential for their survival against neurotoxic electrophiles such as inorganic arsenic (iAs). Subtoxic iAs exposure induced an increase in de novo glutathione (GSH) synthesis and a reduced intracellular environment in primary cortical astrocytes. iAs toxicity was potentiated by inhibition of de novo GSH synthe- sis, and the neurotoxicity was mediated by relief of drug resistance associated proteins (MRP)s, which enhanced arsenic accumulation. NMR metabolomics revealed that iAs induced the cataplerotic generation of glutamate via the tricarboxylic acid (TCA) cycle, which contributed to de novo GSH synthesis. iAs exposure increased the dependency of mitochondrial oxygen consumption on fatty acid oxidation (FAO). Interestingly, while inhibition of mitochondrial pyruvate transport (MPC1), the electron transport chain (ETC) or FAO had no effect on astrocyte viability, it enhanced arsenic accumulation and iAs toxicity while inducing a moderate decrease in cellular bioenergetics. This demonstrated that mitochondrial metabolism is important for both xenobiotic detoxification. iAs exposure led to a substantial accumulation of extracellular glutamate that was mediated by the reversal of excitatory amino acid transporters EAAT1/2. Accordingly, inhibition of glutamate transport enhanced iAs toxicity. NMR-metabolomics also demonstrated that extracellular glutamate uptake and metabolism is required for de novo GSH synthesis upon iAs exposure. These results revealed that astrocytic mitochondrial metabolism support detoxification of neurotoxic electrophiles by GSH, via the synthesis of its precursors. Furthermore, they suggest that mitochondrial-dependent glutamate synthesis compensates for intracellular glutamate depletion triggered by astrocytic glutamate transport reversal upon iAs exposure.

**2018 Endoplasmic Reticulum (ER) Stress Inhibition Protocols against Pyrethroid-Induced Disruption of Hippocampal Neurogenesis and Cognitive Deficits in Mice**

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Cognitive impairment is increasing globally; currently more than 16 million people are living with cognitive impairment in the United States. However, age is the major risk factor, environmental exposure has been identified as a significant contributor for developing cognitive impairment. Deltamethrin is a broad-spectrum type II pyrethroid insecticide that has been increasingly used in agriculture and homes for pest control. Previously, we reported that repeated exposure to 3 mg/kg deltamethrin caused a marked reduction in hippocampal neurogenesis and impaired learning and memory in adult mice. Here, we sought to determine whether exposure to NOAEL of 1 mg/kg of deltamethrin elicits similar effects. We used the novel object recognition task, fear conditioning, and Morris water maze to determine the effects of deltamethrin on cognitive behavior. Our data revealed that repeated exposure to NOAEL of deltamethrin for 30 days caused significant deficits in novel object recognition and learning and memory in water maze and fear conditioning tasks. These behavioral deficits were accompanied by impairment of hippocampal neurogenesis as neural progenitor cell proliferation (BrDU+ cells and Ki67+ cells) and immature neurons (DCX+ cells) were significantly decreased in the DG of the hippocampus. Further, deltamethrin-treated mice exhibited remarkable ER stress as the protein levels of ATF-4, CHOP, and GRP-78 were significantly increased in the hippocampus. To determine whether ER stress is associated with impairment of the hippocampal neurogenesis and cognitive deficits, a group of mice were treated with intraperitoneal (i.p.) injections of 1 mg/kg salubrinal (eIF2α inhibitor) 30 min prior to deltamethrin administration. Our data showed that pre-treatment of mice with salubrinal prevented ER stress, restored neurogenesis, and improved cognitive deficits in deltamethrin-treated mice. We also used caspase-12 KO mice for further exploration of the role of ER stress in impairment of hippocampal neurogenesis and cognitive deficits. We found that caspase-12 KO mice showed significant protection against deltamethrin-induced disruption of hippocampal neurogenesis and cognitive deficits when compared to WT mice. Together, these results demonstrate that repeated exposure to current NOAEL of deltamethrin leads to ER stress mediated inhibition of adult hippocampal neurogenesis which subsequently contribute to deficits in learning and memory in mice. Supported by R01ES027481.

**2017 Cholorpyrifos and Chlorpyrifos-Oxon Exposures Lead to the Dysregulation of Basal Glutamate Release in Human-Induced Pluripotent Stem Cell-Derived Glutamatergic Neurons**


Chlorpyrifos (CP) is an organophosphate pesticide and insecticide developed for agricultural and household settings. Chlorpyrifos, or its more toxic metabolite CP-oxon (CPO), causes a wide range of health complications such as neurological dysfunction, immunotoxicity, developmental issues, and cardiovascular damage. The primary target of CP neurotoxicity is typically thought to be acetylcholinesterase inhibition, though emerging data suggest that other neurotransmitter systems can be affected at low doses. Currently, little is known about how CPO(0) affects the glutamatergic (GLUergic) system. The aim of this study is to determine the neurotoxic effects of CP and CPO on glutamate (GLU) release, characterize baseline GLU dynamics in human GLUergic cortical neurons, and address a gap in neurotransmitter monitoring using novel biosensors. GLUergic cortical cultures were differentiated via dual-iPSCs from human induced pluripotent stem cells (hPSCs). Upon maturation (>90%), a baseline collection (in HBSS) for extracel- lular GLU was performed followed by a 24-h sub-cytotoxic exposure to CP (3 µM - 300 µM) or CPO (0.1 µM - 10 µM). Following exposure, cells recovered for 24 h with an additional GLU collection. After the recovery period, whole-cell protein lysates were harvested to correct measured GLU release for cell density by total protein. All collections were analyzed using a commercial GLU assay kit. Cells exposed to CP demonstrated a concentration-dependent increase in net GLU release, while cells exposed to CPO presented no significant effect. The upregulation persisted over 24 h. Thus, it is shown that in addition to altering the acetylcholinergic system, CP and CPO may have unexpected effects on neuronal GLU homeostasis. An additional set of collections with vehicle exposure was performed at 10-min intervals over 60 min to monitor GLU efflux kinetics. GLU release was identified to sharply increase over 0 - 15 min and plateau after 20 min, which is likely to restore extracellular GLU levels. Newly developed enzymatic electrochemical biosensor arrays with cell culture compatibility were utilized to further characterize these findings. The biosensors were created using direct ink writing robotic printing combined with thin film processing and designed for sample delivery via injection or cell culture. Media samples with L-Glutamic Acid (60 µM) were dispensed on cultured cells and collected after 1 - 60 min for combined evaluation through sensors and enzymatic assays. In addition, GLU standards spiked with L-Glutamic Acid (0 µM - 60 µM) were prepared in HBBS, PBS, and BrainPhys for further testing. The novel biosen- sors confirmed earlier GLU-kinetics findings and accurately measured extracellular GLU release in a variety of media types. Overall, this study reveals a potential new mechanism for neurotoxicity, establishes a better understanding of GLU dynamics in vitro, and introduces innovative sensor technology.

**2015 Mitochondrial Metabolism in Astrocytes Is Essential for Their Survival against Inorganic Arsenic Exposure**

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Astrocytes are the first line of defense against xenobiotics crossing into the brain. As primarily glycolytic cells, mitochondrial metabolism in astrocytes is not required for their survival. However, in this work, we demonstrated that astrocytic mitochondrial metabolism is essential for their survival against neurotoxic electrophiles such as inorganic arsenic (iAs). Subtoxic iAs exposure induced an increase in de novo glutathione (GSH) synthesis and a reduced intracellular environment in primary cortical astrocytes. iAs toxicity was potentiated by inhibition of de novo GSH synthe- sis, and the neurotoxicity was mediated by relief of drug resistance associated proteins (MRP)s, which enhanced arsenic accumulation. NMR metabolomics revealed that iAs induced the cataplerotic generation of glutamate via the tricarboxylic acid (TCA) cycle, which contributed to de novo GSH synthesis. iAs exposure increased the dependency of mitochondrial oxygen consumption on fatty acid oxidation (FAO). Interestingly, while inhibition of mitochondrial pyruvate transport (MPC1), the electron transport chain (ETC) or FAO had no effect on astrocyte viability, it enhanced arsenic accumulation and iAs toxicity while inducing a moderate decrease in cellular bioenergetics. This demonstrated that mitochondrial metabolism is important for both xenobiotic detoxification. iAs exposure led to a substantial accumulation of extracellular glutamate that was mediated by the reversal of excitatory amino acid transporters EAAT1/2. Accordingly, inhibition of glutamate transport enhanced iAs toxicity. NMR-metabolomics also demonstrated that extracellular glutamate uptake and metabolism is required for de novo GSH synthesis upon iAs exposure. These results revealed that astrocytic mitochondrial metabolism support detoxification of neurotoxic electrophiles by GSH, via the synthesis of its precursors. Furthermore, they suggest that mitochondrial-dependent glutamate synthesis compensates for intracellular glutamate depletion triggered by astrocytic glutamate transport reversal upon iAs exposure.
Per- and polyfluoroalkyl substances (PFAS) are a large group of chemicals of concern based on their potential persistence, bioaccumulation, and toxicity. A tiered approach to screening and testing using new approach methodologies has been proposed as one of the few tractable approaches for evaluating thousands of PFAS for human health and environmental hazards. Based on previously conducted in vitro screening assays with human cell lines, four PFAS identified as estrogenic (perfluorooctanoic acid [PFOA], 1H,1H,2H,2H-perfluorobutane-1,2-diol [FC-8-diol]; 1H,1H,10H,10H-perfluorodecan-1,10-diol [FC-10-diol]; 1H,1H,8H,8H-perfluoro-3,6-dioxo-3-methyl-3-oxaheptane [HFPDOA]; GenX) were subsequently tested for estrogenic actions in vivo. Male fathead minnows (Pimephales promelas) were exposed to the selected PFAS for 96 h and hepatic expression of four estrogen-regulated genes was examined. Of the four estrogenic PFAS, FC-8-diol, FC-10-diol, and FC-8-DOH, which ranked among the most potent estrogenic PFAS out of over 140 screened, elicited concentration-dependent up-regulation of vitellogenin and estrogen receptor alpha transcripts and down-regulation of insulin-like growth factor 1 and apolipoprotein eb transcripts in exposed male fathead minnows. In contrast, PFOA, which was a substantially less potent estrogen receptor agonist in vitro, and HFPDOA, which was non-estrogenic in vitro, elicited no concentration-dependent effects on the estrogen regulated transcripts. After accounting for differences in measured bioconcentration in plasma, the in vitro and in vivo rank orders of potency aligned. The results provide increased confidence in the use of human cell-based assays to identify estrogenic environmental hazards of PFAS. Results also contributed a predictive limitation for effect of use in screening level assessments of potential PFAS-related estrogenic activity at contaminated sites and provide a basis to identifying structurally related PFAS that should be evaluated for estrogenic activity. The contents of this abstract neither constitute nor necessarily reflect US EPA policy.

2020 Sex-Specific Effects of Perfluorooctanoic Acid (PFOA) Exposure on Human-Induced Pluripotent Stem Cells (hiPSCs)


Exposures to environmental toxicants during early development can influence susceptibility to disease throughout the life course, and likely play critical roles in the pathogenesis of cardiovascular diseases (CVDs). This developmental programming is hypothesized to occur, at least in part, through genetic modifications including changes in DNA methylation. In this study, we utilized hiPSCs to examine the sex-specific effects of perfluoroalkyl substances (PFAS) on cellular proliferation, pluripotency, and expression of genes that regulate DNA methylation and de-methylation. hiPSCs from one male and one female donor were exposed to PFOA, a ubiquitous PFAS, for 72 hours, followed by qRT-PCR for pluripotency markers (POUSF1, SOX2, NANOG), DNA methyltransferases (DNMT1, 3A, 3B), and tet methylcytosine dioxygenases (TET1, 2, 3). Proliferation was assessed using IncuCyte live cell imaging and quantification using IncuCyte Zoom 2018A. PFOA exposure had no significant effect on cell proliferation in the dose range of 0.5 - 200 μM in either male or female-derived cell lines. PFOA (200 μM) exposure significantly decreased POUSF1 in the female-derived line (p=0.029) but not the male. There were significant differences in the baseline expression of pluripotency markers between male and female-derived cell lines in the control as well as in some concentrations of PFOA exposure. PFOA (100, 150 μM) exposure significantly decreased TET2 (p=0.044, 0.027) in the female-derived cell line but not the male. There were significant baseline differences in the expression of DNMTs and TETs between male and female-derived cell lines in the control as well as in some concentrations of PFOA exposure. We also examined if PFOA exposure in hiPSCs affected their ability to differentiate into cardiomyocytes. hiPSCs exposed to PFOA for 72 hours were differentiated to cardiomyocytes using an established protocol. All PFOA-exposed hiPSCs were able to differentiate to cardiomyocytes, regardless of concentration. We’ve discovered potential sex differences in the expression of pluripotency markers and epigenetic modifying enzymes, at baseline and in response to PFOA exposure. This project will contribute to our understanding of the sex-specific effects of PFOA exposure on cardiogenic differentiation and epigenetic programming, which may underlie the risk of later CVDs.
spheroids. These results underscore the importance of leveraging human-relevant animal models to understand mechanisms contributing to the PFOA-induced lipid dysregulation observed in human populations.

**2023 Assessment of Perfluorooctanesulfonic Acid (PFOS) Distribution in an Albumin-Deficient (Alb-/-) Mouse Model**

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Perfluorooctanesulfonic acid (PFOS) is a perfluoroalkyl substance (PFAS) ubiquitously found in the environment, with exposure occurring through the consumption of drinking water and food (via contact materials) and consumer product use/exposure. PFOS is detected in the serum of ~95% of the United States adult population and has a half-life of 4.1-8.67 years. Exposure is associated with increased cholesterol, liver hypertrophy, hepatic peroxisome proliferation, dyslipidemia, and obesity among others. In comparisons of PFOS accumulation in whole blood and plasma, it was revealed that PFOS accumulates in the non-cellular blood fraction. Albumin is a protein synthesized by the liver that aids in moving small molecules throughout the bloodstream and is important for maintaining fluid homeostasis. Albumin is the major carrier for PFOS and has been found to be 99% bound to PFOS in vitro. An albuminemic mouse model was chosen for this study to investigate how albumin contributes to the distribution of PFOS into PFAS relevant tissues, and to investigate how albumin influences liver endpoints such as: ALT, AST, total triglycerides, and liver pathology. It is hypothesized that PFOS concentrations will decrease in PFAS relevant tissues and adverse liver endpoints will be less severe in the albumin deficient mice as compared to wild type. In this study, adult male C57BL/6J albumin null (Alb-/-, n=8) and wild-type mice (Alb+/-, n=8) were orally administered either vehicle (0.5% Tween 20) or PFOS (10 mg/kg BW in 0.5% Tween 20) every day for 7 days. The mice were weighed every day and the dose was adjusted accordingly. A dose of 10 mg/kg BW was chosen to ensure adverse liver endpoints such as increased liver weight and total triglycerides, and hepatocyte vacuolation would be observed at day 7. At the time of necropsy, tissues were collected and snap-frozen in liquid nitrogen. Samples were analyzed for triglycerides, and hepatocyte vacuolation would be observed at day 7. At the time of necropsy, tissues were collected and SNAP-frozen for use.

**2024 Antioxidant Enzyme Activity Responses following Short-Chain Per- and Polyoxyalkyl Substances (PFAS) Exposures in Human Liver, Kidney, Muscle, and Microglia Cell Lines**

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There are lines of evidence pointing to legacy per- and polyoxyalkyl substances (PFAS) inducing the generation of reactive oxygen species (ROS) and compromising antioxidant defense mechanisms in vitro and in vivo. While a handful of studies have assessed oxidative stress effects by PFAS, few specifically address short-chain PFAS. In the present study, we evaluated biomarkers of oxidative stress effects in vitro following exposures to low (1 nM) and high (1 μM) concentrations of five alternative PFAS compounds [undecanoic acid (UFA), perfluorotributylamine (PFTrBz), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), and 6:2 fluorotelomer alcohol (6:2 FTOH)]. We conducted experiments in human cell lines representative of four different tissue types, including liver (HepaRG), kidney (HEK293-TLR2), brain microglia (HMC-3), and muscle (RMS-13). We initially screened for oxidative stress in HepaRG cells using fluorescence microscopy and found evidence of ROS generation in cells exposed to PFBS and PFHxS. Subsequent analysis of short-chain PFAS-exposed HepaRG cells exposed to PFBS and PFHxS did not reveal any significant changes in ROS levels. In contrast, PFOS and PFHxS exposure resulted in a significant increase in ROS levels. HepaRG cells exposed to PFOS and PFHxS also showed a significant increase in the activity of several antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). These results indicate that short-chain PFAS, such as PFOS and PFHxS, are able to induce oxidative stress in HepaRG cells, and this effect is associated with increased antioxidant enzyme activity. These findings further support the use of HepaRG cells as a human liver model to study the effects of PFAS exposure on hepatic antioxidant defenses.

**2025 Investigating the Role of Perfluorooctanesulfonic Acid (PFOS) Exposure on Insulin Biosynthesis in Pancreatic βT-6 Cells.**

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An oxidized redox environment in the endoplasmic reticulum (ER) of pancreatic β-cells is necessary for normal β-cell function. In β-cells, proinsulin undergoes oxidative folding of three disulfide bonds required for export from the ER. However, shifts in the redox status of the ER—either reductive or oxidative changes—can impair protein folding. As β-cells have low endogenous levels of many antioxidant defenses, they are particularly sensitive to toxicant-induced redox stress. Perfluorooctanesulfonic acid (PFOS) is a persistent and ubiquitous legacy environmental contaminant, historically used in numerous consumer products. Epidemiological and animal studies have linked exposure to PFOS to several adverse health outcomes, including β-cell dysfunction and oxidative stress. This study aims to investigate the role of PFOS exposures on proinsulin misfolding. Pancreatic βT-6 cells were exposed to either 0.1% DMSO or 50, or 100 μg/L PFOS for 48h. A proinsulin spheroid assay was used to quantify the misfolding of proinsulin (NAC), a potent enhancer of cellular antioxidant capacity. Following exposures, cells were either fixed/permeabilized for proinsulin and insulin immunofluorescence with confocal imaging or lysed for gene expression of the ER stress markers ATF4 and CHOP. To assess proinsulin misfolding, βT-6 cells were exposed to either 0.1% DMSO or 10 μg/L PFOS for 48h, lysed and split into three aliquots: 1) unmodified, to detect proinsulin aggregates, 2) alkylated with 6 mM AMS - to detect free cysteine (Cys), i.e. unfolded bond partners, and 3) reduced and alkylated - a positive control to show all potential Cys. All samples were then resolved by SDS-PAGE under reducing conditions and immunoblotted against proinsulin and insulin. Additionally, insulin secretion upon glucose stimulation (GSIS) was measured by ELISA. In PFOS treated βT-6 cells, no changes were observed in intracellular proinsulin fluorescence. However, intracellular insulin fluorescence decreased in cells exposed to 50 and 100 μg/L PFOS, by 20 and 30%, respectively. This was accompanied by a decrease in glucose-stimulated insulin secretion. Interestingly, cells exposed to PFOS and co-exposed showed a 30% increase in insulin production (50%) in intracellular insulin fluorescence compared to controls. ATF4 gene expression was increased in all treatment groups, including cells exposed to 100 μM NAC alone. βT-6 cells exposed to PFOS, also showed an increase in proinsulin alklyation, which is an indication of free thiol and mispairing of cysteine residues. Overall, our data support the hypothesis that PFOS-induced changes and co-exposure seem to exacerbate some of the endpoints investigated, highlighting redox stress as an important and often over-looked mode of toxicity. These data demonstrate that exposure to environmental contaminants can disrupt the redox balance necessary to properly synthesize and secrete mature insulin, ultimately contributing to β-cell dysfunction. This work was supported by ROI515028749.

**2026 An Environmentally Relevant Mixture of Perfluoralkyl Substances (PFAS) Impacts Proliferation, Steroid Hormone Synthesis, and Gene Transcription in Primary Human Granulosa Cells**

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Perfluoralkyl substances (PFAS) are a group of synthetic chemicals that are resistant to biodegradation and are environmentally persistent. PFAS are found in many consumer products including non-stick cookware, food packaging materials, upholstery, and personal care products. In addition, PFAS are a major source of environmental contamination. PFAS exposure can cause endocrine dysregulation and disrupt menstrual cycle, cause early menopause via primary ovarian insufficiency (POI), and alter steroid hormone levels in human and animal models. Although virtually 100% of the U.S. population has measurable exposure to several types of PFAS, little research has been done to understand the effects of PFAS mixtures on reproductive health. This study investigated the effects of a PFAS mixture [PFOA, perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS)] on human granulosa cell (hGC) function and transcriptome, hypothesizing that a PFAS mixture would promote cell growth and alter gene expression. Primary hGCs were harvested from follicular aspirates of healthy, reproductive-age women (25-35 yr. old) who were undergoing oocyte retrieval for in vitro fertilization (n=8). The impact of PFAS exposure on hGC cell proliferation was evaluated by the MTT assay. Cells were cultured with vehicle control (DMSO <0.01% final concentration) or PFAS mixture (2 nM PFOHxS, 7 nM PFOA, 10 nM PFOS) for 96h. Increased cell proliferation was observed (P < 0.05) and immunofluorescent staining with the cellular proliferation marker Ki67 and cell cycle analysis via flow cytometry further supported the proliferative action of the PFAS mixture. To measure steroid hormone production, cells were cultured with 100 μM NAC and treated with a substance PAFAS mixture and androstenedione (500 nM). After 48h, cells were treated with follicle stimulating hormone (FSH; 30 ng/mL). Media was collected 48h after FSH treatment, and estradiol and progesterone levels were measured from the media of treated cells. The mixture did not affect FSH-stimulated estradiol secretion but increased both

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Perfluoralkyl substances (PFAS) Induce Platinum Resistance in Ovarian Cancer by Improving Mitochondrial Membrane Potential and Altering Oxidative Stress Profiles

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Perfluoralkyl substances (PFAS) are widespread, persistent environmental contaminants that frequently pollute drinking water supplies worldwide. While these chemicals have been linked to a plethora of adverse outcomes, studies have shown that PFAS exposure can increase risk of infertility and ovarian cancer. Ovarian cancer is the most lethal gynecologic malignancy with a ~65% mortality rate. One contributing factor to its high lethality is resistance to platinum-based chemotherapy, the standard of care for ovarian cancer treatment. Importantly, our recently published study showed, for the first time, that select PFAS induce platinum resistance in two ovarian cancer cell lines. We hypothesized that PFAS-induced alterations to mitochondrial biology were responsible for the observed platinum resistance. In the context of ovarian cancer, platinum-resistant tumor populations often display increased mitochondrial networks and enhanced bioenergetic capacities. To determine the effect of PFAS on mitochondrial and cell health, we evaluated changes in mitochondrial membrane potential and reactive oxygen species (ROS) production. Specifically, we measured superoxide and hydrogen peroxide (H2O2) production. Using the JC-1 dye, mitochondrial membrane potential was quantified after PFAS exposure alone and in combination with carboplatin, mitochondrial membrane potential increased compared to controls by up to 46%, whereas carboplatin alone decreased mitochondrial membrane potential by ~50%. Carboplatin’s mechanism of action includes binding DNA and altering ROS production to promote apoptosis, so decreased mitochondrial membrane potential was expected. Increased mitochondrial membrane potential after PFAS exposure alone and in combination with carboplatin can significantly improve mitochondrial functioning and cellular health, potentially providing a mechanism underlying the observed PFAS-induced platinum resistance. Antioxidant levels in cancer cells are known to influence ROS production because in cancer cells, ROS production is often increased due to enhanced signaling pathway activity and energy production. Alongside increased ROS production, cancer cells increase antioxidant levels to mitigate oxidative stress. Since platinum-based agents increase ROS to induce apoptosis, increased antioxidant levels in cancer cells can contribute to diminished therapy response. To understand how ROS production is affected by PFAS exposure, ovarian cancer cells were exposed to PFAS for 48-hours prior to evaluating superoxide and H2O2 levels. Preliminary data suggest that PFAS exposure does not affect superoxide levels; however, the half-life of superoxide is 5 seconds, so assay sensitivity may be an issue. At the ROS endpoint, we measured H2O2 production. After PFAS exposure alone, H2O2 luminescence intensity increased in ovarian cancer cells, suggesting PFAS increase H2O2 production. Interestingly, after PFAS exposure + carboplatin treatment, H2O2 luminescence intensity decreased compared to the carboplatin only control, suggesting H2O2 production decreased, or antioxidant levels were increased in PFAS exposed cells and mitigated increased H2O2 production. Regardless, our findings implicate alterations to mitochondrial functioning in PFAS-induced chemotherapy resistance. Future work will evaluate changes in mitochondrial DNA copy number and energy production pathways.

Low delivery efficiency of nanoparticles (NPs) to tumors is a critical barrier for cancer treatment. To address this challenge, new artificial intelligence (AI) and machine learning (ML) algorithms may provide new tools to address this challenge. In this study, ML and AI models were generated to predict the significant tumor-related parameters based on physicochemical properties of NPs (e.g., size, shape, etc.) or tumor-related metrics (e.g., tumor size, cancer type, etc.). Then, the ML-predicted parameters were used to define a generic physiologically based pharmacokinetic (PBPK) model in tumor-bearing mice. To predict the delivery efficiency (DE) of NPs to the tumor at 24 h (DE24) and 168 h (DE168) after intravenous administration, as well as the maximum DE (DEmax). ML and AI models were trained to predict uptake and release rate parameters of tumor cells based on in silico Nano-Tumor Database (37 databases of different types of NPs in tumor-bearing mice). The performance of various traditional ML algorithms and deep learning approaches were compared. The PBPK model with optimized kinetic parameters was used to predict the DE of different NPs in the tumor and achieved a determination coefficient of R2 = 0.70 [root mean squared error (RMSE) = 0.46] for DE24, R2 = 0.41 (RMSE = 1.23) for DE168, and R2 = 0.71 (RMSE = 0.26) for DEmax. This study introduces a new methodological paradigm, by integrating ML and AI algorithms with PBPK modeling to construct a robust PBPK model for NPs. These results will help to improve our understanding of tumor DE of nanomedicines and the PBPK model can aid in the design of cancer nanomedicines with enhanced tumor DE.
Humans are exposed to a myriad of environmental pollutants (e.g., pharmaceuticals and personal care products, pesticides, metals, etc.) every day, which may impact human health individually or as a mixture. Exposure and toxicity assessment are important components in understanding the impact of exposure on human health. Our recent advances in novel machine learning and deep learning models in predicting chemical fate and toxicity may leverage time-consuming laboratory and field experiments. Specifically, we predicted pesticide dissipation half-life intervals in the field, which is an important factor in assessing the environmental fate of pesticides and establishing pre-harvest intervals critical to good agriculture practices. Empirically measured pesticide dissipation half-lives are highly variable and the accurate prediction with models is challenging. We hence predicted hundreds of pesticide dissipation half-life intervals in plants using gradient boosting regression tree (GBRT), with extended connectivity fingerprints (ECFP), temperature, plant type, and plant component class as model inputs. We achieved the highest F1-micro score of 0.696 ± 0.010 (GBRT-ECFP) compared with other machine learning models. We also identified important substructures such as aromatic rings, carbonyl group, organophosphate N-containing heterocyclic groups related to pesticide dissipation half-lives through feature importance analysis. In addition to exposure assessment, we developed deep learning models for toxicity prediction. In silico prediction of chemical ecotoxicity ($HC_{50}$) represents an important complement to improve in vivo and vitro toxicological assessment of manufactured chemicals. Recent application of machine learning models to predict chemical $HC_{50}$ yields variable prediction performance that depends on effectively learning chemical representations from high-dimensional data. To improve $HC_{50}$ prediction performance, we developed an autoencoder model by learning latent space chemical embeddings. This novel approach achieved state-of-the-art prediction performance of $HC_{50}$ with $R^2$ of 0.668 ± 0.003 and mean absolute error of 0.572 ± 0.001 that outperformed other dimension reduction methods (e.g., PCA) and machine learning models (e.g., random forest). Our results highlighted the usefulness of utilizing novel machine learning models in exposure assessment and toxicity prediction.

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Manual examination of pathological tissues is the key step in animal studies to determine whether the testing compound would cause injuries to the animals and their extent and severity. This is a laborious process and requires extensive training. In addition, as spontaneous injuries may happen, it is critical to differentiate spontaneous injuries from those caused by the treatment. To address these specific questions, PathologAI was developed as an AI framework for digital pathology analysis at the whole slide image (WSI) level, adopting a weakly supervised classification approach and an image embedding based on Generative Models. It was applied to predict necrosis in liver images from the Toxigenomics Project-Genomics Assisted Toxicity Evaluation system (TG-GATES), on a total of $n=816$ WSi with $n=877$ controls. TG-GATES thoroughly studied 170 compounds at three dose levels (low, middle, and high) with matched controls for four time points ($n=3, 7, 14,$ and $28$ days). PathologAI first obtained a WSI preprocessing embedding transformation module based on a combination of Histolab digital pathology image processing environment with a deep-learning Generative Adversarial Network architecture. This module was trained on $50$ control slides free of pathological findings, $50$ slides with minimal or slight necrosis, and $2$ with moderate or severe necrosis. A convolutional neural network (CNN) classifier was then trained on $120$ control slides free of findings and $113$ with moderate or severe necrosis. PathologAI achieved promising classification accuracy on the validation set: $87\%$ among $87$ control slides free of findings, $83\%$ among $120$ controls with spontaneous necrosis, $67\%$ among $137$ treated animals with spontaneous minimal or slight necrosis, and $59\%$ among $127$ treated animals with minimal or slight necrosis caused by the treatment. PathologAI was thus able to separate spontaneous necrosis from treatment related necrosis and discriminate mild findings and dose levels. PathologAI could provide the much-needed analysis tool to improve the efficiency, consistency, and accuracy of the preclinical toxicological studies.
2034 Association between Exposure to Toxics and Essential Metal Mixtures and Gestational Age in the ELGAN Cohort


Prenatal exposure to individual toxic metals is associated with adverse neonatal outcomes, specifically reduced gestational age, but few studies have investigated whether such outcomes are associated with metal mixtures. This study evaluated the association between umbilical cord metal concentrations and gestational age. Data were collected from 294 children from the Extremely Low Gestational Age Newborn (ELGAN) cohort, a multicenter cohort study. Umbilical cords were collected and analyzed for levels of 11 metals: Arsenic (As), Manganese (Mn), Cadmium (Cd), Lead (Pb), Mercury (Hg), Copper (Cu), Antimony (Sb), Strontium (Sr), Selenium (Se), Barium (Ba), and Zinc (Zn). Metal co-exposures were modeled in three groups: total measured metals (As, Mn, Cd, Pb, Hg, Cu, Sb, Sr, Se, Ba, and Zn), toxic metals (Pb, As, Mn, Cd, Hg, Sr, Sb, Hg, Ba), and essential metals (Cu, Zn, Se). Additional mixed metals analyses were performed using quanile g-based computation including the same groupings of total metals, toxic metals, and essential metals. All models controlled for maternal education, insurance status, maternal age, maternal body mass index, and maternal smoking status. In the co-adjustment models, cord levels of strontium (β: -2.25, p < 0.04) and selenium (β: -6.93, p < 0.03) were significantly associated with decreased gestational age. In the metal mixtures analysis, a combined total metal mixture was related to gestational age with an estimated reduction of 2.75 gestational days (p=0.01). Levels of Pb, Sb, Copper, and As were associated with higher gestational age (mean difference: +1.85 days), while Ba, Mn, Cd, Hg, Sr, Se, Ba, and Zn, were significantly associated with decreased gestational age. In the metal mixtures analysis, a combined total metal mixture was related to gestational age with an estimated reduction of 2.75 gestational days (p=0.01). Levels of Pb, Sb, Copper, and As were associated with higher gestational age (mean difference: +1.85 days), while Ba, Mn, Cd, Hg, Sr, Se, Ba, and Zn, were associated with decreased gestational age (mean difference: -2.25 days). These findings support the concept that exposure to metal mixtures early in pregnancy may be associated with adverse reproductive outcomes.

2035 A Mixture of Epidermal Growth Factor Receptor-Disrupting Chemicals Reduce Cellular Bioenergetics and Alter Mitochondrial Dynamics in Human Primary Cytotrophoblast Cells


During pregnancy, pregnant women are exposed to complex environmental chemical mixtures that reach the fetoplacental unit and directly target the placenta. We and others have demonstrated that certain chemicals can affect activation of epidermal growth factor receptor (EGFR) which is essential for placental trophoblast growth and differentiation. Its native ligand, EGF can also stimulate glycolysis and mitochondrial respiration in placental trophoblast cells. However, it is still unknown, whether EGFR-disrupting chemicals impair trophoblast cells' cellular bioenergetics. To test this hypothesis, we isolated human primary cytotrophoblast cells (hCtBs, 5 primary cell cultures) from healthy pregnancies at term and exposed them for 24 h to a chemical mixture (Chem-Mix: atrazine, bisphenol S, niclosamide, PCB126, PCB153, and trans-nonachlor; doses: 1, 10, and 100 ng/ml) demonstrated by us in prior study to blunt EGFR activation. The oxygen consumption rate (OCR) was measured using XFe96, and thymidine incorporation using MitoStain, a mitochondrial stress test protocol. None of the doses employed induced cytotoxicity in hCtBs. Chem-Mix did not affect basal OCR but reduced the maximum respiratory capacity in a dose dependent manner. To investigate whether the effect was EGF mediated, hCtBs were exposed to 100 ng/ml Chem-Mix with or without EGF for 24 h. We then conducted mitochondrial and glycolytic stress tests and evaluated ATP production, glucose consumption, and lactate synthesis. The respiratory capacity and ATP production were increased by EGF, while Chem-Mix reduced both EGF- and non-EGF-mediated OCR and ATP production of hCtBs. A similar pattern was observed in the glycolytic medium acidification, with EGF increasing the acidification, and Chem-Mix blocking EGF-induced glycolytic acidification. EGF also induced glucose consumption and increase lactate production. Interestingly, Chem-Mix blocked EGF-induced glucose consumption, but media lactate was increased by Chem-Mix independent of EGF. These findings were recapitulated in a human first trimester extravillous trophoblast cell line (HTR8/SVneo). To determine if changes in OCR and ATP production were due to changes in mitochondrial remodeling, HTR8/SVneo trophoblasts were exposed to Chem-Mix and immunostained with the outer mitochondrial membrane protein TOM20. Highly inclined and laminated optical sheet (HILO) illumination and stochastic optical reconstruction microscopy (STORM) super-resolution imaging revealed that Chem-Mix reduced the mitochondrial network. This was further confirmed by the reduction in OPA1, a mitochondrial dynamic GTPase involved in mitochondrial fusion. In conclusion, we demonstrated that a mixture of EGFR-disrupting chemicals alters mitochondrial remodeling, resulting in altered cellular bioenergetics, reducing the capacity of human cytotrophoblasts to generate energy. Future studies should investigate the mechanism by which mitochondrial dynamics are altered and its pathological significance to the placenta and to pregnancy complications. Supported by NIH/ES R01 ES027863 to A.V.L.

2036 Role of Alkylated Polycyclic Aromatic Hydrocarbons in Mixture Toxicity from a Legacy Creosote Site

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Creosote is a pesticide used to preserve wood products. Typically derived from distillation of coal tar, creosote is a complex mixture containing mostly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. While creosote is a common contaminant, the toxic effects of weathered creosote are poorly understood. In particular, alkylated PAHs are abundant constituents of creosote and many petroleum products and are known to become enriched relative to their respective parent compounds through weathering. Less is known about the toxicity of alkylated PAHs compared to their parent compounds. Despite this, alkylated PAHs have been shown to contribute substantially to the toxicity of PAH mixtures in the environment. The goal of this study is to understand the contribution of alkylated PAHs to the toxicity of a complex, weathered mixture from a legacy creosote site. This study utilizes low density polyethylene passive samplers deployed at a former wood treatment facility to accumulate freely dissolved organics in the surface water. Passive samplers are extracted and analyzed by gas chromatography - tandem mass spectrometry for unsubstituted and alkylated PAHs. To assess toxicity, embryonic zebrafish are exposed to passive sampler extracts in 96-well plates and are observed at 24 and 120 hours post fertilization for a suite of behavioral and morphological endpoints. A twelve-month sampling campaign at the site has demonstrated substantial temporal variability in chemical abundance and toxicity with sum PAH concentrations in two adjacent months varying by greater than a factor of two and LC50 values varying by a similar magnitude. Alkylated PAHs constituted the majority of measured PAHs in environmental extracts. Ongoing studies to determine the drivers of toxicity and the role of alkylated PAHs by fractionation of field collected mixtures in an effect-directed analysis framework. Fractionation by gel permeation chromatography revealed that three fractions together recapitulated the toxicity of the whole mixture. The fraction containing the alkylated PAHs caused the majority of the toxic effect while the fraction containing many unsubstituted PAHs is caused less than 10% mortality. A third unidentified fraction caused high incidence of notochord malformation in zebrafish at low concentrations. While further toxicant confirmation is necessary, these results suggest that highly abundant alkylated naphthalenes and phenanthrenes are responsible for driving toxicity rather than the routinely monitored parent PAHs. Understanding the role of alkylated PAHs can inform remediation efforts and improve our ability to protect human health and water quality.
Regulating PFAS in drinking water and other media presents a major challenge, in part because they typically occur as complex mixtures. There are a very large number of PFAS (>10,000) and toxicologic data are absent or sparse for most. Regulatory bodies in the USA and EU have tended to set limits based on health effects or removal methods. However, in our study, the combined effect was estimated by the sum of the water concentration (or dose) of a handful of PFAS. On the other hand, a relative potency factor (RPF) approach has been proposed for PFAS. This paper examines whether the assumptions for RPFs have been met, comparing and contrasting two recent lines of research. First, RPFs for PFAS have been proposed based on in vivo liver toxicity, stating that dose-response relationships were parallel (including assuming equal maximal effect (efficacy)), a necessary but not sufficient requirement for the RPF model. Second, several papers have examined PPARα activation by PFAS, thought to be an important molecular initiating event (MIE) for these compounds. Most recently, concentration-response curves for individual PFAS were measured using COS-7 cells containing hPPARα, driving a luciferase reporter system. Using the individual concentration-response curves, the response of mixtures was estimated via three mixture models: RPFs, generalized concentration addition (GCA) and effect summation; the predictions were compared to empirical results. hPPARα activation by the tested PFAS showed variation in both potency and efficacy, with the sulfonic acids tending to have lower efficacy than the carboxylic acids. GCA, which allows differences in efficacy, provided the best fit of the data. The equal efficacy assumption of RPFs (“parallel concentration-response curves”) was violated. However, approximate low-dose RPFs that depend on both potency and efficacy may perform better. Additional data suggest multiple MIEs for PFAS. Prediction of the combined effect resulting from the merger of signals from multiple MIEs is not well understood and can be consistent with concentration addition, but not guaranteed. In the face of these uncertainties, the current approach of summing of some PFAS may be an appropriate interim procedure, although an additional uncertainty factor for the mixture should be considered. An alternative approach might use extractable organofluorine as a warning signal regarding unexplained organofluorines that might contribute to mixture effects.
Wood smoke particulate (WSP) exposure causes respiratory disease including asthma, COPD, infections, and cancer. The number of individuals exposed to WSP is increasing due to climate change and increased development at wildland urban interfaces, yet our knowledge of the cellular and molecular mechanisms leading to adverse respiratory outcomes is not. We hypothesized that exposure to WSP would disrupt the normal function of epithelial cells and engender an oxidative stress and inflammatory response in directly exposed epithelial cells and indirectly exposed fibroblasts. We developed an in vitro organotypic model that combines both airway epithelial cells (16HBEs) and airway fibroblasts (IMR90s). We found that the epithelial cell layer displayed a high electrical resistance, low compound permeability, and polarized tight junction proteins, which recapitulates in vivo physiology. However, after 24 hours of exposure to WSP markers of epithelial barrier integrity decrease, indicating that WSP exposure impacts normal epithelial cell function. We then investigated the effects of WSP exposure on the kinetics of gene expression for targets related to the oxidative-stress response and pro-inflammation in both epithelial cells and fibroblasts as RNA-sequencing indicated a large increase in transcripts expression related to these responses. We found the early exposure response (2-6 hours) in epithelial cells involves cytokine induction (IL-8, IL-6, IL-1α, and IL-1β), and the early exposure response in fibroblasts involves redox-sensitive targets (HMOX-1, GCLM, SQSTM-1). The late exposure response (12 and 24 hours) involves enzyme induction (HMOX-1, GCLM, COX-2) in cell types. These results indicate a differential exposure response wherein directly exposed epithelial cells undergo an early inflammatory response, and indirectly exposed fibroblasts attempt to mitigate redox stress. To discover the molecular events leading to changes in target expression, we then investigated the role of the NRF2, ERK, and P38 signaling pathways, as these are known to activate oxidative-stress and pro-inflammatory responses. We observed a significant increase in both the stabilization of NRF2 protein and the translocation of NRF2 to the nucleus; however, no significant increase in the phosphorylation of ERK1/2 or p38 was observed, indicating that WSP exposure induces a NRF2-mediated response rather than MAPK-mediated. We then investigated whether the NRF2-NRF2 response mediates intercellular signaling by inhibiting NRF2 activity solely in fibroblasts, as we determined previously that the fibroblasts had an early redox-sensitive response. We found that NRF2 inhibition in fibroblasts attenuated the induction of redox-sensitive targets and changed the expression of pro-inflammatory cytokines (IL-8, IL-1α) in fibroblasts. The inhibition of NRF2 in fibroblasts also increased the expression of pro-inflammatory cytokines (IL-8, IL-6, IL-1α, IL-1β) without any change in redox-sensitive targets in epithelial cells, suggesting that the fibroblast NRF2-response mediates the WSP exposure response of epithelial cells. We found that WSP exposure disrupts normal epithelial barrier function and induces a NRF2-mediated response in both epithelial cells and fibroblasts. Furthermore, the NRF2 response in fibroblasts modulates the epithelial response to WSP, as loss of NRF2 signaling in fibroblasts increases the induction of pro-inflammatory targets in epithelial cells. These results elucidate the complex multicellular response to WSP exposure in an effort to bridge the gap between environmental exposure and adverse respiratory outcomes. Does not reflect EPA policy.
Inflammatory Macrophage Accumulation in the Lung following Ozone Exposure in Humans Is Associated with Increases in MCP-1 and CXCL10

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Ozone (O3) is a ubiquitous air pollutant that presents a concern for public health. O3 exposure in rodents induces a well-characterized and persistent increase in proinflammatory macrophages in the lung. Inhibiting the activity of these cells mitigates toxicity, demonstrating that these cells play a key role in the pathogenic response to O3. However, the role of inflammatory macrophages in O3 toxicity in humans remains poorly characterized. To address this, we examined the phenotypes of lung macrophages in induced sputum from healthy human subjects following exposure to air or O3. Subjects were recruited for this study at the Controlled Exposure Facility located in the Environmental and Occupational Health Sciences Institute at Rutgers University. Each subject (n=37) produced a sputum sample for initial analysis to ensure their ability to generate sufficient quantities of material. Subjects were exposed to air (filtered, 3 hr) and O3 (0.2 ppm, 3 hr) 7 days apart, the order of which was determined through a randomized cross-over design. Sputum samples were obtained after air exposure and 24 (n=13), 48 (n=12), and 72 (n=12) hr post-O3 exposure. Mucus plugs were manually separated from sputum, weighed, and incubated in dithiothreitol (0.1%). Recovered cells were immunostained and analyzed by flow cytometry; sputum supernatants were analyzed for monocyte chemoattractants MCP-1 and CXCL10 by ELISA. Viable, CD45+ cells were classified into 3 discrete populations: resident macrophages (MHL-DR+/SS-hi), recruited macrophages (HLA-DR+/SS-low), and granulocytes (CD14-/HLA-DR+/CD11b+). Results presented are mean ± standard error of mean.

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<th>Variable</th>
<th>Air Post-O3</th>
<th>O3 Post-O3</th>
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<tr>
<td>Resident Macrophages</td>
<td>0.42±0.07</td>
<td>0.35±0.05</td>
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<tr>
<td>Recruited Macrophages</td>
<td>0.58±0.06</td>
<td>0.65±0.06</td>
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<tr>
<td>Granulocytes</td>
<td>0.24±0.03</td>
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As a result of exposure to O3, there was a significant increase in the number of recruited macrophages and granulocytes in the sputum, while the number of resident macrophages remained relatively constant. These findings indicate that exposure to O3 leads to increased activation and recruitment of inflammatory macrophages and granulocytes in the lung, potentially contributing to the pathogenic response of ozone exposure in humans.

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2046 Acute Toxicity of Tobacco and Menthol Flavored E-cigarette Aerosols in Normal and Diseased Differentiated 3D Mcucollary Lung Tissues


Tobacco and Menthol flavored electronic cigarettes are widely available with little information on pulmonary toxicity data. Exposure to e-cigarette aerosol constituents in healthy (normal) or individuals with pre-existing respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) may affect differently. At present, the toxicity of exposure to these products in users with pre-existing lung diseases is unknown. We hypothesized that exposure to e-cigarette aerosols in asthma and COPD tissues would have greater cytotoxicity and cause immunosuppression compared to normal lung tissues. Differentiated mucociliary 3D tissues from (EpiAirway, Mattek) from healthy (normal) and asthma and COPD donors who were non-smokers and smokers were exposed to propylene glycol/vegetable glycerin (PG/VG), tobacco, menthol, and spearmint from 10-90 puffs (55 mL, 3 puffs/min, 30s inter puff interval) using Vitrocell air-liquid interface aerosol exposure system. Further, EpiAirway3D tissues were treated with predominant flavoring chemicals of these flavors, pulegone, and eugenol at 500 μM. Twenty-four hours later, mucosal rinse, conditioned media, and tissues were collected. Inflammatory mediators in conditioned media and mucosal rinse were measured by Luminox assay. 3D tissues were stained with H&E and PAS stain. LDH assay on conditioned media was performed for cytotoxicity. RNA and protein were collected for gene expression analysis and western blotting. Exposure to PG/VG, menthol, and tobacco flavored aerosols elicited dose-dependent cytotoxicity and inflammatory responses. In healthy non-smokers a significant increase in inflammatory cytokines, tumor necrosis factor (TNF), CCL2, CCL5, PAF, and RAGE, PAI-1, mucins (MUC5AC), and the sensory receptor (TRPM8) were augmented with aerosol exposures. Comparative assessments of cytotoxicity, inflammatory mediators, and lung injury markers of E-liquid aerosols of tobacco and menthol/mint flavors were assessed in normal and diseased lung tissues. Including hucromatins (PG/VG), menthol/mint (coughing), and tobacco flavor aerosols with disease (asthma/COPD) phenotypically differently compared to healthy (normal) tissues. Mucosal immunity played a significant role in the elicited immune response. Pre-existing conditions exacerbated the injurious response, and smokers exhibited impaired immune responses. Our data suggest that vaping e-cigarettes or switching to e-cigarettes adds a modified risk to lung disease pathogenesis in smokers and non-smokers, especially under pre-existing lung disease conditions. The study concludes that comparative hazard characterization is crucial in the regulatory toxicological profiling of flavors in normal and disease models. This study was supported by K99ES033835 and US4CA228110.

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characterization of the lung at the cellular level and provides the basis for future studies to address cell-specific responses following exposure to environmental pollutants. Support in part: T32 ES007059, T32 HL007013, R21 ES030276.

2049 The Role of Myeloid Heme Oxygenase 1 in Ozone-Induced Pulmonary Injury, Inflammation, and Antioxidant Responses
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Ozone (O\textsubscript{3}) is a ubiquitous criterion air pollutant associated with increased incidence and exacerbation of cardiopulmonary diseases. Acute O\textsubscript{3} inhalation causes increased pulmonary oxidative stress as well as inflammation, driven in part by the activation of alveolar macrophages. To combat O\textsubscript{3}-induced oxidative stress, the lung up-regulates antioxidant response elements such as heme oxygenase 1 (HO-1). HO-1 is an inducible transcription factor that is essential for clearing damaged free heme into anti-inflammatory mediators. It is highly expressed in macrophages, which recognize and degrade free heme bound to the plasma glycoprotein, hemopexin. It is known that O\textsubscript{3} increases HO-1 expression in the lung. However, it is unknown if macrophage expression of HO-1 dampens O\textsubscript{3}-induced inflammation and oxidative stress. We hypothesize that HO-1 expression in macrophages is essential for dampening pulmonary inflammation and oxidative stress following exposure to O\textsubscript{3}. We exposed male LysM-Cre driven heme oxygenase 1 knockout (LysM-HO-1\textsuperscript{-/-}) and WT mice to either filtered air (FA) or 1 ppm O\textsubscript{3} for 3 hours (proportional to a human exposure during an O\textsubscript{3} action day). Mice were necropsied at 24 hours following exposure to collect bronchoalveolar lavage (BAL) fluid, blood, and lung tissue. We measured cell differentials in BAL samples, as well as total protein, albumin, and hemopexin via enzyme-linked immunosorbent assay (ELISA). We also measured the antioxidant response via mRNA expression in lung tissue. In WT mice, O\textsubscript{3} exposure significantly increased BAL total protein (p=0.001), albumin (p=0.013), and total cell counts (p=0.002). In LysM-HO-1\textsuperscript{-/-} mice exposed to O\textsubscript{3} compared to their respective FA controls. In LysM-HO-1\textsuperscript{-/-} mice, O\textsubscript{3} exposure significantly increased BAL albumin (p=0.007) as well as lung tissue expression of Glutamate-Cysteine ligase catalytic subunit (GCLC, p=0.002), HO-1 (HMOX1, p=0.042), and nuclear factor erythroid 2-like 2 (NFE2L2, p=0.014). However, O\textsubscript{3} exposure did not significantly alter the pulmonary expression of NAD(P)H quinone oxidoreductase 1 (NQO1, p=0.052) or nitric oxide synthase 2 (NOS2, p=0.34). We observed similar trends for LysM-HO-1\textsuperscript{-/-} mice exposed to O\textsubscript{3} compared to their respective WT controls. In LysM-HO-1\textsuperscript{-/-} mice, O\textsubscript{3} exposure significantly increased lung tissue NQO1 expression (p=0.042) in LysM-HO-1\textsuperscript{-/-} mice, whereas it was not significantly changed in WT mice (p=0.052). To determine if the altered antioxidant response in the LysM-HO-1\textsuperscript{-/-} was driven by decreased heme metabolism, we measured BAL hemopexin. However, BAL hemopexin levels were not different between LysM-HO-1\textsuperscript{-/-} and WT mice (p=0.25). Thus far, our data indicate myeloid expression of HO-1 mediates pulmonary antioxidant responses to O\textsubscript{3}, exposure, with implications for altered resolution of lung inflammation and injury. Future studies will focus on the role of myeloid HO-1 expression in the resolution of O\textsubscript{3}-induced inflammation at successive time points as well as macrophage-specific heme metabolism.

2050 Cross-Species Transcriptomics in Liver Spheroids Identifies a Dog-Specific Mechanism of Hepatotoxicity for Amcenestrant
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Amcenestrant is an orally available selective estrogen receptor degrader (SERD) that demonstrated dose-limiting hepatotoxicity at 100 mg/kg/day. No notable hepatotoxicity was observed in rat toxicity studies conducted with amcenestrant, and no dose-limiting compound-related hepatic observations were seen in clinical studies with amcenestrant. Whole genome transcriptional profiles of rat, dog, and human liver spheroids were generated by RNA sequencing for amcenestrant and RA15400562 (an N-dealkyl metabolite observed at higher levels in dog than in human or rat) to investigate the differences in hepatotoxicity observed between these species. Spheroids were treated with these compounds at a single concentration of 12 μM for amcenestrant and 50 μM for RA15400562 (approximately = IC\textsubscript{50} for 6, 24, and 72 hours. The transcriptional profiles in liver spheroids revealed key differences in biological response to both amcenestrant and RA15400562 between rat, dog, and human. Strong FXR antagonist activity with profound downregulation of protective hepatic bile acid exporters was observed with both compounds in the dog, and to a weaker extent in the rat, but not in human. Bile Salt Export Protein (BSEP) was 7.4-fold downregulated at 72 hrs. by amcenestrant in the dog, and organic solute transporters alpha and beta (OSTalpha, OSTbeta) were also suppressed. In contrast, PXR agonist activity was observed in the human following amcenestrant treatment, but not in the dog or the rat. RA15400562 did exhibit PXR agonist activity in rat. Together, these data suggest that dogs may be uniquely susceptible to cholestatic hepatotoxicity following administration of amcenestrant. In vitro hepatic spheroid cultures coupled with transcriptional profiling present a powerful tool for mechanistic evaluation of species differences in toxic response.

2051 Organotypic 3D Primary Human Nasal Tissue for Infection with SARS-CoV-2 Variants and Drug Safety and Efficacy Studies
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The nasal mucosa serves as the first line of defense against inhaled chemicals, drugs, and respiratory infections in the nasal cavity. Here we developed a novel in vitro primary human cell-based 3D human nasal epithelial tissue (NET) cultured on microporous membrane cell culture inserts at an air-liquid interface (ALI). The NET was characterized by histology, barrier function (TEER), viability (MTT), infection with viruses, and response to toxic chemicals. Histological and immuno-histochemical evaluation of the in vitro NET model showed a polarized, ciliated, and multilayered epithelial cell morphology. The NET model was infectable with SARS-CoV-2 variants (Beta, Delta, and Omicron) and the maximum infection was noted around Day 4, which is slightly faster than the 5 days observed for tracheal-bronchial tissues (EpAirway). A dose dependent response was noted when the infected NET tissues were treated with drugs such as Remdesivir and EIDD1931. Tissue toxicity was monitored by Cell Counting Kit-8 (CCK-8). To evaluate the utility of the nasal tissue model for toxicological studies of respiratory irritants, we tested the effect of a known mucous membrane irritant, butylamine, following 4 hr topical exposure to 0.5 and 2 mg/mL of the test article. The data showed reduction in barrier by 12.3 ± 5.8 and 33.2 ± 0.0, respectively, compared to the vehicle control (corn oil), which is indicative of toxicity caused by the test article. In short, this novel NET model can be added to the toolbox of 3D respiratory tissue models to predict viral infection, drug safety and efficacy, and toxicity of chemical inhalants at an important respiratory entry site.

2052 Microphysiological Systems Detect Hepatotoxicity More S sensitively Than Current Safety Assessment Models

In clinical trials, prime drug candidates are often discontinued because of hepatotoxicity that was inferiorly detected in preclinical or in vitro models. Currently, early drug safety assessments utilize preclinical rodent and non-rodent animal models to evaluate hepatotoxicity, yet gaps still exist in our ability to detect clinical drug-induced liver injury (DILI). Recent developments in microphysiological systems (MPS) combine human multicellular complexes with fluids to better recapitulate a human liver with the goal of predicting drug toxicities in clinical and real-world settings. Herein, troglitazone induced a ~70-fold increase in cytotoxicity in a liver MPS, whereas no hepatotoxicity was detected in a Flat Culture model. Our data also demonstrate that adverse effects were not observed in the MPS model close to the clinical NO\textsubscript{\textsubscript{90}}, thus narrowing the safety/efficacy window. Transcriptional comparisons of human primary hepatocytes and liver non-parenchymal cells (NPCs) in Flat Culture and MPS indicate that multiple biological processes, including pathways associated with drug metabolism and cytokine interactions, differed significantly between the two models. Additionally, further analysis of inflammatory signaling highlighted the communication between the various channels of the MPS, as LPS treatment of NPCs affected cytokine response in hepatocytes. This could explain as to why DILI was more sensitively detected in MPS when compared to Flat Culture. Overall, the performance and relevance of MPS to enhance the detection of hepatotoxicity indicate that these models could innovate current safety standards and disease models by better predicting human response to drugs, thus, ensuring improved translational efficiency for drug candidates entering clinical trials.
Adverse cardiovascular events, particularly arrhythmias, remain among the most frequent toxic responses to pharmaceuticals. For instance, many classes of chemotherapeutics (e.g., anthracyclines) have long-term cardiovascular side effects. Current safety assessment relies on labor-intensive in vivo models that are limited by species-specific physiology and targeted in vitro assays that only capture a single arrhythmia mechanism. Moreover, these assessments are often limited to examining acute toxicity at high drug concentrations, which do not reflect chronic, in vivo exposure conditions. To address these limitations, we examined the concentration responses of the action potentials (APs) of human cardiac microtissues under both acute and chronic exposure conditions to identify concentration- and time-dependent proarrhythmic effects of pharmaceutical compounds with known chronic cardio toxicity. We examined doxorubicin, a common chemotherapeutic which is known to cause heart failure due to direct cardiomyocyte toxicity, and pentamidine, an antifungal known to inhibit ion channel trafficking and increase arrhythmia risk after long-term exposure. Self-assembled microtissues were generated by combining human induced-pluripotent stem cell-derived ventricular cardiomyocytes and primary human cardiac fibroblasts in a 90:10 ratio in agarose round-bottom microwells. Microtissues were cultured for one week prior to drug exposure. APs were recorded via optical mapping with the voltage sensitive dye RH110 in the presence of 1 Hz pacing. Eight metrics quantifying changes in the AP (i.e., excitability; stimulation delay; rise time; AP duration (APD) to 30%, 50%, 80%, and maximum repolarization (mAPx)); and AP triangulation) were measured after 1-hour, 1-day, and 3-days exposure to doxorubicin (0.1, 0.3, 1, 3 μM) or pentamidine (0.1, 0.3, 1, 3 μM) and compared to vehicle controls (0.1% v/v dimethyl sulfoxide in 3:4 molds per condition (n = 90-136 microtissues)). After 3 days, microtissues treated with high dose doxorubicin (3 μM) lost excitability. Lower doxorubicin doses (1 μM) had significantly shortened APDs to 30%, 50%, 80%, and maximum repolarization (APD90: 302.2 ± 35.3 ms vs 410.0 ± 44.3 ms in controls). This shortening of APD and loss of excitability is in line with cardio myocyte toxicity and echocardiographic injury observed in vivo after doxorubicin treatment. Contrastingly, high dose (3 μM) pentamidine-treated microtissues had statistically significant increases in APDmxr (489.7 ± 49.5 ms versus 410.0 ± 44.3 ms after doxorubicin treatment). These changes in APD and triangulation are distinct from doxorubicin responses and are associated with delayed late-stage repolarization due to inhibited HERG channel trafficking. Taken together, these results demonstrate that human cardiac microtissues can detect proarrhythmic changes in AP after chronic exposure to compounds arising from multiple mechanisms and suggest their potential utility in predicting cardio toxicity of pharmaceuticals in vitro.

The efficacy of chemotherapy is often limited by the poor pharmacodynamic properties which often result in narrow therapeutic windows with a high occurrence of adverse events in patients. The ability to assess systemic tissue injury induced by chemotherapeutics will have a significant impact on pharmaceutical development and in clinical oncology. The goal of this study was to investigate the feasibility of using 99mTc-Duramin as an imaging agent which detects membrane reorganization as a surrogate marker for apoptosis/necrosis, for assessing systemic tissue injury induced by anticancer drugs using Duramin (DXR) as a model. Biodistribution techniques were used to study the uptake of 99mTc-duramin in control versus DXR-treated rats. The biodistribution of 99mTc-Duramin in control rats were established as baseline (n = 15), and in drug-treated animals (n = 15). The elevation in 99mTc-Duramin uptake, a surrogate marker for tissue injury, was quantified using t-test and rank sum test. The ratios between each treated animal and the basal level were also calculated to determine the individual susceptibility to drug toxicity. Whole-body dynamic scans were then performed to investigate the spatio-temporal dynamics of DXR-induced tissue damage. Histopathology and tissue analysis for apoptosis are currently underway to validate the findings. Significant elevation in 99mTc-Duramin uptake was detected in multiple organs/tissues in treated animals compared to control concurrent with known toxicities reported in the literature. Whole-tissue based caspase activation assays established a positive correlation between the level of apoptosis and 99mTc-duramin uptake. Additionally, our imaging technique detected nonapoptotic tissue injury that was not detectable by caspase assay but was accompanied with a prominent elevation in 99mTc-duramin uptake. For DXR treated rats, susceptible organs/tissues identified using 99mTc-duramin uptake as indices were consistent with known toxicological effects of the drug. These studies indicated that changes in 99mTc-duramin as a result of drug toxicity are detectable and quantifiable, thus providing critical proof-of-concept for the utility of this technology. The imaging technique also revealed gender-based differences in uptake indicating differing degrees of damage and susceptibility post-treatment with DXR. Minimally invasive imaging of DXR in animals provides useful information and population levels for gauging systemic tissue injury induced by chemotherapeutics. This approach has potential to generate a real impact on pharmaceutical development, drug discovery, and clinical oncology.

Aryl Hydrocarbon Receptor (AhR) is a receptor that responds to xenobiotics to induce metabolic pathways including the CYP1A1 and CYP1A2 genes. Sustained AhR activation has been associated with carcinogenicity and other toxicities in rodents and humans. Thus, identification of this liability is of the utmost importance to ensure patient safety. We assessed AhR activation in rats using CyPA1 and CyPA2 transcriptional induction in liver as sensitive and specific biomarkers. To define a biological threshold for carcinogenic risk, we conducted 1-week rat studies with an interim peel-off using two AhR-activating carcinogens and three AhR-activating non-carcinogens. These studies identified an “alerting” risk level based on a scoring range that separated AhR-related carcinogenic risk for carcinogens from non-carcinogens, while also demonstrated sustained and/or elevated transcriptional activation of AhR-activating carcinogens over time. This alerting scoring range level flagged only 6% of our compounds as requiring further investigation of carcinogenic risk. An alerting AhR score for Merck A was identified in a 4-day rat study which further increased in a 1-week study. We then assessed an in vivo AhR luciferase assay with parent drug but did not observe AhR activation. To confirm the in vivo CyPA1 and CyPA2 transcriptional response were indeed mediated by AhR, we compared responses in AhR knockout (KO) and wildtype (WT) rats. We observed CyPA1 and CyPA2 induction for Merck A and PCB-126 in WT, but not in KO rats. In addition, we used chromatin immunoprecipitation with a rat AhR specific antibody on samples from the 1-week rat study to demonstrate that AhR binding to CyPA1 and CyPA2 promoters in rat livers was induced in animals treated with Merck A. Finally, in vitro studies were conducted in a metabolically competent model where CyPA1 was robustly induced by Merck A, and the response was robustly reduced by P450 knocked down with Por/CyB5 siRNA. Together, these findings support that the observed in vivo AhR response was metabolite driven and provided a mechanistic understanding of the AhR-mediated response for Merck A. This provided a path forward using a metabolically competent model to avoid AhR activation and associated carcinogenic risk for backup compounds from the same target.
RO7497987 (FLT3L-Fc) is a cytokine-Fc fusion, agonizing receptor-type tyrosine-protein kinase FLT3 (fms-related tyrosine kinase 3; also known as CD135). FLT3 is expressed on dendritic cells (DCs) as well as myeloid and lymphoid progenitors. Agonism of this pathway promotes DC proliferation, differentiation, mobilization, and survival. RO7497987 is a potential treatment for cancer, especially in the combination setting in patients receiving checkpoint immunotherapy. Nonclinical pharmacodynamics and safety were assessed in Sprague-Dawley rats and cynomolgus monkeys. RO7497987 was well tolerated at doses of up to 10 mg/kg IV given every 3 weeks for 2 doses with no adverse findings. RO7497987 induced marked expansion of blood cDC1, cDC2, and pDC cells (up to 301-fold in rats and 378-fold in monkeys), peaking at 8-10 days after first dose. The major clinical pathology findings were expansion of white blood cell (WBC) populations including lymphocytes, monocytes, neutrophils, basophils, and large unstained cells, which were most pronounced after the first dose. The WBC changes in both species correlated microscopically with histiocytic and monocellular cell infiltrates in multiple organs in all dose groups examined and associated with minimal to mild decrease in platelet counts and microscopically with increased splenic megakaryocytic extramedullary hematopoeisis in rats as confirmed by digital quantitation. All changes with regards to the immunophenotyping, clinical pathology and anatomic pathology data were considered related to anticipated pharmacodynamic effects. Based on the lack of adverse findings with RO7497987, the NDAA as monotherapy be the highest dose tested in repeat-dose GPL toxicity studies (10 mg/kg and 3 mg/kg in rats and cynomolgus monkeys, respectively). The totality of nonclinical safety data of RO7497987 support the Phase Ia Study for RO7497987 and provide sufficient safety margins.

Colorectal cancer (CRC) is the 3rd leading cause of cancer-related deaths in both men and women and is expected to cause over 52 thousand deaths in 2022 in the United States alone. Due to the limited success of advanced targeted therapies, there is a need to develop new chemotherapeutic strategies. 5-fluorouracil (5-FU)-based chemotherapy regimens (e.g., FOLFOX and FOLFIRI) have been the mainstay treatment for most of the advanced-stage CRC. However, its clinical utilities are hampered by systemic toxicities and dose limitations. Because only a small percentage (~5%) of the 5-FU gets converted into active metabolites (FDUMP), and the rest of the metabolites are either ineffective or responsible for toxicities. This underscores the need for better agents to improve progressive disease, emphasizing the urgent need to develop more potent and safer fluoropyrimidine (FP). In this regard, we are developing a new nanoscale FP polymeric drug formulations (CF10) comprising ten repeating FDUMP nucleotide monomers of single-stranded DNA that form ternary complexes with endogenous cytidylyl synthase (TS) and DNA topoisomerase-1 (Top1) to overcome SFU resistance and exert potent anticancer activity respectively. Our target evaluation studies demonstrated that CF10 treatment causes the formation of TS-CF10 ternary complexes and Top1-DNA cleavage complexes and increased replication stress in CRC cell lines, as indicated by increased levels of FANCD2 mono ubiquitination, phosphorylation of CHK1, CHK2, and H2AX. Clinogenic survival assays confirmed that CF10 is 1000 times more potent than 5-FU in killing CRC cells and causes excessive DNA lesions compared to 5-FU. As a primary patient-derived cell line better represent the tumor heterogeneity, the evaluation of CF10 in three primary patient-derived colon cancer cell lines showed concentration-dependent inhibition of the growth of colon cancer cells. In order to examine CF10 efficacy and safety in in vivo, we used mouse CRC flank xenograft models (HCT-116, HT-29, and CT-26). The in vivo studies show CF10 selectively targets malignant cells (spare non-malignant cells) and does not significantly decrease the average length of intestinal villi compared to 5-FU treated mice. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine were similar in both the control and treatment group after 6 weeks treatment, suggesting CF10 does not cause any liver or kidney toxicities. The biodistribution and the quantification of FP metabolites from mouse plasma following intravenous CF10 or 5-FU injection suggest better tumor localization of CF10 which contributes to its robust anticancer effects. Additionally, CF10 treatment significantly reduced tumor sizes and improved survival (84.5 days vs 32 days; P < 0.001) related to 5-FU in an orthotopic T116 GEO CRC mouse model that spontaneously metastasized to the liver. This led us to hypothesize further that CF10 could be inhibiting the TS-mediated EMT phenotype in CRCs. We used the Tdt-on system to regulate TS expression in HCT-116 cells to further investigate the significance of enhanced TS as a factor in CRC metastatic progression and 5-FU resistance. We noticed that TS overexpression promotes EMT phenotypes and enhances cell migration and invasion. Strikingly, CF10 suppresses the growth of TS-expressing primary CRC organoids, as seen by the reduction of colonies formation. Collectively, our results demonstrate that CF10 is thousand times more potent than 5-FU in killing CRC cells and showed superior in vivo and in vitro anticancer efficacy and favorable toxicity profiles in treating metastatic models of CRC.
embryonic toxicity assay (ZET), based on the OECD TG 236 Fish Embryo Toxicity assay with an exposure period from 6 to 120 hours post fertilization (hpf), and the General and Behavioral Toxicity (GBT) assay, which has an exposure period from 72 to 120 hpf. By 72 hpf organogenesis and body patterning is largely complete making the model more a measure of systemic toxicity compared to teratogenicity. The models were designed to fit within the Canada Council on Animal Care guideline’s that define the one-day or first stage of fish embryonic development as an in vitro model. Within the context of the move away from animal testing, in 2018 Health Canada partnered with the NRC to refine the ZET and GBT assays to be robust alternatives to mammalian models for generating hazard data required in quantitative risk assessments for human health and the environment. The ZET and GBT assays were expanded to include the additional assessments of the stability, uptake and metabolism of the chemicals being tested allowing for a more whole transcriptome gene expression analysis. The development of the transcriptomics platform allows for an evaluation of the whole endocrine system, which differs from the historical focus on developmental and reproductive effects. Additionally, inclusion of the stability, uptake and metabolism paradigm allows for the correlation of data across chemical bioactivity with available developmental, behavioral and transcriptional effects of the compounds tested with the morphological, behavioral and transcriptomic effects. A set of 20 substances was tested while developing these models, including, Dechlorane Plus, Bisphenol S, Triphenyl Phosphate, Tricresyl Phosphate, Tris (dichloro-isopropyl) phosphate, Raloxifene HCl, Testosterone Propionate, Permethrin, Thiabendazole, Benzenophone, Bisphenol A, Valproic Acid, Acldaric Acid, Amoxicillin, Pyrene, Resorcinal, Pyrroxyphen, Propofol, 3,4-dichoroanilene and Tetramethylbisphenol A. The testing has shown that the stability, uptake and metabolism profiles were compound specific. In addition, the transcriptomics data revealed pathway specific effects that may be linked to the mechanism of action. Together the four components of the refined testing platform make the zebrafish larval models highly informative in their predictive capacity and the platform is now moving into the validation phase. This will include a cross-validation study with the US EPA using a set of 80 additional chemicals. The proposed application of the refined ZET and GBT platform for chemical risk assessments by Health Canada is as a first-tier screening tool for generating hazard data. As such, the zebrafish platform has significant potential to generate the hazard data required for robust human health and environmental risk assessments as we move away from mammalian testing and uphold the 3 Rs in chemical risk assessments.

The embryonic zebrafish is a useful vertebrate model for assessing the effects of genotoxic and non-genotoxic developmental and reprotox. However, developmental toxicity outcomes can vary, and the incidence of altered phenotypes may not be directly comparable between laboratories. To address these limitations for gaining broader adoption of the zebrafish embryo model for toxicological screening, we established the Systematic Evaluation of the Application of Zebrafish in Toxicology (SEAZIT) program to investigate how experimental protocol differences can influence developmental toxicity assessments. As such, the zebrafish platform has significant potential to generate the hazard data required for robust human health and environmental risk assessments as we move away from mammalian testing and uphold the 3 Rs in chemical risk assessments.

Pericardial edema is a common phenotype observed in zebrafish embryo-based chemical toxicity screens, yet the mechanisms underlying chemically-induced pericardial edema remains unclear. One of the potential mechanisms underlying edema may be a disruption in embryonic osmoregulation. Therefore, the objective of this study was to identify whether triphenyl phosphate (TPHP) - a widely used aryl phosphate ester-based flame retardant - induces pericardial edema via impacts on ion transport and the skin of embryonic zebrafish. In addition to TPHP-induced effects on the morphology and organization on the embryonic yolk sac epithelium, an increase in ionic strength of exposure media exacerbated the impact of TPHP on pericardial edema when embryos were exposed from 24-72 h post-fertilization (hpf). However, there was no difference in embryonic sodium concentrations in situ with TPHP-exposed embryos relative to embryos exposed to vehicle (10% DMSO) from 24-72 hpf. We also found that increasing the osmolarity of the exposure media with mannitol (an osmotic diuretic which mitigates TPHP-induced pericardial edema) and increasing the ionic strength of the exposure media (which exacerbates TPHP-induced pericardial edema) did not affect embryonic doses of TPHP, suggesting that TPHP uptake was not altered under these varying experimental conditions. Our findings demonstrate that TPHP alters the structure of the epithelium of the yolk sac, leading to disruption of embryonic osmoregulation and formation of pericardial edema. In addition, our findings suggest that chemically-induced edema within zebrafish embryos is dependent on exposure media composition, underscoring the importance of further standardization of zebrafish-based chemical toxicity screening assays around the world.

The field of bioprinting has great potential for developing in-house, customizable organ models that would contribute greatly to aid in in vitro predictive toxicology (PvT). Bioprinting technology utilizes both traditional additive manufacturing techniques with the relevant living cells to create physiologically relevant organ models that would contribute greatly to aid in the field of predictive toxicology. Thus, the objective of this study was to develop a 3D bioprinted model of the hepatic epithelium of the yolk sac and determine the safety of the GM crop when consumed. Previous work has demonstrated the utility of using human-derived intestinal epithelial cell (IEC) lines cultured as polarized monolayers on permeable Transwell® filters to differentiate between hazardous and non-hazardous proteins. The current work uses this experimental platform, specific altered phenotypes in the DRF study, where head was the primary altered site (27 substances, neither of testing laboratory); 10 out of 27 substances were found to induce head defects of zebrafish embryo consistently in all three laboratories. With the developed methods in the DRF phase, including the database/data analysis pipeline development, and zebrafish phenotype ontology mapping, we can compare zebrafish developmental toxicity results across laboratories at the level of specific phenotypes rather than just the gross developmental toxicity. These methods shall be applied to the Def phase to help elucidate the contribution of experimental conditions on affecting developmental toxicity outcomes and to improve the outcome translatability in zebrafish community.

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The field of bioprinting has great potential for developing in-house, customizable organ models that would contribute greatly to aid in in vitro predictive toxicology (PvT). Bioprinting technology utilizes both traditional additive manufacturing techniques with the relevant living cells to create physiologically relevant organ models that would contribute greatly to aid in the field of predictive toxicology. Thus, the objective of this study was to develop a 3D bioprinted model of the hepatic epithelium of the yolk sac and determine the safety of the GM crop when consumed. Previous work has demonstrated the utility of using human-derived intestinal epithelial cell (IEC) lines cultured as polarized monolayers on permeable Transwell® filters to differentiate between hazardous and non-hazardous proteins. The current work uses this experimental platform, specific altered phenotypes in the DRF study, where head was the primary altered site (27 substances, neither of testing laboratory); 10 out of 27 substances were found to induce head defects of zebrafish embryo consistently in all three laboratories. With the developed methods in the DRF phase, including the database/data analysis pipeline development, and zebrafish phenotype ontology mapping, we can compare zebrafish developmental toxicity results across laboratories at the level of specific phenotypes rather than just the gross developmental toxicity. These methods shall be applied to the Def phase to help elucidate the contribution of experimental conditions on affecting developmental toxicity outcomes and to improve the outcome translatability in zebrafish community.
previously developed and tested with commercially purchased hazardous and non-hazardous proteins, to identify whether insecticidal proteins elicit evidence of hazardous properties when interacting with human IECs. Two proteins isolated from *Alcaligenes faecalis* and showing insecticidal bioactivity when used in combination (AF1P-1A and AF1P-1B) were evaluated separately and together in the IEC experimental platform. The proteins were applied to the apical surface of differentiated S1-Chips at high concentrations. The effect of AF1P-1A on the S1-Chips epithelium was determined by analyzing the amount of urea, which were about 10 µg/mL, in the S1-Chips effluents. Effluents must be diluted by 5-fold to effectively reduce such interference, but this made it difficult to observe the percentage of reduction after drug treatment, as urea levels neared 1000 µg/mL and incubated for 48 hours. Effects on monolayer integrity (TEER, flux of FITC-inulin, & flux of HRP) and cell viability (MTT reduction & LDH release) were evaluated. A seven-day bioassay was conducted with western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera: Chrysomelidae) to confirm the bioactivity of AF1P-1A and AF1P-1B mixtures. Aggregate in vitro assay results were compared with those obtained from adult female mosquitoes using a method approximately 80% mortality as well as adverse health outcomes later in life. A number of hypotheses have been proposed to explain the etiology of preterm birth, including exposure to environmental contaminants. Epidemiological studies have identified a number of chemicals, such as heavy metals, pesticides, flame retardants, and polyfluorinated chemicals as potential contributing factors; however, the mechanistic data are lacking to establish causality. In recent years, few physiological human cell-based models that can reproduce the complexity of multi-cellular tissue architecture that comprises the fetal membrane/decidual interface (feto-maternal interface (FMI)) in utero have been developed. A FMi organ-on-chip (FMi-OOC) model, which mimics the multi-cellular layers composed of four fetal-maternal cell types (immortalized maternal decidua cells, fetal chorion, amnion mesenchymal, and amnion epithelial cells) by using planar concentric cell culture chambers interconnected by arrays of microchannels has been recently developed. This study used this previously developed FMi-OOC model to test the hypothesis that polybrominated diphenyl ether 47 (PBDE-47), perfluorooctanoic acid (PFOA), and dichlorodiphenyltrichloroethane (DDT), chemicals linked to preterm birth in epidemiological studies, can cause cell death and inflammation predisposing to preterm birth. We first conducted dose-response studies of toxicants in the maternal decidua cells (the maternal site of exposure) to determine the dose range to be used in the FMi-OOC experiments. This was done by assessing the cell viability (determined using CellTitre-Glo) and cytokine release (IL-6, IL-8, IL-10, GM-CSF, and TNFα) for up to 72 hours. Results from these decidual exposure studies indicated that at 72 hours there was a dose-dependent inflammatory cytokine response. The doses for experiments using the FMi-OOC were selected based on >80% cell viability over 72 hours. Next, we evaluated chemical transport across the chip with and without cells present. Finally, we performed experiments in the FMi-OOC by exposing test chemical(s) (n=3 per treatment group) to the maternal decidual cells in the chip, and then determining the cell fate and inflammatory responses (cell morphology and cytotoxicity) and then determining the cell fate and inflammatory responses (cell morphology and cytotoxicity) and then determining the cell fate and inflammatory responses (cell morphology and cytotoxicity) and then determining the cell fate and inflammatory responses (cell morphology and cytotoxicity).
The X-Species DILI Validation Consortium is a pre-competitive consortium of pharmaceutical companies and technology providers established to develop cross-species drug testing and validation strategies for rapid and reliable screening and prediction of drug-induced liver injury (DILI). In a multi-step strategy, compounds with well-documented cross-species in vivo data will be tested using species-specific 3D InSight™ Liver spheroid models, and the two datasets will be compared. To elucidate differences and similarities in toxicological mechanisms across species, the primary in vitro toxicology endpoint (e.g., cytotoxicity, histological changes) of DILI-specific phenotypic responses will be complemented with comprehensive molecular profiling using transcriptomics, proteomics, and lipidsomics approaches. Here we present preliminary results from the establishment and initial application of a single-spheroid (microtissue) RNA-seq method, a quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based method for up to 47 bile acids in liver microspheres and supernatants, and an MS-based data-independent approach (DIA) method for global protein profiling. We implemented a novel RNA-seq approach that can robustly quantify transcriptome changes in single liver microspheres (approx. 2500 cells). This method comprises mRNA polyA capture and library preparation followed by sequencing. For demonstration, we treated human liver microspheres with the acute phase (DMSO), media, 5 μM chlorpromazine, or 5 μM silicagel for 48 h and 72 h. Both drug treatments induced gene expression changes compared with the vehicle control (DMSO). We also confirmed the method's applicability to rat and dog liver microspheres. To comprehensively evaluate effects on bile acid levels, we established an LC-MS/MS method that can quantify up to 47 bile acids from 3 liver spheroids and their supernatants. It was employed to assess the effects of 1-, 6-, or 24-h treatment, with 75 μM bosentan or 6.4 μM chlorpromazine treatment on human liver microspheres. Compared with the vehicle control (DMSO), both drugs significantly affected several bile acids, and the analysis revealed differential effects of bosentan and chlorpromazine (e.g., on glycochenodeoxycholic acid levels). For quantitative proteome profiling, our MS-based DIA approach effectively quantified proteins from liver microspheres across four species—human, rat, dog, and monkey—and captured the acute phase protein response of lipopolysaccharide-treated human microspheres. The combination of these established high-resolution molecular measurements is expected to provide further insights into common and unique mechanistic properties of toxicity across species. In vitro testing methods based on three-dimensional (3D) reconstructed skin equivalents are reliable, rapid tools frequently used to screen actives and formulations for efficacy evaluation, including potential anti-inflammatory activity. Presented here are data generated using an established in vitro assay based on the EpiDerm™ Human Cell Construct (MatTek Corporation) that was optimized to reduce the time of inflammatory cascade induction that may be more appropriate for the screening of certain actives or product lines. In the standard assay, the EpiDerm™ tissues are exposed topically for 6 hours to materials intended to counteract the inflammation induced by the test compound (PMA) applied to the culture media. The tissue viability and IL-8 secretion in collected culture media were the assay endpoints evaluated at the completion of the exposure time. To accommodate the evaluation of products that need to be tested for short exposure times to the tissues depending on consumer uses or other considerations, the assay was optimized in two phases. In the first phase, kinetic inflammatory events were investigated to determine if a shorter exposure of the tissues to PMA would induce significant expression of IL-8 to allow reliable data interpretations. In these experiments, untreated EpiDerm™ tissues were first pre-incubated overnight in media without PMA; then 6 sets of duplicate tissues were transferred to media containing PMA for exposure times varying in hourly increments from 1 to 6 hours followed by 5 to 6 hours post-exposure to media without PMA, respectively. The culture media was collected at the completion of the exposure to PMA and then again every hour until the end of the post-exposure time in media without PMA, for all groups. This allowed for the evaluation of IL-8 signal depletion in the absence of the inflammation inducing agent. The results showed a time-dependent response of IL-8 expression by 1 hour of exposure to PMA (from 127 pg/mL) with a maximum at 6 hours followed by a gradual decrease over the next 5 hours (up to 6 hours). We also identified a time-dependent reduction of the cytokine signal in the post-exposure period, more significant between the first and second collection times. Based on these results, the treatment groups included in this phase, the tissues incubated in PMA-containing media for 3 hours followed by a 3-hour post-exposure without PMA showed a significant induction of the IL-8 (558 pg/mL) and reduction of the cytokine expression in the post-exposure period (369 pg/mL) to allow for reliable subsequent data analysis. Therefore, this treatment regimen was selected for the second, confirmatory phase of the experiments. In this second phase, the untreated tissues were considered to be the assay negative control, while a commercially available Class 2 (high potency) anti-inflammatory cream was used as the assay positive control. These experiments confirmed the performance of the optimized assay based on the controls data. Our data demonstrated that the reduction of the IL-8 level between the first two collection time points was 3-fold higher for the tissues treated with the assay positive control compared to the negative control-treated tissues, similar to results obtained in the standard protocol. This optimized approach not only shortens the assay length with similar efficacy to the standard but also accommodates testing of products that require a customized testing strategy that may be based on shorter exposure times to the inflammatory agent and/or prototypes investigated.
Exposure to chemical substances from environmental or occupational settings could cause aberrant or toxic responses in immune system. Influences of chemical exposure have been evaluated through epidemiological or in vivo approach using laboratory animals. Meanwhile worldwide restrictions in animal use for research have increased the efforts to develop alternative test methods. Development of alternative test method for screening immunotoxic chemical substances has not been actively changed, therefore no official test guideline is available internationally. The IMMUNOTOX-T assay method was recently developed using THP-1 dendritic cell line to in vitro screen immunotoxic potential of chemicals. Criteria for categorization of test substances to toxic categories were specified on profiling of 27 cytokines production from THP-1 cell line activated with lipopolysaccharides in the presence or absence of test chemicals. Total 63 chemical substances were used for developing the IMMUNOTOX-T assay method, including 7 immunosuppressant drugs, 15 non-immunosuppressants in vivo, and 41 immunomodulatory chemicals in vivo. Various parameters predicting immunotoxicity potential were adopted including relative cytokine production level versus vehicle control, mean cytokine production value for the immunosuppressants, acceptable cytokine production ranges. Among the 41 immunomodulatory test substances, 40 substances (97.6%) were predicted as immunomodulant. According to the modular approach to alternative test validation, 27 chemicals were specified on cytokine reporting ability, between-laboratory reproducibility (BRL), and predictive capacity are core modules for pre-validation process. Strategy for semi pre-validation process on the IMMUNOTOX-T assay will be introduced, in which 3 research laboratories are involved. Briefly, 9 test substances including 3 immunosuppressants, 3 non-immunosuppressant immunotoxic and 3 non-immunosuppressant immunomodulatory substances will be tested in the key laboratory, and BRL will be calculated following at least duplicate experiments. The IMMUNOTOX-T assay will be transferred to one GLP laboratory and 3 reference chemicals will be used for confirmation of successful transferability. Concerning on BRL, the same 9 test substances as used in the key laboratory are used in the laboratory for at least duplicate experiments. The predictive capacity of IMMUNOTOX-T assay will be determined using 16 reference substances (3 immunosuppressants, 7 non-immunosuppressants, and 6 immunomodulators). Supported by grant #2022R1A2C1091555, National Research Foundation of Korea, and the Ministry of Environment-Chemical hazards and risk educational training program.

### 3012 Semi-modular Approach for Prevalidation on the IMMUNOTOX-T In Vitro Alternative Assay Method Developed for Screening Chemicals with Immunotoxicity

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3016 Assessing the Utility of the Genomic Allergen Rapid Detection (GARDskin) Assay to Detect Dermal Sensitization Potential in UVCBs and Formulated Lubricant Products
A. R. Greninger, K. Goyak, T. Lindberg, O. Larne, R. Gradin, and A. Forrey

Advances in new approach methods and their combinations into defined approaches can provide clarity and confidence in concluding on skin sensitization potential. However, challenges remain in utilizing these approaches for difficult to test materials such as those with challenging chemical properties (low water solubility, hydrophobic substances) or complex compositions like Unknown or Variable Composition Complex reaction products and Biological Materials (UVCBs) and formulated mixtures. The previously developed available non-animal test methods for skin sensitization based on key-events of the adverse outcome pathway (AOP) have clearly defined requirements for test material properties that impact feasibility or confound reliance on negative results particularly for difficult to test materials and impede the application of defined approaches to conclude on skin sensitization hazard. A set of difficult to test materials were evaluated in the recently validated GARDskin assay since it offered advantages such as a broader applicability domain, availability of additional validated test solvents for poorly soluble materials while providing mechanistically relevant information on key events across the skin sensitization AOP. The aim of the study was to evaluate the accuracy of the GARDskin assay for a set of synthetic base oils (UVCBs), lubricant additives (UVCBs/poorly soluble substances) and fully formulated lubricants/geraese (mixtures) as well as to provide additional information to assist in a weight of evidence determination given that several of the test materials had borderline conflicting data within the skin sensitization AOP. All test items were adequately solubilized in one of the following solvents, Ethanol (0.1% final), DMSO (0.25% or 0.1% final), or Xylenes ( 0.1% final). SenzaCells were incubated in triplicate under standard conditions with the test items at a max concentration of 500µM for those with a known molecular weight or 100 ppm (w/v) for those without a known molecular weight. Following cell stimulations, RNA was isolated and endpoint measurements were performed using the GARDskin genomic profile signature. Based on the results of this study, the accuracy for prediction of skin sensitization hazard was 100% for synthetic base oils (n=4), 83% for lubricant additives (n=6), and 66% for formulated lubricants/geraeses (n=7). A pairwise comparison of these test items detected by the GARDskin assay and the SenzaCell assay concluded that no test method is perfect. This method should be considered as a replacement for SBS as it negates the necessity for animals and improves infectious agent detection. This method can be used regardless of animal cage type and provides a means to either monitor individual small- or across studies on the same cage rack during a routine monitoring period.

3017 Integrating In Silico and In Vitro Approaches to Understand Cross-Species Predictions of Chemical Susceptibility for Aromatase
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Endocrine active chemicals are of concern for chemical hazard evaluation, and several new approach methodologies (NAMs) have been developed to rapidly screen chemicals for biological activities leading to endocrine disruption. Aromatase (CYP19A1), for example, catalyzes the biosynthesis of estrogens from androgens, which can be affected by chemicals that alter estrogenic activity. Many NAMs that focus on aromatase currently rely on mammalian-based test systems; however, the applicability of these approaches to non-mammalian targets remains uncertain. This study employs in silico approaches and subsequent in vitro confirmation assays to investigate cross-species predictions of chemical susceptibility for aromatase inhibition. The U.S. EPA’s Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool identifies whether known critical amino acids involved in catalytic function vary across species and makes predictions of susceptibility based on amino acid molecular weight and side chain classification. For this evaluation, SeqAPASS was used to identify amino acid differences between vertebrate species. Predictions then guided the selection of amino acids for in vitro site-directed mutagenesis (SDM) studies. In vitro SDM of the wildtype (WT) human Cyp19A1 gene sequence was used to create six enzyme variants representing amino acid differences identified in other vertebrates. The variant peptides, expressed in cell culture, were subsequently screened for aromatase inhibition. The results of in vitro inhibition assays agreed with SeqAPASS predictions for five of the six mutants. One variant, P308Q, predicted as not a match in SeqAPASS, demonstrated similar enzyme inhibition relative to WT enzyme. Virtual docking of testosterone to aromatase structural models representing the WT and amino acid substituted enzymes support in vitro results, also indicating similar activity between the P308Q variant and WT enzyme. Overall, these results demonstrate the power of multi-tiered approaches for predicting and understanding chemical susceptibility across species and suggest that targeted refinements to in silico tools could be made to enhance current predictions. This abstract neither constitutes nor necessarily reflects US EPA policy.

3018 Sentinel-Free Detection of Rodent-Infectious Agent by Contact Media Agitation in Soiled Bedding

Health monitoring of rodents during studies is a component of IACUC objectives as it reduces study outcome variation and protects animal welfare. Despite the historical use of soiled bedding sentinels (SBS) for infectious agent detection, this method fails to detect or poorly detect the majority of prevalent and commonly excluded rodent conventional pathogens. This is due to the fact that many of these infectious agents have greater sensitivity to SBS than have been developed which can reduce or replaces SBS. A recent approach is to directly sample dust from pooled soiled bedding collected from study cages and agitating with a contact media (filter). As reported procedures for this method are diverse, we sought to compare sampling methods and materials to standardize an optimal process. We evaluated different media exposure schedules and media types. Two 3-4 wk-old female SPF CD-1 contact sentinels were placed in each of two cages of three 6-10 wk-old and one cage of three >14 wk-old female pet shop quality mice. During weekly cage changes, soiled bedding from all pet shop mice was mixed and diluted to ~17% with bedding from naïve SPF CD-1 mouse production isolators contaminated with contact media (6 types) in a sealed colletion box to facilitate efficient media exposure or provided to SBS cages for exposure (three 3-4 wk-old female SPF CD-1 mice). All media and treatment schedule variables were performed in triplicate. Sampling schedules for media varied from monthly to weekly exposure to bedding as well as monthly-pooled Media exposure throughout a 3-month period. Using all available diagnostic methodologies, 42, 31, and 10 different infectious agents were detected among pet shop mice, contact sentinels, and SBS respectively. The two optimal media and sampling schedule detected 28 and 29 agents among triplicate samples by PCR. The total number of positive PCR infectious agent assays (PPIAA) compared to non-depended assay sets detected by the SBS method underscored that no method is perfect. This method should be considered as a replacement for SBS as it negates the necessity for animals and improves infectious agent detection. This method can be used regardless of animal cage type and provides a means to either monitor individual small- or across studies on the same cage rack during a routine monitoring period.

3019 Expanding New Approach Methods for Developmental Toxicity: The DevTox Germ Layer Reporter Platform
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The Frank R. Lautenberg Chemical Safety for the 21st Century Act mandates the US EPA develop new approach methods to detect chemical risks to susceptible populations, including pregnant women, that reduce reliance on the use of vertebrate animals in chemical testing. The DevTox GLR (germ layer reporter) model platform was recently established for high-throughput screening and prioritization of potential developmental hazards (doi.org/10.3390/txx10030392). The model platform utilizes the RUES2-GLR plupotent stem cell reporter line that expresses fluorescent fusion protein biomarkers SOX17 (endoderm), Brachury (mesoderm), and SOX9 (mesoderm and ectoderm) to identify specific abnormal developmental biomarkers. The two reporter lines were validated to screen a large set of developmental toxicants (acetaminophen, folic acid, penicillin G and saccharin) and development stimulants, RNA was isolated and endpoint measurements were performed using the GARDskin genomic profile signature. Based on the results of this study, the accuracy for prediction of skin sensitization hazard was 100% for synthetic base oils (n=4), 83% for lubricant additives (n=6), and 66% for formulated lubricants/geraeses (n=7). A pairwise comparison of these test items detected by the GARDskin assay and the SenzaCell assay concluded that no test method is perfect. This method should be considered as a replacement for SBS as it negates the necessity for animals and improves infectious agent detection. This method can be used regardless of animal cage type and provides a means to either monitor individual small- or across studies on the same cage rack during a routine monitoring period.

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has the capability to determine mechanistic potencies of developmental toxicants on cell linesages specified during gastrulation. This abstract does not necessarily reflect EPA policy, nor endorse or recommend any products mentioned.

**Combining In Vitro Quantitative Measurement of Both Skin Irritation and Skin Sensitization in a Single SENS-IS Assay: Application to Medical Devices and Cosmetic Commercial Products**


The SENS-IS assay is the only quantitative in vitro sensitization test. It uses a human 3D epidermis and a genomic signature of 62 biomarkers. Because it uses epidermis as the test system, one can use the SENS-IS assay to test any chemical independent of its solubility and/or hydrophility but also mixtures, finished products, silicones, medical devices, agrochemical products, with an accuracy of more than 90%. To extend the use of the assay by taking advantage of the fact that the 3D reconstructed human epidermis is also used for skin irritation assay with similar application kinetics, we have developed a new genomic signature combined with an algorithm defined by Artificial Intelligence calculations. Skin irritation is defined through an Irritation Index that gives a linear quantification of skin irritation and allows for the classification of irritants according to UN GHS (3 classes, cat 2, cat3 and no cat).

The validation of accuracy was performed on 105 chemicals (30 category 2, 25 Category 3 and 50 no category) with an overall accuracy of 85%. The assay was used to quantify skin irritation and skin sensitization of several cosmetic finished products, households and agrochemical formulations with a very good precision. This combination of two topical toxicity analysis in a single assay will accelerate analysis and decrease the price of the test. It gives the opportunity to use the SENS-IS assay prior to perform assays on human volunteers (HRPT).

**Comparison of Structural Characteristics and Molecular Markers among Rabbit Skin, Pig Skin, and Reconstructed Human Epidermis for an Ex Vivo Human Skin Model**

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The Organization for Economic Co-operation and Development approved a reconstructed human epidermis (RHE) model for in vitro skin irritation and corrosion tests as an alternative to animal testing for cosmetics in the European Union since 2013. However, RHE models have several limitations, such as high manufacturing costs, a loose skin barrier, and inability to simulate all cellular and non-cellular components of the human epidermis. Therefore, new alternative skin models are needed. Ex vivo skin models have been suggested as promising tools. Here, we investigated the structural similarity of the epidermis of pig and rabbit skin, a commercial RHE model (Keraskin), and human skin. To compare the structural similarity, the thickness of each epidermal layer was compared using molecular markers. Among the candidate human skin surrogates, the epidermal thickness of the pig skin was the most similar to that of human skin, followed by rabbit skin and Keraskin. Keraskin showed thicker cornified and granular layers than human skin, while rabbit skin displayed thinner layers. Moreover, the proliferation indices of Keraskin and rabbit skin were higher than those of human skin, whereas the proliferation index of the pig skin was similar to that of human skin. Some or none of the human skin barrier proteins FLG, CLDN1, and CDH1 were expressed in pig and rabbit skin, whereas all human proteins were expressed in Keraskin. Collectively, we propose ex vivo pig skin as the most suitable model for skin irritation testing because of its similarity to human skin.

**Method Development of a Waterpipe Aerosol Exposure System for In Vitro Testing Using 3D Reconstructed Human Airway Tissue**


The study's objective was to investigate the use of an air-liquid interface (ALI) exposure system to evaluate the biological response of 3D reconstructed human airway tissues to waterpipe aerosol. The fluorescent signal was converted to a mass deposition per tissue inserts through a calibration curve. Airway tissues received repeated exposure to waterpipe aerosol for 40 minutes per day according to the puffing regime set in the ISO 22486 standard over 4 days at different concentrations according to air dilution settings. When the aerosol was diluted with an airflow rate of 0.5 L/min, no biological changes were observed across any of the endpoints. In order to investigate exposure conditions under more intense conditions, a 4-fold increase in deposition was achieved by exposing the MucilAir™ tissues to undiluted aerosol. Under these conditions, a 24% cell viability reduction measured by the Resazurin assay and 50% reduction in CBF was noticed while the other endpoints remained unchanged. This preliminary data obtained with a commercialized waterpipe sample suggests that a biological response was observed and dose dependent. This investigation allows us to define the most relevant exposure parameters and biological endpoints for future product stewardship testing using different waterpipe blends.

**Circulated Multi-organoid Platform (CMTOP) as a Nonanimal Methodology for Toxicological Assessment**

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We have developed a novel non-animal platform to conduct rapid and cost-effective toxicological assessments using organoids derived from human induced pluripotent stem cells. To do this, human airway epithelium, liver organoids, and cerebral organoids were cultured in a circulated platform to mimic human circulatory environment. Using this Circulated Multi-Tissue Organoid Platform (CMTOP), we assessed the effects of acute and repeated exposure of diesel exhaust (DE) on human lung, liver, and brain organoid biomarker expression levels. For these experiments, only the airway epithelium was exposed to DE or air (control), while culture medium was circulated from the airway epithelium module to the liver organoid module, then subsequently circulated to the cerebral organoid module.

For the acute exposure, airway epithelium was exposed to 25μg/m³ DE for 4 hours. For the repeated exposure, airway epithelium was exposed to 0.25μg/m³ DE for 10 days, 2 hours per day. Circulation media, airway epithelium, liver, and cerebral organoids were collected to measure expression levels of tissue specific biomarkers under each exposure conditions. Alanine aminotransferase (ALT), albumin, arginase 1 (ARG 1), cytochrome P450 family 3 subfamily A member 4 (CYP3A4), and neurotrophic factor (NTF) were measured in liver organoid media and lysed cerebral organoids. We detected a significant Glu increase (>2-fold) in the lysed cerebral organoids after acute exposure, and a 4-fold increase in lysed organoids after repeated exposure. Additionally, there were trends in decreases in other brain specific markers (5100 calcium-binding protein B and brain derived neurotrophic factor) that were not statistically significant. Furthermore, inflammatory cytokines were not statistically significant in general. Glu accumulation in cerebral organoids following DE exposure demonstrates the utility of CMTOP platform, as it represents the first in vitro model to detect DE induced cerebral Glu uptake.

**Predicting Acute Oral Toxicity Using AcutoX: An Animal Product—Free and Metabolically Relevant Human Cell-Based Test**

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Acute oral toxicity is currently assessed using the rodent LD50 test following OECD test guidelines 420, 423 and 425. These tests are widely criticized on scientific and ethical grounds, as they lack reproducibility (Hoffman, 2010; https://doi.org/10.1016/j.yrtph.2011.08.004) and relevance to human exposure. We have shown that an in vitro cytotoxicity assay can be used to predict acute oral toxicity. The aim of this work was to develop an enhanced, animal product-free, metabolically relevant, in vitro assay capable of predicting EPA and GHS acute oral toxicity category classifications. A cohort of 70 chemically diverse test items, with known, light of a certain wavelength, fluorescence can be used to quantify the amount of deposition measured using a trapping solution to evaluate the exposure to waterpipe aerosol. The fluorescent signal was converted to a mass deposition per tissue extracts through a calibration curve. Airway tissues received repeated exposure to waterpipe aerosol for 40 minutes per day according to the puffing regime set in the ISO 22486 standard over 4 days at different concentrations according to air dilution settings. When the aerosol was diluted with an airflow rate of 0.5 L/min, no biological changes were observed across any of the endpoints. In order to investigate exposure conditions under more intense conditions, a 4-fold increase in deposition was achieved by exposing the MucilAir™ tissues to undiluted aerosol. Under these conditions, a 24% cell viability reduction measured by the Resazurin assay and 50% reduction in CBF was noticed while the other endpoints remained unchanged. This preliminary data obtained with a commercialized waterpipe sample suggests that a biological response was observed and dose dependent. This investigation allows us to define the most relevant exposure parameters and biological endpoints for future product stewardship testing using different waterpipe blends.
in recent years, advancements in engineering and cell biology have enabled increasingly complex and physiologically relevant, human in vitro approaches such as the CatoMOS collaborative acute toxicity modelling suite computational model (Mansouri, 2021; https://doi.org/10.1289/EHP8495), to improve the prediction of acute oral toxicity classification and labelling and is considered to be suitable for inclusion in regulatory practice approaches for acute oral toxicity classification. Acutox assay contributes to a registrants 3Rs initiative by replacing aspects of in vivo acute oral toxicity testing and offers a novel animal-product-free and metabolically relevant, human in vitro approach to acute toxicity.

Well-curated rodent LD50 categories spanning all EPA and GHS classifications, TEER and cell viability measurements. The effects of CdCl₂ were assessed using TEER and cell viability measurements.

In summary, we have developed a novel LoC bronchial epithelial airway model. This healthy human bronchial epithelial model incorporates the dynamic stretching of the native airway, adding additional biologic relevance to the primary airway cultures. The morphology, maturation timeline, barrier integrity, and response to a known toxicant of the cultures were consistent with expected outcomes.

Clinical pathology testing of laboratory animals is crucial for chemical safety evaluation and risk assessment. As the toxicology community and regulatory agencies are moving towards replacement, reduction, and refinement (3Rs) of animal studies, and meanwhile, abundant animal data are available from the public domain, we are exploring an Artificial Intelligence (AI) approach to learn from the existing animal studies to generate the animal data without conducting animal experiments. In this study, we developed AnimalGAN using Generative Adversarial Networks (GANs), which was able to learn from the associations between chemical exposure (the combination of chemical structure, dose, and exposure duration) and clinical pathology findings (i.e., hematologic and clinical chemistry measures) in legacy animal study data to generate synthetic clinical pathology profiles consistent with a diverse population level response to chemical exposure. Implementing AnimalGAN on the Open TG-GATEs database, it successfully inferred hematological and biochemical parameters with high similarity (0.998±0.002) to the corresponding animal testing values under the same experimental design. Furthermore, the generated hematological and biochemical parameters by AnimalGAN could yield similar toxicity identification results comparable with those from animal samples, with an average concordance rate over 90%. Moreover, we challenged AnimalGAN to generate clinical pathology data for treatments reported in DrugMatrix. We found that the generated data could be used to assess toxicity as their corresponding actual animal data is used, with an average concordance rate around 71%, which is comparable with the concordance of toxicity assessment results by using TG-GATEs data and DrugMatrix data for their overlapped treatments. The proposed AnimalGAN framework and its applications demonstrated the potential of utilizing advanced Artificial Intelligence (AI) approaches to produce non-animal models as alternatives to animal studies based on the existing data.
Acute inhalation toxicity assessment is required for chemical formulations, including agrochemicals, and currently is based on results from in vivo rodent studies. In 2016, the US EPA communicated that the agency received approximately 500 sets of acute studies annually. Based on a minimum number of animals used in a guideline acute inhalation toxicity study, this leads to an estimate of approximately 6000 animals per year used for acute inhalation testing. Therefore, an alternative method that does not use animals and accurately predicts inhalation toxicity categories would significantly reduce the use of animals while continuing to protect human health. In a previous study, three formulated pesticide products were evaluated using the EpiAirway™ in vitro human airway model and the results were compared with previous acute in vivo inhalation studies. Evaluation of irritation potential and tissue damage was determined by measuring transepithelial electrical resistance (TEER), lactate dehydrogenase (LDH) release, resazurin metabolism, and histopathology. The results suggested the TEER endpoint was the most sensitive enzymatic indicator of toxicity. Importantly, the histopathology assessment correctly categorized the formulations as having high, moderate, or low inhalation toxicity. To improve the ability of the assay to correctly categorize inhalation toxicity and support use in a regulatory context, the study design was optimized, including increasing exposure time of products to tissues for 4 hours from 3 hours and adding a 20-hour recovery period. In the current study, ten formulated pesticide products were evaluated using EpiAirway™ and the results were compared with previous acute rat in vivo inhalation studies. Evaluation of irritation potential and tissue damage was determined by measuring transepithelial electrical resistance (TEER), lactate dehydrogenase (LDH) release, 4-[4,5-dimethyl-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) viability, and histopathology. Test materials were applied to the EpiAirway™ tissues at three concentrations with four replicates per concentration for four hours. Vehicle controls (deionized water or corn oil), positive controls (0.5% Triton X-100 and 14.7 mg/mL formaldehyde) and negative (untreated) controls were tested in parallel. Initial results indicated that control treatments performed as expected for each functional endpoint. In contrast, the results from the pesticide formulations did not align consistently with in vivo results. Further refinement is needed to optimize the conditions and address the impact of change in the route of exposure.

The in vitro micronucleus (MN) assay is an essential component of test batteries used in toxicological screening for potential genotoxicity. Our previous study adapted metabolically competent HepaRG cells to the high-throughput (HT) flow cytometry-based MN assay for genotoxicity assessment. Given that compared to 2D HepaRG monolayers, 3D HepaRG spheroids have improved metabolic capacity and are more sensitive in detecting DNA damage induced by genotoxicants/carcinogens, the current study explored the performance of HTMN assay in 3D HepaRG spheroids by testing 34 test articles, including 8 direct-acting and 11 indirect-acting genotoxicants/carcinogens as well as 15 performance of the Three-Dimensional HepaRG Micronucleus Assay for In Vitro Genotoxicity Testing


The in vitro micronucleus (MN) assay is an essential component of test batteries used in toxicological screening for potential genotoxicity. Our previous study adapted metabolically competent HepaRG cells to the high-throughput (HT) flow cytometry-based MN assay for genotoxicity assessment. Given that compared to 2D HepaRG monolayers, 3D HepaRG spheroids have improved metabolic capacity and are more sensitive in detecting DNA damage induced by genotoxicants/carcinogens, the present study evaluated the performance of HTMN assay in 3D HepaRG spheroids by testing 34 test articles, including 8 direct-acting and 11 indirect-acting genotoxicants/carcinogens as well as 15 compounds that show different genotoxic responses in vitro and in vivo. Spheroids were exposed to various compounds for 24 h, followed by an additional incubation of 6 days in fresh differentiation medium supplemented with human epidermal growth factor to go through 1.5 cell population doubling. Then the spheroids were lysed and stained for conducting the HTMN assay. The results demonstrated that 3D HepaRG spheroids showed slightly higher sensitivity in detecting genotoxicants/ carcinogens that require metabolic activation, with two compounds [7,12-dimethylbenzanthracene (DMBA) and dimethyltriosamine (DMNA)] inducing higher %MN in 3D spheroids compared to 2D cultures. Benchmark dose analysis demonstrated that these two compounds had significantly lower BMD₉₅ in 3D spheroids than in 2D cultures. These data suggest that the HTMN assay can be adapted to 3D HepaRG spheroids for genotoxicity testing, and that 3D HepaRG spheroids might have slightly improved sensitivity in detecting genotoxicants/carcinogens that require metabolic activation.

3030 Tris (1,3-Dichloro-2-Propyl) Phosphate Disrupts Cellular Metabolism within Human Embryonic Kidney (HEK293) Cells

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Tris (1,3-dichloro-2-propyl) phosphate (TDCIPP) is a widely used, additive flame retardant that migrates from end-use products into environmental media (e.g., surface water, air, and dust), resulting in chronic exposure of human populations around the world. Prior epidemiological studies within the United States have found that human TDCIPP exposure is associated with a decrease in fecundity, a finding that may be driven by TDCIPP-induced implantation failure. However, little is known about whether TDCIPP disrupts the physiology of human embryonic cells at environmentally relevant concentrations. Therefore, the overall objective of this study was to determine whether TDCIPP alters cell viability, cytokine methylation, reactive oxygen species (ROS) levels, and cellular metabolism using human embryonic kidney (HEK293) cells. First, using CellTiter-Glo assays, we found that, relative to vehicle controls, exposure to environmentally relevant concentrations of TDCIPP for 24 or 48 h resulted in a concentration-dependent increase in cell viability, a finding that was likely driven by an increase in the relative ATP abundance. Second, using 5-methylcytosine (5-mc)-specific immunocytochemistry, we found that TDCIPP exposure up to 48 h did not affect global cytosine methylation. Finally, using CellTROX green assays, we detected a significant concentration-dependent decrease in ROS in situ (relative to vehicle-treated cells) after 48 h (but not 24 h) of TDCIPP exposure, suggesting that exposure to 0.1% DMSO alone may increase ROS generation within HEK293 cells and TDCIPP may counteract the effects of 0.1% DMSO. To date, our findings in HEK293 cells demonstrate that 1) TDCIPP increases ATP production; 2) TDCIPP does not affect the abundance of 5-mc; and 3) TDCIPP decreases ROS relative to vehicle-treated cells. Our ongoing and future studies within HEK293 cells will rely on metabolomics, real-time cell analysis, and functional pharmacologic approaches to begin uncovering the mechanism underlying TDCIPP-induced disruption of cellular metabolism. Importantly, our findings with TDCIPP point to a novel mechanism of action that may be relevant to human embryonic cells and/or other organohalogen flame retardants that are frequently detected within human biomonitoring studies.

3031 Bioprinted Pancreatic Tumors: A Novel Animal-Free Approach for Screening Chemotherapy, Radiotherapy, and Immunotherapies against Cancer

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The tumor microenvironment plays a crucial role in the progression of solid tumors. Among the solid tumors, pancreatic ductal adenocarcinoma (PDAC) has had the lowest survival rate. PDAC has a highly aggressive tumor with a dense stroma and a poor vascularization network making it difficult for drug delivery. The complex TME and drug delivery issues have made it difficult to develop new therapies in PDAC for the past 4 decades. The first step of developing an effective therapy is in vitro screening method. Considering the TME of PDAC, there is an immense need to develop reliable in vitro anti-cancer screening models which closely mimic the in vivo microenvironment. The conventional 2D monolayer models lack most of the tumor characteristics. It is evident that a leap from the oversimplified 2D systems to animal models makes it difficult to transform and reproduce the outcomes obtained during in vitro studies. To bridge this gap, 3D tumor models are being extensively explored as an alternative option to 2D systems. In the current study, we developed and characterized bioprinted PDAC tumor models generated using extrusion bioprinting. Importantly, our study is unique in the fact that the bioink mainly replicates the natural stromal environment as observed in human PDAC tissues. The tumor models displayed favorable cell viability and proliferation rate post-printing. Importantly, we characterized the model for important hallmarks of cancer such as hypoxia, tissue invasion, etc. We wanted to test the anti-cancer activity of standard chemotherapeutics and radiotherapy on 2D versus the 3D model. In general, PDAC cells showed higher resistance in a 3D environment than in the 2D monolayer culture. Considering the differences observed and the fact that 3D models were able to mimic factors such as hypoxic environments, our study provides a reliable platform for studying the toxic effects of therapies on PDAC tissues.

3032 Polybrominated Diphenyl Ether (PBDE) and Its Effects on Specific Gut Microbes and Their Associated Metabolic Networks Relating to Cellular Growth and Survival

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Polybrominated diphenyl ethers (PBDEs) were formerly used flame retardants and are persistent environmental toxicants implicated in various metabolic diseases. We have previously shown that oral exposure to the diet enriched PBDE congeners 2,2,4,4-tetrabromo-diphenyl ether (BDE-47) and 2,2,4,4,5-pentabromo-diphenyl ether (BDE-99) produced gut dysbiosis in large intestinal content of mice, including a marked elevation of the short chain fatty acid (SCFA)-producing Akkermansia muciniphila. We also showed that BDE-99 exposure increased the DNA encoding microbial 7-dehydrodolony enzymes for secondary bile acid (BA) synthesis. In this study, we followed up to investigate how BDE-47 and BDE-99 directly regulate specific microbes involved in SCFA- and secondary BA-synthesis. Akkermansia muciniphila is a Bacteroidia scintum bacteria – a well-known gut bacteria involved in secondary BA-synthesis, were exposed to either BDE-47 or BDE-99 at the following concentrations: 0µM (control),10µM, and 100µM. Escherichia coli was exposed in parallel as a positive control. Mass spectrometry analysis was used to observe metabolic changes. Over the time course, both BDE-47 and BDE-99 showed
growth inhibition of A. muciniphila and E. coli; the growth of C. scindens was also inhibited by BDE-47 but was promoted by BDE-209. C. scindens in BDE-47 decreased 2-pyrollidinone but increased uronic acid, whereas BDE-99 increased lactate and decreased kyurenine. In A. muciniphila, BDE-47 increased NADPH while BDE-99 increased 5-aminoevulinic acid. In E. coli, BDE-47 increased both aspartate and 4-aminobutyric acid while BDE-99 increased both 2-pyrollidinone and glucosamine. In conclusion, our study showed that PBDEs affected the growth as well as carbohydrate and amino acid metabolism in important intestinal commensal bacteria. Understanding the direct interactions between orally exposed environmental chemicals and gut microbes may provide additional insights into the mechanisms of toxicity.

3033 Functional Assessment of hiPSC-Derived Brain Organoids to Study the Effects of Chemical Exposure and Electrical Stimulation on Synaptic Plasticity


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Current in vitro brain models are powerful tools to study the molecular pathways of neurotoxicity but are limited when studying the functional changes in network activity because of technological limitations of the field. Advancements in high-density micro-electrode arrays (MEAs) and the optimization of live calcium imaging have made characterizing these functional changes more feasible, and more specifically, the effects of aneurotoxic exposures can have on network activity and synaptic plasticity in vitro. In addition, recent advances in cell culture allow modeling of the cellular processes in vitro in more physiologically relevant settings. Induced Pluripotent Stem Cell (iPSC) derived brain organoids are an example of such microphysiological systems. Here, we aim to functionally characterize network activity and synaptic plasticity in response to chemical exposure and electrical stimuli in our lab’s human iPSC-derived brain organoids. To research this, we have been differentiating brain organoids for 12 weeks and characterizing spontaneous and modulated electrical activity weekly using live calcium imaging and MEAs. We have been modulating electrical activity with acute chemical and electrical stimuli. We also modulated the electrical activity by long-term, low-dose exposures to arsenic, lead, cadmium, chromium and heavy metal mixtures. In addition, we are characterizing early synaptogenesis, including immediate early genes over time in conjunction with the stimulations by immuno-histochemistry and RT-PCR. Our preliminary results show that overall spontaneous firing rate decreased and interburst interval decreased in organoids stimulated with 20µM Glutamate and 20µM Gamma-Aminobutyric Acid. Organoids recovered spontaneous firing rate and interburst interval over 2 weeks of Gamma-Aminobutyric Acid washout. Furthermore, long-term culturing of multiple organoids on a single MEA found evidence of highly interconnected network formation within and between organoids. In the future, we plan to study how long-term potentiation is affected by chemical and electrical exposures in order to develop a model to study the effects on cognition. Moreover, this functional assessment of brain organoids can be used for toxicological endpoints, especially for neurodevelopmental toxicants where network activity and synaptogenesis is of interest.

3034 Synaptogenesis Assay for Developmental Neurotoxicity Testing in a Human 3D Brain Model


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The rapid increase in the prevalence of many neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD), is a major public health concern, with animal models of NDD are useful tools to study the mechanisms of toxicity but are limited when studying the functional changes to recapitulate neurodevelopment and disruptions to synaptogenesis due to chemical exposures in a medium- to high-throughput manner. Thus, this alternative in vitro assay could reduce the burden of animal testing in DNT screening while providing high quality, high-throughput, and relevant data for chemical risk assessment to improve human health outcomes.

3035 Evaluation of the KeratinoSens Assay in the Presence of Acetone as Solvent Control

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The in vitro KeratinoSens™ assay has been well established to cover Key Event 2 (KE2) in the Adverse Outcome Pathway (AOP) of skin sensitization as described in OECD TG 442D. In combination with the assays addressing the first key event (e.g., Direct Peptide Reactivity Assay [DPRA]) and/or the third key event (e.g., Human Cell Line Activation Test [h-CLAT]), the KeratinoSens™ assay can be used to derive a prediction of skin sensitization potential of test substances, which is also an example of such microphysiological systems. Here, we aim to functionally characterize network activity and synaptic plasticity in response to chemical exposure and electrical stimuli in our lab’s human iPSC-derived brain organoids.

On the contrary, for a solvent effect to be evaluated in the h-CLAT, demonstrated weak sensitization potential in the KeratinoSens™ assay under both solvent conditions. Correspondingly, imidazolidinyl urea, a weak sensitizer in the h-CLAT, demonstrated weak sensitization potential in the KeratinoSens™ assay under both solvent conditions. In contrast, EGDMa conformed with a positive KeratinoSens™ assay prediction in the presence of 1% DMSO; however, was predicted weakly negative in the presence of 1% DMSO/1% acetone. EGDMa is a weak sensitizer proficiency substance in the h-CLAT (but not in the KeratinoSens™ assay), did not exhibit sensitizing potential in the KeratinoSens™ assay in either solvent condition. Overall, the use of acetone as a solvent with the proficiency chemicals demonstrated the influence of prospective solvents and the need for additional proficiency substances to make a complete assessment.

3036 How to Get Physiological 3D Peristalsis Movement into In Vitro Models? A Novel Gut-on-Chip Model

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The human gut is a highly mechanically active organ undergoing continuous peristalsis movement. Recent drug discovery research highlighted the importance of incorporating physiologically relevant biomechanical stimuli in vitro models to the in vivo counterparts in the cascade of gut barrier function. Using Cell Line Activation Test (CLAT) organoid and editing, we have introduced the green fluorescent protein (GFP) to tag a pre-synaptic protein, synaptophysin (SYP), in an induced pluripotent stem cell (iPSC) line. After quality control steps, this reporter line was differentiated into brain organoids. We then followed the expression and colocalization of the GFP tag with synaptic markers over time and quantified GFP expression upon exposure the environmental chemicals (Lead, Arsenic, Cadmium, Chromium, and a mixture of the four), which are suggested to perturb neurodevelopment and synaptogenesis. To complete the synaptogenesis assay, a second fluorescent tag is being added to the same GFP-SYP-iPSC cell line to tag a post-synaptic marker, postsynaptic density protein 95 (PSD95), using the same CRISPR/Cas9 techniques. After quality control steps and differentiation into brain organoids, high content imaging quantification of colocalization of the tagged SYP and PSD95 proteins will allow for the assessment of neurodevelopment and disruptions to synaptogenesis due to chemical exposures in a medium- to high-throughput manner. Thus, this alternative in vitro assay could reduce the burden of animal testing in DNT screening while providing high quality, high-throughput, and relevant data for chemical risk assessment to improve human health outcomes.
parameters were set to recapitulate the gut motions during the active daytime and resting nighttime. The model characterization and impact of physiological and biomechanical stimulation were investigated by RT-PCR, immunofluorescence, ELISA, and by measuring Trans barrier Electrical Resistance (TER). To investigate drug efficacy on the gut epithelium, an inflammatory model was developed. We successfully transferred the gut barrier model developed on standard Transwell® to the AX12, showing the capability to easily transfer any existing model to the AX12. The gut monolayers on the AX12 chip showed an increased level of gene expression at early time points compared to Transwell®. Further, reproducible TER increase revealed the barrier formation capacity of our gut-on-chip model. Upon 3D peristaltic stimulation, the barrier function of the monolayers adapted to the stimuli, reflected by TER values closer to in vivo parameters. When using a proinflammatory trigger, the 3D peristaltic stretch-treated cells showed a higher sensitivity compared to the static model. In summary, we successfully developed a new gut-on-chip model including physiological 3D peristaltic motion to better investigate the efficiency and safety of molecules. The results demonstrate that the AX12 barrier-on-chip system is suitable to accurately model different tissue barriers in the gut. The 3D peristaltic stimulation allows an accurate barrier characterization and the inclusion of 3D peristaltic stretch resulted in an in vivo-like barrier and increased the sensitivity of the developed inflammatory model. In summary, this new gut-on-chip model represents an important step forward to better assess toxicity, develop disease models, and perform drug testing studies.

3037 The Potential of PBK Modeling to Inform Agrochemical Safety and Reduce Toxicity Testing in Dogs
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The need to understand the impact of agrochemicals on human health for both acute and chronic exposure currently requires multiple animal studies both short- and long-term. While rodents have historically been the standard laboratory animals used for this purpose, the dog was added in the 1940s as a secondary, non-rodent species to address concerns regarding potential inter-species variability in toxicological response. The utility of these studies, in the dog, has been limited due to the low-throughput nature of the data driven by demands on resources and ethical reasons, as well as a lack of mechanistic understanding. Physiologically-Based Kinetic (PBK) models offer a high-throughput, mechanistic, in silico approach to simulate and predict agrochemical exposure based on physiological properties of the organism and physicochemical properties of the compound of interest. We have developed PBK-based models for the dog based on well-established systems of mathematical equations and physiological parameter values to predict chemical exposure for a range of chemicals in different dosing scenarios. The model not only offers insight into predicted toxicokinetics in the blood and throughout the body, but also provides mechanistic insight into the processes of absorption, distribution, metabolism, and excretion and how they relate to particular properties of the agrochemical. The dog PBK model allowed for the optimisation of dosing regimens to achieve desired levels of exposure during product safety testing, the prediction of long-term exposure for repeat-dose studies, and the comparison of closely related chemicals with mechanistic explanations into their different behaviours and associated risks to health. The dog PBK model has been able to guide in vivo studies and minimise the unnecessary use of dogs by proposing criteria such as appropriate dose ranges with the most relevant a priori assumptions. Mechanistic mathematical modelling, in combination with in vitro-guided parameterisation, offers the most ethical and effective way to reduce animal testing currently and even more so in the future as these technologies develop. In the near-term we believe that these approaches can contribute to the removal and replacement of long-term dog studies for assessing risk of toxicity related to chemical exposure.

3038 Development of New Approach Methodologies (NAMs) for Avian Chronic Risk Refinement
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As part of regulatory requirements for pesticide risk assessment, both acute and chronic avian toxicity tests are conducted. These in vivo studies using zebra finch, northern bobwhite, and mallard, provide relevant endpoints for lower tier risk assessments. However, risk refinements are often limited due to the specific design of these tests. Additional in vitro tests are not recommended due to animal welfare issues. In order to better understand realistic risks and provide refinement options, it is necessary to extend our understanding of avian toxicology (U.S.EPA) and European Food Safety Authority (EFSA) support the development of new approach methodologies (NAMs) as a promising way forward in the risk assessment for vertebrate wildlife. The limited work available in this area highlight the need for developing case studies to illustrate the use of NAMs in improving mechanistic understanding of the internal kinetics. The use of non-pharmacological assay (in vitro, in silico) and historical in vivo avian residue data. In this study, we developed in vitro to in vivo extrapolation (IVIVE) based on mechanistic PBK models for three avian species and thiamethoxam. In vitro kinetic measurements of hen, mallard and bobwhite including metabolic stability and plasma protein binding were collected for the target compound. Those in vitro kinetic measurements were used to parameterize IVIVE-PBK models. An in vivo regulatory residue study using laying hens was used to validate the laying hen model. Mallard and bobwhite simulations were conducted with the same avian PBK model structure but considering differences in species physiology. The time-course of internal concentration predictions by the PBK model will serve as input into Toxicodynamic models (TD) and such an approach will use Dynamic Energy Budget (DEB) theory, to predict the effects on survival, growth or reproduction from exposure under realistic conditions. The integrated DEB-PBK-TD approach can greatly increase the utility of in vivo regulatory studies conducted for higher tier avian risk assessment and allow for a more realistic assessment of risks to individuals and populations in the field.

3039 Developing a Rapid-Throughput Optomotor Behavior Assay in Zebrafish Larvae
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Photo-motor responses to exogenous chemicals in zebrafish larvae can provide insight into neurobehavioral changes caused by chemical and drug exposure. Zebrafish larvae are ideal for high-throughput behavior analysis due to their small size, color vision, and robust photo-motor responses. Ambient light levels, the presence of food, and zebrafish age influence their light/dark preferences. To assess zebrafish larval light/dark preference, an optomotor assay was developed using the Viewpoint Zebrabox. The optomotor assay uses an LCD screen that can be programmed to vary the color and area of stationary light patterns. The LCD is immediately below the microtiter assay plate. Using 7- and 10-days post fertilisation (dpf) larvae, the preference for white light compared to a dark environment (infrared light) was assessed. At this life stage, larvae preferred white light. An area of 25% white light, 75% dark, and one-minute area switching were optimal for the most consistent sample size were assessed, and 5 minutes was determined to be sufficient to detect light/dark preference behavior in wildtype control larvae. To test the optomotor assay, larvae were exposed for 5 min to chemicals known to impact visual-motor behavior: 0.15% ethanol, 10 mg/L caffeine, and 0.05 mg/L benzoiazepine. Next steps include testing the optomotor behavior assay as a low throughput (5-10 dpf) as an effort towards higher throughput. A power analysis will be conducted to identify the minimum sample size needed to detect chemical impact to optomotor behavior. We believe that an automated approach to the optomotor assay will become a rapid tool to quickly uncover place preference abnormalities resulting from chemical exposure, a sensitive endpoint of larval zebrafish toxicology.

3040 Assessing Effects of Simultaneous Hyperglycemia and Cadmium Exposures in a Zebrafish Larval Model Using the GUTS TKTD Modeling Framework

Cadmium is a highly toxic, non-physiological environmental metal pollutant. Humans are exposed to Cd resulting in a wide variety of diseases including diabetes, cancers, genotoxicity, kidney and liver Diseases. Due to the long-term, progressive nature of Cd bioaccumulation in biological tissues, it is a serious risk for chronic disease. We currently have limited understanding of the mechanisms by which Cd causes chronic metabolic imbalances. Understanding global, whole-body changes of bioenergetic imbalances is of much interest in the context of Cd toxicity. In this abstract we used a zebrafish (ZF) embryo model to study the effects of Cd exposures in the presence of hyperglycemia using a general unified threshold survival (GUTS) approach. We used standard A/B wild-type model ZF embryos up to 144 hpf (6 days) for the duration of the study. The embryos were cultured in two glucose concentrations- a). 0 No Glucose and b. 120 mM Glucose and baseline exposure conditions for hyperglycemia. The ZF embryos were then exposed to Cd at 0, 50, 100, 250, 500, 1000, 2000, 3000, 5000, and 7500 µg/L for a duration of 6 dpf in a 12:12 h photoperiod at 28℃ ± 0.5℃. The mortality during the exposure conditions was checked twice daily for 6 days and the mortality curves were prepared by counting the number of dead ZF larvae each day. Three independent replicates of the studies were performed over separate weeks to ensure replicability of the survival curves/data. The survival data after Cd exposures at 6 dpf was used to build the TKTD models using the GUTS-SD and GUTS-IT modeling paradigms. We used the EC50 values obtained from the TKTD survival modeling data to create a biological plausible predictions of the effects of synergism of hyperglycemia with Cd exposures. Using this design, we tested 1. Overall energy expenditure using a high-throughput Alamar Blue based assay. 2. the overall uptake of Cd in the ZF larvae using ICP-MS 3. Whole-body glucose uptake differences using a 2-NBDG assay and 4. NAD metabolite differences. In addition to the biochemical and toxicological parameters, we scored the developmental toxicity effects (heart defects, circulation, edema, hemorrhage, tail morphology, pigmentation, motility, malformations in the eye, brain and liver). We observed a surprising difference in survival outcomes of ZF larvae in the presence of hyperglycemia. ZF larvae hatched in the presence of 120 mM glucose were noted to have much reduced mortality.
compared to larvae grown in normal E3 media. The EC50 values for 0 mM glucose was noted to be 165 μg/mL while 120 mM glucose cultured larvae had an EC50 value of 3500 μg/mL. We propose hyperglycemia provides a survival benefit against Cd toxicity due to bioenergetic compensation. Multiple developmental defects were noted in 0 mM glucose reared larvae which were not observed in hyperglycemic larvae. The current project highlights the role of Cd exposures as a key driver of whole-body metabolic dysfunction in a dose dependent manner.

3041 Superficial Vascular Access in Uremic Animal Models to Test Medical Devices

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To explore the possibility of providing suitable superficial vascular access of varying durabilities, in various animal models and in some, inducing a uremic state in the animal by a technique using percutaneous embolization of both renal arteries using polyvinyl alcohol particles. Chronic kidney disease and stage 5 renal disease is a burgeoning issue worldwide reaching epidemic proportions with regular haemodialysis being the main modality of life saving treatment. Animal model testing remains an important component of preclinical safety evaluation of medical devices contributing to technology advancement. Long term hemodialysis access is commonly done using arteriovenous fistula (AVF) or arteriovenous grafts (AVG). During those interventions, native venous or synthetic grafts are arterialized, and then ready to use. There has been an explosion of new dialysis technologies, some, portable dialysis machines for home usage, others having unique features with significant advantages over existing machines. Concurrently, medical devices have been developed to increase the oral route of inhalation triggering alarms when safety parameters are breached. Though, there have been studies performing such preclinical procedures, none of them have attempted them in uremic animals, and also almost all of them have performed them in the lower limbs, which is not ideal, and does not test the ability to perform its ability to maintain the anephric patient in good health. Testing these devices preclinically needs a suitable superficial vascular access in a uremic/non-uremic animal model. Swine and sheep are common preclinical safety models because the sizes of their cardiac and central vascular anatomical structures being similar to those of humans. American Yorkshire pigs (60-80 Kgs) were the preferred animal, because they resemble humans in size and blood volume, have similar physiology, digestive tract and cardiovascular system and have a better community acceptance as a laboratory animal. AVG was selected as the primary vascular access. In addition, a tunneled Permcutch for shorter length of study and a double lumen Mahmoudialysis catheter for single session of medical device testing. For AVG, a PTFE graft interposition was performed between the carotid artery and the external jugular vein, with the vascular access superficialized subcutaneous and tail vein to test various immune markers to baseline values by 24 wk. In BN rats, resolution of inflammation was further compromised by HDF diet, as many exposure-induced alterations in local/systemic immune markers were still evident in HF/WF animals at 24 wk. Collectively, HDF diet appeared to have a greater impact on global immune status and acute lung injury in SD rats, but a more pronounced effect on inflammation resolution in BN rats. These results illustrate the potential combined impact of genetic, behavioral, and environmental factors in modulating immunological responsiveness and ultimately emphasize the importance of the exposure in shaping biological responses.

3042 Impact of Airway Variability on Particle Deposition in the Lungs of Ferrets


Ferrets have been used as an animal model in inhalation toxicology due to the ease of handling, utility in vaccine research and similarities to humans’ tracheobronchial geometry. Currently, there is no widely available particle dosimetry model for the ferret that can be used to estimate inhaled doses or used for interspecies extrapolation. While transport mechanisms and thus deposition models are similar among most mammals and rodents, lung geometries and physiologies are species-specific. Based on existing ‘single path’ models in the literature, a simplified computational lung geometry for ferrets, which included intraspecies airway geometry variability was developed. A double-path tree branching structure was used for the tracheobronchial tree where one pathway accounted for the conducting airways and the second for the alveolar airways if present (airway generation 5 and beyond). The major advantage of the new lung geometry was that it avoided artificial separation of conducting and alveolar regions based on airway generation number particularly for lung geometries with monopodial structures that are prevalent in rodents. Inclusion of intraspecies airway geometry variability typically seen in rodents increases the utility of the new lung geometry. The proposed geometrical structure can replace typical-path models, often used in the literature, to predict particle deposition more accurately in all species. Ferret-specific lung volume and breathing parameters were used to predict particle deposition in the ferret using the Multiple-Path Particle Dosimetry model (MPPD, Applied Research Associates, Raleigh, NC). Model predictions showed that while regional deposition followed a similar pattern to that of typical-path models, deposition distribution as a function of airway generation number was significantly different. Deposition of micrometer-size particles tended to deposit in proximal airway generations whereas nanoparticle deposition occurred in more distal airways. The developed ferret model provides a more realistic prediction of particle deposition distribution in the respiratory tract of ferrets and will be a useful tool for risk assessment applications. The study was funded by the Cystic Fibrosis Foundation.

3043 Examination of the Exposome in an Animal Model: The Impact of High-Fat Diet and Rat Strain on Local and Systemic Immune Markers following Occupational Welding Fume Exposure


An experimental model was designed to investigate the impact of multiple exposomal factors on susceptibility to acute lung toxicity and subsequent resolution of inflammation following an occupationally-relevant inhalation exposure. To assess the role of genetic influence, two rat strains—Sprague-Dawley (SD) and Brown Norway (BN)—were used. A high fat (HF) “Western diet” (14.8% protein, 40.6% carbohydrate, 44.6% fat) was also incorporated to evaluate the potential impact of behavioral/lifestyle factors. Accordingly, male SD and BN rats were maintained on a HF or (regular) diet for 24 wks. Inhalation exposure to filtered air or stainless steel welding fume (WF, 53% Fe, 24% Mn, 17% Cr, 6% Ni, 0.4% Cu) occurred beginning wk 7 for 5 wks (target concentration of 20 mg/m3 × 3 h/day × 5 days/week × 5 weeks). Rats were euthanized at 7, 12, and 24 wks to evaluate inflammation and immune markers related to the baseline, exposure, and recovery phases of the study, respectively. At 7 wks, HF-fed animals exhibited greater immune markers (blood leukocyte/neutrophil number, lymph node B-cell proportionality) with effects more pronounced in SD than BN rats. Indices of acute lung inflammation were elevated in all WF-exposed animals at 12 wks; however, diet appeared to preferentially impact SD rats at this time point, as lymph node cellularity and bronchoalveolar lavage neutrophils were further elevated in HF rats. Overall, SD rats exhibited the greatest capacity for recovery of altered immune markers to baseline values by 24 wk. In BN rats, resolution of inflammation was further compromised by HF diet, as many exposure-induced alterations in local/systemic immune markers were still evident in HF/WF animals at 24 wks. Collectively, HF diet appeared to have a greater impact on global immune status and acute lung injury in SD rats, but a more pronounced effect on inflammation resolution in BN rats. These results illustrate the potential combined impact of genetic, behavioral, and environmental factors in modulating immunological responsiveness and ultimately emphasize the importance of the exposome in shaping biological responses.

3044 Toxicity and Human Health Risk Assessment of Topical Aloe Vera Extract

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Various formulations of processed or unprocessed Aloe vera Gel (AVG) are indiscriminately used for skin beauty and enhancement, treatment of skin ailments, fading dark spots and anti-aging, among many others. Recently, there have been concerns about skin lesions and even, possibly, potential for skin cancer due to AVG use. There have not been any meaningful toxicity and health risk assessment reports on its topical use. Four groups of rabbits (n = 8) had a 3 cm diameter circular wound shaved at their back to allow for the different preparations of AVG, once daily, for 30 days thus: Group 1 (control): 1 g of petroleum jelly (PJ) alone; Group 2: 1 g AVG + 1 g of PJ; Group 3: 2 g AVG + 1 g of PJ; Group 4: 4 g AVG + 1 g of PJ. On day 31, skin sections were carefully scraped from five of the animals in each group for assessment of skin lesions, dermatitis and erythema. The resulting three rats in each group were left untreated for a further 15 days for reversibility tests. Results showed significant (P<0.05, ANOVA) lesion counts, vs control group. Following reversibility period, lesion parameters were restored to normal levels. Hazard identification and Dose-response relationships do not indicate skin carcinogenesis. Exposure assessment and risk characterisation indicate a hazard quotient of 1.4. In conclusion, prolonged topical exposure to AVG preparations has potential for reversible skin lesions, but not cancer, thereby warranting caution during long-term topical administration in humans.
Various immune therapies against human gliomas have so far shown limited success. Syngeneic glioma cell line models that are commonly used in preclinical immune therapies do not mimic the heterogeneity of human gliomas. Also, most rodent models produce an immune response against the cell lines. In this study, we characterized the immune landscape of rat gliomas induced by N-ethyl-N-nitrosourea (ENU) as a potential model for immunotherapy. ENU (50 mg/kg) was administered intravenously to pregnant female SD rats on gestation day 20. Male pups were euthanized between postnatal days 110-230 days. Based on microscopic morphology, the rat brain tumors were diagnosed as oligodendroglomas, astrocytomas, and/or mixed gliomas and appear to share histological features of low-grade gliomas. To understand histochimistry of the tumors, we used tissue microarrays (TMA) to assess tumor-infiltrating lymphocytes (TILs), tumor-associated macrophages (TAMs), as well as expression of programmed cell death-1 (PD-1) and programmed death-ligand 1 (PD-L1). The results indicated a heterogeneous immune landscape in the rat gliomas examined in this study. The inflammatory score and the number of TILs and TAMs were lower in oligodendroglomas than in mixed gliomas and/or astrocytomas, which suggested that the astrocytoma component may be associated with higher immune cell infiltration. The main infiltrating cells in mixed gliomas seemed to be macrophages that were positive for Iba-1, but mostly negative for CD163 (M2 macrophage marker) and P2RY12 (microglia marker) suggesting their phenotype may be more aligned with M1 macrophages. CD8 and PD-1 expression of lymphocytes was rare in all examined gliomas including astrocytomas that had a lot of TILs. Surprisingly, all gliomas expressed PD-L1 in this study although the intensity was variable across tumors suggesting the immune landscape in these rat gliomas may be immunosuppressive. In conclusion, ENU-induced rat glioma model may have potential consideration for pharmacological evaluation of immune therapies since they resemble human gliomas in terms of tumor heterogeneity and immune landscape.

Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory disease and is characterized by synovial proliferation and joint destruction, resulting in joint destruction. It is associated with chronic pain, loss of function, and disability. The murine model of Collagen Antibody-Induced Arthritis (CAIA) mimics many of the features of arthritis in humans and has been used successfully in addressing questions of disease pathogenesis and to screen candidate therapeutic agents. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that plays a pivotal role in regulating the inflammatory response in chronic autoimmune diseases such as RA. The discovery of the role of TNF-α in the pathogenesis of RA has led to anti-TNF biologic therapies as a breakthrough in the treatment of RA. The objective of this study was to investigate anti-inflammatory effects of MYMD-1®, a small molecule selective inhibitor of tumor necrosis factor-alpha (TNF-α) with easy access to the body including the brain, in the murine CAIA model. The CAIA model was induced by an intravenous injection of a monoclonal antibodies cocktail that are directed to collagen type II on Day 1 ( sensitization), followed by an intraperitoneal injection of the endotoxin LPS on Day 6 (boost immunization). Three oral doses given Bid (twice a day) of MYMD-1® (50, 250 and 450 mg/kg/day) were tested starting at the onset of the disease (Day 8 in this study). Etanercept (a biologic TNF-α inhibitor) and Dexamethasone (a glucocorticoid) were administered subcutaneously twice weekly (10 mg/kg) or by oral daily Etanercept (a biologic TNF-α inhibitor) and Dexamethasone (a glucocorticoid) were tested starting at the onset of the disease (Day 8 in this study). Three oral doses given BID (two times a day) of MYMD-1® at 450 mg/kg/day inhibited arthritis development in CAIA murine model, with in-life data consistent with histopathological findings. Unlike currently available TNF-α inhibitors, MYMD-1® can be given orally and is a promising drug for rheumatoid arthritis.

**3047 Spontaneous Histopathology Findings in St. Kitts African Green Monkeys (Chlorocebus sabeus)**


Nonhuman primate test systems are essential for biopharmaceutical and medical device safety and toxicity testing of many important established and emerging therapeutic strategies, and the St. Kitts African green monkey (SK-AGM) is becoming a more widely used preclinical model for a range of test articles, including gene and cell therapies, biologics, and medical devices. Broader in vivo preclinical use, and ex vivo analyses of collected tissues, has elevated the need for unbiased review and characterization of the background histopathology of the SK-AGM. To fill this gap, we prospectively performed postmortem examinations on 60 male and 60 female SK-AGM of various ages that had no history of significant medical concerns or interventions, or experimental procedures. All animals were humanely euthanized followed by timely sample collection from all major organs, including gross lesions, for formalin-fixation, paraffin-embedding, H&E staining, and histopathologic examination by a pathologist. Common histopathologic findings included lymphocytic, lymphoplasmacytic, and/or lymphohistiocytic infiltrates in the kidney, liver, lung, trachea, thyroid, salivary glands, tongue, esophagus, stomach, small intestines, cecum, colon, rectum, vagina, cervix, prostate, and seminal vesicle; articular hyperplasia; age-related mineralization of hyaline cartilage; thymic atrophy; pulmonary anthracosis; protozoal cysts and metazoan infections; neuronal and myocardial lipofuscinosis; lymphoid germinal center amyloidosis, and myocardio- cate cytosclerosis. Less common or unique histopathologic findings of interest included myocytolysis degeneration and adiocyte infiltration as seen in heart, muscle, and adipose tissues; myocardiomyopathy; pituitary and thyroid cysts; membranoproliferative glomerulonephritis; prostatic fibroepithelial hyperplasia as seen in human benign prostatic hyperplasia; pituitary microadenoma; hemosiderosis; hemorrhachrosis; seminiferous tubule degeneration and aspermatia; adeno- myosis; laryngitis with apical filamentous bacteria, and gastric amyloidosis. These findings help identify histopathologic changes that may be seen in tissues from SK-AGMs used in biopharmaceutical preclinical safety and toxicity or medical device testing that are not test article or device related.

**3048 The MIA Model: A Reliable Translational Tool for Osteoarthritis Research**

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Osteoarthritis (OA) is the most common joint disease, associated with pain, disability, and decreased quality of life. It is characterized by progressive loss of cartilage, remodeling of the subchondral bone and synovial proliferation, and loss of joint function. The chemically induced monoiodoacetate (MIA) model is widely used to study OA. MIA is an inhibitor of glyceraldehyde-3-phosphate that disrupts cellular glycolysis leading to cell death. A better characterization of this model is required for its successful use in drug development. To better elucidate this model, we injected adult Sprague Dawley rats with increasing doses of MIA (0.5, 1, 2, and 3 mg) in the right knee joint and demonstrated that disruption of the knee joint denoted by loss of chondrocytes and structural changes, is dose dependent, consistent with previously reported data. OA follows a biphasic pattern, with an early inflammatory phase and a later less inflammatory phase. To better understand this process, we injected 3 mg MIA in rats knee joints on day 0 and euthanized at different time points in order to follow the histopathological changes and inflammation progression: days 5 and 11 post MIA (early phase) and days 26 and 75 post MIA (late phase). Increased inflammation was apparent at Day 5 and gradually decreased as disease progresses. The inflammatory infiltrate score decreased from 3 to 1. However, damage of the knee joint progressively increased and was most prominent at later phases. The total composite score increased from 0 to 50 at later phases. In parallel to inflammation, cytokines such as IFNγ, IL1β, IL6, TNFa, and the chemokine KC-GRO showed an overall increase at early stages of the disease. TNFa and IL6 maintained high levels throughout disease progression. Using the Dynamic weight bearing assay, we demonstrate that pain, denoted by limited usage of the affected limb appears early and is maintained throughout the progression of the disease. The opioid tramadol and the glucocorticoid dexamethasone, given at early or late stages of the disease, alleviated pain through different mechanisms: tramadol targets CNS pain perception whereas dexamethasone decreases inflammation and modifies disease progression. In conclusion, MIA model of OA reliably recapitulates the disease progression observed in humans. This model delineates the biphasic component of the disease and constitutes an adequate model for the study of the mechanisms of OA and subsequently the use of potential candidates in preventive or therapeutic approaches.
Global contamination by perfluoroalkyl substances is an emerging environmental concern. Due to the known market capacity and the complexity to grow endocrine alterations, this work aimed to investigate the toxicity of perfluorooctanoic acid (PFOA) on sperm kinetics and larval viability on the most economically important fish species in Colombia, Prochilodus magdalenae (bocachico). Semen production in bocachico was induced hormonally in the spawning season to obtain appropriate seminal volume. The semen was activated with Milli-Q water or water containing different PFOA concentrations (1, 3, 10 µM). Motility (total and types), progressive and linear velocity (linear and curvilinear) were assessed by direct microscopy observation and processed using the software Sperm Class Analyzer. One- or ten-days old fish larvae were treated with different PFOA concentrations for 24 h on microplates. Total motility, progressive and linear velocities of spermatozoa were not altered at concentrations lower than 30 µM. However, concentrations greater than or equal to 300 µM significantly affected the sperm kinetics of bocachico, in particular progressive (99 µM, 91±5.6 % vs control, 87±2.1 %) and linear velocity (96 µM, 16±2.1 %/s vs control, 14±1.7 %/s). Similarly, PFOA levels between 10 and 1000 µM were lethal to 1- and 12-days old larvae, effect observed even after 1 h treatment. The results showed that exposure to PFOA causes deleterious effects in the early developmental stages of P. magdalenae, probably increasing the risk of reduction in the fertility and reproductive rate of this species in the wild. Uncartagna (Support to Research Groups and Doctoral Formation, 2021,2022; Grant 114/2020). MinCiencias (Doctoral Formation, 2020-2021). SGR BPIN 20200010362.

3051 Paving with Pathology: Sulfur Mustard Wounds


Gulf War Illness (GWI) is a chronic multisymptomatic illness that persists among the veterans of Gulf War (GW) till date. Among the major symptoms reported by the veterans, cognitive disruption, memory loss and neurological dysfunctions were most prevalent. Transient receptor potential vanilloid 4 (TRPV4), an osmo-mechano sensitive calcium channel is known to be expressed in the brain endothelial cells and associated with blood brain barrier (BBB) regulation. Deletion of trpv4 has been found to exacerbate inflammation via increased oxidative stress. In this study we investigated the role of TRPV4 in GW chemicals pyridostigmine bromide and perethrin induced neuroinflammation in an experimental GW model using adult C57BL/6J and TRPV4 knockout (TRPV4KO) mice. Significant decrease in the expression of tight junction protein Claudin 5 and increased expression of matrix metalloprotease 9 was observed in TRPV4KO mice exposed with GW chemicals compared to GW chemical exposed wild type mice. Increased astrocyte activation, expression of oxidative stress marker cytochrome P4502E1 (CYP2E1) and proinflammatory cytokines IL-1β and IL-6 was observed in GW chemical exposed TRPV4KO mice compared to wild type mice. Further, decreased expression of neuroplasticity marker brain derived neurotrophic factor (BDNF) and increased expression of Tau protein in TRPV4KO mice exposed with GW chemicals as compared to wild type mice group indicated neurodegeneration in TRPV4KO mice. Further, administration of diallyl sulfide (DAS), an inhibitor of CYP2E1 in GW chemical exposed TRPV4KO mice ameliorated BBB disruption, oxidative stress and neuroinflammation which was observed by an increase in the expression of tight junction protein and decrease in the expression of proinflammatory cytokines in this mice group. Increase in expression of BDNF and decrease in expression of Tau protein in TRPV4KO mice exposed with GW chemicals and DAS was also observed. Results suggested that TRPV4 has a mechanistic role in regulating GW chemical induced brain disruption and neuroinflammation by suppressing CYP2E1 mediated oxidative stress which worsened in GW chemical exposed TRPV4KO mice. Hence, TRPV4 based therapeutic strategies could be beneficial in ameliorating GW chemical induced neurological symptoms. Study supported by DoD grant W81XWH18103574 and VA Ment Award 10ICX01923-D1 awarded to Dr. S. Chatterjee.

3052 Downscaled Sinclair vs. Göttingen Minipigs: Similarities and Differences of Toxicological Reference Range Data in Preclinical Safety Studies


Minipigs (MP) are recognized as offering advantages over other established non-ro- dent models such as beagle dogs based upon substantial evidence of similarities in humans with regard to anatomy, physiology, and biochemistry. Currently, MPs are used increasingly in nonclinical CROs and (bio)pharmaceutical industry to support IND enabling toxicology studies. However, similarities and differences in toxicological reference data between the commonly used Gottingen and Sinclair breeds have not been reported. To provide scientific justification for selection of the most appropriate strain of MP for clients’ drug development program, and as part of the Altasciences Historical Control Database initiative, this study was performed to compare reference data for indicators of toxicology including pathology and cross-referenced with physiological and biochemical parameters obtained from Sinclair and Göttingen MP studies conducted at Altasciences’ Columbia site. Data for Gottingen MPs was extracted from electronic data capture system (Pristima®) and compared with the reference data of downsized Sinclair MPs (Book of Normals 2021; SBR), including body weight, clinical pathology (hematology, serum chemistry, coagulation, urinalysis), organ weights and histopathology background lesions of a panel of tissues from nine physiologically relevant organs. Multiple statistical analyses including mean and Standard Deviation (SD), range (min, max), fold difference of average, quartile, interquartile range (IQR) and Tukey fence (upper and lower limit) were used for data comparison. Both Sinclair and Gottingen tissue weight to body weight ratio will be reported and discussed. Based on data comparison in this study, all apparent differences among clinical pathology, organ weight and background microscopic findings between Sinclair vs Gottingen MPs were considered minor (1.5-fold and 2.5-fold, respectively) and females (1.3-fold and 1.4-fold, respectively). The sex differences of organ weight to brain or body weight ratio were also observed, as the heart and adrenal weights to brain ratio were lower, while thymus weight to body weight ratio was higher in male (only) Sinclair MPs as compared to Gottingen MPs. The most common microscopic finding noted in Sinclair MPs was multifocal lymphocytic and monocytic infiltration in various organs, the incidence of which was significantly different as well as similarities of spontaneous histopathological findings and incidence rate will be reported and discussed. Based on data comparison in this study, all apparent differences among clinical pathology, organ weight and background microscopic findings between Sinclair vs Gottingen MPs were considered minor and of no biological significance. This study demonstrates that the use of Sinclair MP strain selection can be justified for preclinical studies using downsized Sinclair MPs, which
The minipig is a valid non-rodent animal model that is infrequently utilized for nonclinical pharmaceutical development relative to either dog or non-human primate (NHP). A 2014 IQ DruSafe survey indicated minipig use was largely limited to studies evaluating small molecule dermal products. The 2022 survey revealed an incremental increase in minipig use, primarily in the development of protein molecules and oral small molecule products. Despite this increased usage, the minipig still represents relatively small percentage (generally 5% or less) of all non-rodents used in pharmaceutical development. Based on survey responses, key challenges to wider minipig use are (1) limited efficacy models in pigs compared to other animal species; (2) lack of limited historical control data; (3) lack of relevant reagents to assess cross-reactivity, pharmacology or toxicity which leads to inconsistent use of minipig for assessing pharmacologic activity and immuno-genicity risk for biologic products and (4) requirement for larger amounts of test article to conduct in vivo studies. IQ DruSafe companies have also expressed concerns regarding in-house capabilities, training and handling of minipigs as well as uncertainties regarding capabilities and experience of using minipig at CROs. The EU- and UK-based member companies commented that there are increased considerations of utilizing minipigs due to NHP shortage as well as ethical considerations related to NHP and dog usage, but this has not translated to more routine minipig implementation. The results of this 2022 IQ DruSafe survey indicate that many of the concerns previously identified in 2014 persist. Focused, ongoing, industry-wide efforts to address these challenges may assist in more frequent consideration of minipig as a species for nonclinical pharmaceutical development.
In silico toxicity protocols are meant to support computationally based assessments using principles that ensure that results can be generated, recorded, communicated, and evaluated, and then evaluated in a uniform, consistent, and reproducible manner. We investigated the availability of in silico models to predict the ten key characteristics (KC) of carcinogens for a nongenotoxic compound, pregabalin, in a single-species carcinogenic producing only one type of tumor, the hemangiosarcomas in mice. The overall goal of this exercise is to test the ability of in silico models to predict nongenotoxic carcinogenicity with pregabalin as a case study. The established mode of action (MOA) of pregabalin is triggered by tissue hypoxia, leading to oxidative stress (KCS), chronic inflammation (KC6), and increased cell proliferation (KC10) of endothelial cells. Of these KCS, the most relevant to the current evaluation is reliability. In silico methods are meant to support computationally based assessments using principles that ensure that results can be generated, recorded, communicated, and evaluated, and then evaluated in a uniform, consistent, and reproducible manner. We investigated the availability of in silico models to predict the ten key characteristics (KC) of carcinogens for a nongenotoxic compound, pregabalin, in a single-species carcinogenic producing only one type of tumor, the hemangiosarcomas in mice. The overall goal of this exercise is to test the ability of in silico models to predict nongenotoxic carcinogenicity with pregabalin as a case study. The established mode of action (MOA) of pregabalin is triggered by tissue hypoxia, leading to oxidative stress (KCS), chronic inflammation (KC6), and increased cell proliferation (KC10) of endothelial cells. Of these KCS, the most relevant to the current evaluation is reliability.
Reproductive toxicity testing is recommended by regulatory agencies during product development and the safety evaluation process. Traditional reproductive toxicity testing in animals, especially guideline multigeneration reproductive toxicity studies, is time-consuming and expensive, which makes it infeasible to test thousands of chemicals in animals. Therefore, machine learning, as a promising alternative approach, should be considered when evaluating the reproductive toxicity of chemicals. We constructed predictive models from seven machine learning algorithms (decision tree, decision forest, random forest, k-nearest neighbors, support vector machine, linear discriminant analysis, and logistic regression) using rat multigeneration reproductive toxicity data of 275 chemicals from Toxicity Reference Database (ToxRefDB). A consensus model was generated based on the seven individual models. The performances of individual and consensus models were evaluated using 500 iterations of 5-fold cross-validations, and the external validation data set curated from the COSMOS database and the literature. The balanced accuracy of the models ranged from 58% to 65% in the 5-fold cross-validations and 45% to 61% in the external validations. Prediction confidence was calculated. The balanced accuracy of the models ranged from 58% to 65% in the 5-fold cross-validations and 45% to 61% in the external validations. Prediction confidence was calculated. The balanced accuracy of the models ranged from 58% to 65% in the 5-fold cross-validations and 45% to 61% in the external validations. Prediction confidence was calculated. The balanced accuracy of the models ranged from 58% to 65% in the 5-fold cross-validations and 45% to 61% in the external validations. Prediction confidence was calculated. The balanced accuracy of the models ranged from 58% to 65% in the 5-fold cross-validations and 45% to 61% in the external validations. Confidence in machine learning models. We demonstrate the importance of using consensus models for harnessing the benefits of multiple machine learning models (i.e., by reducing redundant systems to check validity of outcomes). While we continue to build upon our models to better characterize weak toxicants, there is sound potential in saving resources by eliminating strong reproductive toxicants before investing in in vivo modeling. The modeling approach (machine learning models) is offered for assessing the rat multigeneration reproductive toxicity of chemicals. Our results suggest that machine learning may be a promising alternative approach to include in the evaluation of the potential reproductive toxicity of chemicals. Disclaimer: This abstract reflects the views of the authors and does not necessarily reflect those of the US FDA.
Extractable and leachable chemicals can potentially pass from manufacturing materials, packaging materials and container closure systems into pharmaceutical drug products. They are also important in the context of medical devices, as chemicals can leach from a device into any surrounding tissue. In both cases E&Ls need to be assessed for their toxicological safety, with the major endpoints of concern being mutagenicity/carcinogenicity, sensitisation, and systemic toxicity. This study sought to investigate the role that in silico models, including expert systems and various statistical models, could play in predicting the sensitisation hazard and potency of E&Ls as part of a wider toxicity risk assessment. 790 E&L chemical structures were collected from the published ELsie and PQRI databases, and publicly available dermal and respiratory sensitisation data was located for 297 of these 790. 290 had dermal sensitisation hazard data, 106 had dermal sensitisation potency data, and 47 had respiratory sensitisation hazard data. This in vivo data was used to assess the performance of various in silico sensitisation models and combinations thereof. To explore the predictivity of the available expert knowledge, the skin sensitisation structural alerts from the Derek Nexus expert system were investigated. When used to predict the sensitisation hazard of E&Ls, the balanced accuracy was 79% for dermal sensitisation and 84% for respiratory sensitisation. A hybrid expert knowledge/statistical approach, including an alert-based k-nearest neighbours (k-NN) model and the recently updated Dermal Sensitisation Thresholds and High Potency Category rules, was able to predict whether an E&L is a non-sensitiser, a weak/medium sensitiser, or a strong/extreme sensitiser with 76% accuracy and 100% coverage. To investigate the performance of purely statistical systems, 75 different models were built and evaluated, using 3 training sets, 5 algorithms and 5 chemical descriptors. The model algorithms investigated included self-organising hypothesis networks (SOHN) with 2 sets of hyperparameters, random forests, k-NN models and support vector machines (SVM). The training sets consisted of Local Lymph Node Assay (LLNA) data, alone or in combination with human and guinea pig data. Principal component analysis was used to show that the test set had a good overlap (92-93%) with the chemical space of the different training sets, although there were isolated classes of E&Ls which were not well-represented in the training sets (e.g. phenolic antioxidants and UV absorbers). The balanced accuracies for these models in predicting the dermal sensitisation hazard of E&Ls ranged from 49 to 85%. The best performing model for the test set used in this study, considering both predictivity and coverage, was a SOHN using a circular fingerprint and trained on all available LLNA, human and guinea pig data. This model had a balanced accuracy of 76% and a coverage of 73%. Combining this model with Derek Nexus in a conservative fashion led to an overall balanced accuracy of 80% and a coverage of 100%. As the science involved in the toxicological risk assessment of extractables and leachables advances, in silico approaches are expected to play an increasingly important role. This is especially pertinent in the context of the upcoming ICH Q3E guidelines which hope will bring much-needed clarity and international harmonisation to this area. This study has sought to investigate a wide range of expert knowledge-based and statistical in silico sensitisation models, to see which approaches (on their own or in combination) would be best placed to help assess the sensitisation risk of E&Ls moving forwards.

### 3069 An Integrated Testing Strategy Based on In Vitro Phenotypic and Transcriptomic Data for Selecting Representative Petroleum UVCBs for Toxicity Evaluation In Vivo

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Hazard evaluation of substances of unknown or variable composition, complex reaction products and biological materials’ (UVCBs) has been a major challenge in regulatory science. The chemical composition of UVCBs is difficult to ascertain using analytical techniques. Therefore, alternative data streams have been proposed for grouping UVCBs and selection of potential ‘worst case’ substances for further toxicity evaluation in vivo. Petroleum substances are representative UVCBs and human cell-based data has previously been used to both substantiate and critique current manufacturing process-based groupings; however, the regulatory agencies have questioned the utility of such data. In this study, we hypothesized that a combination of phenotypic and transcriptomic data on petroleum UVCBs can be integrated to increase confidence and make protective decisions as to selection of petroleum UVCBs for additional toxicity evaluation. To test this hypothesis, we used phenotypic and transcriptomic data obtained from testing 141 petroleum UVCBs comprising of 16 manufacturing categories in six human cell types (induced pluripotent stem cell (iPSC)-derived hepatocytes, cardiomyocytes, neurons, and endothelial cells, and MCF7 and A375 cell lines). Benchmark doses for gene-substance combinations were calculated using published data (e.g. the IC30 and IC50) for each cell type, and the phenotype and transcriptome data were used to calculate an integrated testing strategy based on the IC50 derived hepatocytes and cardiomyocytes can inform selection of representative “worst case” petroleum UVCBs from each manufacturing class for further toxicity evaluation in vivo.
cardiomyocytes. Previously published population-based concentration-response profiles from hiPSC-derived cardiomyocytes with 56 drugs and 82 bioanomalous chemical exposures (including flame retardants, food additives, industrial chemicals, metal, PAH, and pesticides) were used to derive point-of-departure (POD) values for eight phenotypes viability and function by using Bayesian modeling. We constructed a probit regression model with the drug dataset, using POD values as predictors for mortality and positive chronotropic effects (TPD) and for forced cessation to assess model performance. Comparisons of the area under the curve (AUC) of receiver operator curves (ROC) demonstrated that using five of eight phenotypes as predictors (cytotoxicity, QT prolongation, positive chronotrope, negative chronotrope, and asystole), we could reasonably categorize TPD risk with an AUC of 0.87, having similar or better performance to previously published hiPSC-based models for pharmaceuticals. We then applied this model, optimized for maximal balanced accuracy (>0.83), to non-chemical compounds, and predicted four pesticides (Rotenone, Deltamethrin, Tebuconazole, and Dichlorodiphenyldichloroethene) and one food additive (Quinone hydrochloride dihydrate) as having potential proarrhythmic risk. We conclude that an integrated in vitro-in silico approach using population-wide hiPSC-derived cardiomyocytes, Bayesian concentration-response modeling, and probit regression, can provide a reasonable approach to predicting the potential proarrhythmic risk for environmental chemicals.

3071 Prediction of Drug-Induced Liver Injury and Cardiotoxicity Using Chemical Structure and In Vitro Assay Data
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Drug-induced liver injury (DILI) and cardiotoxicity (DICT) are major adverse effects triggered by drug candidates and some approved drugs. To provide an alternative to in vivo toxicity testing, the U.S. Tox21 consortium has screened a collection of ~10k compounds in ~20 HTS assays. However, the relative unsuitability of HTS data to build a quantitative high-throughput screening (qHTS) format. In this study, we compiled reference compound lists for DILI and DICT and compared the potential of Tox21 assay data with chemical structure information in building prediction models for human in vivo hepatotoxicity and cardiotoxicity. Models were built with four different machine learning algorithms (e.g., Random Forest, Naïve Bayes, Extreme Gradient Boosting, and Support Vector Machine) and model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC-ROC). Chemical structure-based models showed reasonable predictive power for DILI (best AUC-ROC = 0.75 ± 0.03) and DICT (best AUC-ROC = 0.83 ± 0.03), while Tox21 assay data alone only showed better than random performance. DILI and DICT prediction models built using a combination of assay data and chemical structure information did not have a positive impact on model performance. The suboptimal predictive performance of the assay data is likely due to insufficient coverage of an adequately predictive number of toxicity mechanisms. The Tox21 consortium is currently expanding the coverage of biological response space with additional assays that probe toxicologically important targets and under-represented pathways that may improve the prediction of in vivo toxicity such as DILI and DICT.

3072 An Endpoint-Specific Framework for Read-Across Analog Selection for Human Health Effects
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Integrating computational chemistry and toxicology can improve the read-across analog approach to fill data gaps in chemical safety assessment. In read-across, structural alerts are compared between target and reference compounds using defined test data and one or more materials with sufficient data. Recent advances have focused on enhancing the grouping or clustering of chemicals to facilitate toxicity prediction via read-across. Analog selection purports relevant features, such as physical-chemical properties, toxicokinetic-related properties (bioavailability, metabolism, and degradation pathways), and toxicodynamic properties of chemicals with an emphasis on mechanisms or modes of action. However, each human health endpoint (genotoxicity, skin sensitization, phototoxicity, repeated dose toxicity, reproductive toxicity, and local respiratory toxicity) provides a different critical context for analog selection. Here six endpoint-specific, rule-based schemes are described. Each scheme creates an endpoint-specific workflow for filling the target material data gap by read-across. These schemes are intended to create a transparent rationale that supports the selected read-across analog(s) for the specific endpoint under study. This framework can systematically drive the selection of read-across analogs for each endpoint, thereby accelerating the safety assessment process.

3073 Toxicogenomics Data for Chemical Safety Assessment and Development of New Approach Methodologies: An Adverse Outcome Pathway-Based Approach
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Mechanistic toxicology is emerging as a powerful approach for chemical safety assessment and development. It focuses on the mechanisms through which chemicals induce their effects, hence also providing valuable insight into the development of safe-by-design compounds. Omics technologies are central to dissecting these mechanisms and are widely applied in the context of toxicogenomics. However, despite the immense potential of toxicogenomic approaches, their implementation into the regulatory framework is hindered by the lack of standardization, and uncertainties in the analysis and interpretation of omics data. At the same time, the lack of mechanistic evidence in the context of Adverse Outcome Pathways (AOPs) is promoted for the development of New Approach Methodologies (NAMs) that can offer solutions to the pressing need for faster, cheaper, and more ethical safety assessment of chemicals. We hypothesized, that embedding toxicogenomics into the AOP framework would unleash the full potential of AOPs while also building regulatory confidence towards toxicogenomics. The modelling of AOPs with molecular data would support systematic development of NAMs and reduce the need for multiple testing strategies. Similarly, it would guide the translation of complex molecular signatures captured by omics technologies into meaningful biological interpretations. Hence, we developed a multi-step strategy to systematically annotate molecular events into AOPs through their Key Events (KEs) and applied it to all currently available AOPs relevant for human health risk assessment. Using the resulting associations, we successfully highlighted relevant adverse outcomes for chemical exposures from their associated gene signatures. Furthermore, we established the concept of an AOP fingerprint and showed strong in vitro to in vivo convergence with independent toxicogenomic data sets for drugs in clinically validated cardiovascular effects in vitro and cardiac biomarkers for pulmonary fibrosis. These results suggest that the established associations strongly support meaningful interpretation of toxicogenomic data and guide the development of data-driven computational methodologies for chemical safety assessment.

3074 Physiologically Based Kinetic Modeling--Based Prediction of the Relative Potency of Pyrrolizidine Alkaloid N-Oxides at Realistic Low-Dose Levels
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Pyrrolizidine alkaloids (PAs) are plant toxins found in food and feed, often existing in their N-oxide form (PA N-oxides). PA N-oxides are often considered to be less toxic than their parent PAs because PA N-oxides first need to be reduced by liver CYP450 and gut microbiota to the corresponding PAs. The PAs can convert to reactive pyrolyte metabolites that form adducts with proteins and DNA, ultimately causing toxic effects. In a worst-case scenario, full conversion of PA to PA N-oxide to their parent PAs is considered, and PA N-oxides are assumed to be equally toxic as their parent PAs (relative potency or REP value of 1 compared to the respective parent PA). On the other hand, based on experimental data from in vitro studies, the PA N-oxides are reported to be significantly less toxic than the corresponding PAs (REP value of 0.01) mainly because these in vitro studies do not consider reduction by gut microbiota. In vivo studies at realistic dose levels tend to show the potency of the PA N-oxides to be lower than that of their parent PAs with REP values varying between 0.61 and 0.88. The present study aimed to define the REP value of macrocyclic diester PA N-oxides compared to their corresponding parent PA at low dose levels using in vitro and in vivo-derived parameters, physiologically based kinetic (PBK) models for PA N-oxides (a sub-model for PAs) were built for both rats and humans. The REP value was subsequently calculated as the ratio of the PBK model predicted area under the concentration-time curve (AUC) 0.24h of the parent PA upon oral dosing of the PA N-oxide and the predicted PA AUC 0.24h upon oral administration of an equimolar dose of the corresponding PA. In both rats and humans, the REP value thus obtained appeared to decrease with increasing dose levels. At low dose levels, the predicted REP value of seneconine N-oxide relative to seneconine was higher than at dose levels used in the animal experiments, amounting to 0.89 for humans and 0.84 for rats. The modeling also confirmed the experimental observation reported for seneconine N-oxide that the REP value varies with the endpoint used. At the high dose levels used in animal experiments, the REP value based on the ratio of the plasma AUC 0.24h for the parent PA is higher than the REP value calculated based on the level of the amount for pyrolyte-protein adduct formation. Incorporation of GSH pyrolyte adduct formation in the PBK models revealed that at high dose levels, saturation of the GSH-dependent detoxification capacity results in relatively higher levels of pyrolyte-protein adducts. This may happen more easily at an equimolar dose of the PA than of the PA N-oxide since the plasma concentrations and maximum plasma concentration (Cmax) of the PA are higher upon dosing the PA than upon dosing the PA N-oxide. This causes a lower REP value when calculated.
based on the amount of pyrrole-protein levels than what would be expected based on the plasma PA levels, and the REP values will be independent of the endpoint used. Taken together, our PKB modeling provides insight into factors influencing the REP values of PA N-oxides relative to their parent PAs and allows prediction of these REP values in humans and at realistic low dose levels which would be experimentally less accessible.

### 3075 Identification of Adverse Outcome Pathway (AOP) Network Leading to Pulmonary Fibrosis and Its Application to Inhalation Toxicants Screening: Integrative Data Mining Approach Using Comparative Toxicogenomics Database and AOP Wiki


An adverse outcome pathway (AOP) framework can be applied as an efficient tool for the rapid screening of environmental chemicals. For the development of an AOP, a database mining approach can support an expert derivation approach by gathering a wider range of evidence than in a literature review. In this study, data from various databases were integrated and analyzed to supplement the AOP leading to pulmonary fibrosis by analyzing additional evidence using a data mining approach and establishing an application domain for chemicals. First, we collected chemicals, genes, and phenotypes that were studied and related to pulmonary fibrosis through the Comparative Toxicogenomics Database (CTD). CGP-tetramers constructed by linking each related chemical, gene, phenotype, and disease can provide the basic components for the assembly of putative AOPs. Next, an AOP network was established by connecting eight existing AOPs for pulmonary fibrosis developed by expert derivation from the AOP Wiki. Finally, the pulmonary fibrosis AOP network was proposed by integrating the AOP network from AOP Wiki and the CGP-tetramers from the CTD. To prioritize potential chemical stressors in the AOP network, 61 chemicals were ranked using the relevance of the chemical to the AOP and chemical exposure information from the CompTox Chemicals Dashboard. The approach proposed in this study can guide the utilization of available evidence from various databases as well as the literature in constructing AOP networks related to specific diseases. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through ‘Environmental Health R&D Program’ (202100331005).

### 3076 ToxRefDB V2.1: A Minor Update to the Toxicity Reference Database


A component of building scientific confidence in new approach methodologies (NAMs) for toxicology is comparison to results from in vivo studies. However, these efforts require NAM and animal study data to be computationally accessible and interoperable. The Toxicity Reference Database (ToxRefDB) aggregates in vivo data from nearly 6000 studies for over 1000 chemicals. Developed via a manual curation workflow, ToxRefDB serves as a resource for study design, quantitative dose response, and endpoint testing status information. Recently, we added guidelines from the US EPA and National Toxicology Program (NTTP). Study coverage includes a variety of repeat dose study designs, such as chronic, subchronic, subacute, prenatal developmental, and multisgenerational reproductive, utilizing various administration routes. Many of the studies come from registries submitted to toxicity studies known as data evaluation records (DERs) from the US EPA’s Office of Pesticide Programs (OPP), but continued curation efforts have expanded ToxRefDB to include toxicity studies from additional sources, including NTP reports, peer-reviewed primary research articles, and pharmaceutical pre-clinical studies. An important component of ToxRefDB is its controlled vocabulary for studies and effects observed for enhanced data quality. ToxRefDB v2.1 is a data update of ToxRefDB v2.0 to correct issues discovered with the compilation script that caused some extracted values to not import properly from AccessDB curation files, such as failure to import some effects. No additional curation was performed for the v2.1 update. Although the overall number of studies and chemicals remains unchanged, the v2.1 update includes additional data as previously curated studies with extracted dose treatment groups and effects are now fully accessible. This added data can improve the utility of ToxRefDB as a resource for curated legacy in vivo information by providing more comprehensive information of the past animal studies conducted. Moving forward, an application-driven workflow with the Data Collection Tool (DCT) will be utilized to create a more sustainable process for loading curated information to a database and support a more regular release cycle. Continued development increases ToxRefDB’s utility as a resource for retrospective analyses that lay the foundation for acceptance of NAMs, as well as development of new predictive tools. This abstract does not necessarily reflect US EPA policy.

### 3077 Data Fusion by Matrix Completion for Exposome Target Interaction Prediction

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Exposure to toxic chemicals presents a huge health burden and disease risk. Determining the molecular target(s) of toxic chemicals is key to understanding and predicting which chemicals cause specific toxic effects. Given that over five hundred new chemicals enter regulatory review each year in the US under the Toxic Substances Control Act, it is infeasible to perform a comprehensive safety assessment for all of these new chemicals due to limited resources. Thus, a robust computational method for discovering targets of toxic exposures, which can then be used to prioritize chemicals for further analysis, is a promising direction for public health research. In this study, we implemented a novel matrix completion algorithm named coupled matrix-matrix completion (CMMC) for predicting exposome-target interactions. The low rank matrix completion problem can be described as predicting missing values in incomplete under-oberved or partially observed matrix. Different from previous matrix completion algorithms based on matrix factorization, the CMMC algorithm incorporates two coupled matrices in form of auxiliary information to help better optimize the distance metric and maintains a fast runtime. The two coupled matrices represent the relationships among the chemical exposures and gene targets, respectively, and help jointly analyze incomplete data sets. We collected and processed relevant data from the Comparative Toxicogenomics Database (CTD) as a benchmark dataset for implementing and testing our method and compared it to alternative prediction methods. Three benchmark datasets of different sizes were used to compare our method with the CMMC, Collaborative Matrix Factorization (CMF), Graph-Regularized Matrix Factorization, and WGRMF (Weighted Graph-Regularized Matrix Factorization) on the same benchmark datasets. The area under the ROC curve (AUC) was used to compare the performance of the aforementioned methods. Our CMMC achieved the highest AUCs for all three sizes of input datasets with an average AUC of 0.893 ± 0.012 using the 400×600 dataset. A case study with bisphenol A (BPA) and three of its structurally related replacements: BPF, BPF, and BPS, was used to test our method for predicting target genes of chemical exposures without any prior knowledge of biological effects. To perform a comprehensive case study, we generated 200 different datasets, each with 200 randomly selected chemicals (300 chemicals in total including the case chemical) and 500 genes from a total 5000 chemicals and 10,000 genes for each case-study chemical, respectively. In all 200 chemical-gene interaction matrices for each case-study chemical, all known interaction values were removed to mimic a real-life scenario where we are assessing a new untested chemical. Six known target genes (i.e., ESR1, ESRI, AR, NR1I2, PGR, PPARG, and PPARD) for the four bisphenol chemicals were successfully predicted by CMMC. The case study shows that CMMC can be used to discover potential molecular targets of novel chemicals without any prior bioactivity knowledge. Our CMMC approach is a powerful method for predicting the target genes of toxic exposures.

### 3078 Integrating Adverse Outcome Pathway and Biological Pathway Knowledge in Interpretable Deep Learning Modeling Framework for Hepatotoxicity Predictions


Current machine learning approaches to evaluate hepatotoxicity are often black-box and incapable of explaining toxicity mechanisms. To address this challenge, we developed a domain network (DNN) approach that utilizes the adverse outcome pathway (AOP) framework and the Reactome pathway hierarchy to construct DNN architecture for predicting compound hepatotoxicity and inferring potential toxicity pathway mechanisms. We curated 500 compounds with known hepatotoxicity classifications as the modeling set and further randomly split it into training (400 compounds) and test sets (100 compounds). Experimental data of these compounds, which represent interactions with receptors or enzymes as molecular initiating events in AOPs and gene expression regulations, were collected from ToxCast/Tox21 and L1000 programs. The structural, biological, and transcriptomic data of the training set compounds were used to train a DNN model of hepatotoxicity using biological pathway information obtained from Reactome. Five-fold cross-validation using 400 training set compounds and external validation using 100 test set compounds showed good predictivity with the area under ROC curve values of 0.77 and 0.67, respectively. The resulting DNN model can predict new compounds as potential toxicants to the liver if they show active responses in the key events represented as neurons within the network. The pathway from the toxicant to a toxic event contains regulatory functions that can modulate gene expressions and, in turn, key events that induce hepatotoxicity can reveal plausible toxicity mechanisms. This study provides an interpretable machine learning strategy to utilize public high-throughput screening and transcriptome data to characterize hazards and prioritize potential toxicants.
Toxicology is one of the biggest drivers for compound attrition and identifying toxicity signals at the early discovery stages of the project and guiding to safer space significantly increases the chance of its success. We present here a computational "safety by design" toolbox (deployed on Live design) for various toxicological endpoints that can be employed during the lead generation stages. Based on the requirement of the projects we create global/local models in the toolbox, to lead molecular disemery to a chemical space likely to be safe and effective. Our toolbox consists of several validated machine-learning and structure-based models that can be used by medicinal chemists. In this presentation, we are going to show one case study that will demonstrate safety by design in action. We have chosen a kinase project for this presentation, we have leveraged historical data and framed hypotheses, and the "safety by design" toolbox helped the project address selectivity against various off target kinase and prioritize chemotype in lead generation stage. Overall, the developed toolbox is used by medicinal chemists and chemical toxicologists interactively for safe and effective molecule discovery.

3082 Evaluating Performance of In Silico Models in Predicting Dermal Sensitization to Support Occupational Toxicological Data Gap Filling Approaches
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Occupational skin diseases are one of the most common workplace diseases with an estimated 2.5 million cases in the US each year, potentially leading to chemical skin. Such exposures can result in irritant or allergic contact dermatitis. Therefore, it is important to assess dermal hazards in an occupational setting to protect worker health. Given the advancement in testing methodologies as well as an ever-growing goal of moving away from conducting animal studies, it is imperative that alternative hazard assessment methods are utilized for assessing dermal exposure related hazards. The OECD 497 test guideline provides guidance on the application of in silico models to predict skin sensitization. In the pharmaceutical industry, in silico methods are especially informative when relevant in vivo data to identify occupational hazards such as dermal sensitization and irritation are unavailable for novel compounds. With in silico tools, three in vivo (clinical and rule-based) were evaluated to determine their ability to predict dermal sensitizers in the Roche pharmaceutical chemical space. Eighty-nine compounds with historical in vivo experimental sensitization data were selected for comparison with in silico model predictions. Model predictions were evaluated for sensitivity, specificity, and balanced accuracy. An expert review was also incorporated to interpret the results. Model sensitivity ranged from 60-75%, and specificity ranged from 68-77%, respectively. Balanced accuracy results ranged from 68-76% demonstrating good concordance with in vivo outcome. Notably, the percentage of compounds outside the models applicability domain (17-51%), that is where a model was unable to make a prediction, illustrated that there is room for improvement in the chemical space coverage perspective. This work highlights the favorable performance of dermal sensitization in silico models in the pharmaceutical chemical space as well as the need to enhance dermal sensitization models from a structural coverage perspective. Through collaboration with model developers, companies have the ability to assist in improving model performance and subsequent utilization for applications within pharmaceutical safety.

3083 Integrating High-Throughput Toxicokinetics Data and Knowledge-Based Deep Neural Network (K-DNN) Methodology to Advance a Computational Adverse Outcome Pathway Framework for Assessing Hepatotoxicity
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Traditional experimental approaches that use animals to identify hepatotoxins are costly and time-consuming. Pathway-based alternatives, such as those that use in vitro assays, are promising for evaluating chemical toxicity. Use of these approaches has proven successful in the development of pathway-based models for simple biological pathways, such as endocrine disruption. However, hepatotoxicants can act through multiple targets and toxicity mechanisms, many of which remain to be fully elucidated. For example, classic nuclear receptors (e.g., pregnane X receptor) and steroid hormone receptors (e.g., estrogen and androgen receptors) are potential off-targets for drugs acting leading to the dosing of liver injury. In this study, we aimed to design a knowledge-based deep neural network (K-DNN) for assessing hepatotoxicity. To this end, we used 869 chemicals with known hepatotoxicity classifications as probe compounds to search for relevant PubChem assay data. We then ranked the assay data by their correlation to hepatotoxicity.
We also obtained high-throughput exposure assay data for 56 compounds and implemented potential exposure calculations using a high-throughput toxicokinetics (httk) toolkit. The average plasma concentrations at steady state (i.e., C_s), which were outputs of the httk toolkit, were implemented as one of the layers of the k-DNN approach. The underlying k-DNN architecture incorporates assay response information from off-target drug binding, cellular responses, and potential liver exposure for predicting whether a given compound is likely to be hepatotoxic. The hidden toxicity pathways can be revealed by identifying the molecular markers from the activated neurons within the network. This approach can accurately prioritize hepatotoxic compounds (AUC = 0.75) and provide valuable insights into the underlying mechanisms, such as oxidative stress and disruption of mitochondrial function. Furthermore, this novel approach applies artificial intelligence to predict likelihood of chemical toxicity risk using integrating toxicokinetic, chemical, and mechanistic biological data. Supported by National Institute of Environmental Health Sciences (NIEHS) grants (R01ES031080, R15ES023148, R35ES031709, and P30ES035022) and National Center for Advancing Translational Sciences (NCATS) grant (UL1TR003017).

**3084 Best Combination of Machine Learning Algorithms and Molecular Fingerprints for Development of Target-Specific Bioactivity Prediction Models Using ToxCast Bioassay Data**


The development of toxicity classification models using the ToxCast database has been extensively studied. Machine learning approaches are effective in identifying the bioactivity of untested chemicals. In this study, the best models for 737 ToxCast assays with target information were identified to develop an effective bioactivity prediction model based on the ToxCast data. For each assay dataset, 30 models were trained by combining six algorithms (gradient boosting tree, random forest, multilayered perceptron network, k-nearest neighborhood, logistic regression, and naive Bayes) and five molecular fingerprint representations of the chemical structure (MACCS, Morgan, layered, RDKit, and pattern). The synthetic minority oversampling technique (SMOTE) was used to balance the data. Of the 737 best models for each assay, 35 models with acceptable performance were selected based on the F1 score and accuracy. Finally, the selected models were analyzed according to the number and type of training data, and the type of algorithm and molecular fingerprints used for learning. It is important to utilize and study existing HTS data because biological activity data enable toxicity evaluation based on the mechanistic of toxicity. We believe that our results can be used as a cornerstone for a wide range of in silico toxicity prediction studies. Acknowledgement: This work was supported by a grant from the Korean Ministry of Environment through ‘Environmental Health R&D Program’ (20210031005).

**3085 Integration of ToxCast Bioassay to Hazard Assessment by Comparison of Human Equivalent Doses with In Vivo Data: Case Study with Phthalates**


The paradigm of toxicology is shifting from observational science to predictive science. New approach methodologies (NAMs) provide a new feasibility for replacing traditional methods; however, low confidence of NAMs is the current main bottleneck for acceptance in regulatory decision-making. Herein, to establish confidence in NAMs for chemical risk assessment, we conducted a retrospective case study on four major phthalates including Diethylhexyl phthalate (DEHP), Dibutyl phthalate (DBP), Diethyl phthalate (DEP) and Di-n-octyl phthalate (DnOP). In our analysis, all the active ToxCast in vitro data were reviewed based on shared mode of action (Acute lethality, Endocrine, Cancer, Cardiotoxicity, DART) annotated by US-NTP to characterize and quantify the contribution metabolism plays are still evolving. A major challenge lies in the lack of a standardized database of human xenobiotic metabolism pathways for environmental chemicals. To address this issue, we developed a metabolic simulation framework called MetSim, comprised of three main components. First, we propose a harmonised graph-based representation for managing xenobiotic metabolism pathway information between different in silico tools and empirical evidence from the literature. This schema is implemented in a MongoDB database to store, retrieve and analyze large-scale metabolic graphs. Second, MetSim includes a standardised application programming interface (API) for available metabolic simulators, including BioTransformer, the OECD Toolbox, and Tissue Metabolism Simulator (TIMES). Third, MetSim includes functions to systematically evaluate the performance of metabolism simulators using recall, precision and overall accuracy on benchmark data sets. Here we report on the overall architecture of MetSim, and performance results for two data sets: (a) 59 drugs (mostly NSAIDs) and their 179 published metabolites, and (b) 718 diverse substances in the EPA Distributed Structure-Searchable Toxicity (DSTox) database and their 1639 metabolites. The 59 drugs were processed with MetSim using BioTransformer (CypReact model with 3 cycles of human Phase I metabo-lism), TIMES (in vivo rat simulator model) and OECD Toolbox (in vitro rat Liver 9), producing 11202, 590, and 393 metabolites, respectively. The recall for BioTransformer, TIMES and OECD Toolbox was 0.62, 0.41 and 0.52, respectively. For the larger DSTox dataset, two cycles of human phase I (CypReact) and one cycle of phase II metabolism were modeled using BioTransformer, and both TIMES and OECD Toolbox using the same two rat liver models, producing 60097, 6654, and 5204 metabolites, respectively. The recall for BioTransformer, TIMES and OECD Toolbox was 0.16, 0.41 and 0.38, respectively. We summarized the performance for each of the 16 chemical class (using ClassyFire) and metabolic simulator. All tools performed well for phenaneroids, piperadines, lactams, and azoles but poorly for pyrrolines, organonitrogen compounds, and nucleotide analogues. BioTransformer performed well for benzoazoxines, benzoazinepines, and quinolines, but poorly for steroids, benzoazinones, and diazines. Conversely, TIMES and the OECD Toolbox performed well for steroids, benzoazinines, and diazines, but poorly for benzoazoxines, diazmines and organooxygen compounds. MetSim provides useful data and insights on the performance and limitations of in silico metabolism tools, which will inform our subsequent efforts in characterising metabolic similarity. This abstract does not reflect EPA policy.

**3086 MetSim: Integrated Programmatic Access and Pathway Management for Xenobiotic Metabolism Simulation Tools**


Metabolic similarity is a key consideration in read-across but approaches to characterizing and quantifying metabolic similarity are still evolving. A major challenge lies in the lack of a standardized database of human xenobiotic metabolism pathways for environmental chemicals. To address this issue, we developed a metabolic simulation framework called MetSim, comprised of three main components. First, we propose a harmonised graph-based representation for managing xenobiotic metabolism pathway information between different in silico tools and empirical evidence from the literature. This schema is implemented in a MongoDB database to store, retrieve and analyze large-scale metabolic graphs. Second, MetSim includes a standardised application programming interface (API) for available metabolic simulators, including BioTransformer, the OECD Toolbox, and Tissue Metabolism Simulator (TIMES). Third, MetSim includes functions to systematically evaluate the performance of metabolism simulators using recall, precision and overall accuracy on benchmark data sets. Here we report on the overall architecture of MetSim, and performance results for two data sets: (a) 59 drugs (mostly NSAIDs) and their 179 published metabolites, and (b) 718 diverse substances in the EPA Distributed Structure-Searchable Toxicity (DSTox) database and their 1639 metabolites. The 59 drugs were processed with MetSim using BioTransformer (CypReact model with 3 cycles of human Phase I metabolism), TIMES (in vivo rat simulator model) and OECD Toolbox (in vitro rat Liver 9), producing 11202, 590, and 393 metabolites, respectively. The recall for BioTransformer, TIMES and OECD Toolbox was 0.62, 0.41 and 0.52, respectively. For the larger DSTox dataset, two cycles of human phase I (CypReact) and one cycle of phase II metabolism were modeled using BioTransformer, and both TIMES and OECD Toolbox using the same two rat liver models, producing 60097, 6654, and 5204 metabolites, respectively. The recall for BioTransformer, TIMES and OECD Toolbox was 0.16, 0.41 and 0.38, respectively. We summarized the performance for each of the 16 chemical class (using ClassyFire) and metabolic simulator. All tools performed well for phenaneroids, piperadines, lactams, and azoles but poorly for pyrrolines, organonitrogen compounds, and nucleotide analogues. BioTransformer performed well for benzoazoxines, benzoazinepines, and quinolines, but poorly for steroids, benzoazinones, and diazines. Conversely, TIMES and the OECD Toolbox performed well for steroids, benzoazinines, and diazines, but poorly for benzoazoxines, diazmines and organooxygen compounds. MetSim provides useful data and insights on the performance and limitations of in silico metabolism tools, which will inform our subsequent efforts in characterising metabolic similarity. This abstract does not reflect EPA policy.

**3087 Mitotic Figure Detection in Rat Liver Using Supervised Deep Learning—Based Object Detection Models**

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Accurate identification and discrimination of small, rare findings in liver, i.e., single mitotic figures, remains a challenge in the rat liver. Mitotic figures and hematoxylin and eosin (H&E) stained histological sections in toxicologic pathology studies. However, visual grading of those rare events can be challenging and can be aided with quantitative analysis. AI object detection (OD) models can be trained with peer-reviewed annotations of rare findings on whole slide images and can detect them on unseen slides and studies in an efficient and standardised manner. This method was applied to mitotic figure detection in rat liver tissue, showing high precisions and recalls. 175 whole slide H&E images of rat livers across 7 studies were reviewed and annotated by pathologists in Patholytix Study Browser software. 576 annotations in the format of rectangular boxes around mitotic cells were created. The dataset was split into a Train/Test/Validate set with a ratio of 70/15/15 slides respectively. OD models took these annotation boxes as inputs and would output prediction boxes around the rare cells to highlight them. Three classic OD algorithms were investigated here including FrCNN, RetinaNet and EfficientDet. FrCNN is a state-of-art two-stage model with a region proposed network while the other two are one-stage detectors. Faster R-CNN model gave the best performance out of the three algorithms explored based on a higher average precision over 0.95 than the other two models. Crossfold validation at the tile level showed that a good prediction performance was achieved with an average recall of 0.98. Mitotic figures of various mitotic phases were detected reliably. This method-ology enables reliable detection of mitotic figures in the rat liver slides, helps the toxicologic pathologist to focus on specific areas of interest within tissues more rapidly and opens up the possibility of computer-assisted object counting enabling exact mitosis quantification.
New approach methodologies (NAMs) to allow for the extrapolation and application of in vitro results to in vivo exposures are being explored for both chemicals and drugs. However, special situations such as developmental toxicity (DevTox) will require additional considerations as a result of both biokinetic and biodynamic factors. Estimation of DevTox potential will necessitate the ability to account for changes occurring in maternal biology during gestation and fetal development. These approaches must also address whether injury occurs via direct fetal toxicity or indirectly resulting from maternal toxicity. Another unique concern is the timing of exposure with regards to development (windows of susceptibility). Therefore, we are developing a workflow to use for in vitro and in vivo data combined with in silico dosimetry to support prioritization of chemicals based on their potential for DevTox. This workflow, representing our first step, will account for in vitro assays able to estimate DevTox-specific endpoints and toxicokinetic (TK) information and models able to account for maternal-fetal differences over the course of gestation.

To start, we selected 26 chemicals representing a variety of physicochemical properties and potential DevTox modes of action. We used the results of those chemicals in a panel of 5 in vitro DevTox assay families (STM_H9_OrnuCystisNorn, ratio_dn, CCTE_Mundy_HCI, CCTE_Padilla, Tanguay_ZF, and TOX21_SBE/TOX21_SHH) together with dosimetry and in vitro ratio_dn, CCTE_Mundy_HCI, CCTE_Padilla, Tanguay_ZF, and TOX21_SBE/TOX21_Chemicals in a panel of 5 to start, we selected 26 chemicals representing a variety of physicochemical properties and potential DevTox modes of action. We used the results of those chemicals in a panel of 5 in vitro DevTox assay families (STM_H9_OrnuCystisNorn, ratio_dn, CCTE_Mundy_HCI, CCTE_Padilla, Tanguay_ZF, and TOX21_SBE/TOX21_SHH) together with dosimetry and in vitro kinetic modeling using the htk R package to evaluate potential fetal toxicants. For this preliminary investigation, chemicals with at least one DevTox assay result at a shift concentration of 5.0 toxicocytotoxicity burst lower concentration were considered “possible” developmental toxicants, while chemicals with at least one DevTox assay AC₅₀ at or below the 95th percentile of all ToxCast assays (POD₉₅₀) and below the cytotoxic burst were considered “likely” developmental toxicants. Relevant AC₅₀ values were then converted to oral doses (OEDs) for in silico dose-response analysis. For 26 chemicals were classified as “likely” and 7 as “possible” fetal toxicants. Fifteen chemicals did not have a DevTox assay AC₅₀ below the cytotoxic burst. The in vitro concentrations measured in the Stemina assay were adjusted to free concentrations using the Amritage_eval function in htk. The median estimated free fraction was 0.924 (range: 3.80x10⁻⁴-0.999) of the nominal concentrations for the 26 chemicals. The median OED based on the lowest DevTox assay for each chemical was 0.589 (r: 0.008-2.68) mg/kg/day for females aged 16-49y. For the 7 “possible” fetal toxicants, the most sensitive DevTox assay concentration was 454-fold higher (r: 3.1845-fold) than the POD₉₅₀ on average, suggesting that maternal toxicity might occur at a lower maternal effective concentration in these cases. While the chemicals described in this project cover a variety of characteristics, additional work will be performed to improve the generalizability of the workflow and to further evaluate the validity of the results. In particular, the grouping of chemicals as likely/possible/ unlikely developmental toxicants will be refined in future iterations of this work. Additional work will include the most recent DevTox Tier (v3.5) which includes another DevTox assay (CCTE_Deisenroth_DEVTOX), free chemical concentration estimations for the other DevTox assays, and a comparison of high throughput approaches with more in-depth evaluations with in vivo data and bespoke developmental physiologically-based pharmacokinetic models. This work was funded by the ACC LRI.

DASS App: A Web Application for Applying Defined Approaches for Skin Sensitization to Predict Hazard and Potency Categorization

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Defined approaches (DAs) are methods for evaluating data from specific information sources to derive toxicity predictions. Guideline 497 (GL 497), Defined Approaches for Skin Sensitisation (DASS), issued in 2022 by the Organisation for Economic Co-operation and Development (OECD), was the first internationally harmonized guideline to describe a non-animal approach that can be used to fully replace an animal test to identify skin sensitzers. GL 497 describes two validated DAs to identify potential skin sensitizers, the 2 out of 3 (203) and Integrated Testing Strategy (ITS). A third DA, Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS), has been accepted by the U.S. Environmental Protection Agency. The data interpretation procedures implemented in these DAs vary in logical complexity and can be time-consuming to apply manually. Computational approaches can be used to apply the DAs more efficiently but require fluency in computational programming.

We have developed an open-source web application, the DASS App, to facilitate application of these DAs by a wider audience. The DAs available in the DASS App integrate data from three in vitro assays: the direct peptide reactivity assay (DPRA), human cell line activation test (h-CLAT), and the KeratinoSens assay. These assays represent three key events within the skin sensitization adverse outcome pathway. The three DAs implement rule-based approaches for integrating multiple assay results to predict hazard and/or potency. The 2o3 predicts hazard by taking the consensus prediction across the three in vitro assays. The KE 3/1 STS first evaluates quantitative outcomes from h-CLAT to predict whether the chemical should be classified as a Strong or Weak sensitizer; if the h-CLAT is negative, DPRA results are evaluated to determine whether the chemical should be classified as a Weak sensitizer or Not Classified. The ITS predicts hazard identification and potency by scoring results from DPRA, h-CLAT, and in silico predictions. GL 497 defines two versions of ITS, using either DEREK Nexus or OECD QSAR Toolbox predictions. The ITS DA also outlines a data interpretation procedure for cases where data are only available for two information sources. When used on a test set of 150 chemicals, the DASS App generated hazard predictions in less than one second. The DASS App enables users to implement non-animal approaches to evaluate chemical skin sensitization without the need for additional software or computational expertise. The app supports upload and analysis of user-provided data, includes steps to identify inconsistencies and formatting issues, and provides hazard predictions in a downloadable format. The DASS App is available on the US Corp, Raleigh, NC; 221

Neurotoxic Chemicals: Case Study of Four Pesticides

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The neurotoxic pesticides: Cyfluthrin (CYF), βCYF, endosulfan (ENDO) and chlorpyrifos oxon (CPF0) were applied in a case study to see if the open access new approach methodology (NAM) tools could predict in vitro modes of action (MOA). It is known that CYF/ βCYF block Na⁺ channels in brain leading to decreased motor activity; ENDO blocks chloride channels in brain at GABA receptors causing hyperactivity and CPF0 inhibits acetylcholinesterase (AChE) in brain leading to decreased motor activity, cognition, and motor function. The NAMs we employed in this study included: 1) ToxCast in vitro bioassay database from the CompTox Chemicals Dashboard. The focus of assay target selection was their relevance to neurotoxic pathways where active hit calls were reported as activity at 50% concentration (AC₅₀) with AC₅₀ below the cytotoxicity lower limit and with two or fewer cautionary flags, 2) Z-Score calculations to assess chemical-target specificity. The DASS App is available on the US Corp, Raleigh, NC; 221
3) In vitro to in vivo extrapolation (IVIVE) to calculate Administered Equivalent Dose (AED mg/kg/d) using the selected ToxCast neurotoxicity AC50s as data inputs. The AEDs were generated from the Integrated Chemical Environment (ICE) by using a 3 compartment (3C) or a 7 compartment (physiologically based toxicokinetic: PBTK) model. To evaluate NAM predictions between the AEDs and reported in vivo regulatory neurotoxicity points of departure (POD), fold differences (FD) were calculated. Since the in vitro studies were performed on immortalized and iWNV chronically infected human neural cells, the in vivo PODs were adjusted for interspecies animal to human variability with a 10x factor. The adjusted PODs were 0.147 mg/kg/d for CYF/BCYF, 0.180 mg/kg/d for ENDO and 0.106 mg/kg/d for CPF0. Results showed most active hit calls for each chemical were in 3 categories - effects on neuronal firing, bursting, neural network connectivity. These directly were linked to the related MOA involving acute neurotoxicity, developmental neurotoxicity, neurotoxicity and other neurotoxicity-related targets (e.g., AChE). For CYF, BCYF and CPF0, the in vitro targets supported decreased neuroactivity, while for END0, acute in vitro and in vivo neuroactivity was increased. BCYF however is known to have twice the toxicity of CYF in vivo but was less toxic in the in vitro assays (e.g., higher AC50 and no cytotoxicity lower limit). A default of 100 UMs was assumed as the cytotoxicity limit for CYF, by using the reported 7.01 µM cytotoxicity lower limit for CYF. The reason for this difference is not clear but may be due to cell metabolic capability for BCYF or assay design. Z-Scores greater than 3 indicated chemical-target specificity. The percent assays with Z-Scores greater than 3 for CYF, BCYF, END0 and CPF0 were 91%, 100%, 67% and 64% respectively. While the AEDs over- or underestimated the in vivo PODs, the actual values were quite close. For example, with the ICE 3C model, a majority of the selected neurotoxicity assays had a FD <10 (CYF: 91%; BCYF:71%; END0: 76%); for the remaining assays that had FD of >10 they were not higher than 27. Although, for CPF0 the PBTK model had better AED predictions than the 3C only 50% of the FD to 10 were still in line with FD <10. The NAMs available for this case study identified the main neurotoxicity targets and the ICE models were highly predictive and user-friendly. The limitations for this approach will be discussed. Overall, the NAMs help in facilitating mechanistic insights and could be used in informing a point of departure in risk assessment for chemicals with unknown toxicity. This study is an exploratory effort that does not necessarily reflect the position of OEHAH.

3092 MIEML: Predicting Molecular-Initiating-Events by Integrating Machine Learning with Concentration Response Analysis of High-Throughput Transcriptomic Chemical Screening Data


The cost of RNA sequencing has decreased considerably since its inception. Simultaneously, the throughput of this technology has improved, and it is now possible to efficiently profile gene expression changes across thousands of samples in a single study. The falling costs and improving scalability of these technologies has contributed to their proposed use in regulatory contexts as chemical screens. However, the scale and volume for high-dimensional transcriptomic datasets presents a formidable obstacle for utilizing these data in a regulatory decision-making context. As transcriptomic chemical screening data accumulates in the public domain, there is a growing need to develop new bioinformatics approaches for ingesting, integrating and using these datasets. Here, we present mieml (Molecular Initiating Event prediction with Machine Learning), a bioinformatic framework for using transcriptomics data to predict the underlying molecular initiating event (MIE) associated with a chemical exposure. We demonstrate the utility of this framework in predicting MIE activation by training models using gene expression profiles from a large bioactivity screen conducted in the breast cancer-derived MCF7 cell line. We trained binary classifiers to predict activation of 20 distinct MIEs using transcriptomic profiles from reference chemicals known to be associated with each MIE. Of these, four sets of MIE classifiers were validated using permutation testing followed by an empirical significance of mieml. Validated classifiers were then used to generate MIE activation predictions for 1,784 test chemicals at each concentration. Predictions were then used as inputs for concentration-response modeling using the tcplfit2 R package. Chemicals that showed concentration-responsive MIE prediction scores were identified as candidate activators of that MIE. For further validation, we compared our MIE activation predictions to the ToxCast targeted high-throughput screening assay dataset, revealing general agreement between ToxCast bioactivities and mieml predictions. Specifically, mieml predictions showed concordance with ToxCast data for estrogen receptor agonism, aryl hydrocarbon receptor agonism, and glucocorticoid receptor agonism. A subset of test chemicals were predicted to be bioactive by mieml, but were not captured by the currently available ToxCast dataset. High-confidence, novel MIEs predicted may be useful for enhancing the permeability of the BBB to predict MIEs that are not well-represented within the MCF7 cell line. This framework does not necessarily reflect US EPA policy. Company or product names do not constitute endorsement by US EPA.

3093 Computational Associations between Urethane Exposure and the Development and Progression of Endometriosis


Endometriosis is a disease characterized by the presence of endometrial tissue outside of the uterus, leading to chronic inflammation and severe pain. Although endometriosis affects approximately 10% of women globally, the exact cause of endometriosis remains unknown; however, many chemical species have implicated in its development and/or proliferation. Utilizing a multi-data, integrative analytical framework, the role of urethane, an organic compound found in fortified foods and alcoholic beverages, was investigated in the development of endometriosis. Through quantitative structure activity relationship (QSAR) analysis, urethane was identified as a potential mutagen. Computational analysis using online databases and genomic platforms also identified several pathways linking urethane exposure to endometriosis including the mutagenesis of KRAS and activation of MAPK1. Urethane was also linked to epigenetic changes in GATA2/6 that may promote endometriosis through enhanced inflammation, cellular proliferation, and estrogen signaling. Furthermore, urethane exposure was also shown to broadly upregulate growth factors that play critical roles in cell growth and survival and downregulate factors promoting apoptosis. Clinical analysis of the identified chemical-gene-disease- linkage-based computational linkage identified. Ultimately, through the modulation of several different cellular pathways, urethane exposure is likely a chemical progenitor and aggravator of endometriosis and may also be linked to the development of uterine cancers.

3094 Integrating Structure Annotation and Machine Learning Approaches to Develop Graphene Toxicity Models


Modern nanotechnology is a prominent technology that can provide efficient and cost-effective nanomaterials (NMs) meeting the needs for sustainable development. Increasing usage of NMs in consumer products arouses great concerns regarding nanotoxicity in humans, resulting in urgent demands for risk assessments of NMs. Traditional animal testing of nanotoxicity is expensive, time-consuming, and labor-intensive. The quantitative nanotoxicity structure activity relationship (QNAR) modeling studies to develop computational nanotoxicity models, such as the ones based on machine learning (ML) approaches, are promising alternatives to direct evaluation of nanotoxicity based on nanotoxicity features. However, NMs, including two-dimensional nanomaterials (2DNMs) such as graphenes, have complex structures compared to small molecules, making them difficult to annotate and quantify the nanostuctures for modeling purposes. To address this issue, we constructed a virtual graphenes library using new nanostucture graph theory annotation technique. The irregular graphene structures were generated by modifying the number of vertices, edges, and functional groups on virtual nanosheets. The nanostuctures were digitalized from the annotated virtual graphenes and saved as Protein Data Bank (PDB) files. Based on the annotated nanostuctures, geometrical nanodescriptors were computed using the Delaunay tessellation approach and further combined with additional surfactant descriptions for ML model developments. The partial least square regression (PLSR) models for the graphenes were built and validated using a leave-one-out cross-validation (LOOCCV) procedure. The resulted models showed good predictivity in all available toxicity endpoints including cell membrane integrity, mitochondrial enzymatic activity, oxidative stress, and apoptosis with the coefficient of determination (R^2) ranging from 0.558 to 0.822 obtained from cross validations. This study provides a novel nanostucture annotation strategy that can be applied to generate high-quality nanodescriptors for ML model developments, which can be widely applied to nanoinformatics studies of graphenes and other NMs.

3095 Safety Assessment of Modifying the Blood-Brain Barrier by Targeting Claudin-5 in a Cyromolgus Monkey Model

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The blood-brain barrier (BBB) is a major obstacle to drug delivery to the central nervous system (CNS). In the BBB, the spaces between adjacent brain microvascular endothelial cells is sealed by multiprotein complexes known as tight junctions (TJs). Claudin-5 (CLDN-5) is considered to be the main TJ protein in the BBB. Accordingly, CLDN-5 modulation by CLDN-5 binders, such as anti-CLDN-5 monoclonal antibodies (mAbs), may be useful for enhancing the permeability of the BBB to
allow efficient drug delivery to the brain. To begin translating the benefits of CLDN-5 binders for clinical use, studies demonstrating the safety of these binders are required in non-human primates with similar brain structures and functions to the human brain, as are proof-of-concept studies confirming that CLDN-5-targeted drug delivery strategies enhance the permeability of the BBB to enable efficient drug delivery to the brain of non-human primates, whose brain structures and functions resemble those of the human brain. In this study, the in vivo safety and efficacy of CLDN-5 binders were examined in a non-human primate, the cynomolgus monkey, using anti-CLDN-5 mAbs. Rat anti-CLDN-5 mAb (R9) or rat IgG was administered intravenously to cynomolgus monkeys. Twenty-four hours later, the monkeys were intravenously administered 20 mg/kg of sodium fluorescein (FNa), and their cerebrospinal fluid (CSF) and plasma were collected. Administration of 3.0 mg/kg R9 increased FNa concentrations in the CSF, however, no behavioral changes or changes in plasma biomarkers for inflammation, liver, or kidney damage were observed. A monkey that received R9 at 6.0 mg/kg experienced convulsions, and subcutaneous administration of the anti-inflammatory agent prednisolone at 10 mg/kg showed no effect. The convulsions were attenuated only after the intravenous administration of the intracranial hypotensive agent mannitol at 10 ml/kg/30 min. After necropsy, histopathological examination of this animal revealed vasodilation in the brain and perivascular edema in the frontal lobe; fibrin was detected in the subarachnoid space. A possible explanation for the toxicity of R9 at high doses is the induction of antibody infusion-related adverse effects, such as the release of inflammatory cytokines or the induction of antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity. Indeed, rat IgG2b, which is in the same subclass as R9, has been shown to induce these two forms of cytotoxicity, suggesting that R9-bound vascular endothelial cells can become targets of the same immune responses. In conclusion, CLDN-5 might be a target for enhancing drug delivery to brain tissue; however, the therapeutic window of R9 is narrow. Therefore, CLDN-5-targeted strategies for drug delivery to the brain will be suitable for severe diseases without therapeutic options, such as traumatic brain injury and brain tumors.

3096 Polo-Like Kinase 4 (PLK4) Safety Review: Distilling the Risks with a Rapid Augmented Intelligence Approach

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Polo-like kinase 4 (PLK4) is a unique member of the Polo-like family of kinases with an essential role in centriole duplication. PLK4 turn-over must be strictly controlled to prevent centriole amplification. PLK4 inhibitors have potential in cancer treatment, rendering cells unstable and more sensitive to chemotherapy. Using automated data mining techniques, together with expert curation and interpretation, which are the backbone of the technology-enabled KnowledgeScan™ Target Safety Assessment framework, we reviewed the safety risks associated with PLK4. PLK4 synonyms were used to create a corpus, from which we analysed S88 literature records and created profiles against known data from sources such as Ensembl. Finally, PLK4-relevant information and risks associated with PLK4 modulation were organized by organ/system. Consistent with the broad expression of PLK4, the evidence suggested potential risks in most organ systems (excluding haematopoietic and urinary systems). For several risks, it is possible to identify underlying mechanistic processes (like cell proliferation and cytokinesis) which may play a key role in their pathogenesis. Augmentation of PLK4 function suggests a risk of carcinogenesis. Supported by evidence of impacting DNA damage responses and hyperplasia in the pancreas, skin, and hair. Given the development activities on this target in the oncology space, this is unsurprising. Attenuation of PLK4 function was associated with potential risks of neuropenia, inflammation, reproductive impacts and impaired liver regeneration. Human PLK4 mutations (e.g. loss-of-function) have been associated with microcephaly, chorioretinopathy and growth failure. The risks associated with modulating PLK4 are diverse. The KnowledgeScan workflow quickly and concisely distilled the key biological risks, together with the intelligence surrounding known PLK4 inhibitors and therapies. This enabled us to logically present the collected and reviewed information in a fully hyperlinked dossier for PLK4. Looking prospectively, the industry is placing more emphasis upon the importance of in silico methods coupled with weight of evidence reviews. In silico approaches, including the augmented intelligence methodologies highlighted in this project, support the 3Rs campaign and enable toxicologists to make early, informed, evidence-based decisions regarding safety and other toxicological concerns. This research application discusses the importance of methodological structure, data traceability and accountability enabling significant improvements to comprehensive data collection, curation and interpretation. The long-term impact of studies within this area, together with the application of these promising techniques, will help ensure this emerging field remains unbiased and comprehensive.

3097 PKSmart: A Public Tool to Predict In Vivo Pharmacokinetics of Small Molecules

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It is not only the biological activity of a compound that determines its mechanism of action but also the exposure. Drug exposure can be defined using human pharmacokinetics (PK) parameters that affect the blood concentration profile of a drug, such as steady-state volume of distribution (VDss), total body clearance (CL), half-life (t½), fraction unbound in plasma (fu) and mean residence time (MRT). Early assessment of PK properties is crucial, such as in DMTA cycles, and this is what we enable in this work with models needing only chemical structure as input. In this work, we used molecular structural fingerprints, physicochemical properties, and predicted animal PK data to model the human PK parameters VDss, CL, t½, fu and MRT for 1,283 unique compounds. First, we trained models on 372 compounds using molecular structural fingerprints and physicochemical properties to predict animal PK parameters for rodents, rabbits, and dogs. Second, we trained Random Forest models on 1,283 unique compounds using Morgan fingerprints, Mordred descriptors and predicted animal PK parameters (obtained using the models above) in a Random Forest algorithm to predict human CL and VDss, where the model was validated using repeated nested cross-validation and an external test set. When using repeated nested cross-validation, human VDss achieved a median R² = 0.54, (Geometric Mean Fold Error, GMFE=2.11), CL with a median R² = 0.31 (GMFE=2.47), 1% with a median R² = 0.43 (GMFE=2.49), fu with a median R² = 0.55 (GMFE=2.49), and MRT with a median R² = 0.28 (GMFE=2.49) over 25 held-out test sets for models combining Morgan fingerprints, Mordred descriptors and predicted animal PK parameters. Using the external test set validation, 60.0% of compounds for VDss, 84.3% of compounds for CL, 53.4% of compounds for fu, and 55.3% of compounds for t½ were within a 3-fold error of the observed values. The models also provide an associated fold error estimate (and a range of predictions) based on the similarity to the chemical space of the training data. PKSmart (a web-hosted application: https://serve.scilifelab.se) which users can access using a web browser with all code also downloadable for local use. To the best of our knowledge, this is the first work that releases PK models publicly that can predict human and animal PK parameters using inputs of chemical structure alone.

3098 Advancing the Representation and Biological Interpretation of Cell Painting Readouts for Toxicity Prediction

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Cell Painting features are a numerical representation of the morphological changes in cells that are subjected to a certain perturbation (such as exposure to a compound) and have previously been shown to be predictive of a compound’s biological activity. However, compared to structural fingerprints or features from e.g. gene expression experiments, Cell Painting features remain challenging to interpret. On the practical side, they are known to improve the performance of predictive models related to safety- and efficacy-relevant endpoints and their information content are at least partially independent of structural descriptors. In this work, we developed an algorithm to improve the interpretation of a recent Cell Painting dataset published by the Broad Institute for toxicity prediction tasks. Toxicity assays (from ToxCast) representing eight biological processes, such as proliferation, oxidative stress, and mitochondrial disruption, were used in this work. We created a feature matrix representation (of the data) which we enable to predict compound behavior using features correlated across compound treatments placed in the vicinity. Next, we trained convolutional neural networks (CNN) on them that leverage neighbour-hood correlation in the matrix representations. Subsequently, we generated visual explanations for the predictions using gradient-weighted class activation maps (Grad-CAM), which was more information-rich than selecting individual features and easier to interpret. F1 scores for detecting toxic compounds ranged from 0.20±0.11 using CNN models compared to RF models 0.25±0.1, with no significant difference in distribution (using paired sample t-test) over the nested cross-validation for 7 out of 8 prediction tasks. Feature matrix representations (created without endpoint data) showed that features were correlated across compound treatments by the measuring module (intensity, granularity, radial distribution, etc.) rather than the object that where the measurements were taken (cells, cytoplasm, or nuclei). We observed compounds with similar mechanisms of action exhibit similar information areas in the Cell Painting matrix representation. For example, microtubule disruptors (paclitaxel, daunorubicin, cycloheximide, parbendazole) had a similar area of interest in the Cell Painting matrix representation. For example, microtubule disruptors (paclitaxel, daunorubicin, cycloheximide, parbendazole) had a similar area of interest in the Cell Painting matrix representation.
In vitro cell, tissue and organ culture is an important tool for scientists to study diseases and test the efficacy, ADME and toxicological properties of new drugs. With the increasing complexity of available cell culture models, much progress has been made to approximate the human in vivo situation, thereby reducing the need for animal experiments in research and development. However, despite being considered a major alternative for animal experimentation, in vitro cell culture systems often require animal supplements. Especially total liver organoids (TLOs) are a unique and valuable platform to study organ specific metabolism and function. Total liver organoids (TLOs) are a unique and valuable platform to study organ specific metabolism and function. A good example of this is the Organ on a Chip system developed by the company Organovo, which allows for recapitulation of the human intestinal GI tract compared to the 2D transwell system resulting in a faster and more robust assessment of drug-induced damage in the GI tract. Developing in vitro kidney injury model systems remains a challenge due to the high level of complexity of the organ. In this study, we adapted the Takeda iPSC-derived kidney organoid protocol to generate an advanced image-based high-throughput testing method for chemicals. The newly generated kidney organoid protocol enabled high-throughput experimentation including glomerulonephritis, proximal tubule, and distal tubule. Furthermore, this adapted protocol allows us to create large quantities of kidney organoids with high compatibility with live cell confocal imaging. We also established a panel of CRISPR-engineered GFP reporters for various cellular stress response pathways. As a proof-of-concept for application of these reporters in kidney organoids, we first evaluated a DNA damage response reporter line for the biomarker protein p21 (p21) was clearly upregulated upon exposure to specific nephron regions. We anticipate that these kidney organoids can be used as an in vitro high throughput test system to monitor chemically-induced nephrotoxicity.

In small molecule drug discovery, potential safety issues in a molecule can be identified using structure alerts associated mostly with reactive metabolite formation. Chemical motifs flagged by such structural alerts can be potentially bioactivated by metabolic enzymes, resulting in unexpected side effects in animals and humans, such as drug-induced liver injuries. A straightforward chemical strategy is to replace the flagged chemical motif with a safer one. However, this strategy is not always feasible for various reasons, such as a negative impact on the primary target activity. Many approved drugs contain structural alerts, which are safely used in many cases. Therefore, in addition to chemical modification strategies, a deeper understanding of the drugs having structural alerts is needed. Furthermore, information on adverse events and pharmacokinetics profiles are important to properly address the potential risk of a drug candidate with structural alerts. In Takeda, structures of marketed and withdrawn drugs and their clinical safety and pharmacokinetic information from multiple databases were curated and integrated to enable a systematic analysis of drugs with structural alerts. For example, through an analysis of drugs having a phenol moiety, the percentage of drugs reported showing severe hepatobiliary disorders was 12.6% (n = 15) among the total phenol drugs (n = 95) in our database, which was similar to that of non-phenol drugs (12.8%, total n = 1035). On the other hand, the percentage of drugs showing severe hepatobiliary disorders was higher (24.9%, n = 60) in the analysis of aniline drugs in comparison with other drugs (9.6%, total n = 909), which suggests the potential risk derived from an aniline as a structural alert can more often manifest as side effects compared to phenol drugs. A deeper analysis, however, indicated that even among phenol drugs, the likelihood of showing severe hepatobiliary disorders increases with phenol drugs with a daily dose of 150 mg or higher and shorter than 1 year of use. The integration of information is essential when making a decision on developing such compounds having structural alerts. Several case studies will be presented to show the importance of chemical safety group review, which enables us to conduct a systematic analysis of compounds with structural alerts.

Bone marrow toxicity is one of the most frequent side-effects of many chemotherapeutic agents. Targeted therapies have the potential to overcome many of the toxicities associated with traditional chemotherapy, such as chemotherapy-induced cytopenia against rapidly dividing cells. Some targeted therapies, like the CD33 targeting antibody lintuzumab, still encounter hematotoxicity clinically, which may be due in part to the target expression on hematopoietic stem cells. Developing improved targeted therapies will involve optimization of multiple dimensions of the immune...
therapy, including target selection, binder comparison, construct design, and
amorning strategy, to enable potent tumor eradication while sparing critical normal
hematopoietic stem and progenitor cells. While assays for preclinical assessment
of hemotoxicity for traditional (small molecule) chemotherapeutic agents are well
establiished, methods to evaluate on-target off-tumor toxicity for other modalities
are lacking. The colony forming unit (CFU) assay is considered the gold-standard
approach to assess the effect of non-specific hematotoxicity. However, high-throughput
in vitro assay would be desirable to facilitate early compound screening. Here, we
adopted the HemaTox assay for immune-based modalities such as cell therapy
cell engagers. In contrast to the CFU assay, the HemaTox assay is a liquid
culture-based stem cell differentiation assay with a high-throughput capacity and
shorter turnaround time. It is suitable for screening multiple therapeutics at various
concentrations and has the ability to delineate lineage-specific toxicity. During
assay development, variables such as assay conditions (time points, concentra-
tions, media components, cell densities) and procedures (co-culture setup, effector
cell removal, assay readout) were optimized. Autorolous αβ chimeric antigen receptor
t细胞 (CAR T cells) with clinically relevant tool binders were generated and
tested in the HemaTox assay. After 7 days of differentiation, untreated human bone
marrow CD34+ cells yielded 1362±0.1640 myeloid cells, while only 93-406 myeloid
cells were developed if cocultured with tool constructs for 24 hours at 3:1 effector
to target (E:T) ratio. When compared with the CFU assay, the relative myelosuppres-
sion among different constructs in the HemaTox myeloid assay correlated with the
inhibition on the granulocyte-macrophage progenitor (CFU-GM) colony develop-
m which we conducted
the results of this assay informed target selection and
conduct design for novel cell therapy development. The HemaTox assay delivers
a fast, high throughput approach for bone marrow safety assessment, enabling
"safety by design" in early drug discovery.

### 3104 Impact of Target Expression on the Adverse Event Profile of Antibody-Drug Conjugates (ADCs): Implications for Target Safety Assessments

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Several antibody-drug conjugates (ADCs) are already approved in oncology indica-
tions. However, expression of the antibody target in normal tissue may pose a
potential safety risk where cytotoxic payloads are utilized. The aim of this study
was to guide target safety assessments (TSAs) by determining if target expres-
sion data for ADCs correlates with reported clinical toxicity. TSAs use target
biology, gene and protein expression data, genetic information from humans and
animals and competitor compound intelligence to understand and mitigate the
potential safety risks associated with modulating a drug target. Using publicly
available data from drug labels for the twelve regulatory approved oncology
ADCs, common clinical adverse events (AEs) were collated from the black box
warnings, warnings and precautions and adverse reaction sections. The twelve
oncologyADCs studied in our study covered ten individual targets (CD33, CD30,
HER2, CD22, CD79, NECTIN-4, TROP-2, BCMA, CD19, tissue factor). Data on target
mRNA and protein expression levels in normal tissues were obtained from publicly
available datasets deposited by the Human Protein Atlas consortium, categorized
as high, medium, low or negligible expression across key organ systems. For
organ systems with high or medium expression of the target, we conducted an analysis to determine any correlation with clinical toxicity as defined by reported
AEs. Across all 12 ADCs, AEs occurred in 70 % of organ systems that had high
expression of the target. Half of the instances of high target expression occurred in
the hematologic and immune organ system. However, all twelve ADCs had hematol-
logic and immune AEs regardless of target expression level, suggesting a strong
contribution of non-target related toxicity. Beyond the hematologic and immune
organ system, AEs were observed in two out of five (40 %) organ systems with high
target expression. In these two cases, target expression was high in the skin and
skin-related AEs were documented for two ADCs (sacituzumab govitecan target-
ing TROP-2 and ticarutumab Herceptin targeting tissue factor). Across all ADCs,
AEs occurred in 45 % of organ systems (in direct opposition to payload related target
expression). Overall, as expected, commonly occurring clinically identified AEs were more
frequent in organ systems where there was high expression of the antibody target.
Similarly, there was concordance but at a lower level in organ systems with medium
expression of the target. In conclusion, expression levels of the antibody target
are associated with clinical AEs in that tissue. However, the likelihood of toxicity
is dependent on many factors not yet assessed in this study including the distribution
of the ADC, the expression level of the target in normal versus diseased tissue, the
payload used and the proliferation rate of the target tissues. When considering
TSAs for new ADCs, expression of the antibody target in normal tissues should be
highlighted. However, rigorous experimental assessment will be required to determine if high levels of target expression translate into clinical and/
or non-clinical adverse effects.

### 3105 Is There an Easy Way for Unraveling Drug Toxicities? Bioinformatics Approach Answers This Question

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For every 100 drugs entered into phase 1 clinical testing in oncology, fewer
than 10 will ultimately receive regulatory approval, while a majority of approved
cancer drugs provide substantial clinical benefit. The aim of this study was to
predict potential toxicities of drug targets before they enter the clinical trials using
preexisting data. In order to do this, we generated a comprehensive database
and software, including internally curated and various publicly available data on
proteins, biologically-active chemicals, their interactions, pathways and patholo-
gies. We catalogued 4 million references, supporting each database entry, with
hyperlinked interactions to appropriate PubMed articles as support, while proteins
and chemicals are hyperlinked to EntrezProtein and PubChem, respectively. In
order to support data integration and capture different levels of histopathological
observations, we developed toxicity ontology with over 2500 toxicity endpoints.
We validated the system by exploring mTOR (mechanistic target of rapamycin
kinase) as a potential drug candidate for cancer treatment. mTOR inhibitors exhibit
a promising efficacy in preclinical studies and early clinical trials of advanced renal
cell carcinoma and other types of cancer, however, some inhibitors have serious
adverse effects in patients and have to be discontinued. Hence, elucidation of the
putative role of mTOR in different organ systems can help in predicting adverse
effects that may arise following mTOR inhibitors treatment. Mapping mTOR
onto Gene Ontology (GO) knowledge base function, within the system, identified
mTOR biological function in phosphoprotein binding, cell growth, heart trabecular
formation, response to nutrients and cell surface receptor protein binding and
fibrillar organization. Analysis function within our computational tool demonstrated
an association of mTOR with several diseases like angiosarcoma, Horton disease
(Giant cell arteritis), and cerebral cavernous malformation in which is mTOR dysregu-
lated and/or correspond to potential application of mTOR inhibitors. Mapping
mTOR, together with its protein interaction network onto organ/tissue pathologies
within our system demonstrated association with several pathologies related to
circulatory system (hypertension, heart hypertrophy, atherosclerosis and cardiomy-
opathy) which correspond to potential toxicities induced by mTOR inhibitors; and
reproductive system (ovary carcinoma, endometrium carcinoma, mammary gland
carcinoma and prostatic carcinoma) and urinary system (urothelial carcinoma and kidney carcinoma) which correspond to potential application of
mTOR inhibitors and/or their toxicity. Analyzing mTOR together with its chemical interaction partners (agonists and antagonists) identified mTOR role in renal cell
carcinoma that corresponds to mTOR inhibitor therapeutic application. Indeed,
scarcity of potential targets identified (organ failure, heart failure, hypertension
and/or hypertension) associated with the use of mTOR inhibitors everolimus and
temsolimus. In this example, we showed that our tool can contribute to a better
understanding of therapeutic potential of a target inhibition and quick toxicity
assessment of a target.

### 3106 A Pilot Pharmacology/Toxicity Study of an Anti-SCF Monoclonal Antibody in Cynomolgus Macaques

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Stem Cell Factor (SCF) binds to and activates KIT (CD117, c-KIT), a receptor tyrosine
kinase required for the differentiation, chemotaxis, maturation and survival of mast
cells. KIT is also expressed and has function in in several other cell types, includ-
ing melanocytes. This poster describes a pilot study of a neutralizing monoclo-
nal antibody to SCF in cynomolgus macaques. Prior to the initiation of the study,
baseline samples were collected from three animals (one male, two females) which
included blood samples for clinical pathology, buccal swabs and skin biopsies from
each ear pinna. Test article (TA) was administered on Day 1 and on Day 8, at a dose
level of 75 mg/kg/dose by a slow intravenous push. Longitudinal serum samples
were collected over the 57 days of the study for pharmacokinetic analysis, hematol-
ogy, clinical chemistry and coagulation. Skin biopsies, from the ear pinna and buccal
swabs, were again collected on day 30 and 57. The test article was well tolerated
with no noted adverse clinical observations. Pharmacokinetic analysis revealed
drug exposure consistent with the expected pharmacokinetics for a humanized
monoclonal antibody. One animal developed detectable anti-drug antibodies (ADA)
by the end of the study period. A mild transient decrease in mean corpuscular
hemoglobin concentration (MCHC), with a corresponding slight increase in mean
corpuscular volume, was seen at day 4, following the first dose. This brief hematol-
ogy change was not seen following the second dose on day 8. Toluidine blue
staining of ear punch biopsies revealed decreased mast cell numbers, relative
to Day 1, which partially reversed by day 57. This observation was consistent with
Nonstring RNA analysis of mast cell genes from skin biopsies. Nanostirng
analysis of the biopsies also showed decreases in melanocyte genes consistent
with known pharmacology of KIT inhibition in melanogenesis, as well as changes
in the expression of several genes in RNA samples derived from the buccal swabs.
In conclusion, administration of a humanized monoclonal antibody to SCF, to cynomolgus macaques, at 75 mg/kg/dose on days 1 and 8, was well tolerated with observations consistent with the expected pharmacology of the molecule, with the lack of clinically adverse findings.

Cell therapy is a rapidly advancing field of research with great promise to treat multiple diseases. However, multiple challenges, from isolating the correct cell type, determining its mode of action, manufacturing and establishing appropriate quality control criteria, must be overcome to reach its potential. In recent years, cellular energy metabolism has emerged as an important consideration when developing cell therapies. The efficacy of CAR-T therapy and/or modulating T cell metabolism reprogramming has been proven crucial for their survival and response. Here we evaluate a set of bioluminescent cell-based assays for measuring rate of metabolite uptake, monitoring activity for key metabolic pathways and determining changes in intracellular metabolite levels during T cell transition from naive to activated to memory phenotypes. The assays developed are robust and well-adapted to current and future high throughputs. We show that upon activation of naive CD8+ T cells with CD3/CD28 beads (anti-CD3 and anti-CD28 antibody coated beads) as the cells start to increase in size and prepare for rapid expansion, the changes in key metabolic pathways can be rapidly measured. We show that during the first 72 hours after activation, ATP levels in activated T cells increase by approximately 1.7-fold per cell as compared to naive cells. The changes in ATP, are accompanied by a greater than 20-fold increase in reducing capacity of live cells measured using RealTime-Glo Viability Assay and differential response to metabolic pathway inhibitors. Naïve cells treated with 2-deoxy-glucose (2DG), a glycolytic pathway inhibitor, showed only a slight (less than 10%) decrease in ATP levels as compared to more than 50% decrease when treated with the oxidative phosphorylation (OXPHOS) inhibitors rotenone/antimycin, indicating their dependence on OXPHOS for ATP generation. In contrast, activated T cells showed a greater than 50% decrease upon 2DG treatment and about 20-25% decrease upon rotenone/antimycin treatment, in-line with increased dependence on glycolysis. As T cells expand, they continue to retain increased glycolytic activity. More than a 5-fold increase in glucose uptake and a 6-fold increase in lactate secretion was measured in activated as compared to naïve cells after 10 days of expansion. Difference in metabolic phenotype of naïve and activated cells was also reflected in intracellular metabolite levels. Activated T cells showed more than a 10-fold increase in lactate secretion during T cell transition from naive to memory phenotypes. The metabolic changes were commensurate with the changes in ATP levels. The assays are applicable to different cell types including 3D models in addition to the T cells demonstrated here.
development. Overall, this presentation will highlight our experience in the use of 3D P Ts over the past few years, including benefits and challenges, for drug safety assessment in the pharmaceutical industry.

3111 Nitrosamines and Carcinogenic Potency: Learnings from Druckrey
M. Kenyon, O. Dirat, C. McWilliams, and K. Dobo. Pfizer Inc., Groton, CT. Sponsor: J. Cook
To limit the presence of nitrosamines (NAs) in drugs, health authorities (HAs) have established acceptable intake (AIs) for numerous common NAs, many of which are based on read-across to the simple, potent animal carcinogen nitrosodibutylamine (NDEA) for which a robust carcinogenicity study exists. Although there is animal carcinogenicity data available for many NAs, most studies were designed to explore the relationship between structure and potency, not to support quantitative dose response analysis. Many carcinogenicity studies employed a limited number of treatment groups and animals. Although much of this data cannot be used to derive TD50s (dose causing tumors in 50% of animals), the potency information about a series of NAs may be useful to help in establishing an AI when one or more compounds in the series has an established AI from another robust study. The goal of this assessment was to establish AIs for several alkyl NAs that were tested in rats by Druckrey et al (1967) via similar but limited study designs, and to use this data and structural activity relationships, such as degree of steric hindrance, to establish an AI for a complex drug-related nitrosamine (NDSRI) of high molecular weight. Data was collected for alkyl NAs including those with straight chains of different lengths (e.g., ethyl, methyl, propyl and butyl), as well as branched chain (isopropyl and isobutyl) and a piperidine. For each of the NAs, daily dose, total dose, and average tumor induction time data were summarized and from this data general potency groups were identified. These potency groups were anchored to AIs derived from robust animal carcinogenicity studies for NDEA, nitrosodimethylamine (NDMA), nitrosopiperidine (NPIP) and nitrosodibutylamine (NDBA), which were also tested by Druckrey. Based on the rodent carcinogenicity data published by Druckrey, it is possible to differentiate the AIs of alkyl NAs based on their relative potency. This is in contrast to many of the limits published by the HAs which often default to 26.5 ng/day. Methylethynitrosamine has a similar potency to NDMA, while ethylisopropylamine and diisopropylamine were of similar potency to NPIP. Increased steric hindrance from the presence of one or more isopropyl groups decreased the potency further; ethylisopropynitrosamine and diisopropylnitrosamine were of similar potency to NDBA (TD50 = 8 mg/kg/day) and the presence of a t-butylnitrosamine reduced the potency even further. In the absence of robust animal carcinogenicity studies, the AIs for the isopropyl and t-butylnitrosamines were aligned with the threshold of toxicological concern defined in ICH M7, as was that for the complex NDSRI that contained a t-butylnitrogropeptide alpha to the NA on one side and a large R group on the other. The use of all available animal carcinogenicity data, even when not sufficient for TD50 derivation, together with an understanding of how structural features affect metabolic activation of a NA, is an important step in establishing AIs for complex NDSRIs. Carcinogenicity data for a class of compounds all tested in experimental by a similar protocol can be used to gain an understanding of relative potency to aid in setting an AI that is protective of patients. This then allows for control and remediation efforts to be commensurate with the risk of the NA which may have impacts on the availability of important medicines.

3112 Quantifying Mortality Risk for Rodent Carcinogenicity Studies
Pharmaceuticals whose expected clinical use is for at least six continuous months may need to be evaluated for carcinogenic potential. The most common approach is to conduct a two-year rodent bioassay. Current trends demonstrate that swine models for rodent carcinogenicity studies have a low potential for surviving the full 104-weeks of the study. Using a large historical database and machine learning tools, we developed a model that assigns mortality risk for animals participating in rodent carcinogenicity studies. This has the potential to improve animal welfare, prevent data loss, and improve study logistics by forecasting animal mortality risk. We developed two data pipelines for this study: 1) model training applying Cox proportional hazards regression in MFlow to a large historical database and 2) using these results to assign mortality risk for animals participating in ongoing carcinogenicity studies. For model training, several attributes were matched to the intended target studies, such as study design, species, stock, and dosing route. The training model used features such as body weight loss and onset of several clinical observations to quantify mortality risk. We then applied the results from this model to ongoing studies to generate probabilities for survival for each animal at various time points. The data are visualized using Power BI. The results allow one to generate survival curves for animals at several levels of granularity: from the individual animal to across the entire study. The survival curves can be modified by applying different threshold levels to estimate how many animals will survive at different time points of the study. These results can be segmented by variables such as sex and dose group to plan for early study termination. In conclusion, we developed a tool that quantifies mortality risk for rodent carcinogenicity studies. It has the potential to be used to inform euthanasia decisions for individual animals, improve animal welfare, and prevent data loss from unscheduled deaths. It can also be applied to the entire study to improve logistics, e.g., identifying when the control group will reach a critical threshold for early study termination.

3113 An IQ Consortium Analysis of Starting Dose Selection for Oncology Small Molecule First-in-Patient Trials Suggests an Alternative NOAEL-Based Method Can Be Safe While Reducing Time to the Recommended Phase 2 Dose
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The first-in-patient (FIP) starting dose for oncology agents should be responsibly safe and provide potential therapeutic benefit to the patient. For late-stage oncology patients, this dose is often based on the ICH S9 guidance, which was developed primarily based on experience with cytotoxic chemotherapy agents using the rodent Severely Toxic Dose in 10% of the animals (STD10) or non-rodent Highest Non-Severely Toxic Dose (HNSTD) and an appropriate safety factor. With the increase in molecularly targeted chemotherapeutics, it is prudent to re-evaluate how the FIP dose is derived to ensure that the appropriate balance between risk and therapeutic benefit to the patient is achieved. Blinded data on 92 small molecule oncology compounds from 11 pharmaceutical companies who are members of the IQ Oncology Consortium were gathered to investigate if a NOAEL-based starting dose without a safety factor would have been tolerated in the FIP trial and if so, estimating how many dose escalation cohorts could have been reduced. Our analysis suggests that the NOAEL-based alternative starting dose would have been tolerated in most cases evaluated, with an anticipated mean reduction of 2.3 cohorts. Of the 12 cases where the NOAEL approach would have exceeded the Maximum Tolerated Dose/Recommended Phase 2 Dose (MTD/ RP2D), none of the nonclinical toxicities in these cases were considered irreversible and would be monitorable in all but one instance. Most non-tolerated cases were within 2 to 3-fold of the MTD/RP2D, with the clinical adverse effects (AEs) considered manageable and mitigated by dose de-escalation. No one method of FIP dose calculation will likely be appropriate for all oncology small molecules and starting dose selection should be performed using a case-by-case approach. However, the NOAEL-based method that does not utilize a safety factor should be considered when appropriate to minimize the number of patients exposed to sub-therapeutic doses of an investigational oncology agent and accelerating development to RP2D.

3114 Comparison of the Dermal Histopathological Structure between Bama and Göttingen Minipigs
X. Zhao, T. Zhou, S. McPherson, and X. Li. WuXi AppTec, Suzhou, China.
The use of the minipig as a non-rodent species in non-clinical research has continued to increase and although its use is still considerably less than that of the dog or the non-human primate (NHP), for certain areas of research, the physiological similarities between swine and humans make it the species of choice. A recent European RETHINK project has taken place to assess minipigs (in general not as a specific species) as models for the toxicity testing of new medicines and chemicals and concluded that there is great utility in their use in various areas of research. The minipig is often the species used for dermal studies. Swine skin shows many similarities to human skin. This has resulted in the extensive use of swine in burn and radiation research as the skin of other research animals such as rodents and dogs differs significantly from that of man. The epidermis of these animals is much thinner with a flat epidermal-dermal interface which does not have rete ridges and papillary projections, with relatively loose dermal structures and an underdeveloped vascular system. In comparison, swine skin is composed of three layers: epidermis, dermis, and subcutis. The epidermis contains the strata seen in human skin while the dermis contains superficial venules and arteriolar plexuses, collagen, elastic fibers, hair follicles, sebaceous glands, and apocrine sweat glands and the subcutis contains loose connective tissue and fat tissue. A comparison of skin samples (H&E) taken from the back of Göttingen and Bama minipigs showed that the structure of the two strata was very similar. The only difference of note was a slight difference in thickness of the dermis (slightly thicker in the Göttingen animals), but this may be related to the age of the animals examined. In conclusion, both Bama and Göttingen minipigs have a similar skin histopathological structure and their structures are comparable to humans.
The use of the minipig as a non-rodent species in non-clinical research has continued to increase and although its use is still considerably less than that of the dog or the non-human primate (NHP), for certain areas of research the physiological similarities between swine and humans make it the species of choice. A recent European RETHINK project has taken place to assess minipigs (in general not as a or the non-human primate (NHP), for certain areas of research the physiological... increases and although its use is still considerably less than that of the dog or the non-human primate (NHP), for certain areas of research the physiological similarities between swine and humans make it the species of choice. A recent European RETHINK project has taken place to assess minipigs (in general not as a or the non-human primate (NHP), for certain areas of research the physiological similarities between swine and humans make it the species of choice. A recent European RETHINK project has taken place to assess minipigs (in general not as a or the non-human primate (NHP), for certain areas of research the physiological similarities...
period in males and females receiving 2, 5 or 10 µg/dose compared to concurrent controls. These findings, except for the spleen weights in females, were fully reversible and did not impair the functionality of the organs and were therefore considered not to be adverse. We attribute the findings to adaptive changes of the immune system, in particular redistribution of lymphocytes, which were reversible and can be considered consistent with text article pharmacology and benign in nature. The No Observed Adverse Effect Level (NOAEL) of Lactobacillus ATG-K2 did not show any local tolerance at the same doses as the intravaginal repeated-dose toxicity study. In conclusion, the no-observed-adverse-effect level (NOAEL) of Lactobacillus plantarum ATG-K2 was 12×10^9 CFU/head/day and no target organ was identified in female rats. Our findings are the first to suggest that Lactobacillus plantarum is safe for use as an intravaginal treatment with no adverse effects observed in toxicological testing and has potential application as a therapeutic agent or for other biological uses.

**3119 Shedding Light on Lipid-Based Formulation Development on a BCS II Molecule Enabling an Animal Toxicology Study**

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In recent years, an increasing number of drug candidates discovered by high throughput screenings are poorly water-soluble compounds and show limited bioavailability following oral administration. Nowadays, LBFs have been successfully applied to several commercial products, ranging from lipid solutions, lipid emulsions, lipid dispersions, self-emulsifying drug delivery systems (SEDDS) and microemulsifying drug delivery systems (SMEDDS). In particular, SEDDS and SMEDDS are comprised by mixtures of lipids and surfactants that self-assemble spontaneously in aqueous media to form fine emulsions (SEDDS) or microemulsions (SMEDDS). The present work focused on the development of orally administered lipid-based formulations for a BCS class II, highly lipophilic (logP 5), and neutral compound to conduct animal toxicity study and Phase I clinical trials. Firstly, an excipient solubility screening was conducted including oils, surfactants and solubilizers suitable for oral delivery and selected based on internal knowledge of tolerability in dogs. Based on the excipient screening results, twelve vehicles were designed and tested to identify the most stable lipid-based formulations able to self-emulsify in biorelevant media. Taking into consideration the self-emulsification properties and the appearance of the formulations, the size of the micelles formed upon dispersion and the API target concentration of 50 mg/g, five prototype lead formulations were selected for in vitro lipolysis studies. The lipolysis study showed that formulation F12 and F14 were the least susceptible to digestion, F6 presented good solvent capacity but, it was susceptible to enzymatic digestion. F9 appeared to be the least robust as only 15% of the API was still in the micellar phase at the last time point. At the conclusion of the lipid digestion testing, the formulation prototypes F6, F12 and F14 were less prone to enzymatic digestion and could form SMEDDS upon dispersion in water. Therefore, these formulations were assessed using an in vitro bi-phasic dissolution. Based on the robustness against the enzymatic digestion, the progressive release of the API during the dissolution assay and the chemical stability of the formulation up to 14 days, the prototype F14 prepared at 90 mg/g concentration was considered an adequate prototype to conduct pharmacokinetic/pharmacodynamic studies (PK/PD). In addition, formulations F6 prepared at 75 mg/g was selected for PK studies since suitable chemical stability and the in-vitro performances were shown. Indeed, this formulation presented good tolerability following a 28 days tox study in beagle dogs and demonstrated to be stable as a liquid-filled hard capsule (LHFC) drug product for up to one year. Besides the tolerability concerns often related to the administration of high amount of lipid excipients in animals, this study demonstrated that through a knowledgeable design of lipid-based excipients a safe and successful formulation could be developed and the advantages of these excipients to enhance the bioavailability of a highly lipophilic compound exploited.

**3120 Evaluation of Intravaginal Toxicity of Lactobacillus plantarum ATG-K2 Powder in Female Rats**

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Bacterial vaginosis (BV) is a microbial dysbiosis that shifts the paradigms of vaginal flora from lacticobacilli to opportunist pathogens. Globally, BV is treated with antibiotic therapy and recurrence rates are > 70 % occurring within 6 months due to antibiotic resistance against pathogenic bacteria. An incorporation of lacticobacilli orally or intravaginally for the re-colonization of healthy microbes in vagina is the suggested way of treatment. Although lacticobacilli are suggested as a novel therapeutic for women’s BV, evaluation of safety and toxicity have not been well understood previously. Therefore, in this study, we aimed to evaluate the safety profile of Lactobacillus plantarum ATG-K2 in subacute intravaginal animal toxicity under OECD guidelines and GLP regulations. Toxicological assessments were performed at doses of 4×10^9, 8×10^9, and 12×10^9 CFU/head/day in a 2-week intravaginal repeated-dose toxicity study of Lactobacillus plantarum ATG-K2 in 10 female SD rats assigned per group. In addition, a local tolerance study was performed. No toxicological changes in clinical signs, body weights, water and food consumption, urinalysis, hematology, clinical biochemistry, gross findings, or histopathological examinations were observed in the intravaginal repeated-dose toxicity study. An excipient screening study ATG-K2 did not show any local tolerance in the dogs at the same doses as the intravaginal repeated-dose toxicity study. In conclusion, the no-observed-adverse-effect level (NOAEL) of Lactobacillus plantarum ATG-K2 was 12×10^9 CFU/head/day and no target organ was identified in female rats. Our findings are the first to suggest that Lactobacillus plantarum is safe for use as an intravaginal treatment with no adverse effects observed in toxicological testing and has potential application as a therapeutic agent or for other biological uses.

**3121 Refinement of Nonrodent Restraint in Preclinical Inhalation Studies**

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Nonrodent species restraint for the inhalated route of administration is necessary in nonclinical safety assessment studies. In order to minimize variability in the delivered dose, it is critical that animals be as calm as possible, and that the habituation process is an important part of any such study. As part of Charles River’s (CR) continued commitment to the 3Rs (Replacement, Reduction, and Refinement), refining preexisting processes and improving animal welfare is a constant focus. Our previous project needed to address concerns on day by day for increasing increments up to the intended dosing durations (which could be as much as 240 minutes in the case of dogs or 120 mins for NHPs) were reevaluated to identify areas for improvement. Several opportunities for improvement of the pre-existing non-rodent masks (both dog and NHP) were identified: fit (i.e., the gasket around the nose), expiratory gas flow, and harnessed an animal brought into the laboratory for an air trial and a mock dosing. These steps can be performed in a single session and rewards provided to recognize desired behavior such as not touching the mask, staying calm, and not moving. New masks were also tested for each species. A custom dog mask was fabricated for CR and made of a certified silicone seal attached to a clear resin body. For the NHP, a human nasal CPAP mask (commercially available) was trialed. Each mask was found to create a better fit to the animal. It was noted during the validation, and subsequently on production studies, that animals that used the new mask and habituation processes were calmer during dose administration.

**3122 Procedure-Related Findings in a 13-Week Intravenous Infusion Study in the Rat: Comparison of Methods Using a Vascular Access Harness and a Vascular Access Button**

J. Ducroq1, A. Tamellini2, T. Tighenifi1, V. Croccianti1, P. Vignand1, and S. Buler2. 1Charles River Laboratories, Saint Germain Nuelles, France; and 2Charles River Laboratories, Reno, NV.

Two different implantation procedures are commonly used for the treatment of rats by intravenous infusion in preclinical toxicity studies: the vascular access harness (VAH) and the vascular access button (VAB). Since access to the implantation site is different, the impact of the procedure could be different. The aim of the present study performed in male Wistar rats was to compare procedure-related findings noted with both methods in a 13-week intermittent infusion study with two different vehicles (0.9% NaCl or 50 mg/mL mannitol) commonly used for parenteral administration. Mannitol is a sugar-alcohol derived from mannose with an increased risk of bacterial contamination due to its sugar nature. In this study, four groups of 9 male rats were infused once daily (5 days a week) for 2 hours at 1 mL/kg/hour as follows: 0.9% NaCl or 50 mg/mL mannitol, 11/18 males rats equipped with a VAH had catheter obstructions that were resolved by flushing with saline, whereas only 3/18 males equipped with a VAB had a catheter obstruction. The other perfusion abnormality concerned only VAH animals and consisted in the disconnection of the catheter from the infusion line (10/18 rats). For three of these 10 rats, the extremity of the catheter retracted under the skin and a second surgery under anesthesia was needed to reconnect the catheter. All lesions due to the harness were noted for 3/18 VAH rats, correlating in 2 rats with skin ulceration and inflammation at histopathological examination. At terminal euthanasia, lower white blood cell counts were noted in the rats equipped with VAB and necropsy findings of firm consistency of the implanted vena cava and pale focus on the kidneys were only noted in males equipped with a
Tuberculosis (TB), a disease that primarily affects the lungs, is caused by the bacterium Mycobacterium tuberculosis (Mt). Roughly 25% of the global population is infected with TB, and it is the second leading cause of mortality from infectious disease after COVID-19. Multi-drug resistance in treating TB has been a growing concern in recent years. Mt has a synergistic relationship with HIV, often resulting in severe HIV-TB co-infections that are difficult to treat. The emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) strains of TB further narrows treatment options. Development of novel therapies targeting TB is necessary. Our collaborative efforts between the University of Texas Southwestern Medical Center and the University of Texas Health Science Center at Dallas, with hope of identifying a new class of therapeutics for TB, are currently under study.

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A Smooth Muscle Cell Maturation Protocol for Higher-Throughput Analysis of Contraction Force

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Cardiovascular diseases (CVDs) are the leading cause of death globally with 1.3 billion people of the world’s population suffering from hypertension, causing a tremendous public health issue. Despite this global burden, preclinical drug development lacks a relevant cell-based assay system employing human smooth muscle cells for the assessment of CVD-related hypertension. Here we assessed two aortic smooth muscle cell (HASMc) cultivation protocols and compared the maturation process of the cells on phenotypic level with immunostainings and on functional level with the FlexCyte 96 technology. Primary HASMcs were cultured on 25T plates for several days in recommended maintenance medium. After preculture, the cells were dissociated and plated on flexible membranes of the FlexCye 96 plates to ensure a human heart related physiological environment. Subsequently, the cells were cultured for 5 days in either maturation or maintenance medium before functional assessment of contractile properties. Here, compounds with positive inotropic effect were used. For phenotypic characterization, the cells were fixed and stained with Alexa Fluor 488 Phalloidin directly within the plate. The results demonstrate the effect of the maturation protocol on HASMcs with a stronger contraction (+30% compared to non-matured control cells) and more pronounced actin structures. The assessment of an optimal preculture protocol for HASMcs ensures ideal conditions for further development. The use of HASMcs on the FlexCye 96 96-well plate cell viability and toxicity in vitro, standardizing a new standard cell-based assay system for the analysis of CVD related hypertension.

Role of Respiratory Uncoupling in Drug-Induced Mitochondrial Permeability Transition


Mitochondrial injury contributes to severe drug-induced liver injury. Particularly, mitochondrial permeability transition (MPT) is thought to be relevant to cytotoxic hepatitis. However, the mechanism of drug-induced MPT is unclear and prediction of MPT is not adequately evaluated in the preclinical stage. In a previous study, we found that troglitazone, a drug withdrawn due to liver injury, induced MPT via mild depolarization resulting for the first time (Takemura et al., J. Toxicol. Sci., 2019). Herein, we investigated whether other drugs that induce MPT share similar properties as troglitazone, using isolated mitochondria from rat liver. Of the 22 tests drugs examined, six drugs (amiodarone, benzbrromarone, diclofenac, nimesulide, ticlopidine, and troglitazone) induced MPT and showed an uncoupling effect. A new operating characteristic (ROC) analysis was conducted to predict the MPT potential from the respiratory control ratio, an indicator of uncoupling intensity. Results showed that 2.5 was the best threshold that exhibited high sensitivity (1.00) and high specificity (0.81), indicating that uncoupling was correlated with MPT potential. Activation of calcium-independent phospholipase A2 appeared to be involved in uncoupling-induced MPT. Furthermore, a strong relationship between MPT intensity and the uncoupling effect among similar compounds was confirmed (thiazolidinediones anti-diabetes group, salicylic acid-non-steroidal anti-inflammatory drugs (NSAID) group, and fenamic acid-NSAID group). These results may help in predicting MPT potential using cultured cells and modifying the chemical structures of the drugs to reduce MPT risk. These data have already published in Toxicol. Appl. Pharmacol, 2021, 427, 115659.

Uridine Triacetate Rescues Intestinal Toxicity by DHODH Inhibition in Mice


Recently, the interest in DHODH as a drug target for haematological malignancies was renewed after evidence showing the efficacy of DHODH inhibitors in preclinical models of acute myeloid leukemia (AML). However, one of the key challenges for these inhibitors is the potential for off-target toxicities such as mucositis and myelosuppression. JNJ-74856665 is an orally bioavailable, potent, and selective DHODH inhibitor that mediates anti-leukemic activity in vitro and in vivo. As non-transformed cells can survive under DHODH inhibition by using the pyrimidine salvage pathway, we hypothesized that treatment with the nucleoside uridine could prevent the mucosal on target toxicity by DHODH inhibition. As a proof of concept, we designed an investigative study in mice dosed with JNJ-74856665 at 10 mg/kg/day PO daily for 7 days with or without uridine triacetate (UTA), an oral prodrug of uridine, at 2000 mg/kg TID PO daily for 7 days. Previously, we showed in a mouse PK study that this UTA dosing regimen resulted in an exposure to uridine comparable to that of IP uridine regimens preventing 5-Fluorouracil lethality in mice. Overall, mice dosed with JNJ-74856665 and UTA showed better tolerability compared to JNJ-74856665 alone, with a lower incidence of clinical observations (rough haircut) and less BW loss. The intestines of these mice were examined at the histopathology level as a readout for the efficacy of UTA preventing mucosal injury after DHODH inhibition. JNJ-74856665 alone resulted in minimal to moderate crypt epithelial necrosis in the small and large intestines and villus atrophy in the small intestine. In contrast, no microscopic findings were noted in the intestines of mice dosed with JNJ-74856665 and UTA. In agreement, histomorphometry studies revealed that villus shortening in the jejunum of JNJ-74856665-dosed mice was completely prevented by UTA. Finally, we confirmed by toxicokinetic analysis that the effects of UTA were not related to a modification of JNJ-74856665 exposure. These preclinical results suggest that uridine treatment could potentially mitigate mucositis in patients treated with DHODH inhibitors. Further studies are required to explore the best regimen of uridine treatment to protect the normal mucosa without compromising the anti-leukemic effects of DHODH inhibitors.

Assessment of Drug-Induced Liver Mitochondrial Dysfunction in Primary Human Hepatocytes Using XF MitoTox Assay

Y. Yam, L. Winner, G. W. Rogers, and N. Romero. Agilent Technologies Inc., Lexington, MA. Sponsor: Y. Yam, American Association for Cancer Research. Mitochondrial dysfunction is known to play a central role in drug-induced hepato-toxicity, and thus early detection of mitochondrial toxicity is highly desired in early drug development. Agilent Seahorse XF MitoTox Assay is a novel solution designed to study mitochondrial toxicity in vitro. This technology standardizes a new standard cell-based assay system for the analysis of CVD related hypertension.

Validation of a Serum-Free Approach to Facilitate the Development of High-Throughput Immunogenicity Screening Assays In Vitro

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The generation of anti-drug antibodies (ADA) towards a therapeutic agent can have severe implications in terms of drug safety and efficacy following administration in a clinical setting. Identification of high-risk candidates during the initial phase of development is therefore, imperative in order to improve both patient outcomes and downstream attrition rates. As species often fail to mimic the complexity of the human immune system the immunogenic potency of therapeutic agents, in terms of their potential to elicit a human-specific proinflammatory response, can be grossly underestimated during traditional in vivo safety assessment. As part of an early de-risking strategy, we performed a high-throughput, in vitro approach to immunogenicity screening using two distinct models: i) peripheral blood mononuclear cells (PBMC) and ii) monocyte-derived dendritic cells (MoDC) isolated from the blood of healthy human donors. The use of serum-free culture conditions was also explored to improve assay variability and facilitate a move towards a more robust screening assay. A variety of compounds were screened for immunogenic effects in each model, including monoclonal antibodies, small molecules associated with delayed-onset hypersensitivity reactions and oligonucleotides. Compound immunogenicity was assessed according to the ability of the test article to induce lymphoproliferation and cytokine release after a period of 6 days and 72 hrs.
respectively. For MoDC, an exposure period of 24 hrs was used to measure cytokine secretion. For in vivo experiments, in each mouse, the PK/PD parameters for the SCD product/substrate ratio were monitored. For this purpose, an assay was developed to measure the SCD product/substrate ratio in the Harderian gland and tear fluid of the rats and dogs. In addition, histopathological analysis was performed to determine the SCD product/substrate ratio in the Harderian gland and tear fluid of the rats and dogs. The histopathological analysis showed that the SCD product/substrate ratio was lower in the Harderian gland and tear fluid of the rats and dogs than in the controls. These results suggest that the SCD product/substrate ratio in the Harderian gland and tear fluid of the rats and dogs may be influenced by the SCD product/substrate ratio in the Harderian gland and tear fluid of the rats and dogs. Therefore, the SCD product/substrate ratio in the Harderian gland and tear fluid of the rats and dogs may be an important factor in the development of ocular and adnexal effects due to sebaceous and meibomian gland atrophy.

3133 Validation of Brief-Access Taste Aversion (BATA) as a Preclinical Test for the Palatability Evaluation of Pediatric Formulations

The taste of an oral medication is a critical quality attribute for therapeutic adherence and patient compliance, especially in children. Indeed, the unpalatable taste is often cited as the primary cause of failure of therapeutic regimes. The importance of building palatability into pediatric medicines is now regulated by the Pediatric Investigation Plan (PIP). Palatability studies with human taste panels are carried out at the latter stages of formulation development if at all. Thus, there is a great need to develop novel strategies to assess the palatability and effects of medicines at early stage of development. The brief-access taste assessment (BATA) is an in vivo screening tool with great promise in providing the assessment of APIs palatability that may predict human taste responses. The BATA procedure was used to compare conditioned licking responses to a concentration array of compounds that humans describe for example as "sweet" (sucrose), "salty" (NaCl), "sour" (citric acid), and "bitter" (quinine). The BATA assay operationally defines aversive taste as suppression of the rate at which a rodent licks from sipper tubes that deliver tasting solutions or suspensions. To validate a BATA model in our facility, independent groups (n=14) of naive male Sprague-Dawley rats were used. Different groups of animals were given access to the different compounds with a randomized sequence of presentation across animals. We performed different trials with quinine hydrochloride and sucrose. The animals were mildly water-deprived and then put into a special experimental cage. The instrument recorded the number of licks (lickometer) that the rodents make if the API was presented in different concentrations in several sipper tubes. Animals only had a very short period of time (between 5 and 10 s) to test each solution. Typically, a high number of licks indicated a pleasant taste whereas a low number of licks an aversive taste. With this procedure, a full doseresponse (concentration-aversion) curve of licks was recorded over a short period of time. Independent experiments performed demonstrated the validity of the model to discriminate increasing concentration of bitter taste using quinine hydrochloride solutions while no differences were found when comparing different increasing concentration of sucrose with water. Further experiments are still ongoing in order to expand the list of compounds and edulcorants.

3134 Inhibition of Steroaryl-CoA Desaturase Causes Ocular Effects in Rats and Dogs

Steroaryl-coenzyme A desaturase (SCD) is a microsomal enzyme that catalyzes the formation of a cis-double bond at the delta-9 position of saturated fatty acyl-CoA esters. The oxidation of saturated lipid products are major components of triglycerides, cholesterol esters, and membrane phospholipids. Lipids play a critical role in the pathology of metabolic disease, and SCD dysregulation has been implicated in the development of dyslipidemia, diabetes, cancer, obesity, fatty liver, and other metabolic disorders. Considerable evidence has therefore been placed on the development of small molecule SCD inhibitors. However, adverse taste triggered receptor mediated transcytosis of bitter taste can be induced in the skin and eye (dry eye symptoms) have been reported in rodents following systemic exposure to such molecules. Skin and eye effects due to sebaceous and meibomian gland atrophy have also been observed in SCD1 knockout mice. The ocular changes have been attributed to degeneration of the meibomian and/or Harderian glands in rats and mice with depletion of SCD-derived lipophilic fluids for tears. Strategies to overcome these effects have been attempted, including liver-targeted molecules to reduce systemic exposure. In this study, MK-8245, a potent, reversible, orally active, small molecule SCD inhibitor with a 90-fold liver to plasma ratio and low nanomolar potency was investigated in a GLP repeat-dose oral rat and dog toxicity study. The studies were 1 month in duration (rats: 0, 10, 100, 1000 mg/kg/day, 10/sex/group; dogs: 0, 3, 20, 375 mg/kg/day, 3/sex/group). The studies included routine antemortem parameters and determination of the lipid desaturation index (DI = ratio of SCD product/substrate) in the Harderian gland in rats and in the tear fluid of dogs. Despite the liver-targeted strategy, MK-8245 produced ocular and/or adnexal effects at the highest dose in both species. In dogs, after 4 weeks, there were gross ocular findings (redness of bulbar conjunctiva and cloudy eye) and ophthalmoscopic findings (corneal neovascularization with/without edema, and bulbar conjunctival hyperemia). Histomorphologic findings included meibomian gland atrophy with squamous metaplasia and secondary corneal erosion and neovascularization of the corneal stroma. Histological changes in the skin of mice after treatment with MK-8245 were consistent with the clinical signs observed in the dogs.
The objective of this study was to examine the in vivo retinal microanatomy of findings observed during pretest opthalmic examination in laboratory rabbits. Five-to-fourteen-month-old Dutch-Belts rabbits were evaluated via complete ophthalmic examinations that included slit lamp and indirect ophthalmoscopy, fundus photography and confocal scanning laser ophthalmoscopy and spectral domain-optical coherence tomography (cSLO/sd-OCT). Normal and affected eyes were collected and evaluated microscopically after in vivo imaging. Five male and two female rabbits presented unilateral or bilateral, oval, pink hued lesions superior to the optic nerve head. Lesions were autofluorescent under AF mode, and OCT showed focal retinal detachment, autofluorescent material, likely lipofuscin, accumulated between photoreceptor and RPE layers. Hematoxylin and Eosin-stained histology sections showed retinal detachment, outer retinal atrophy, and subretinal accumulation of amorphous amphophilic material. Serial Two-Photon Plus system (STP+) showed in 2D and 3D images the location of the lesions and detached retina with material accumulation in the subretinal space. In conclusion, fundus abnormalities often observed during pretest ophthalmic examination and broadly categorized as choriorretinal scars have been further characterized to identify specific microarchitectural changes. This is the first report correlating in vivo microanatomical and histological description of these retinal findings in Dutch-Belts rabbits.

SkinEthic HCE Time-to-Toxicity: The First New Approach Methodology Adopted by the OECD for Discriminating On its Own the Three UN GHS Ocular Hazard Categories

For more than two decades, scientists have been trying to replace in vivo rabbit eye irritation test with non-animal methods. So far, several in vitro new approach methodologies (NAM) have been implemented into regulations, however none of them is able to replace the test completely due to the complexity of the endpoint and the classification schemes applied by the regulation under the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS).

Taking into account our expertise on tissue engineering on the SkinEthic™ HCE model (test system of OECD TG 492) and our knowledge on associated protocols, the SkinEthic™ HCE Time-to-Toxicity test method was established and adopted by OECD as the first stand-alone NAM for discriminating the three UN GHS ocular hazard categories (OECD TG 492B). The test method evaluates the hazard potential of a chemical in vivo on its ability to induce cytotoxicity and necrosis. The methodology, a set of skinEthic protocols, one for liquids and one for solids. Based on the viability observed for the different exposure periods (from 5 to 120-min) a classification was assigned. The method, initially developed with 74 training chemicals, was challenged with 52 test chemicals selected on the basis of important criteria were selected for testing in a three-phase study using a common set of non-animal test methods. Criteria included availability of historical rabbit data or ocular irritancy classification information, representation of common agrochemical formulation types, and a range of U.S. Environmental Protection Agency (EPA) and United Nations Globally Harmonised System of Classification and Labelling of Chemicals (GHS) hazard classifications. In Phase 1, six formulations classified as non-irritating (EPA Category IV; GHS Not Classified [NC]) or severely irritating (EPA Category I; GHS Category 1) were evaluated in seven in vitro or ex vivo eye irritation test methods as a proof-of-principle to determine which methods might be useful in the defined approach. Ten additional formulations representing the full range of classifications (EPA Categories I, II, III, and IV; GHS Categories 1, 2A, 2B, and NC) were tested in Phase 2. Based on an assessment of the results and considering the relevance of each method to humans, four in vitro or ex vivo assays were selected to proceed with Phase 3 testing of an additional twelve formulations classified as moderately (EPA Category II; GHS Category 2A) or mildly irritating (EPA Category III; GHS Category 2B). Additionally, a subset of thirteen formulations were tested in a fifth method. A total of 29 formulations have now been tested in as many as five methods: Bovine Corneal Opacity and Permeability (with histopathology and quantitative analysis); Organisation for Economic Co-operation and Development Test Guideline (OECD TG 437), EpilOcular (OECD TG 492), SkinEthic Time-to-Toxicity (OECD TG 492B), and Ex Vivo Cornea Culture Models. Data generated in this study were used to analyze alignment across these five non-animal methods and the rabbit test. Consensus predictions for each formulation were determined based on majority alignment among individual assay results. Consensus predictions were achieved for 26 and 27 of 29 formulations for EPA and GHS classification systems, respectively, and were demonstrated for the majority of the remaining formulations. These data form the basis of ongoing work to develop a defined approach for assessing eye irritation potential of agrochemical formulations. This project was funded in whole or in part with federal funds from the NIH, NIH under Contract No. HHSN273201500010C.

Phenylarsine Oxide–Induced Corneal Injury Involves Oxidative Stress–Mediated Unfolded Protein Response in Human Corneal Epithelial Cells and Rabbit Ex Vivo Cornea Culture Model

Arsenicals are highly toxic inorganic and organic derivatives of arsenic developed during World War I and II that can cause severe injuries in many tissue systems such as the lungs, skin, liver, and eyes. Phenylarsine oxide (PAO), an analog of lewisite, is a highly toxic trivalent arsenical and a potential chemical warfare agent. PAO-induced toxicity has been studied in lung, liver, and skin tissues but not on ocular tissues, even though eyes are uniquely vulnerable to injury by vesicants. Dendritic human corneal and rabbit corneal in vitro models such as lewisite have been shown to cause edema of eyelids, inflammation, massive corneal necrosis, and blindness. Accordingly, we first studied PAO-induced toxicity in human corneal epithelial cells, and further validated the findings in the rabbit ex-vivo cornea culture model of PAO exposure. Human corneal epithelial cells were exposed to different concentrations of PAO (50-300 nM) for 4, 6, and 24 h. MITT and Trypan Blue assays revealed that PAO decreased the viability of human corneal cells in a concentration- and time-dependent manner; concentrations of 100 and 200 nM and a time-point of 24 h were found to be optimal for the mechanistic studies. As arsensical-induced injuries in different tissues are often associated with oxidative stress, we found that PAO treatment induced strong oxidative stress in corneal epithelial cells, and a simultaneous treatment with 10 mM N-acetyl cysteine (NAC) reversed the PAO-induced toxicity in human corneal epithelial cells. Oxidative stress induction by PAO was accompanied by unfolded protein response (UPR) signaling activation. Further investigations using a rabbit ex-vivo cornea culture model revealed similar results as treatment of PAO (5 and 10 µg) for 3, 5, and 10 min caused moderate to extensive corneal epithelial layer degradation (~50-90%, P<0.05) and reduced the epithelial layer thickness (~20-70%, P<0.05) in a dose- and time-dependent manner. Similar to human corneal cells, injuries by PAO in rabbit corneas were also associated with oxidative stress and UPR signaling activation as evidenced by enhanced expression of heme oxygenase (HO)-1 and activating transcription factor (ATF)-6.
Sulfur mustard (SM; bis[2-chloroethyl] sulfide) is a strong blistering, highly reactive, lipophilic chemical warfare agent known to cause toxicity to skin, respiratory system, and eyes. Cornea is one of the most important structures in the eye, and a small change in the structural integrity of the cornea can cause blurred vision. Therefore, to better understand SM injury mechanisms in the limbal tissue and Dex efficacy against SM-induced injuries, proteomics analysis was performed on tissues obtained from the New Zealand White rabbit corneas. Treatment groups: Group 1, non-treated control eyes; Group 2, SM exposed eyes; Group 3, SM exposed and Dex (0.1%) treated eyes. Dex was applied topically as an eye drop (commercially available) beginning at 2 h after SM exposure and given every 8 h up to 28 days (tissue harvesting time point) post SM exposure. Raw spectra were interpreted against Oryctolagus cuniculus protein sequences downloaded from UniProt. From the database of 45,894 (reviewed and unreviewed) proteins, 1868 proteins were identified in the limbal tissue samples (FDR <0.01), one-way ANOVA followed by Tukey’s post hoc confirmed 83 proteins that were significantly different between the three groups (FDR 0.05, p<0.05). In the group wise comparison, 69 proteins were significantly changed in SM vs control group whereas 40 proteins were significantly different in SM+Dex vs SM, and 15 proteins were significantly different in SM+Dex vs control group. Out of 33 proteins upregulated by SM exposure, 30 proteins were downregulated by Dex treatment, and out of 36 proteins downregulated after SM exposure, 35 were upregulated by Dex treatment. Top 3 predicted pathways generated by pathway analysis included carbohydrate metabolism, endoplasmic reticulum stress, and CAR signaling pathway. These results suggest that SM increases the oxidative stress and immunological responses in limbal tissue. We hypothesize that the efficacy of Dex treatments against SM-induced ocular injuries involves decreased oxidative stress via increasing glutathione-dependent pathways as well as immune responses by decreasing DEAS proteins. Supported by U01 EY030405.

**3139 Developing an Ex Vivo Human Cornea Culture Model of Nitrogen Mustard–Induced Injury and Exploring Dexamethasone as an Effective Therapeutic Intervention**

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Even a century since sulfur mustard (SM) was first used in warfare during World War I, threats of SM exposure are impending from stockpiles and improperly discarded munitions. Ocular, dermal, and inhalation are predominant SM exposure routes. Eyes are most susceptible to SM toxicity due to their high rate of cellular turnover and water content. Nitrogen mustard (NM) is a bifunctional alkylating agent and a potent analogue of SM that can be used in laboratory setting to study vesicant-induced ocular toxicity. Previously, we have established SM- and NM-induced corneal injury models in rabbit corneas (in vivo and ex vivo rabbit corneal cultures). We have also shown that dexamethasone (DEX), a steroid anti-inflammatory drug, alleviates vesicant-induced corneal injuries in these models. In the present study, we developed an ex vivo human cornea culture model of NM exposure and found new molecular mediators of DEX action. Human corneas were subjected to three NM exposure conditions: 100 nmol for 2 h, 100 nmol for 4 h, and 200 nmol for 2 h, with one eye exposed to NM and the other eye exposed only to the culture media (control). NM-induced injuries were assessed 24 h post exposure using biological parameters (epithelial degradation using H&E staining). The 100 nmol for 2 h condition was found to be optimum for NM exposure. Both 100 nmol for 4 h and 200 nmol for 2 h exposures caused detrimental damage to the human corneal epithelium, leading to near complete denudation that was not ideal to study DEX efficacy. Thus, the 100 nmol NM for 2 h was used for all further studies. Histopathological analyses showed increased epithelial degradation (~47%) and epithelial-stromal separation (2-fold increase) 24 h post exposure compared to the controls (n=3-5). Of the 87 proteins assessed, 24 proteins had a ≥40% change in expression as compared to a 24 h post NM exposure to assess treatment efficacy. DEX administration was initiated at 2 h post NM exposure and every 8 h thereafter, until the study endpoint of 24 h post exposure. Cultured corneas were exposed to 100 nmol of NM for 2 h (both eyes in a pair) and only one eye (left or right selected randomly) received the DEX 8 h treatment. Histopathological analyses showed decreased epithelial-stromal separation (50%) upon DEX treatment 24 h post NM exposure. Of the 24 proteins identified, 6 proteins were found to have a significant DEX treatment effect (Student’s t-test, p<0.05) 24 h post exposure. These proteins were delta like canonical Notch ligand 1 (DLL1); endostatin, erb-b2 receptor tyrosine kinase 4, fibroblast growth factor 2 (FGF basic); CD54 and chemo kinase ligand 7 (CCL7); notably, these proteins are known to play a significant role in angiogenesis, leukocyte infiltration, inflammatory responses as well as cell differentiation and proliferation. Taken together, this study suggests that DEX targets critical pathways of wound healing, particularly reversing vesicant-induced neo-vascularization (DLL1 and FGF basic) and leukocyte infiltration (CD54 and CCL7), that have not been explored before and are important in understanding the underlying mechanism of action of DEX to CounterACT vesicants-induced injuries in human cornea. Supported by U01 EY030405.

**3140 Proteomic Analysis to Investigate Sulfur Mustard–Caused Limbus Tissue Insults and Dexamethasone Efficacy in the Rabbit Ocular Injury Model**

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Sulfur mustard (SM; bis[2-chloroethyl] sulfide) is a strong blistering, highly reactive, lipophilic chemical warfare agent known to cause toxicity to skin, respiratory system, and eyes. Cornea is one of the most important structures in the eye, and a small change in the structural integrity of the cornea can cause blurred vision. However, corneal exposure to toxins like SM can cause mild to severe injury that may eventually lead to total blindness. Ophthalmic manifestations associated with SM exposure involve persistent epithelial defects, dry eye condition, cornea gas keratopathy, limbal tissue ischemia, limbal stem cell deficiencies, and limbal epithelial defects. Thus, the unique ability to regenerate corneal epithelial layer depends upon the limbal epithelial stem cells (LESC) residing in the limbal region that circumvent the cornea, and act as a boundary between cornea and conjunctiva. SM exposure crafts a void in the regenerating ability of corneal epithelial by creating LSEC; however, mechanism of action of SM induced injuries in the limbal region is still unknown. Also, whereas dexamethasone (Dex) is shown to be effective in SM-induced corneal injuries, its therapeutic effects in the limbal tissue have not been investigated. Therefore, to better understand SM injury mechanisms in the limbal tissue and Dex efficacy against SM-induced injuries, proteomics analysis was performed on tissues obtained from the New Zealand White rabbit corneas. Treatment groups: Group 1, non-treated control eyes; Group 2, SM exposed eyes; Group 3, SM exposed and DEX (0.1%) treated eyes. DEX was applied topically as an eye drop (commercially available) beginning at 2 h after SM exposure and given every 8 h up to 28 days (tissue harvesting time point) post SM exposure. Raw spectra were interpreted against Oryctolagus cuniculus protein sequences downloaded from UniPort. From the database of 45,894 (reviewed and unreviewed) proteins, 1868 proteins were identified in the limbal tissue samples (FDR <0.01), one-way ANOVA followed by Tukey’s post hoc confirmed 83 proteins that were significantly different between the three groups (FDR 0.05, p<0.05). In the group wise comparison, 69 proteins were significantly different in SM vs control group whereas 40 proteins were significantly different in SM+Dex vs SM, and 15 proteins were significantly different in SM+Dex vs control group. Out of 33 proteins upregulated by SM exposure, 30 proteins were downregulated by Dex treatment, and out of 36 proteins downregulated after SM exposure, 35 were upregulated by Dex treatment. Top 3 predicted pathways generated by pathway analysis included cancer metabolism, endoplasmic reticulum stress, and CAR signaling pathway. These results suggest that SM increases the oxidative stress and immunological responses in limbal tissue. We hypothesize that the efficacy of Dex treatments against SM-induced ocular injuries involves decreased oxidative stress via increasing glutathione-dependent pathways as well as immune responses by decreasing DEAS proteins. Supported by U01 EY030405.

**3141 Proteomic Analysis Shows Dexamethasone Targets ACTin Polymerization Associated Pathways to Reverse Sulfur Mustard–Induced Toxicity in Rabbit Corneas**

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Sulfur mustard (SM), a vesicating chemical warfare agent, primarily causes injuries to organs that are most exposed to the external environment including eyes, skin and respiratory system, where eyes are the most sensitive sensory organ susceptible to SM-induced corneal and conjunctival injuries. Upon exposure to SM, dry eye disease, persistent epithelial defects, blepharitis, cornea thinning, corneal opacity and ulceration, corneal vascularization, limbal tissue ischemia and limbal stem cell deficiency are the injuries that appear in the ocular tissues. Despite intense efforts from toxicologists, mechanism of action of SM-induced injuries has not been comprehended completely. Also, we have previously shown that dexamethasone (Dex, FDA approved commercially available drug) is a potent therapeutic intervention against SM-induced corneal injuries; however, its mechanism of action is also not well known. Therefore, to investigate the obscured evidence regarding the mechanism of action of SM-induced corneal injuries and Dex efficacy against them, we used an LC-MS/MS based Accurate Mass and Retention Time (AMRT) Library strategy to perform protein quantitation on a 6550 Q-TOF with database searching in PEAKS Studio X+ and quantitation performed in Profinder v.10 software on the corneal tissues from rabbit eyes from three groups, namely: Group 1, untreated control eyes; Group 2, SM exposed eyes; Group 3, SM exposed and Dex (0.1%)
Acrolein is the simplest unsaturated aldehyde, commonly used as a broad-spectrum biocide for controlling plant and mollusk growth in water systems, as well as in industrial applications, such as the manufacturing of metals, resins, and plastics, as well as pharmaceuticals and perfumes, rely on acrolein as an intermediate. Given its availability and prior use as a chemical weapon in World War I, acrolein has been recognized as a chemical of concern. However, despite the eyes being uniquely vulnerable to injury by exposure to chemical toxins, there has been limited study on ocular injury resulting from acute acrolein exposure. While slit lamp biomicroscopy is the standard clinical evaluation technique for ocular chemical exposures, our prior work indicated that high-resolution anterior segment optical coherence tomography (AS-OCT) was able to effectively assess chemical eye injury over time, providing information about structures beyond the ocular surface and allowing for identification and measurement of key biomarkers predicting injury progression. This study applied the same techniques, aiming to characterize the ocular injury and wound healing response caused by acute acrolein exposure. Following an IACUC-approved protocol, an acute injury model of acrolein exposure was performed in a mouse model through topical application of 0.5 mg/mL (n=15) and 2.5 mg/mL (n=10) acrolein for 30 s. Anterior segment optical coherence tomography (AS-OCT) was performed prior to the injury and up to 28 days following the induction of injury. In addition, fluorescein angiography (FA) was performed on day 28. A custom segmentation algorithm automatically measured the corneal thickness measurements corroborated this observation, indicating that swelling peaked at day 2 with an average 20% increase in corneal thickness, which persisted into day 7 before beginning to reduce slowly over the next two weeks. In addition, 80% of the mice were affected by limbal neovascularization, beginning as early as day 14. In subsequent weeks, angiogenesis spread centrally into the cornea from the limbus. While there was no significant difference in corneal thickness, a trend indicating a mild 20% increase in corneal opacity was also seen. This trend paralleled that of corneal swelling, with a similar peak in corneal opacity at day 3 that would gradually reduce over time. Furthermore, preliminary histology indicates the possibility of corneal stromal fibroblast loss and the presence of collateral damage beyond the anterior segment of the eye. In this study, we described the progression of chemical injury and wound healing following ocular exposure to acrolein. We demonstrated that topical exposure of the eye to acrolein leads to a concentration-dependent response including mild corneal edema accompanied by limbal and corneal neovascularization. This contrasts with observations from other chemical eye injury models such as mustard gas or sodium hypochlorite where severe corneal edema, in addition to other pathologies like corneal opacification and Descemet’s membrane detachment, frequently preceding the development of neovascularization has been observed. With only mild signs of ocular injury several weeks before the start of neovascularization, we believe this is the wound healing response may be the result of the formation and accumulation of DNA adducts, a primary mechanism of acrolein’s effects observed in other types of tissues. For the long time, assessment of a serious eye damage/eye irritation relied on the use of laboratory animals - in vivo Draize eye irritation test, OECD TG 405. In 2015, a new test guideline (OECD TG 492) was accepted which enables the use of an in vitro procedure based on reconstituted human cornea-like epithelium (RhCE) to distinguish between chemicals (substances and mixtures) not requiring classification and those that must be labeled for eye irritation or serious eye damage. However, this method could not distinguish between chemicals causing serious eye damage and less-severe eye irritation. Recently, OECD TG 492b has been accepted which describes in vitro procedure allowing the identification of chemicals that: a) do not require labeling for serious eye damage or eye irritation (No Category or Cat 0), b) can cause serious eye damage (Category 1 or Cat 1), and c) are eye irritants (Category 2 or Cat 2) according to the UN GHS ocular hazard categories. The Episcleral™ time-to-toxicity test method was developed for eye hazard identification of liquid and solid chemicals according to the three UN GHS categories. The method is based on the results of several specialized projects. The CON4EI project resulted in the prediction models for liquids and solids based on a set of 50 chemicals (38 liquids and 42 solids). Additional chemicals were tested within the ALTAEI project, and another set of chemicals was added in 2022. This resulted in a robust final set of 144 reference chemicals - 78 liquids and 66 solids, to confirm the proposed testing strategy. The performance criteria established by the OECD expert group in 2009 for phototoxic and photocytotoxic chemicals were confirmed. Using proposed testing strategy for liquids, we were able to correctly identify 78.7% of Cat 1 (N=27), 65.3% of Cat 2 (N=26) and 82.0% of No Cat (N=25). Using proposed testing strategy for solids, we correctly predicted 75.0% of Cat 1 (N=28), 59.4% of Cat 2 (N=16) and 80.3% of No Cat (N=22). Overall, 76.8% of Cat 1 (N=55), 51.8% of Cat 2 (N=47) and 87.5% of No Cat (N=47) were predicted. The Episcleral™ time-to-toxicity test method is a novel approach for subcategorizing both liquid and solid compounds. The developed prediction models have proven to be capable of successful distinguishing of chemicals (substances and mixtures) into 3 UN GHS ocular hazard categories: No Cat, Cat 0, and Cat 1.
Chloropicrin (CP), a choking and lacrimating agent, has been used as a warfare chemical agent, mainly during World War I. It is currently a popular pesticide and fumigating agent; thereby facilitating its easy acquisition and potential use as a chemical attack agent in addition to its accidental and occupational exposures. Exposure to CP results in severe ocular injury, especially to the corneal epithelium. Studies on corneal injury progression and underlying mechanisms in a relevant in vivo animal model are lacking. This has mainly hindered the development of effective therapies to treat the acute and long-term ocular CP effects. To study the clinical and biological effects of CP on ocular exposure, we tested different CP concentrations in mice to develop a relevant murine ocular injury model. This model will aid in identifying the mechanism of CP-induced ocular injury and in efficacy studies to develop effective treatments. The left eye of male BALB/c mice was exposed to CP using a vapor cap (20% CP for 1 min or 30 sec (0.4 mg of CP) or 10% CP for 1 min (0.2 mg of CP)), and the right eye served as a control. Mice were euthanized at day 25 post CP exposure, and the eyes were harvested to study the corneal injury further. CP-exposures caused a significant increase in corneal ulceration and eyelid edema, which resolved by day 14 post-exposure. However, CP exposure caused an unresolved significant increase in corneal opacity and neovascularization.

Development of hydrops (corneal Bullae due to corneal edema) and hyphema (bleeding into the anterior chamber) was observed after CP-induced effects. Histopathological analyses showed a significant CP-induced decrease in corneal epithelial thickness and increased stromal thickness with more pronounced injury, including stromal fibrosis, edema, neovascularization, anterior and posterior synechia and infiltration of immune cells. Loss of the corneal endothelial cells and Descemet membrane could be associated with the CP-induced corneal edema with fluid buildup causing hydrops that can contribute to cloudy vision, corneal scarring and eye pain. Damage to the uveal vessels from CP might be further responsible for corneal opacity and hyphema. Although exposure to 20% CP for 1 min caused more eyelid edema, ulceration and hyphema, other effects were similar in all CP-exposed eyes following CP exposure. A mouse model of CP-induced ocular injury will be helpful in ocular injury model development and pathophysiological studies to identify molecular targets for therapeutic interventions.

The simultaneous measurement of the cardiovascular and respiratory systems has gained recent popularity. However, due to its interdependent interaction, many models fail to clearly determine the contribution of each system when both cardiovascular and respiratory indices are altered. In this study, we aimed to extrapolate the previously created rat model to rabbits and create an anesthetize model that allows for simultaneous cardiovascular and the respiratory measurements to determine specific in vivo liabilities for active toxic effects. To validate the interdependency of the model, a known neuromuscular blocker (cistatracurium) and negative inotrope/vasodilator (verapamil) were utilized. Eight anesthetized (isoflurane, ketamine, and fentanyl) and mechanically ventilated male New Zealand White rabbits were acutely instrumented to simultaneously assess systemic/left ventricular hemodynamics (via catheterization) as well as skeletal and diaphragmatic (respiratory) force (via strain gauge). In four rabbits, data were collected both before and during verapamil (three 0.2 mg/kg IV boluses) and cisatracurium (2 µg/kg/min for 30 min IV or 0.01 to 0.05 mg/kg bolus). In the other four, the stability of the prep was tested up to 8 hours. Verapamil dose-dependently decreased systemic pressures, elevated filling pressures and decreased indices of both systolic (e.g., dp/dtmax) and diastolic cardiac function (slowed tau). Verapamil moderately depressed diaphragmatic (via fentanyl) and negative inotrope/vasodilator (verapamil) were utilized. Eight anesthetized (isoflurane, ketamine, and fentanyl) and mechanically ventilated male New Zealand White rabbits were acutely instrumented to simultaneously assess systemic/left ventricular hemodynamics (via catheterization) as well as skeletal and diaphragmatic (respiratory) force (via strain gauge). In four rabbits, data were collected both before and during verapamil (three 0.2 mg/kg IV boluses) and cisatracurium (2 µg/kg/min for 30 min IV or 0.01 to 0.05 mg/kg bolus). In the other four, the stability of the prep was tested up to 8 hours. Verapamil dose-dependently decreased systemic pressures, elevated filling pressures and decreased indices of both systolic (e.g., dp/dtmax) and diastolic cardiac function (slowed tau). Verapamil moderately depressed diaphragmatic function. On the other hand, cisatracurium elicited a reduction in diaphragmatic and skeletal muscle force by -77 ± 6% and -86 ± 5%, respectively without causing a change in the main cardiovascular parameters (systemic pressure, dp/dtmax and TAU, etc.). Time-control experiments proved the preparation was relatively stable for up to 8 hours - with a slight reduction in dp/dtmax (from 3626 ± 317 to 3023 ± 310 mmHg/s), slight increase in diaphragmatic force (from 48 ± 4 to 54 ± 7 g), a reduction in skeletal muscle force (from 8.2 ± 0.5 to 6.0 ± 1.4 g). Taken together, these results/observations demonstrate an in vivo anesthetized and mechanically ventilated rabbit model is capable of detecting neuromuscular (diaphragmatic and skeletal) and cardiovascular alterations in situ. The rabbit preparation was shown to be both stable and responsive to independent cardiovascular/neuromuscular changes triggered by either verapamil or cisatracurium. Hence, the utility of this model could be extended to screen toxic compounds and determine liabilities of both cardiovascular and respiratory systems in a species larger than a rodent.

| P5 | 3145 Progression of Chloropicrin-Induced Ocular Injury: Clinical and Biological Effects |
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Chloropicrin (CP), a choking and lacrimating agent, has been used as a warfare chemical agent, mainly during World War I. It is currently a popular pesticide and fumigating agent; thereby facilitating its easy acquisition and potential use as a chemical attack agent in addition to its accidental and occupational exposures. Exposure to CP results in severe ocular injury, especially to the corneal epithelium. Studies on corneal injury progression and underlying mechanisms in a relevant in vivo animal model are lacking. This has mainly hindered the development of effective therapies to treat the acute and long-term ocular CP effects. To study the clinical and biological effects of CP on ocular exposure, we tested different CP concentrations in mice to develop a relevant murine ocular injury model. This model will aid in identifying the mechanism of CP-induced ocular injury and in efficacy studies to develop effective treatments. The left eye of male BALB/c mice was exposed to CP using a vapor cap (20% CP for 1 min or 30 sec (0.4 mg of CP) or 10% CP for 1 min (0.2 mg of CP)), and the right eye served as a control. Mice were euthanized at day 25 post CP exposure, and the eyes were harvested to study the corneal injury further. CP-exposures caused a significant increase in corneal ulceration and eyelid edema, which resolved by day 14 post-exposure. However, CP exposure caused an unresolved significant increase in corneal opacity and neovascularization.

Development of hydrops (corneal Bullae due to corneal edema) and hyphema (bleeding into the anterior chamber) was observed after CP-induced effects. Histopathological analyses showed a significant CP-induced decrease in corneal epithelial thickness and increased stromal thickness with more pronounced injury, including stromal fibrosis, edema, neovascularization, anterior and posterior synechia and infiltration of immune cells. Loss of the corneal endothelial cells and Descemet membrane could be associated with the CP-induced corneal edema with fluid buildup causing hydrops that can contribute to cloudy vision, corneal scarring and eye pain. Damage to the uveal vessels from CP might be further responsible for corneal opacity and hyphema. Although exposure to 20% CP for 1 min caused more eyelid edema, ulceration and hyphema, other effects were similar in all CP-exposed eyes following CP exposure. A mouse model of CP-induced ocular injury will be helpful in ocular injury model development and pathophysiological studies to identify molecular targets for therapeutic interventions.

| P5 | 3146 Establishment of an Ocular Irritation Safety Assessment Approach Using OECD 492 EpiOcular and OECD 437 Bovine Corneal Opacity and Permeability Methods |

Regulatory safety assessment approaches such as EU REACH chemical registrations require the assessment of chemicals and substances for the potential to cause eye corrosion or irritation. Historically such assessments were performed using in vivo models such as the OECD 405 Draize Test conducted using rabbits. More recently in vitro and ex vivo approaches have gained regulatory acceptance and public support. One such approach is the use of the OECD 492 EpiOcular™ reconstructed human cornea-like epithelial model in combination with the OECD 437 bovine corneal opacity and permeability (BCOP) test. These models are accepted under an Integrated Approach for Testing Assessment (IATA) for ocular irritation and neovascularization by the Centre d'Expertise des substances. The Centre d'Expertise des substances has adopted the establishment of both the OECD 492 EpiOcular™ and OECD 437 BCOP methods and generated data in accordance with the laboratory proficiency requirements of the respective OECD guidelines. EpiOcular™ tissues were sourced from MatTek and exposed to test agents (n=2 per test agent for a period of 2 to 30 min for liquids and 2 to 4 hours for solid materials, following which tissue viability was measured using MTt reduction and compared to concurrent untreated controls. Each chemical was assayed in triplicate experiments and all experiments achieved the required assay acceptability criteria. For OECD 437 BCOP studies, bovine eyes were sourced from a local abattoir and quality verified by visual inspection prior to use. Corneas were excised and placed within cornea clamps (n=3 per test item), with test substances exposed for 10 min for liquids and 4 hours for solid materials. Changes in opacity were measured using a laser light based opacimeter (LLBO) (Peira 180) and permeability was assessed using sodium fluorescein dye. Three independent experiments were performed. Measurements of opacity and permeability were then used to calculate LLBO irritation score (LIS) and LUX to determine a category 1 or 2 based on the OECD 437 guideline. Of the 15 substances used to determine the laboratory proficiency for the EpiOcular™ OECD 492 assay, 14 produced the expected outcome for prediction as either a non-irritant (7 of 15) or not classifiable as a non-irritant (7 of 15) in all experiments. One material, methylnitroglycolate, was identified as an MTt non-irritant. Corrections were subsequently made to account for this and this substance then produced the expected non-classified result in the remaining 2 experiments. For the BCOP test, 13 substances were used to assess laboratory proficiency: five category 1 eye irritants; three category 2 irritants for which BCOP is not suited to derive a prediction, and 5 non-irritants. All 5 of the category 1 irritants where correctly predicted in the BCOP studies conducted at Gentronix. The 5 non-irritants incorrectly produced the expected outcome of non-standalone prediction. From these results, establishment as a proficient laboratory as per the OECD 492 and OECD 437 guidelines was achieved, enabling the use of the in vivo/ex vivo IATA for ocular irritation and severe eye damage safety assessments.
Environmental and occupational hazards usually result in different health complications. Haemostatic balance is necessary for normal vascular flow which is important for healthy living. Toxic metals accumulations have been associated with reduced fibrinolysis through various interactions such as oxidative stress. Whereas a balance between coagulation and fibrinolysis is necessary for healthy blood flow, reduced fibrinolysis results in thrombotic conditions like ischemia, atherosclerosis, obstructive sleep apnoea (OSA), etc. The objective of this study is to evaluate tendencies to thrombosis among Benin Bronze Casters (BBC), Environmental Cohorts (EC) in Benin bronze locations and Unexposed Participants (UP). Two hundred participants were recruited for the study from Benin City, South-South, Nigeria comprising of 100 BBC, 40 age-and-sex matched EC and 60 UP. Blood levels of the toxic metals (Lead, chromium and cadmium and Mercury) were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Levels of Human Plasminogen activator-inhibitor-1 (PAI-1) and α-2-antiplasmin (α2-AP) were also determined using enzyme linked immunosorbent assay (ELISA), Elabscience: E-HL-H1204 (PAI-1) and E-HL-H0968 (α2-AP) technique. Data were analysed using ANOVA at α=0.05. Results from the study showed a significantly higher level of toxic metals in the bronze casters and environmental cohorts compared with the controls. The blood lead levels in the BBC (39.98±1.63µg/ml), EC (26.15±0.75µg/ml) were significantly higher than UP (8.09±1.46µg/ml). The blood mercury levels in the BBC (0.08±0.02µg/ml), EC (0.10±0.01µg/ml) were significantly higher than UP (0.01±0.00µg/ml), the blood cadmium level in the BBC (0.99±0.29µg/ml), EC (0.65±0.29µg/ml) were significantly higher than the controls (0.19±0.01µg/ml) and the blood chromium levels in the BBC (27.22µg/ml), EC (17.80±0.51µg/ml) were significantly higher than the controls (5.51±0.10µg/ml). Also, the levels of PAI-1 in BBC (1.03±0.09µg/ml), the levels of α-2-antiplasmin in BBC (10327±0.06µg/ml) and EC (1.06±0.14ng/ml), were significantly higher than Unexposed (0.44±0.07ng/ml), the levels of α-2-antiplasmin in BBC (10327±0.06µg/ml) and EC (1.06±0.14ng/ml), were significantly higher than UP (5.51±0.10µg/ml). Also, the levels of PAI-1 in BBC (1.04±0.14ng/ml) and EC (1.06±0.14ng/ml), were significantly higher than UP (0.17±0.02µg/ml) and Unexposed (0.10±0.01µg/ml). The slight QT prolongation observed in the controls (0.19±0.01µg/ml) and the studies. These results suggest that pretomanid used alone or in combination with other QT-prolonging drugs may likely to increase the risk or cause clinically meaningful effects on the rate of ventricular repolarization.

Microbotuline polymerization inhibitors (MIs) have mitotic inhibitory effects. MIs that bind to microtubules via vinca alkaloid-binding sites often induce cardiovascular toxicity. Clinical trials of combretastatin A4 (CA4), an MI that binds to microtubules via colchicine-binding sites, have been conducted in human and veterinary medicine. However, CA4 has not been marketed because the effects of the effective dose and the cardiotoxic dose is insufficient. Meanwhile, bromodomain-containing protein 4 (BRD4) binds to the lysine residues of acetylated histones and controls chromatin structure, thereby regulating the expression of inflammatory signals. BRD4 inhibitors have been reported to improve the prognosis of heart failure. BRD4 has been reported to be required for recovery from anti-microtubule drug-induced mitotic arrest. Based on these reports, we hypothesized that the combined use of CA4 and BRD4 inhibitors can attenuate CA4-induced myocardial damage and enhance CA4's antitumor effect. Therefore, in this study, the cardiotoxicity and antitumor effect of CA4 when used in combination with a BRD4 inhibitor (JQ1) were evaluated. First, we made CA4DP-induced cardiac injury model rats and evaluated cardiotoxicity when JQ1 was used in combination. Vehicle, CA4 disodium phosphate (CA4DP; a produg of CA4), JQ1 or CA4DP+JQ1 was administered to rats. JQ1 25 mg/kg was intraperitoneally administered once a day from Day 1, and CA4DP 100 mg/kg was administered on Day 3. Echocardiography on Day 6 showed that the heart rate, Cardiac Index (cardiac output/total body surface area) and heart function parameters were decreased in the CA4DP and CA4DP+JQ1 groups. The decrease trended to be greater in the CA4DP group. Histopathological examination of heart samples showed that degeneration/necrosis of cardiomyocytes and infiltration of inflammatory cells were more severe in the CA4DP group than in the CA4DP+JQ1 group. Next, we evaluated the antitumor effect when CA4 and JQ1 were used in combination. CA4 and/or JQ1 was applied to a cultured canine mammary tumor cell line (CHMP 13a) for 72 hours and cell viability was evaluated. Both CA4 and JQ1 resulted in a concentration-dependent decrease in cell viability when added alone and cell viability was further reduced by the combined use. We then transplanted CHMP 13a into nude mice to create a xenograft model, and vehicle, CA4DP, JQ1 or CA4DP+JQ1 was administered to evaluate the antitumor effect. JQ1 was administered intraperitoneally once a day from Day 1, and CA4DP was administered on Days 3 and 8 intraperitoneally. On Day 10, tumors were extracted and weighed. As a result, compared with the vehicle group, tumor weight tended to be lighter in the CA4DP and CA4DP+JQ1 groups, while being significantly lighter in the CA4DP+JQ1 group. Our results suggest that the concomitant use of CA4DP and JQ1 can attenuate CA4DP cardiotoxicity while enhancing its antitumor effect. While in vivo studies are still required to verify the dose relationship of CA4DP and JQ1 on the cardiotoxicity and antitumor effect, we were able to secure a margin between the cardiotoxicity and bedaquiline or moxifloxacin.
3153 Development and Validation of an Analytical Method for Quantitation of Raloxifene and Its Metabolites in Rat Plasma and Heart by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Raloxifene is commonly used to prevent osteoporosis in postmenopausal women. It undergoes extensive first pass metabolism to form raloxifene-4-glucuronide (R4G) and raloxifene-6-glucuronide (R6G), but the kinetics and distribution are not well understood. The Division of Translational Toxicology is conducting short-term studies to evaluate cardiotoxicity following oral exposure of Hsd:Sprague Dawley®Rats (HSD) rats to raloxifene. Generating systemic exposure data are integral to calculating toxicokinetics. However, there are limited methods in the literature to simultaneously quantitate raloxifene and its metabolites in rodent matrices without extensive sample clean-up procedures. The objective of this work was to validate and implement a simple LC-MS/MS method to quantitate raloxifene and its metabolites in rat plasma and tissues. Matrix calibration standards were prepared by fortifying 50 μL of rat plasma with 10 μL of a solution containing raloxifene, R4G, and R6G in methanol and 10 μL of internal standard solution (raloxifene-d1). Samples were extracted by protein precipitation with acetonitrile, dried with water, and analyzed by LC-MS/MS in positive ion mode. The method was successfully validated in male Sprague Dawley rat plasma over the concentration range 0.5/5 to 100/1000/200 ng/mL for raloxifene/R4G/R6G. Calibration curves were linear (r ≥ 0.99), and accuracy, determined as percent relative error (%RE), was ±14.9% for standards at all levels. The limits of detection were 0.15, 1.35, and 0.201 ng/mL for raloxifene, R4G, and R6G, respectively. Intra- and inter-day precision, determined as % relative standard deviation (RSD), was 11.1% and %RE was ±18.0% for quality control standards prepared at 1/10/2, 5/50/10, and 50/500/100 ng/mL. The method was evaluated for HSD rat plasma and heart homogenate (ratio of 1:2 heart:water). For raloxifene, %RE values were ≤ ±10.0% and RSD ≤ ±5.5%. For both plasma and heart homogenate, suppression of the internal standard response but not for the metabolites, the metabolites quantitated high in the HSD matrices, especially in the heart (%RE ≤ 44%, %RSD ≤ 6.0%). Analyte stability in extracted plasma was demonstrated for 5 d at ambient and refrigerated temperatures as well as in plasma stored at -80°C for up to 42 d. Stability of calibration standards and standards in plasma for both plasma and heart homogenate, and matrix suppression of the internal standard response were determined under analytical conditions. These data demonstrate that the method is suitable for the quantitation of raloxifene and its metabolites in rat plasma, but a more suitable internal standard should be used for the metabolites to compensate for matrix suppression in the heart. The method validated here can be adapted to other species and matrices.

3154 High-Content Screening and High-Throughput RNA Sequencing Using hiPSC-CMs for the Assessment of Functional and Structural Cardiotoxicity

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Cardiotoxicity remains one of the most commonly reported adverse drug reactions that lead to drug attrition during pre-clinical and clinical drug development. Drug-induced cardiotoxicity can affect all components of the cardiovascular system and may develop as a direct change in cardiac electrophysiology (acute alteration of the mechanical function of the myocardium) and/or as a structural change, resulting in dysfunction of the heart. The current approach to the cardiotoxicity assessment models with better predictive value and cutting-edge in vitro strategies are needed to significantly improve cardiac safety, pharmacology and clinical outcome. To this end, the effects of 42 reference compounds in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were investigated in a combined risk assessment strategy. hiPSC-CMs were cultured in 384-well plates for 10 days and treated with compounds in triplicate at an eight-point dose range response for acute or 24 h. Compounds were selected across several therapeutic indications and included 12 structural and 14 functional cardiotoxicants, 7 structural/functional cardiotoxicants and 9 non-cardiotoxicants. In this study, functional cardiotoxicity was assessed through kinetic monitoring of calcium transients (CaT), while structural morphology changes and gross cytotoxicity were monitored using high-content imaging (HCI) and cellular ATP measurements. Additionally, whole genome high-throughput RNA-sequencing (ScreenSeq™) was performed in matched-sister plates. Data was analysed to determine differentially expressed genes (DEGs) and an assessment of cardiotoxicity was done after 2 and 24 hours to address short- and long-term effects. Ten of the fourteen tested SMKis resulted in a biological relevant decrease in either beating rate or base impedance (cell number index), emphasizing cardiotoxicity as one of the most safety liabilities of SMKis. Pearson’s correlation analysis indicated a good correlation between the different comparable concentrations in relation to Cmax values. UMAP analysis of transcriptional compound response clustered cardiotoxicants and mechanism of action. Projection of HCS and CaT readouts on ScreenSeq™ UMAP showed direct relations between compound-induced phenotypes and transcriptome state. Finally, the DEGs in compound-treated cells were analysed for pathway enrichment, and MECs for significant pathway enrichment normalized by compound-specific Cmax values. The analysis generated a list of 10 major functional categories such as cardiomyocyte functionality, glycolysis and gluconeogenesis and mitochondrial function, amongst others. In summary, this combined multi-endpoint approach was capable of detecting and predicting a range diverse cardiotoxicity mechanisms and allows the early de-risking of New Chemical Entities (NCEs) in drug discovery. 1. Laverty, H. et al. (2011). How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? BJP, 163(4), 675-693.

3155 Sex and Heart Health: Differential Effects of Juvenile 2,3,7,8-Tetrachlorodibenzop–Dioxin (TCDD) Exposure on Cardiovascular Function in Female Zebrafish

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Exposure to environmental contamination is a significant factor in the global rise of infertility rates and is an important modifier of cardiac development and health. Approximately 20-30% of congenital heart disease is thought to be caused by exposure to environmental pollutants. Many contaminants affect multiple organs in the body, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent dioxin congener and aryl hydrocarbon receptor (AHR) agonist, which is classified as a reproductive toxicant and causes lethal cardiac phenotypes in embryonic zebrafish. However, the mechanisms by which developmental exposure to dioxins may contribute to adult cardiac dysfunction is unknown. Furthermore, it is not known whether TCDD-induced damage to the ovary, exacerbates TCDD-induced cardiac injuries. To address these important questions, we are using the zebrafish model. Zebrafish is an excellent model for studying cardiovascular and ovarian health as many of the critical genes that orchestrate heart and ovary development are evolutionarily conserved between humans and fish. Here, we show that juvenile exposure to sub-lethal levels of TCDD (50 parts-per-trillion) at 20- and 50- days post-fertilization results in long-term cardiac dysfunction. We monitored the cardiac health of exposed and control fish using electrophysiology and echocardiograms and found that electrical conduction in the heart was significantly altered by TCDD exposure, with females being more significantly impacted than males. While echocardiograms showed that TCDD-exposed individuals are able to functionally compensate over time and maintain normal blood flow, preliminary histologic analysis of cardiac development 60 days post exposure indicated a pathologic remodeling in females. The epicardium, the outermost layer of the heart, has critical roles in cardiac development, disease progression, and repair. Previous work demonstrates that the progenitor cells which give rise to the epicardium, as well as the epicardium itself, are cellular targets of TCDD. Our observations indicate that the epicardium continues to be sensitive to TCDD exposure and also suggest a critical and uninvestigated interaction between sex and cardiovascular health in the response to environmental contamination exposure.

3156 Cardiac Safety of Kinase Inhibitors: Improving Understanding and Prediction of Liabilities in Drug Discovery Using Human Stem Cell–Derived Models

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Many small molecule kinase inhibitors (SMKis) used to fight cancer have been associated with cardiotoxicity in the clinic. Therefore, preventing failure in clinical development is a priority for preclinical discovery. Our study focused on the integration and concurrent measurement of ATP, apoptosis dynamics and functional cardiac indexes in human stem cell-derived cardiomyocytes (hSC-CMs) to determine kinase-mediated cardiac dysfunction. Commercially available hSC-CMs hold great promises for their utilization as a tool in drug development bridging single receptor-based in vitro results and the clinical data. Cellular-based assays with hSC-CMs proved predictive for main functional cardiotoxicity hazards as arrhythmia, chronotropy, inotropy and kinase inhibitor-induced functional cardiotoxicity, supporting cardiotoxicity screening during drug development. CardioExcyte® 96 (Nanion Technologies GmbH, Germany) is a screening platform combining impedance and extracellular field potential (EFP) recordings, which allows label-free simultaneous assessment of electrical activity, beating and electromechanical coupling in a variety of multi-cellular cardiac preparations. In the present study, we used CardioExcyte® 96 for multi-parameter profiling of endogenous responses to 14 SMKs in hSC-CMs. Complementary to the functional effects on the cellular beat signal and dynamics in culture, measurement of apoptosis over the whole compound incubation time of 24 h was performed with the Caspase 3/7 assay and determination of the cells ATP content with the CellTiter-Glo Luminescent Cell Viability Assay. A perturbation analysis was done after 2 and 24 hours to address short- and long-term effects. Ten of the fourteen tested SMKis resulted in a biological relevant decrease in either beating rate or base impedance (cell number index), emphasizing cardiotoxicity as one of the major safety liabilities of SMKis. Pearson’s correlation analysis indicated a good correlation between the different
read-outs of functional importance. Detailed investigation of the cellular signals facilitated multi-parameter evaluation allowing integrative assessment of cardiovascular myocyte behavior. The resulting multiplex signatures can be used as a fingerprint tool to highlight changes in cardiac function and potentially to categorize drugs based on their mechanisms of action.

### 3157 Comparing the Impact of Different Heart Rate Correction Methods on the Sensitivity of QTc Assessment in Nonhuman Primates

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Assessment of rate corrected QT interval duration is an integral part of nonclinical safety assessment. Even in cases where there is no compound-driven effect on heart rate the large circadian changes in rate make correction a necessity. Any residual error from the correction method could introduce additional bias into the statistical comparison model and impact the sensitivity of the overall QTc assessment. This sensitivity is a critical question since nonclinical QTc evaluations are used to support an integrated proarrhythmic risk assessment under the newly released ICH E14/S7B Q&As. The current analysis used a large study (n = 48) that is representative of the methodology and allows multiple rate correction models to be compared while all other study conduct aspects were the same. All 48 nonhuman primates (NHP) received 4 different treatments: vehicle occasion 1, vehicle occasion 2, moxifloxacin (80 mg/kg) occasion 1, moxifloxacin (80 mg/kg) occasion 2. Post-dose QTc interval data were recorded for 48 hours on each treatment day. Linear QTc-RR corrections are commonly used. These generally involve determining the slope of the QT-RR relationship under predose or vehicle-treated conditions and use this slope to correct the QT interval across all treatment occasions. This model assumes that while the slope is individualized to subject it is constant across time for all treatments. We have recently illustrated that the same linear QT-RR relationship under predose and vehicle-treated conditions can be exploited under the assumption that the intercept of the relationship is constant across time for each individual. The common "slope-based method" assumes that any compound effect is on the intercept, and not the slope, of the relationship (or the subsequent QTc-RR relationship would not be zero). The recently described "intercept-based method" assumes drug effect is on the slope and not intercept. It is known that HERG blockers increase the slope of the QT-RR relationship suggesting an "intercept-based method" may be most appropriate. Each of the two methods can be applied at a study (or population) level, an individual animal level or individual treatment period level. We applied each method at each of these 3 levels. A fixed correction method, Bazett’s (QTcB), was also used as a comparison against the different rate correction methods (MDD, 80% power at p = 0.05) for the statistical treatment comparison was used to compare methods. The "intercept-based method" was generally superior. There was also a trend for improved performance of correction going from study level to individual treatment level. In vehicle versus moxifloxacin treatment comparison the two methods gave similar MDD values. QTcF corrections were used to compare methods and the "intercept-based method" was generally superior. For the purpose of this study, we focused on genes associated with GO cluster "Negative regulation of angiogenesis" and signaling pathway "Acute myocardial infarction". We firstly performed centrality analysis to obtain data on interaction between molecules. Detailed analysis of "Negative regulation of angiogenesis" GO cluster revealed three directly associated genes (TIE1, NRP1 and ANGPT2) and 40 additional genes indirectly associated with negative regulation of angiogenesis. Review of annotated public data identified each of these genes to play a role in the regulation of angiogenesis. However, since NRP1 cannot be detectable in the blood compared to TIE1 and ANGPT2, we established idea of using of TIE1 and ANGPT2 as potential angiogenesis biomarkers by blood sampling. We also performed mRNA expression analysis of TIE1 and ANGPT2 in post-MI human cardiac pericytes (hcPCs) isolated from three patients. The hcPC cultures were treated with inhibitor of ERK1/2 signaling (PD0325901, specific MEK1/2 inhibitor), a small molecule that induced the hcPCs differentiation into vascular smooth muscle-like cells (VSMC-like cells) in order to promote reparative vascularization of the ischemic heart, or with DMSO control. We showed that TIE1 and ANGPT2 were expressed at similar levels in DMSO control, and at elevated levels of TFPI were found in patients with MI. In addition to the previous, we have established idea of use of TFPI as a prognostic biomarker for recovery after MI - expecting decreased levels of TFPI in patients in post-MI recovery phase. In this example, we showed how our developed tool can help in analysis of potential angiogenesis and post-MI recovery biomarkers.

### 3158 Comparing the Sensitivity of Crossover and Parallel Study Designs for QTc Assessment: An Analysis Based on a Single Large Study of Moxifloxacin in 48 Nonhuman Primates

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The cardiovascular safety pharmacology (SP) study conducted to satisfy ICH S7A and S7B has commonly used a cross-over (X-over) study design where each animal receives all treatments. In an increasing number of cases X-over designs are not possible and parallel studies must be used. The QTc evaluation in toxicology studies also involves a parallel design. Parallel SP studies can seldom be as large as 8 animals/dose to match an n=8 X-over. Animals in parallel designs receive only one treatment. Thus, parallel studies will have a different sensitivity to detect changes. This sensitivity is a critical question since the nonclinical QTc evaluations to support an integrated proarrhythmic risk assessment under the newly released ICH E14/S7B Q&As. The current analysis used a study large enough (n = 48) to be analyzed both as a crossover design to directly compare the performance of the two experimental designs coupled to different statistical models while all other study conduct aspects were the same. All 48 NHP received 4 different treatments: vehicle occasion 1, vehicle occasion 2, moxifloxacin (80 mg/kg) occasion 1, moxifloxacin occasion 2. Post-dose QTc interval data was recorded for 48 hours on each treatment day. Data were analyzed using 12 animals randomly selected for each treatment or as a X-over study. Different statistical models were used. The primary endpoint was the residual error from the models applied to hourly time intervals. The residual error was used to determine the minimal detectable difference (MDD, 80% power at p = 0.05) for the study design-statistical model combination. Two statistical models were applicable to either study design. They gave similar MDD values. In X-over designs the individual animal identification (ID) can be used in the statistical model. This enabled the smallest MDD value. Simple statistical models for analysis were identified: Treatment + Baseline for parallel designs and Treatment + ID for X-over designs. The statistical sensitivity of parallel study designs is reasonable (MDD for n=6 of 12.5 ms), and in combination with the MDD measures higher than 10.7 ms, the MDD should be used in an integrated risk assessment. When sensitivity of the in vivo QTc assessment is close the X-over design enables a higher sensitivity (MDD for n=6 of 7.5 ms).

### 3160 Inhalation of Micro- and Nanoplastics during Gestation and the Impairment of Pregnancy-Induced Hypertrophic Recovery

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Dynamic physiological changes occur during pregnancy to support the development of the fetus, including a reversible cardiac hypertrophy to support tissue growth and placental and fetal demands. When the heart fails to return to its pre-pregnancy size following parturition, a rare form of heart failure may develop called Peripartum Cardiomyopathy (PPCM). Currently, the diagnostic criteria for PPCM includes development of heart failure in the last month of pregnancy or up to 5 months postpartum; absence of preexisting heart disease, an undefined cause, and irregular echocardiograph findings including left ventricular ejection fraction of < 45% and fractional shortening < 30%. The etiology of PPCM is currently unknown. Epidemiological studies identify an association between environmental exposure to airborne particulate matter and the development of cardiovascular disease. We hypothesized that exposure to aerosolized particulate matter during pregnancy can impair pregnancy induced hypertrophic recovery. To investigate the consequences of particulate matter, specifically micro and nano plastics (MNPs), on the heart during pregnancy, we exposed female Sprague Dawley rats to bulk Polyamide 12 (i.e., nylon) powder in our whole-body inhalation chamber from gestational day (GD)
Doxorubicin (DOX) is a chemotherapeutic agent with known cardiotoxic effects that is often used to treat pediatric and adult malignancies. Current methods for diagnosing DOX induced cardiotoxicity are limited and robust markers for early detection of DOX induced cardiac dysfunction are needed to prevent heart failure. Preclinical models may help in reducing their exposure, managing their impact on the environment. Traditional environmental hazard assessments have not been enriched for cardiovascular toxicity, particularly measuring functional changes. In vitro methods utilizing human cells may provide unique insights into chemical-induced biological perturbations relevant to potential cardiovascular hazards. Here, we evaluated a broad spectrum of CV-relevant cellular activities using whole cell patch clamp analyses, transient mechanical changes, mitochondria membrane potential (MMP), cardiomyocyte contractility and field potential changes, high-content imaging for cellular phenotypic changes and cytotoxicity. We chose chemicals from various classes including botanicals, flame-retardants, insecticides, polycyclic aromatic hydrocarbons, quaternary ammonium salts and PFAS. Whole-cell patch clamp analysis of three main acute ion-channels INa (HNav, peak), ICa (Nav1.2), and IKr (HERG) was performed. Quaternary ammonium salts showed significant inhibition of HNav and HERG channel activity (up to 100%) and a lesser hNav 1.5 inhibition (25-50% maximum). Flame retardants, 2-ethylhexyl diphenyl phosphate and 2,2′,4′-tetrabromodiphenyl ether, showed up to 20% inhibition of hNav 1.2 and HERG channels but no hKv11.1 inhibition. PFAS chemicals showed minor inhibition of ion-channels at higher doses (10 µM). Calcium transients, MMP and multielectrode array measurements on human iPSC-derived cardiomyocytes (hiPSC) aligned with whole-cell patch clamp ion-channel blockage showing dose-dependent changes to action-potential duration, contractility, beat rate and mitochondrial depolarization. Benchmark concentrations (BMC’s) derived from concentration-response data showed a unified safety margin (100×) with predicted human exposure concentrations. This high-throughput compatible in vitro screening approach identified potential agents that cause arrhythmias, mitochondrial damage and changes to cardiomyocyte homeostasis that could cumulatively increase cardiovascular disease risk.

3162 Endothelial and Smooth Muscle Cell Dysfunction in the Thoracic Aorta of Mice Treated with Clinically Relevant Doxorubicin Doses via Time Release Pellets

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Doxorubicin (DOX) is a chemotherapeutic agent with known cardiotoxic effects that is often used to treat pediatric and adult malignancies. Current methods for diagnosing DOX induced cardiotoxicity are limited and robust markers for early detection are needed to prevent heart failure. Preclinical models may help screen candidate markers, yet they often rely on DOX injections that are unable to recapitulate equivalent clinical doses. To this end, we used subcutaneous pellets for the time-release of DOX to automate the necessary safety margins by 100× (100µM) with predicted human exposure concentrations. This high-throughput compatible in vitro screening approach identified potential agents that cause arrhythmias, mitochondrial damage and changes to cardiomyocyte homeostasis that could cumulatively increase cardiovascular disease risk.
with previous findings that BPA exerts an inhibitory effect on the L-type calcium channel current. BPA shortened FPD and APD more severely than either BPF or BPS, but to a lesser extent than estradiol. This study characterized the effects of bisphenols on cardiac electrophysiology and expanded mechanistic insights using a clinically relevant model. These findings can inform regulatory decisions on the use of replacement chemicals and provide a detailed assessment of how bisphenol exposure may precipitate adverse cardiovascular outcomes.

**3164 miRNA Species as Systemic Mediators of Environmentally Persistent Free Radical (EPFR)–Induced Endothelial Dysfunction**


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Particulate matter containing environmentally persistent free radicals (EPRFs) is formed by incomplete combustion of organic pollutants when organic pollutants are converted to soot by the combustion process. In this study, we selected several chemicals from data containing redox-active transition metals. We generated EPFR particles in the laboratory using a combustion reactor, and to investigate dose-dependent effects of free radicals on vascular function, we generated particles over varying free radical concentrations, e.g., EPFRr, (10e11 radicals/g particles) and EPFRr, (10e13 radicals/g). We first exposed C57BL/6 mice male mice by inhalation with filtered air compared to 250 µg/m³ EPFRr 4th for 5 d to investigate their effects on vascular endothelial function. Our studies demonstrated that EPFRr inhalation results in a dose-dependent reduction in endothelium-dependent vascular relaxation. Using Nanotexting digital transcript counting, we next conducted microRNA (miRNA) profiling and identified 35 miRNAs out of 57 that were dose-dependently increased in the plasma after EPFRr exposure. Ingenuity Pathway Analysis (IPA) showed that five of the 35 dysregulated miRNAs are linked with endothelial function, suggesting that miRNA may be a systemic mediator promoting EPFRr-induced endothelial dysfunction. Our recent data also suggest that any hydrocarbon receptor (AhRx) signaling at the air-blood interface in the lungs plays a critical role in EPFRr-induced endothelial dysfunction. Thus, to definitively link miRNA release with EPFRr-induced endothelial dysfunction, ongoing studies are using mice deficient in AhRx at the air-blood interface to test whether miRNA release is attenuated.

**3165 Nano-titanium Dioxide Inhalation–Induced Circulating Xanthine Oxidoreductase Removes Epigenetic Breaks on Vascular Inflammation**

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Nano-titanium dioxide (nano-TiO2) is broadly used in products from biomedical devices to surface coatings and inhalation exposure may occur during the life cycle of such products. We have previously reported elevated oxidant production, and vascular inflammation in nano-TiO2 inhalation exposed rats. However, the underlying mechanisms remain poorly understood. An inflammation-related and oxidant producing enzyme, xanthine oxidoreductase (XOR), increases in the circulation following toxicant insults. It is speculated that circulating XOR can bind to the vasculature and promote dysfunction, but this has not been definitively shown to date. As such, we hypothesized that nano-TiO2 inhalation elevates XOR in the circulation and downstream XOR binds to endothelial cells elevating oxidant production and causing epigenetic alterations in inflammatory gene regulation. Male and Female Sprague-Dawley rats 8-10 weeks of age were exposed to sham-air or nano-TiO2 (2±10µg/m³) for 3 consecutive days and assessed 24 hours after final exposure. XOR activity measured in the plasma shows a greater than 5-fold increase in males and a greater then 3-fold increase in females exposed to nano-TiO2 compared to sham-air controls (both p<0.05). Next, we aimed to determine if XOR could bind to the endothelial surface of mesenteric arterioles. Printed with permission from Alexaﬀ et al.7 Surface expression of 3166 Meta-analysis of General Toxicology Studies with Integrated Implantated Telemetry Demonstrated Minimal Impacts of Surgical Instrumentation on the Toxicology Data for Cynomolgus Monkeys

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Increasing numbers of general toxicology studies with integrated implanted telemetry are being conducted due to the constrained supply of nonhuman primates and demands for higher method sensitivity of electrocardiograms (ECG) parameters, especially for the QT interval, in the ICH S7B/Q. Our previous study demonstrated that these integrated studies (N = 10 or 12) generally had better sensitivities for the ECG parameters than the stand-alone cross-over cardiovascular studies (N = 4 or 6). Additional advantages of the integrated design included repeat-dosing, recording at multiple time points, ability to correlate safety pharmacology data with toxicology data, and concurrent hemodynamic and body temperature measurement. However, there is a concern about the impact of surgical instrumentation on the quality of toxicity data. To address this, we performed a meta-analysis over > 6 months of instrumentation of the M11 device and comprehensively reviewed the toxicity reports of integrated studies conducted from 2017 to 2022 at our Testing Facility. Results from the 5 animals indicated that there were no changes in any of the standard clinical pathology parameters and no signs of infection or inflammation over a 6-month period with instrumentation. The meta-analysis results showed that the instrumentation had minimal to no impacts on clinical signs, body weights, food consumption, clinical pathology parameters, cytokines, immunophenotyping, or histopathology results in monkeys. Occasionally, there were mild fibrosis and regeneration of muscle proximate to the implant site, which were non-adverse, local, and can be clearly distinguished from the test article effects. Our refinement of surgical procedure from intra-peritoneal placement to intramuscular placement also significantly reduced the chance of device erosion. We also obtained summary metrics for the surgical recovery time, type of device, study duration, animal cohort with instrumentation, and pharmacological class of test article, which could guide future study designs. In conclusion, implanted telemetry integrated into a toxicology study does not compromise the study objective, study execution, or toxicology data interpretation and can obtain high quality telemetry data to evaluate test article effects on the cardiovascular system.
contaminant of concern due to its harmful health effects and has been specifically established as a cardiotoxicant. The purpose of this study was to examine the effect(s) of iAs exposure on the maternal heart during pregnancy, with a focus on physiological and molecular changes using in vivo exposure paradigms. We sought to examine the impact of environmental insult on the maternal heart as this is a relatively understudied area and can highlight potential hazards for pregnant women. Pregnant female C57BL/6J mice were exposed to either 1000, or 0µg/L iAs (Control) after conception at embryonic day 2.5 (E2.5) with exposure ending at parturition. Dams were harvested at postnatal day 12 (P12), and while we previously found that the iAs-exposed pregnant dams did not show typical pregnancy-induced hypertrophy compared to controls, the postpartum maternal heart was enlarged compared to controls as measured by heart weight / tibia length. Transcardiac echocardiography revealed that postpartum mice exposed to 1000 or 1000 µg/L iAs had increased ejection fraction, fractional shortening, cardiac output, and stroke volume compared to controls. We next examined contractility in isolated cardiomyocytes from dam hearts harvested at P16 by measuring calcium transients and sarcoplasmic shortening using the Ionoptix system. Interestingly, we found that the iAs-exposed cardiomyocytes from this group showed a significant decrease in calcium transient amplitude and sarcoplaemic shortening, along with impaired relaxation kinetics, which contrasts with the enhanced contractile parameters noted in vivo with echocardiography. Together, our findings suggest that exposure to iAs during pregnancy affects the postpartum remodeling in the maternal heart, with effects noted at the level of the cardiomyocyte. Furthermore, these findings underscore the detrimental impact of iAs on the cardiovascular system, and specifically highlight potential hazard potentials posed by iAs exposure during pregnancy. Supported by NHLBI T32ES007141 (NT) and RO1HL13649 (MK).

3169 Functional Testing of Human iPSC-Derived 3D Cardiac Tri-c culture Microtissues


The adult human heart is a complex organ providing highly regulated processes of pumping blood throughout the body. Heart tissue contains various types of cells, including (not limited to) cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts. Although cardiomyocytes may occupy about 75% of the tissue volume, they only constitute 40-50% of the total cell count. Recent publications show that 3D cell cultures of cardiac spheroids (or microtissues) enhance the maturation and functional activity of cells compared to 2D cultures of cardiomyocytes, thus more closely mimicking actual heart physiology. In this study, we developed a tri-culture model by mixing human iPSC-derived cardiomyocytes, cardiac fibroblasts, and endothelial cells in defined ratios in ultra-low attachment (ULA) spheroid forming plates. Importantly, 3D microtissues were created by directly thawing cryopreserved vials of each individual cell type and culturing them together in an optimized media formulation. Cells formed spheroid-like clusters that started to connect (establishing the so-called panmural connexions) irregularly after 4-5 days. Spheroid formation, size, and morphology was tracked over time using live cell imaging. Additionally, the different cell types in the tri-culture were immunostained using anti-Troponin T (for cardiomyocytes), COL1A1 (for cardiac fibroblasts), and platelet endothelial cell adhesion molecule (for endothelial cells). In vitro beating activity of 3D microtissues was investigated by recording the calcium oscillations on a high-throughput kinetic screening instrument (i.e., FLIPR Penta or FDSS/uCell). Pharmacological modulation of this functional activity was performed by exposing the cells to a set of known small molecules. Importantly, we found that compounds like isoproterenol and dobutamine significantly accelerated the oscillation rate of cardiomyocytes (both types) and increased the peak amplitude (positive inotropic response). Several other compounds, including HERG inhibitors and ion channel blockers, demonstrated changes in the calcium oscillation patterns consistent with expected mode of action. The data presented here highlights the utility and biological relevance of using iPSC-derived cardioid cell types in 3D microtissues as a promising in vitro model for future cardiotoxic effects on human heart tissues in high throughput format.

3170 Effects of Cadmium on Pulmonary Arterial Hypertension

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Cadmium (Cd) causes toxicity by producing reactive oxygen species (ROS) within the body and is known to trigger the apoptotic pathway through the impairment of the mitochondrial and several of its transport pathways. Humans are exposed to Cd through environmental pollution, cigarette smoke, food, and other occupational and consumer products. Past studies have shown that Cd toxicity can lead to vascular damage, endothelial dysfunction, increase in lipid peroxidation, and produce myocardial damage. Pulmonary arterial hypertension (PAH) is a cardiovascular disease characterized by the inflammation, proliferation, and remodeling of the right ventricle (RV) of the heart. There are multiple factors that might cause PAH such as disbalance in oxidative stress, inflammation, and repeated fibrosis and there is currently no definitive mechanism identified for PAH. Previous studies have shown the link between Cd toxicity and cardiovascular diseases (CVDs) and Cd may exacerbate and enhance the severity of pre-existing CVDs. Thus, the goal of this study is to determine whether Cd exposure can cause PAH.

At six weeks of age, 40 male C57/6J mice were split into two initial groups: 20 which received 5ppm Cd in their drinking water (Cd group n=20), and 20 without any Cd in their drinking water (Control group n=20). After 8 weeks, transcardiac echocardiography was used to analyze the diastolic and systolic functions of both the heart and right ventricle. RV free wall thickness (RVFW), tricuspid annulus plane systolic excursion (TAPSE), and systolic excursion (TAPSE), RV Outflow Tract Velocity Time Integral (RV VOTI), and other variables were calculated to examine the Cd effects on the heart. These measurements were considered as the baseline of this study (Week 0) to determine whether exposure to Cd alone could cause PAH and/or RV dysfunction. Our results showed that chronic exposure to Cd for 8 weeks did not cause RVFW, RV VOTI, or TAPSE changes in mice. After these baseline measurements, 10 mice within both control and Cd groups were given a combined treatment with VEGF inhibitor Semaxinib (SUS416) and 10% hypoxia for 4 weeks to induce PAH (SuHx). This was done to determine whether Cd exposure can worsen SuHx-induced PAH and/or RV dysfunction. Repeated transcardiac echocardiography measurements (Weeks 2, 3, and 4) and invasive RV pressure measurement (Week 5) were performed to quantify the dynamic changes in the RV and pressure in the pulmonary artery. After this, the mice were euthanized to collect heart and lung sample for histological and biochemical examinations. Current echocardiographic analysis showed that the RV systolic function was significantly increased in the mice exposed to Cd alone for 12 weeks when compared to Control. Mice with 12-week exposure to Cd plus 4-week SuHx treatment significantly decreased RV cardiac output when compared to mice with SuHx treatment alone and Cd alone. Results also showed that after 13 weeks of Cd exposure, the Cd-SuHx mice had lower RV systolic pressure (Rsvp), an indicator of RV failure, and the Cd-suHx mice had worse RV diastolic function. These data suggests that Cd exposure to Cd alone and Cd plus SuHx might stimulate RV systolic function, but Cd might worsen RV systolic function during the PAH pathogenesis. These cardiac structural and functional results need the confirmation and explanation with the ongoing histological and biochemical analysis. Therefore, our study will help understand how common environmental pollution might be playing a role in causing or worsening the PAH pathogenesis, which will provide us the potential clue for developing treatment methods. Supported in part by NIEHS P30ES030283.
Smoking uptake and with a public health emergency known as E-cigarette or Vaping Use-Associated Lung Injury (EVALI). The US Food and Drug Administration, Center for Tobacco Products (FDA/CTP) is responsible for regulating the devices and consumable materials associated with tobacco products including ENDS. State and federal regulations regarding addition of flavoring compounds in ENDS liquids (e-liquids) may be circumvented when users and vendors employ refillable refillable reservoirs with non-compliant e-liquids. The relative toxicant exposure potential of third-party versus manufacturer-supplied reservoirs is not well understood. This study investigated the effect of third-party refillable reservoirs, or ‘pods’ on the Total Particulate Matter (TPM) and nicotine emissions from a popular ENDS in comparison to the manufacturer’s provided disposable (not refillable) reservoirs. Emissions were collected on glass fiber filter pods using a Programmed Emissions System (EPS) for a JUUL brand Control Unit (PCU) across a controlled variety of puff flow rates and durations, which have been previously demonstrated to affect TPM and nicotine yield delivered to the mouth of a user. Emissions samples were collected and analyzed using gravimetric and GC-FID techniques for JUUL manufactured disposable pods and third-party Blankz refillable pods. Results were analyzed to assess the impact of pod manufacturer on the effective operating envelope of the ENDS and compare TPM and nicotine yield between pods and e-liquids. Results demonstrate that emissions vary significantly as a function of puff duration, flow rate, e-liquid composition, and pod manufacturer. It was observed that the third party pod exhibited e-liquid aspiration into the flow stream at a puff flow rate of 65 mls/sec in comparison to the manufacturer pod at 85 mls/sec. The maximum TPM yield per puff was 5.6 times higher for the third-party pod (12.4 mg/puff) in comparison to the manufacturer’s pod (2.2 mg/puff), while the maximum TPM concentration was over 7 times higher for third party (0.200 mg/ml) versus manufacturer (0.028 mg/ml) pod. The mass ratio of nicotine present in the aerosolized Nicotine (NGP) TPM was found to be similar to the same as the mass ratio of the e-liquid (mg Nic/mg e-liquid) for both pods. In conclusion, toxicant exposure may be dramatically impacted when consumers use third-party pods in conjunction with ENDS devices. Furthermore, availability of refillable pods is a significant barrier to regulatory enforcement regarding limitations of potentially toxic additives to e-liquids. It is confirmed that e-liquids obtained from both manufacturer-supplied and third-party pods depend jointly on the puff flow rate, duration, e-liquid composition, and pod design even when used with the same fixed power PCU. It is recommended that FDA/CTP require experimental characterization of emissions of third-party pods used in conjunction with each PCPU they are compatible with across the range of the product. Third-party pods proposed for marketing approval should be required to demonstrate no increased potential toxicant exposure in comparison to manufacturer-supplied pods.

Health assessors and regulatory agencies generally assess hazard or risk from environmental exposures on a chemical-by-chemical basis that is time and resource-intensive. However, many commercial chemicals are structurally related compounds that may have similar toxicological effects. As such, there is a growing need for class-based assessment methods. The National Academies of Sciences (NAS) recently published a report outlining a class-based approach for evaluating the toxicity of Organohalogen Flame Retardants (OFRs) within 14 chemical classes. In this study, we present in vitro mechanistic studies of Organohalogen Flame Retardants (OFRs) using a streamlined method to collaboratively study a large number of these chemicals. The OHAT systematic review framework was followed to develop systematic evaluation of evidence maps and rating of the product. Third-party pods proposed for marketing approval should be required to demonstrate no increased potential toxicant exposure in comparison to manufacturer-supplied pods.
3176 Application of in Silico Methods for the Definition of Occupational Exposure Banding

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In silico methods may complement the toxicological profile of data-poor substances (when toxicological information or GHS categorization are not available) to support an overall risk assessment. They can be employed to support hazard characterization, including assignment of hazard notations that are in turn used as input to assign an occupational exposure band (OEB) for data-poor substances. This is the range of airborne concentration levels expected to be protective of the worker’s health. Such an approach is a semi-quantitative methodology that involves assigning the OEB based on criteria aligned with specific GHS categories and the associated airborne concentration range of the band is considered to provide adequate control. Firstly, it is noted that a unique feature of this approach is that it does not exist for banding categorization and a literature review has been conducted to identify available banding schemes that categorize chemicals based on their corresponding hazard classifications. Different banding schemes are referenced and presented in the poster alongside a new combined scheme that integrates different approaches. Secondly, a workflow that uses in silico methods is applied for the derivation of the banding categories of some selected data-poor substances. More specifically, the workflow combines in silico methods to estimate potential toxicity of the test chemicals including mutagenicity, carcinogenicity, skin sensitization, irritation (skin/eye), reproductive and developmental toxicity and acute toxicity. As a first step, evaluation of genotoxicity is conducted and this is driven by the principles of the ICH M7 guidance, that provides specific recommendations for assessing drug impurities, including the use of in silico methods to predict bacterial mutagenicity. A positive outcome would assign the test chemical to a low-exposure banding category. A negative outcome prompts for additional in silico predictions to estimate the toxicity arising from other endpoints. The endpoint-specific criteria of the underlying OEB scheme are then used to assign the OEB category. The test chemicals discussed in the poster include an example (RN: 9753S-67-5) that is predicted genotoxic. The band for a data-poor substance that is predicted non-genotoxic (RN: 1508261-86-6) is also estimated using predictions from in silico tools. How read-across (i.e., toxicity of a substance is inferred from the known toxicological profile of a similar compound) might support such predictions is also demonstrated. The results of the toxicity profiling of the chemicals show that the range considered safe for the workers might slightly differ depending on the banding scheme that is adopted. In conclusion, the analysis of the present work illustrates the advantages of the use of in silico methods to provide a tiered approach to identify and evaluate the toxicity arising from other endpoints. The endpoint-specific criteria of the underlying OEB scheme are then used to assign the OEB category. This abstract presents a draft approach and does not reflect views or policies of the US EPA.

3177 Addressing Uncertainty: A Tiered Testing Approach to Develop a Biologically Based Read-Across Hypothesis for Five Branched Primary Alcohols

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Five branched primary alcohols, comprised of hydrocarbon chains ranging from C8 to C13, have been subject to ongoing regulatory reviews resulting in toxicology data generation in a phased approach in order to characterize the hazard profile of these substances. Although both toxicokinetic and subchronic toxicity studies suggested that the two substances at the lowest and highest ends of the carbon chain length-distribution range (isooctanol and isododecanol) elicited distinct toxicological effects, subsequent testing on an intermediary alcohol (isoundecanol) provided key data necessary to elucidate a pattern of predictable toxicological effects. A new hypothesis was developed in order to interrelate the in vivo data with isopropyl to 2-propanol and 2-pentanol to isoundecanol in a tiered approach. This abstract presents a draft approach and does not reflect views or policies of the US EPA.
substances and evaluate the dose-temporal concordance of the major findings (liver and thyroid activation). The resulting read across hypothesis is that this group of primary alcohols elicit qualitatively similar effects after subchronic oral exposure in rats (i.e., effects on the liver with compensatory thyroid response), due to similarity in substance identity and ADME characteristics. Similarities in biotransformation as observed, with metabolism occurring via oxidation and Phase II conjugation reactions mediated by the constitutive and xenobiotic receptor (ARE/ARNT). All three alcohols activate the liver, as evidenced by liver enlargement with corresponding induction of CYP2B and CYP3A activity (enzyme activity measured in isoucanol and isoutercanol only) and increased incidence/severity of hepatocellular hypertrophy, while the thyroid was activated only by isoucanol and isoutercanol (reduced serum T3 and T4, increased thyroid organ weights, follicular cell hypertrophy/hyperplasia). Quantitatively, the magnitude of the liver responses increases with increasing carbon-chain number. MOA analysis supports the hypothesis that markers of liver activation occur at low doses/early time points, followed in a dose-temporal manner by markers of thyroid activation, with med/high concordance. Mechanistically, this MOA is well established, with high biological plausibility. Indicators of thyroid activation were absent even at high doses of the lowest end of the carbon-chain distribution (isooctanol), consistent with the lower incidence and magnitude of liver effects. The application of the read-across approach is to predict both systemic toxicity and reproductive and developmental toxicity associated with the untested substances in this group. Following the guidelines in the European Chemical Agency's Read Across Assessment Framework, this read-across hypothesis is considered by the authors to be “accepted with minor reservations” and thus can be used to fill the remaining regulatory information requirements without further animal testing. If successful, evidence-based read across is a pragmatic approach to balance scientific uncertainties with the legislative intent to use animal testing only as a last resort.

3180 Demonstrating the Reliability of Metabolomics-Based Chemical Grouping: Toward Acceptable Practice
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Metabolomics has reached a critical point in determining its value to regulatory toxicology. Building on 20 years of research, the first metabolomics data to support grouping/read-across was recently submitted to ECHA, metabolomics best-practices for various applications including grouping were published in Nature Communications, and the OECD Omics Reporting Framework has been developed. Given these and other examples of its increasing relevance to chemical safety regulations, an assessment of the reproducibility of metabolomics in support of chemical grouping is required. The aim of the Cellic-funded MATCHING study (MetAbolomics ring-Trial for CHemical groupING) is to determine whether this technology can demonstrate high reproducibility in grouping, and hence high reliability, or whether refinements in analytical or data analysis practices are needed. Through this fully blinded evaluation, the second aim is to propose ‘accept-able practice’ for metabolomics-based grouping. The international consortium comprises seven industrial, government and academic metabolomics ring-trial partners, BASF SE, and the European Chemicals Agency (ECHA) as an independent advisor. First, 8 substances were selected for the trial, and all ring-trial partners were fully blinded to their identities, modes of action, and the number of categories. Plasma samples for the ring-trial were then derived from 28-day rodent tests (8 substances, each ‘low’ and ‘high’ dose, plus vehicle controls), aliquoted, and distributed to partners. Each metabolomics laboratory then applied their preferred LC-MS metabolomics workflows to acquire, process and statistically analyse the data. This included attempting to group the 8 substances into categories based on their metabolomics signatures, and then reporting their findings to ECHA to ensure the blind conditions of the trial were respected. To date, five ring-trial partners have reported, and all have discovered the identical grouping of the 8 substances into 3 unique categories (removing two reports expected by December 2022). Further analyses into the metabolic biomarkers driving the groupings are underway. In addition, both the consistent and differing elements of the consortia’s metabolomics workflows are being examined to propose acceptable practice for metabolomics-based grouping. In conclusion, the findings from the MATCHING study have demonstrated high reliability of metabolomics-based chemical grouping.

3181 Evaluating New Chemicals in the US under the Toxic Substances Control Act: Application of New Approach Methods to Evaluate Eye Irritation
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US Environmental Protection Agency’s Office of Pollution Prevention and Toxics, New Chemicals Division (NCD) is responsible for conducting risk assessments under the Toxic Substances Control Act (TSCA). In 2016, EPAs amended under the Frank R. Launtenberg Act directed EPA to “promote the development and implementation of alternative test methods and strategies to reduce, refine, or replace vertebrate animal testing and provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment.” To incorporate NAM data for eye irritation hazard identification, NCD is collaborating with colleagues from National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Institute for In Vitro Sciences, Inc. and PETA Science Consortium International e.V. to develop a decision framework to evaluate eye irritation hazards for new chemicals under TSCA. This framework proposes to prioritize use of data from NAMs over in vivo studies in animal models for the prediction of eye irritation in humans. In using the framework, acceptable NAMs data on the new chemical substance from OECD test guideline or other acceptable studies are considered first. If information on the new chemical substance is not available or acceptable, a close analogue that has information is utilized. To evaluate eye irritation of the new chemical substance. Under the framework, all information is collected and evaluated in the following order: (1) data from human cell/tissue-based test methods that have been demonstrated to be reproducible and relevant to eye irritation; (2) data from in chemico or non-human in vitro and/or in vivo test methods that have been demonstrated to be reproducible and provide information on the mechanisms of toxicity relevant to eye irritation in humans; and (3) data from in vivo animal studies. If no acceptable information on eye irritation is available, the framework allows for consideration of skin irritation data that predict irritating or corrosive properties to make inferences about eye irritation hazard of the new chemical substance. The decision tree framework as well as case studies will be presented. These views were developed by the authors and do not reflect views or policies of the US EPA or other respective organizations. This project was funded in part with federal funds from the NIEHS, NIH under contract HHSN27220150001TC.

3182 Reliability Assessment of Guideline-Based Studies Using Systematic Review Critical Appraisal Tools
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Systematic review methods and critical appraisal tools (CATs) have been implemented by authoritative and regulatory bodies in recent years to increase the transparency and data quality used in hazard and risk assessments. To assess (a) how well CATs capture data quality and gaps of guideline studies and (b) how CATs that were developed for other disciplines are being modified to address study elements which are important to toxicology, we surveyed risk assessments conducted by authoritative and regulatory bodies, as well as those published in the literature, which used systematic review methods and included CATs to evaluate reliability of guideline (and similar) studies. Most assessments identified similar study elements and outcomes, and (a) how environmental chemicals or food ingredients; CATs included Klimisch categorization and risk of bias-assessments. Using a recent evaluation of hexavalent chromium (Cr(VI)) as one of the case studies, results demonstrates that several studies conducted similarly or similar to guideline studies were defined to have low reliability. Rationale, however, was not due to limitations with methodological or reporting quality - notably, the internal validity was determined to have a very low risk of bias, which typically would support a high level of reliability. Rather, the lack of reliability was based on dose selection, despite being the dose determined via the guideline design. Rationale was not consistent with the CAT guidance, which describes parameters associated with the utility of the exposure design for the endpoint of interest; many of the studies determined to be unreliable were designed specifically to evaluate the mode of action associated with occurrence of a tumors at the given doses and were appropriate given the context of the scientific question under investigation. Such apparent disconnects between CAT criteria and scientific context highlights the importance of subject matter expertise in assessing the reliability of a study in context of the needs of the given risk assessment. In other case studies with food ingredients and pesticides, prompting questions from other CATs were added to risk of bias questions to provide a more granular and directed assessment of each domain. These examples demonstrate many instances where study elements relating to construct and external validity were included into risk of bias questions to fit the needs of the risk assessment or, in some cases, specific study types or subsets of studies. In addition to investigating the applications, a generic comparison of guideline study designs often mandated by authoritative agencies was compared to CAT criteria, demonstrating that several elements
Over 200 million U.S. citizens live in areas with legal access to medical and recreational cannabis. Exposure to cannabis and its contaminants represents a potentially serious health risk to susceptible patients with neurological diseases. Yet, due to the status of cannabis as an illicit Schedule I substance at the federal level, there are no unified regulatory guidelines mitigating the health risk of exposure to contaminants in cannabis. Pesticide and metal contamination have led to safety concerns and multiple recalls. Parkinson’s Disease (PD) is the most common motor disorder in the U.S., affecting 1% of the population over 65-year-old. Approximately 25% of PD patients are reported to use cannabis and cannabis-related products, including cannabidiol - to alleviate symptoms of PD, such as anxiety, pain, sleep problems, and tremors. Here, we examined the state-level guidance for cannabis use in PD patients as well as regulatory testing requirements in the U.S. As of November 7th, 2022, 15 of the 38 state-level cannabis programs listed PD as a qualifying condition for medical use. However, organophosphate pesticides, pyrethroid pesticides, and metals were not required for regulatory testing by five, four, and one of those 15 programs. Next, we conducted an online survey of 36 neurologists and movement disorder specialists who primarily treat PD patients. The response rate was 49% from eight states (AZ, CA, FL, MA, MN, WI, PA, and NM). When asked if they were aware of any substances other than canabinnoids in cannabis products, we found that 88.9% of physicians were unaware of any contaminants commonly found or thought there weren’t any present at all. When asked about their perception of the riskiness of cannabis use in PD patients, 44% reported that the risk is unknown, 27.8% reported low risk, 22.2% reported moderate risk, and 5.6% reported high risk. Toward the end of the survey, physicians were informed of the contaminants commonly found in cannabis products and asked to provide their opinions on the potential risks to their patient population and to inform possible policy changes. Common themes of perceived riskiness included: known long-term effects of pesticides and metal toxicity reported by 88.9% of participants and exacerbation of PD-related comorbidities reported by 55.6% of participants. Common themes of opinions to inform policy changes included: implementing strict standardized testing regimens reported by 38.9% of participants and investing in further research reported by 50% of participants. The results suggest a need for policy intervention as physician training - to improve cannabis safety in PD patients. Further interviews will collect physicians’ opinions on comorbidity risk, potential drug interaction, and education policies.
In our exploratory analysis, our findings suggest that the cancer potency ECHA due to Co exposure. Our results indicate that the Marsh et al. (2017) study was greater increase in lung cancer due to Co exposure and all four exposure groups exposure group are 1.34, 2.32, and 6.23 respectively. We determined that the Marsh data with at least two post-intervention measures would help to elucidate the effective half-life of lead estimated from secondary intervention studies. Although our analysis and the Rust et al. (1999) model have limitations, these results suggest that bone lead mobilization may contribute (6 months) follow-up time. Although our analysis and the Rust et al. (1999) model have limitations, these results suggest that bone lead mobilization may contribute to the effective half-life of lead estimated from secondary intervention studies. Testing the Rust et al. (1999) model with additional secondary intervention study data with at least two post-intervention measures would help to elucidate the complex kinetics of lead in the blood and bone of children.
exposure and preeclampsia. Disclaimer: The findings and conclusions in this report are those of the authors and do not represent the official position of the Centers for Disease Control and Prevention.

**3190 A Systematic Analysis and Data Mining of Opioid-Related Adverse Events Submitted to the FAERS Database**

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With the increasing prescription of opioid medications for various purposes, the opioid epidemic has become a serious national crisis which affects public health as well as social and economic welfare in the United States. An in-depth systematic analysis of opioid-related adverse events (AEs) is required to globally clarify the risks of the opioid exposure and the associations among various opioids. In this study, 92,016 AEs were identified from the list of all FDA-approved drugs, annotated by RxNorm and were classified into 13 opioid classes, including buprenorphine, codeine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, meperidine, morphine, oxycodone, oxymorphone, tapentadol, and tramadol. A total of 14,970,399 AE reports were retrieved and downloaded from the FDA Adverse Events Reporting System (FAERS) from 2004 Quarter 1 to 2020 Quarter 3. After the normalizations, we obtained 20,178,515 pairs of drug-AEs, originated from 69,889 unique drugs and 22,260 AEs, including 78,874 pairs of opioid-AEs of 13 classes of FDA-approved opioids and 14,374 unique AEs. Empirical Bayes Geometric Mean (EBGM), a representative Bayesian and widely used approach for disproportionality analysis, was then applied to have identified 3,317 pairs of potential risk signals for the 13 opioid classes. Based on these potential safety signals, comparative analysis was pursued to global overview the status of opioid-related AEs of all 13 classes of FDA-approved prescription opioids, and the top 10 most reported AEs of each opioid were listed. Both network analysis and hierarchical clustering analysis were conducted in order to further explore the full profiles of potential risk signals of AEs. The results of network analysis and hierarchical cluster analysis were not only consistent and validated with each other, but also provided a better and deeper understanding on the association and relationships of the 13 opioids from various aspects. These results may be potentially applied in improving pain treatment and management especially on chronic pain to avoid severe AEs caused by long term use and co-exposure with other drugs.

**3191 Assessment of a Modified Sandwich Estimator for Generalized Estimating Equations with Application to Opioid Poisoning in MIMIC-IV ICU Patients**

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Longitudinal regression models for correlated binary outcomes are frequently fit using generalized estimating equations (GEE). The Liang and Zeger sandwich estimator is often used in GEE to produce unbiased standard error estimation for regression coefficients in large sample settings, even when the covariance structure is misspecified. The sandwich estimator performs optimally in balanced designs when the number of participants is large with few repeated measurements. The sandwich estimator’s asymptotic properties do not hold in small sample and rare-event settings. Under these conditions, the sandwich estimator underestimates the variances and is biased downwards. Here, the performance of a modified sandwich estimator is compared to the traditional Liang-Zeger estimator and an alternate approach proposed by authors Morel, Pan, and Mancl-DeRouen. Each estimator’s performance was assessed with 95% coverage probabilities for the regression coefficients using simulated data under various combinations of sample sizes and outcome prevalence values with independence and autoregressive correlation structures. We demonstrated in simulations with sample sizes of 100 subjects and an autoregressive covariance structure with higher correlation settings (0.10 and 0.15) that all the sandwich estimators produced coverage probabilities that fell below 95%. This was not observed in our earlier simulations with low correlation values. As the sample sizes dropped under these same correlation conditions, the Liang-Zeger continued to perform abysmally while the Rogers-Stoner and Pan estimators recovered, almost achieving 95% coverage probabilities at 40 subjects and lower. In our limited simulation settings, the Rogers-Stoner sandwich estimator outperformed the other estimators and typically outperformed all other estimators as both the prevalence and sample size decreased. This approach provides a method for modeling rare events in finite samples on the effects of medications, drugs, and poisons.

**3192 RxNorm for Drug Name Normalization: A Case Study of Prescription Opioids in the US FDA Adverse Events Reporting System**


The US Food and Drug Administration (FDA) Adverse Events Reporting System (FAERS) is the largest adverse events (AEs) database containing over 16 million reports from 1969 to the present to support the FDA’s post-marketing safety surveillance program. Numerous studies have been conducted on the database to assess post-marketing reporting rates for drug safety review and risk assessment. However, the drug names in the adverse event reports from FAERS were heterogeneous due to a lack of uniformity of information submitted mandatorily by pharmaceutical companies and voluntarily by patients, health care professionals, and the public. Therefore, the studies using FAERS database without drug name normalization may encounter incomplete collection of adverse event reports from non-standardized reporting and the results might be affected. In this study, we demonstrated the way using RxNorm, developed by the National Library of Medicine, for drug name normalization in FAERS. Using prescription opioids as a case study, we used RxNorm API to map all FDA-approved prescription opioids from the collection of FAERS adverse event reports to their equivalent RxNorm Concept Unique Identifiers (RxCUIs) and RxNorm names. The different names of the opioids were then extracted, and their usage frequencies were calculated in our collection of more than 1.49 million AE reports for 13 FDA-approved prescription opioid classes, reported over 17 years. The results showed that a significant number of different names were consistently used for opioids in FAERS reports, with 2,086 different names used at least three times and 842 different names used at least ten times for 92 RxNorm names of FDA-approved opioids. RxNorm API mapping was confirmed to be able to help reducing the heterogeneity of drug names significantly in the adverse event reports in FAERS. The RxNorm API is expected to have a broad application to different sets of drug names from any database where drug names are diverse and unnormalized. Further research is expected to improve the application of RxNorm API and build up a database to strengthen drug safety review and assessment in pharmacovigilance.

**3193 Arsenic Exposure–Related Hypertension is Linked to Reduced Circulating Nitric Oxide**

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Hypertension is a major cause of death worldwide and is increasing in prevalence. Although arsenic exposure has been associated with the risk of hypertension, this association appears non-uniform due to inconsistent results from studies conducted in different populations and in dose-response relationships. Moreover, hypertension is a complex syndrome with multiple underlying mechanisms. In this study, we investigated the hypothesis that arsenic-promoted impaired production and bioavailability of vascular nitric oxide (NO) is implicated in the pathogenesis of cardiovascular diseases and hypertension and is sensitive to arsenic inhibition. Thus, we investigated the hypothesis that arsenic-promoted decreases in vascular NO was associated with hypertension in a Bangladeshi population exposed to a broad range of arsenic in their drinking water. There were 828 participants, with 641 from high- and 187 from low-arsenic exposure areas. Arsenic levels in drinking water, hair, and nails of each participant were measured by inductively coupled plasma mass spectrometry. Total serum nitrite concentrations, reflecting NO levels, were measured by immunoassay. Hypertension, defined as systolic (SBP) value of ≥ 140 and a diastolic (DBP) value of ≥ 90 mmHg, was approximately 4.5 times higher in the high arsenic areas than that in the low-exposure area. Arsenic exposure showed dose-dependent positive associations with blood pressure after adjusting for age, sex, BMI, education, and smoking habits. The increased odds ratios for hypertension were dose-dependently increased by arsenic even in the participants with moderate concentrations (10-50 µg/L) of water arsenic. As we have observed in cells and mice, arsenic decreased NO availability, and NO levels were inversely associated with risk of hypertension. Additionally, causal mediation analysis showed a significant mediating effect of NO loss on arsenic-related hypertension. Thus, the results of this study suggest that arsenic exposure dose-dependently increased risk of hypertension by impairing vascular NO availability in this population. Supported by the grants from Rajshahi University (No. A-701/6-109[Research]), NEHS (R01033519) and JSPS KAKENHI (No. 21KK0170).

**3194 Paraben Exposure and Adiposity-Related Measures: A Systemic Review of Population-Based Studies**


Parabens are alkyl esters of p-hydroxybenzoic acid. Human exposure to parabens is ubiquitous, including from using pharmaceutical and cosmetic products as well as from food consumption. There is a growing public health concern that...
paraben exposure can have an adverse impact on human health. We conducted a systematic review of epidemiological studies published in the last five years to assess paraben exposure in relation to adiposity-related measures at three different life stages: in utero, between birth and adolescence, and adulthood. A recent study measured paraben exposure in pregnant women and found a significantly elevated risk of subsequent adiposity-related measures, no definitive conclusions can be made due to potential effect modification by factors like gender. Only six studies examined the association between paraben exposure and adiposity-related measures in adolescents. Most of the studies were cross-sectional; therefore, the causal inference is limited. In addition, no potential confounding dietary factors were considered in these studies. Foods containing the most parabens may also be the most caloric. The association between paraben exposure during adulthood and adiposity-related measures was investigated in 12 studies. One-third of the studies found no association between paraben exposure and BMI. Moreover, many studies were statistically underpowered, especially for sub-analysis by gender or other potential effect-modifying factors. Among the remaining eight studies, all but one demonstrated an inverse relationship between paraben exposure and BMI. Paraben exposure is influenced by age, race, and gender. The composition of parabens profiles in human samples is also population and/or region-specific. It is important to note that very few studies reviewed here were originally designed to evaluate the health impact of environmental chemical exposure, including parabens. The environmental exposure components were often added at a later research stage, resulting in single-spot biological samples being collected at various time points, which could potentially lead to exposure misclassification. This review reinforced the necessity for larger, long-term prospective studies with repeated measurements of paraben exposures across a broad range of developmental periods to elucidate the effects of parabens exposure on childhood and adult health outcomes.


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Chronic kidney disease (CKD) affects more than 1 in 7 adults in the US. Although glyphosate is the most widely used herbicide, there is limited epidemiological evidence that examined its potential effects on kidney functions. This study aimed at investigating the effect of glyphosate exposure on kidney functions among US adults, based on the 2013-2014 National Health and Nutrition Examination Survey (NHANES) datasets. The multivariable linear regression models were fitted to evaluate associations of urinary glyphosate with kidney function biomarkers such as estimated glomerular filtration rate (eGFR), serum uric acid, serum albumin, blood urea nitrogen (BUN), and urinary albumin-creatinine ratio (ACR). In addition, multivariable logistic regression was conducted to assess the association between urinary glyphosate and CKD. CKD was defined as eGFR < 60 mL/min/1.73 m². Irrespective of age, albuminuria levels were used to define CKD before the analysis. Demographic, socioeconomic, health, and behavioral factors were included in the model for adjustment. Urinary glyphosate was positively associated with ACR (β 2.92, 95% CI 0.97, 4.86) and BUN (β 0.50, 95% CI 0.04, 0.95), and negatively associated with eGFR (β -1.64, 95% CI -2.82, -0.44), after adjusting for covariates. Each doubling of urinary glyphosate levels was significantly associated with an elevated risk of CKD (OR 1.39, 95% CI 1.12, 1.71), after adjusting for covariates. Our study showed that exposure to glyphosate may negatively affect kidney functions, suggesting a potential association with chronic kidney disease.

3197 Phthalates Exposures Are Associated with Liver Steatosis and Fibrosis in Adult NHANES 2017–2018

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Phthalates are ubiquitous plasticizers that are widespread environmental pollutants and have the potential to affect mitochondrial and insulin resistance, both of which are commonly associated with nonalcoholic fatty liver disease (NAFLD). We wanted to determine if phthalates are associated with risk of NAFLD. We hypothesize that phthalates are associated with hepatic steatosis, fibrosis, and high-risk nonalcoholic steatohepatitis (NASH) in the general adult population. Participants ≥18 years old from the 2017-2018 National Health and Nutrition Examination Survey (NHANES) were included. The exclusion criteria were active viral hepatitis B or C; daily alcohol consumption >15g for men or >14g for women; missing/unreliable elastography data; and <60% (n=4) phthalates detectable in the population. Vibration Controlled Transient Elastography (VCTE) was performed by Fibroscan®. Urinary phthalates were measured by SPE-HPLC-TIS-MS. Mono-carboxyisonyl (CNP), mono-carboxyisoctyl (COP), Mono-2-ethyl-5-carboxyethyl (ECP), Mono-2-ethyl-5-carboxypentylterephthalate (ECPT), Mono-2-hydroxy-iso-butylic (HIBP), Mono-n-butylic (MBP), Mono-(3-carboxypropyl) (MCP), Mono-ethyl (MEP), Mono-3-hydroxy-n-butylic (MHBP), Mono-(2-ethyl-5-hydroxyhexyl) (MHH), Mono-2-ethyl-5-hydroxyterephthalate (MHH), cyclohexane dicarboxylic acid mono (HIDC), Mono-2-ethyl-5-carboxypentyl (ECP), Mono-2-ethyl-5-oxohexyl (MOH), Mono-ooxioisonyl (MONP), mono-benzylic (MNP). R-software was used to conduct univariate and multivariate associations between exposure (phthalates) variables with the liver disease outcome variables using VCTE for controlled attenuation parameter (CAP), liver stiffness measurement (LSM), and Fibroscan-AST (FAST) score. FAST score was calculated using variables LCAP, CAP, and AS. The mean age in this study was 51.7 ± 17.66 years, 50% of participants were women; 36% were non-Hispanic White; and 22% were non-Hispanic Black. The median CAP and LSM were 269.1 ± 122.52 dB/m and 7.53 ± 9.68 kPa, respectively. HIBP was positively associated with CAP, while COP, HIBP, MHBP was positively associated and MHBP was negatively associated on multivariable regression analysis. On univariate analysis, ECP, MHPT, positively associated with LSM; while ECP, ECPT, MHPT, were associated with LSM on multivariable regression analysis. On univariate analysis, ECP, ECPT and MHPT were associated with FAST, while ECP and MHBPT were positively associated on multivariable regression analysis. Phthalates are associated with risk of hepatic steatosis, fibrosis, and high-risk NASH. Further studies are needed to understand the pathophysiology of this association.

3198 Assessing the Health Impact of Land Applied Biosolids in Virginia

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The Virginia Department of Health (VDH) simultaneously conducted a quantitative microbial risk assessment (QMRA) of land applied biosolids in VA and an epidemiological study of residents living near where biosolid samples were recently applied. Biosolids refers to solid, semisolid, or liquid materials removed from municipal sewage and treated to be suitable for recycling as fertilizer. Class A biosolids have...
received a level of treatment that virtually eliminates disease-causing organisms and are often sold alongside commercial fertilizers. Class B biosolids have less restrictive standards for content of disease-causing organisms. Class B biosolids are considered protective of health when coupled with specific application restrictions, such as distance between land with biosolids and any wells and streams, access restrictions for people and livestock, and certain crop exclusions. Biosolids samples were collected from 34 application sites, four storage facilities, and two wastewater treatment facilities in VA. VDH contracted with the University of Arizona to test biosolids samples for pathogens which included seven microorganisms or classes of microorganisms. Pathogens tested included bacteria, viruses, and helmith ova. A total of 40 biosolids samples including 39 Class B biosolids and one Class A biosolids samples were sent for analysis. The biosolids treatment types identified for the 40 samples included: aerobic digestion (10), anaerobic digestion (12), lime stabilization (16), and thermal hydrolysis (1). Five anaerobically digested biosolids from different producers each had fecal coliforms greater than 2,000,000 MPN/g. One lime stabilization treated sample Salmonella content was reported as 5 MPPg/4g compared to six aerobic and one anaerobically digested biosolids samples that had Salmonella content greater than 3MPPg/4g. Measurable concentration of pathogenic viruses were reported in five aerobic and one anaerobic digested biosolids samples. Ascaris was not detected in any samples. The laboratory analysis shows that Class B biosolids generated by lime stabilization have fewer pathogens than aerobic or anaerobically generated Class B biosolids. VDH used the QMRA model developed by the USDA to calculate the probability of aerosol infection by each pathogen using the laboratory results. QMRA uses what is known about pathogen infectivity, human anatomy and physiology, human behavior, and air transport mechanisms of pathogens to quantify risk of infection. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The transport mechanisms of pathogens to quantitate risk of infection. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. The acceptable limit of infection risk from biosolids exposure used was less than 1 in 10,000. 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cancer. The results from the pre-1980 analyses indicate that legacy hair dyes may be associated with increased risk of bladder cancer in hairdressers and/or barbers. In contrast, the post-1980 study results indicate that modern hair dyes are not associated with an increased risk of bladder cancer in hairdressers and/or barbers. Overall, the available epidemiological evidence indicates that the risk of bladder cancer is not elevated among hairdressers and/or barbers employed after 1980.

3202 Organophosphate Esters and Fetal Growth in the LIFECODES Fetal Growth Study

A. G. Rundle

Organophosphate esters (OPEs), such as those used as flame retardants, represent emerging exposures of concern. OPEs have been associated with adverse reproductive outcomes, including changes in fetal growth. Previous studies have been inconsistent with respect to the direction and magnitude of these associations. However, this literature has been limited by small sample sizes. Using data from the LIFECODES Fetal Growth Study (n = 901), we estimated associations between OPEs and size-for-gestational age. The LIFECODES Fetal Growth Study is a case-cohort comprising 249 small-for-gestational age (SGA), 411 appropriate-for-gestational age (AGA) and 241 large-for-gestational age (LGA) births sampled from 2008-2018. We measured concentrations of 8 OPE metabolites at three timepoints and used their geometric mean to estimate average exposures during pregnancy. We used multinomial logistic regression models to estimate the odds ratio (OR) and 95% confidence interval (CI) of an SGA or LGA birth associated with an interquartile range increase in each metabolite. Of the 8 metabolites quantified, we detected 5 frequently (> 60% of participants). The metabolites were weakly correlated with one another (-0.13 < ρ < 0.27) and we identified temporal trends in their concentrations across the study period. For example, concentrations of bis(1,3-dichloro-2-propyl) phosphate (BDCPP) and bis(1-chloro-2-propyl) phosphate (BCPP) declined, while concentrations of bis(1-chloro-2-propyl) phosphate (BCPP) increased across the study period. After adjusting for potential confounders, both bis(1,3-dichloro-2-propyl) phosphate (BDCPP; OR: 1.30, 95% CI: 1.00, 1.68) and BCP (OR: 1.25, 95% CI: 0.99, 1.57) were associated with higher odds of an LGA birth. Associations with SGA births were null. Metabolites of several OPEs were associated with higher odds of an LGA birth. Though the literature is inconsistent, these findings align with previous studies reporting that these metabolites are associated with higher birthweights. Further research is needed to fully examine the relationships between OPEs and adverse fetal growth outcomes.

3203 Inverse Associations of Cord Blood Mitochondrial DNA Copy Number with Adiposity Trajectories in Children

A. Reddam

Mitochondrial DNA copy number (mtDNAcn) is a biomarker representing the average number of mitochondrial genomes in a cell. Significant changes in mtDNAcn may indicate mitochondrial damage and dysfunction and therefore can be used as a measure for mitochondrial toxicity or cellular stress. Alterations in mtDNAcn have been implicated in dysregulated pathways associated with cardiometabolic diseases, diabetes, and obesity. While studies have shown inverse associations between mtDNAcn and adiposity, research has mainly focused on adults and not on children. Our study sample included a cohort of Dominican and African American children from Northern Manhattan and South Bronx, NY recruited from 1998 to 2006. mtDNAcn was measured in cord blood buffy coat and dichotomized into low (< median) and high (> median) categories. Children were followed up with measurements of height and body mass index (BMI) at ages 5, 7, 9, and 11 years. Mixed-effects models with random intercepts for participants were used to assess associations between mtDNAcn in cord blood and child BMI and BMI z-scores. Interactions between mtDNAcn categories and child age or child age squared were used to assess associations between age and BMI and BMI z-score trajectories at different levels of mtDNAcn. Adjusting for relevant covariates (maternal age, ethnicity, BMI, completion of high school, previous birth, environmental tobacco smoke exposure, receipt of public assistance, sex, age, age squared, and DNA input concentration), BMI was on average 1.1 times lower (β = -1.1, 95% CI: -1.9, -0.37) and BMIz was on average 0.32 times lower (β = -0.32, 95% CI: -0.54, -0.10) in individuals with low mtDNAcn compared to individuals with high mtDNAcn in cord blood. BMI trajectories also differed by mtDNAcn level - where children beginning life with higher cord blood mtDNAcn tended to follow a trajectory of lower BMI and BMI z-scores than those with lower mtDNAcn. Similar to the literature in adults, these results suggest that higher cord blood mtDNAcn is associated with lower childhood adiposity. Further research into associations between mtDNAcn and childhood adiposity in children of diverse background is needed to better understand the mechanisms behind this relationship.

3204 The Joppa Environmental Health Project: Application of Community-Level PM2.5 Monitoring to Evaluate the Air Quality in an Historic Freedmen’s Town in South Dallas

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The Joppa Environmental Health Project (JEHP) was formed as a collaborative community-based project with a primary focus to evaluate the air quality in Joppa using PurpleAir (PA) monitors as a citizen-science tool to quantify PM2.5 concentrations in Joppa. PA-HSD monitors were placed in Joppa in which the primary real-time PM2.5 cf/ATM data were processed to estimate monthly PM2.5 averages. Presently, the Environmental Protection Agency’s (EPA) standard for annual average of primary PM2.5 is 12 μg/m³. Monthly averages from November 2021 to April 2022 ranged from 10.45 μg/m³ to 19.13 μg/m³. Based on these results, PurpleAir monitors are considered as an easily accessible citizen-science tool that promotes community advocacy and informed-decision making in environmental justice communities such as Joppa. Moreover, JEHP has initiated community health surveys and airway inflammation assessments in children residing in Joppa. Future work will build on these partnerships in collaboration with the Texas A&M Superfund Research Center to assess hyperlocal VOC concentrations. Other approaches will be vital for long-term goals of formulating policy-oriented neighborhood planning in Joppa and serving as a template for other EJ communities.

3205 Increased Cardiovascular and Respiratory Disease–Related Hospitalizations and Mortality in an Area Surrounding a Hazardous Waste Incinerator

L. Baconguis, and S. Cormier

Clean Harbors, LLC operates a thermal treatment plant near the city of Colfax, LA in Central Louisiana. Its regular open burning of explosives causes significant community disturbances. We hypothesized that airborne pollutants produced from this facility increased the disease burden of individuals residing in Colfax compared to surrounding areas. We gathered hospitalization and mortality rates from the years 2000-2017 from the Louisiana Vital Records Registry, Tumor Registry, and Department of Health. We found greater incidence rates of cardiovascular and respiratory diseases in Colfax compared to the rest of Grant Parish or Louisiana. The incidence rate of hospitalization for hypertension was 932.931 per 10,000, 1.165 (95% CI: 1.14 to 1.19) times that of surrounding regions, while for asthma it was 99.0163 per 10,000, 1.165 (95% CI: 1.14 to 1.19) times that of surrounding regions. Significantly higher mortality rates were also found in Colfax for hypertension (2.060 times that of surrounding regions, 95% CI: 1.71 to 2.48) and ischemia (1.828 times that of surrounding regions, 95% CI: 1.59 to 2.10). Most hospitalized patients lived under 10km from the burn site and most lived northwest to it, consistent with prevailing wind directions over the study period. Despite the small sample size and limited demographic information present in the dataset, we describe a clear trend of increased hospitalization and mortality in Colfax compared to surrounding areas; these are consistent with previous literature on the effects of long-term pollution exposure. This suggests the need for a detailed epidemiological study and exposure analysis within the community.
such as demographic and socioeconomic factors, BMI, diabetes, hypertension, history of alcohol consumption, exposure to smoke via cigarette or other regu-

3207 Women in Toxicology in the United States
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Since the toxicology field was established, women have played a critical role in it. Six females from different social classes and education backgrounds were the pioneers of modern Toxicology. Despite these differences, they overcame similar obstacles in gender, politics, and scientific barriers to disseminate their research. This discussion will start with Ellen Swallow Richards, who, besides being the pioneer in sanitary engineering, founded the home economics movement that applied science to the home. The discussion will continue with Alice Hamilton, a contributor to occupational health, a pioneer in the field of industrial toxicology, and an example of generosity to social movements and those in need. Subsequently, the most famous woman we discuss in this paper is Rachel Carson, whose fundamental work in environmental Toxicology is evidenced in her important book Silent Spring. This work also features Elizabeth Miller, a biochemist known for her fundamental research in cancer carcinogenesis, followed by Mary Amdur. Nowadays much of what we know about air pollution comes due to Mary, who paid from her own pocket for her experimental animals to investigate Donora smog. The most famous female who made significant contributions in carcinogenesis and chemotherapy drugs who worked for 40 years at the National Cancer Institute. Here, we discuss the aforementioned women’s careers and personal struggles that transformed toxicology into the field we know now.

3208 Temporal Trends in Household Phthalate Dust Concentrations in an Agricultural Community Were Associated with Respiratory Health Outcomes
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Phthalates are a group of chemicals commonly found in consumer products to increase the flexibility/durability of plastics. They can accumulate in house dust as they deteriorate. Regulatory restrictions of various phthalates over the years may contribute to changing exposure profiles of phthalates. Analyzing household dust can approximate cumulative phthalate exposure mixtures. Dust data was analyzed from a longitudinal cohort of 97 households for a suite of 18 phthalates in the Lower Yakima Valley of Washington State, USA. We determined whether children’s product reporting requirements and regulations at the state and federal level are associated with longitudinal changes in phthalate concentrations. Data were analyzed for differences in concentrations between 2005 and 2011, and also seasonally. Due to high non-detection rates of 2 phthalates, a subset of 16 phthalates were included in the final analyses. Significant differences were observed in the composition of phthalates in dust over 2005 to 2011. Significant differences were also observed by season for each sampling year. Additionally, recent studies have demonstrated an association between house dust phthalates exposure and development of asthma and allergic disease. Approximately 20% of the children in this cohort have been diagnosed with asthma, which is higher than the national average of 8%. Several factors can contribute to these differences, including the higher asthma rates seen in Hispanic populations; however, the differences seen are greater than would be explained by these differences and correlate with the increasing phthalate dust concentrations. Based on house dust samples from 97 households with respiratory health survey data, we examined differences in total phthalate concentration in dust between households with and without a child with respiratory health issues across time, and significant differences (p<0.1) were found. Understanding the role of environmental exposures is an important step toward prevention and public health applications.

3209 A Translational Model Using In Vitro and Urinary Biomarkers to Inform Bladder Cancer Risk from Arsenic Exposure in South Texas
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Exposure to arsenic (As) from drinking water is a worldwide public health concern since As is toxic and a known human carcinogen with the strongest association to bladder cancer. In the U.S., 5 million people drink water with As concentration over the EPA standard limit of 10µg/L. Since private wells are not regulated, millions of people are highly exposed from this source, including rural residents in Texas. Texas is ranked number seven in the U.S. of people affected by As contamination. Moreover, bladder cancer is highly prevalent in South Texas. Based on risks for As exposure and bladder cancer, there is a critical need for novel biomarkers to identify individuals at risk and to evaluate preventive interventions in South Texas. To address this, a paired in vitro and human translational study was conducted. First, we exposed SV-HUC-1 uroepithelial cells to 0µM (control), 5µM, or 10µM of NaAsO2 for 24 hours (acute exposure) to derive phenotypic endpoints, including cytotoxicity, inflammation, and oxidative stress. Next, to model chronic exposure levels, SV-HUC-1 cells were treated with 0µM, 0.1µM, or 0.5µM of NaAsO2 for 10 weeks. Urine samples were collected from participants in three South Texas counties (N=29) where the mean As level in drinking water samples was 11.2µg/L prior to household intervention with water pitcher filters. PDGF-BB was the most predominant cytokine measured in vitro and in participant urine samples. Water filtration intervention showed a significant decrease in urinary As levels (p<0.0001). Analysis of biomarkers post-intervention is ongoing. Initial findings support a combined experimental and field-based approach can enhance the development of biomarkers to inform As mitigation strategies.

3210 Utilize Dose-Response Modeling to Advance Gene and Pathway Identification for Risk Estimation of Substance Use Disorder
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Substance use disorders (SUDs) that have imposed a significant burden on public health are extremely difficult to treat, therefore, it is critical to understand the underlying etiology of SUD to advance the development of SUD prevention strategies. Genetic epidemiological methods and large accessible biobanks have built the foundation for large-scale genome-wide studies to explore the potential risk factors for SUD at the molecular level. The genome-wide association studies (GWAS) have been widely applied to identify single nucleotide polymorphisms (SNPs) that are potentially associated with SUD through whole genome sequencing but have difficulty providing consistent results due to the limited sample size. This project aims to integrate dose information of substance use to advance the identification of SUD-associated genes and pathways given a limited sample size, and further characterize the SUD risk with adequate uncertainty quantification. The rationale arises from a biologically plausible assumption that SUD risk and cumulative dose are positively associated. Four steps are conducted for the dose-response modeling-based gene and pathway identification: (1) screening out invalid data and data not adequate for dose-response modeling using trend tests, (2) converting the genome data to the dose-response data for benchmark dose (BMD) calculations with adjusted effects of confounders, (3) applying the BMD modeling methodology to estimate BMD for the identified SUD-associated SNP, and (4) estimating pathway BMD to derive a reasonable reference dose for the studied substance. We used the Study of Addiction: Genetics & Environment (SAGE) dataset (phs000092.v1.p1) from dbGaP containing dose levels of cigarettes/day and the nicotine dependence traits to validate the method. 343,938 SNPs with valid BMD estimates related to 12,618 genes among 1 million measured SNPs of 284 subjects have been identified. The most sensitive 15 SNPs ranked by the BMD values are found directly related to smoking and SUD-induced diseases, for example, one of the identified SNP rs219353 is related to the gene “CDCP1” and the substance-related disorder disease. The pathways identified using the proposed method have 11 significant ones directly related to the drug response, for example, one of the significant pathways GO:0035690 is the cellular response to a drug. Employing the BMD method to utilize the dose-response information for SUD-associated gene identification is highly innovative with two important features: requires a smaller sample size and is more sensitive to detect the trend. Future research will be conducted to derive a reference dose for the designated SUD in support of the development of effective prevention strategies.
Emerging evidence indicates that COVID-19-related stressors have increased alcohol consumption. This study aimed to: (1) estimate the prevalence of binge drinking before and after the COVID-19 pandemic declaration and (2) determine the association between binge drinking and sociodemographic characteristics among a nationally representative sample of noninstitutionalized adults in the United States (U.S.). We used the 2020 Health Information National Trends Survey (HINTS), a cross-sectional survey. HINTS data were collected from February through June 2020 (N = 3,865). Stratified by the COVID-19 pandemic declaration, we estimated the weighted prevalence of binge drinking (defined as 4 (female) or 5 (male) or more drinks at an event within two hours) by sociodemographic characteristics and other variables of interest. Weighted multivariable logistic S models were used to assess the association between binge drinking and participants’ characteristics before and after the pandemic declaration. The prevalence of extreme binge drinking (6 or more drinks) increased during the pandemic, although overall binge drinking decreased by 0.82% compared to before the pandemic. However, it increased among specific subpopulations, including individuals aged 18-25 years (during= 61.88% vs. before= 60.60%), males (during= 48.77% vs. before= 47.65%), Hispanic individuals (during= 60.61% vs. before= 55.72%), divorced/separated individuals (during= 49.80% vs. before= 41.10%), those with high school (during= 62.00% vs. before= 47.23%) or college/higher education (during= 38.66% vs. before= 36.46%), and current smokers (during= 64.56% vs. before= 57.57%). Multivariable logistic regression indicated that odds of binge drinking were 1.02 times lower during the pandemic compared to before the pandemic. Simultaneously, it was found that Hispanic individuals (OR= 3.07, 95% CI= 1.53, 6.18), lesbians/gays/bisexuals (OR= 3.37, 95% CI= 1.42, 8.00), former smokers (OR= 2.16, 95% CI= 1.38, 3.39), and current smokers (OR= 4.17, 95% CI= 2.11, 10.53) had higher odds of binge drinking during the pandemic. While overall binge drinking decreased during the COVID-19 pandemic decreased, it increased in certain subpopulations in the U.S. These results indicate the importance of examining the disparate behavioral impact of COVID-19 on subpopulations and the need to develop more tailored and targeted interventions.

Methamphetamine (METH) is one of the most widely abused drugs. METH has been reported to induce cardiac dysrhythmia, leading to sudden death. In forensic autopsies, several cardiac lesions, such as myocardial hystrogy, are often observed. However, the mechanism of METH-induced cardiotoxicity has not been clarified fully. In this study, we investigated the effects of METH on gap junctions (GJ) and ion channels, which are associated with heart function, in the heart of METH-exposed rats. Rats (5 weeks, Wister, male) were injected intraperitoneally with either saline (Saline/METH group) or METH (METH/METH group, 0.25-5 mg/kg body weight; alternate days) for 12 days. After 2 days, in both groups, the rats were injected 10 mg/kg for 4 times (METH challenge), and then asphyxiated with CO2 at 0, 12, and 24 hrs. Protein and RNA were extracted from the hearts. The expression levels of GJ-associated protein (connexin43 (Cx43), zonula occludens (ZO)-1, and ion channel proteins (Kir2.1 and Kir6.2) were analyzed by RT-PCR. The protein levels of Cx43 were analyzed by western blotting and immunostaining. The expression level of microRNA-1 (mir-1), a negative regulator of Cx43 and Kir2.1, was also examined by RT-PCR. Rat body weight changes during the treatment period and heart weight at each dissection showed no difference between the two groups. RT-PCR analyses resulted in Cx43, Kir2.1, Kir6.2, and ZO-1 expression levels increased at 12 or 24 hrs after the METH challenge in both groups. The increments of Cx43 expression level were higher in Saline/METH group than METH/METH group. Western blotting and the quantitative analyses revealed that protein levels of Cx43 were remarkably increased in 12 hrs and continued until 24 hrs by METH challenge in both groups. Immunostaining for Cx43 showed that Cx43 was localized in the intercalated discs of the cardiomyocyte. The fluorescence of Cx43 was dramatically increased in 12 hrs and continued until 24 hrs after METH challenge in both groups. mir-1 expression levels were significantly reduced at 12 hrs and returned to the baseline level at 24 hr after METH challenge in Saline/METH group, but not METH/METH group. In conclusion, the treatments of high concentration of METH altered the heart physiology, suggesting that GJ remodeling and ion channels are associated with METH-induced cardiac dysfunction. Furthermore, mir-1 expression may be partially involved in the regulation of these changes. The responses to a high concentration of METH treatment were smaller in rats treated with continuous METH than in those treated with saline, suggesting that some feedback mechanism may regulate the responses.

In recent decades, illicit opioid use has exploded in the US, accounting for two out of every three drug overdoses. Moreover, the use of highly potent, synthetic opioids such as fentanyl has increased exponentially (over 8,802% increase from 2009 to 2017), and while overdose rates for other opioids have plateaued, fentanyl-associated overdoses increased 45% in 2016-2017 alone. Despite the overdose, fentanyl still remains one of the most effective pain relievers available to patients, and diversion of the drug from healthcare settings adds to the large amount of illicitly manufactured fentanyl currently in the US. Of growing concern is that the number of illicit fentanyl users is skewing towards people of childbearing age, potentially exposing the fetus to the drug. There is evidence to suggest that fentanyl triggers downstream pathways distinct from other opioids, and newborns exposed prenatally to fentanyl present with different symptoms than those exposed to other opioids. Considering also that chronic, developmental fentanyl exposure is a relatively recent phenomenon, it is unclear what to expect from exposed children. To fill this gap, we developed a model of prenatal fentanyl exposure that mimics the use patterns of human users. We hypothesized that developmental fentanyl exposure would alter birth outcomes as well as long-term behavior. Female mice were orally gavaged with fentanyl (74 ug/kg) or saline twice daily prior to breeding. Drug administration continued up through parturition. Fentanyl did not affect dam body weight during gestation; however, fentanyl-exposed litters were significantly smaller than unexposed did not differ (P≤0.05) from their postnatal day (P0), fentanyl-treated males were significantly larger than all other groups at P30 and P120, suggesting changes in homeostatic mechanisms. Behaviorally, fentanyl did not significantly alter any measures of cognition, locomotor activity, or anxiety tested. However, fentanyl-exposed mice of both sexes performed significantly worse than their control counterparts in the tube test, a powerful and ethologically validated test of social dominance and hierarchy. Given that disruptions in this test are often associated with social deficiencies, such as those found in autism spectrum disorder and Rett’s syndrome, it is possible that early fentanyl exposure may result in long-term social deficiencies later in life. Further testing is underway in determining the underlying neural mechanisms for these developmental maladaptations.
drug screens is indicative of use relatively recent to sampling and the presence of BZ alone is typically indicative of consumption several hours before the sample was taken.

3215 Characterization and Validation of Conditioned Place Preference Paradigm to Assess Reinforcing Properties of New Compounds under GLP Compliance
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The Conditioned Place Preference (CPP) paradigm in rats is a method widely accepted for assessing the reinforcing properties of a test item, thus it is part of the safety evaluation program for novel compounds with the potential for abuse in accordance with FDA and EMA guidelines. The CPP is designed to assess whether an association between the subjective state elicited by a drug substance (e.g. feeling good) and the specific environmental stimulus presented during the period in which a drug substance is active (e.g. a black coloured compartment) can be implemented and become a conditioned stimulus. In other words, when conditioned place preference is established, an association between environmental stimuli and the effect of the drug substance is learned. In this work, we describe the results of CPP model validation establishing the motivational properties of known abused drugs using independent groups of naïve male Sprague-Dawley rats. Our CPP model consisted of a 10-day schedule with three distinct phases: preconditioning, conditioning, and postconditioning. After a pre-conditioning session, each rat was treated for 8 consecutive days with alternate injections of different comparator drugs or saline and confined to the conditioning chambers. The association to each chamber was counterbalanced within side, treatments and days. On Day 10, rats were tested for the acquisition (postconditioning) of CPP under identical conditions to those of the preconditioning phase. We performed four independent trials with different drugs at ascending doses: the first two trials with cocaine up to 15 and 20 mg/kg and the fourth with LSD and midazolam up to 0.5 mg/kg and 5 mg/kg, respectively. CPP data from the first experiment demonstrated that cocaine induced strong CPP for drug-paired compartment only at 15 mg/kg, while, in the second experiment, all the cocaine doses tested (10-20 mg/kg) induced CPP. Data from the third experiment showed that morphine-induced CPP at 5 and 10 mg/kg while ketamine did not induce place preference at any tested dose. Data from the fourth experiment showed no place preference in animals administered with LSD and midazolam.

The aim of this work was to set up a conditioned place preference paradigm that could be used to assess the reinforcing properties of a new drug as an alternative method to the abuse liability schedule (e.g. a drug that self-administration of the test item is not feasible, e.g. due to low solubility). The results obtained demonstrated the reliability of this model in assessing the reinforcing properties of cocaine and morphine at different doses as already reported in the literature and confirmed that cocaine or morphine may be used as comparators (positive controls) while testing the reinforcing properties of a new drug. Negative results (i.e. no development of place preference) were obtained after treatment with ketamine, LSD or midazolam under our experimental conditions. Further studies are needed to test different doses and pretreatment time, in order to expand the list of comparators available.

3216 Agreement between Self-Reported Recreational Drug Use and Detection in Plasma, Urine, and Hair in Persons with Active Drug Use Undergoing Hepatitis C Treatment
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The primary route of Hepatitis C virus (HCV) transmission is through shared needles, syringes, or other paraphernalia used to prepare and inject recreational drugs. While HCV is curable with direct acting antiviral (DAA) therapy, active drug use was historically considered exclusionary for DAA treatment due to theoretical concerns for diminished adherence and reinfection. The INCLUD trial found both DAA adherence and treatment success to be high in active drug users. Despite these findings, concerns that self-reported drug use may underestimate actual drug use represents a barrier to care for persons with HCV who use drugs. This sub-study of INCLUD compares self-reported recreational drug use against objective measures to determine the utility of these measures to objectively confirm drug use. Agreement between hair and either self-report, plasma, or urine aggregated over the prior 4 weeks was moderate (54 to 80%). Hair was more likely to be positive for stimuliators (94%, predominantly due to detection of methamphetamine) vs. self-report (54%, agreement: 60%). This observation is likely due to the extended half-life of methamphetamine (90 days) in hair. Cannabis use was detected in only 29% of hair samples, compared to 63% self-reported use during the same person-visits (62% agreement), consistent with prior reports of poor incorporation of cannabinoid into hair. Opioids were found in 28% of hair samples, compared to 14% self-reported use (75% agreement). These data indicate that the utility of hair sampling to determine remote drug use is class dependent, limiting the broad utility of this matrix for assessing remote drug use. The clinical success of DAA in active drug users is coupled with the observation that plasma and urine can objectively confirm recreational drug use. The support the use of DAA in this high-risk population.

3217 Field Forward Collection and Analysis: Utilizing Pressure-Sensitive Adhesive Paper Combined with Portable Mass Spectrometry for Detection of Threats
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Every day, dangerous illicit and synthetic drugs are shipped through the postal service and private carriers originating both from the United States and internationally. These opioid and synthetic substances are contributing to an increased number of overdose deaths, in large part due to higher potency in comparison to cocaine or methamphetamine. This increase level of potency contributes to difficulties when detecting trace quantities and poses a threat to first responders who may unknowingly expose themselves while responding to an incident. Highly sensitive and accurate field detection techniques are required to give first responders fast and actionable assessments of sites. Robust sample collection and trace threat identification in a field setting are challenges facing first responders and law enforcement, but several instrumental and collection methods have been developed in recent years. However, detection of trace amounts of material within complex matrices is still a challenge for field-focused techniques. Previous research has shown the benefits of utilizing pressure-sensitive adhesive (PSA) coated paper, e.g. yellow sticky notes, for collection combined with tandem mass spectrometry (MS/MS) for the identification of trace amounts of small molecules. In this work, the analysis of fentanyl, carfentanil, and cocaine captured on a PSA substrate via portable instruments were examined. The BaySpec Continuity with an atmospheric pressure chemical ionization (APCI) source was used for this work. Collection surfaces consisted of solid metal/glass surfaces as well as cloth. Identification was determined by isolating the precursor ion of the agent and utilizing collision induced dissociation (CID) to fragment the ion into the product ions. Utilizing these sampling and analysis methods, identification of the drugs or adding cutting agents inhibit identification. The sampling method was shown to be robustness for various sampling schemes. Finally, a counterfeit oxycodone pill made up of 99% acetaminophen and 1% fentanyl was investigated by grinding the pill, sampling the powder, and performing MS analysis. It was found that the high concentration of acetaminophen did not mask or inhibit the identification of the threat. The MS analysis required less than 10 seconds to provide an identification and displayed the results in an easy to interpret Red light/Green Light readout. This testing showed the utility of combining PSA substrate sampling with portable MS analysis.

3218 Applying Wastewater Surveillance to Create a Predictive Model of Fentanyl Overdose Deaths and Assessing Locations of Interest
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Wastewater-based epidemiology (WBE) is an innovative and cost-effective approach to construct drug use trends on a community scale. The INCLUD trial couplage measures human excreted chemicals (parent compounds or metabolites) or biological surrogate molecules in wastewater that can be used as near real-time information about health and lifestyle behaviors of a population without the need for individualized surveys or testing. There is growing acceptance of WBE in the field of public health and public health to monitor illegal drugs which allows for analysis of temporal consumption trends in varied settings such as large urban communities. This approach complements traditional, yet important, self-reporting methods and biospecimen testing with the advantages of providing additional information critical to identifying ‘hot spots’, new drug emergence, and shifts in drug
consumption trends. Therefore, WBE can rapidly produce large temporal data sets at low cost which makes it an excellent real-time indicator of changing community drug use trends. This is particularly important for drugs, such as opioids, that are associated with fatal overdoses that have been on the rise in the past decade, with a 56% increase from 2019 to 2020 alone. While the quantification of opioids such as fentanyl in wastewater is not novel, using such data to develop predictive overdose death models is lacking. Furthermore, with large data centers, such as the National Drug Early Warning System (NDEWS) Coordinating Center, collate illicit drug data that can be used as an early warning system, wastewater surveillance is rarely included. To address these gaps, we collected weekly wastewater samples for nine months from three major urban cities and 1 small city. Samples were extracted by solid phase extraction (SPE) and run on a liquid chromatography mass spectrometer (LC/MS-MS) and converted to average consumption rate (mg fentanyl/1,000 people) for each site. Variable trends were evident between sites and temporally at each site ranging from 0 - 250 mg fentanyl/1,000 people. We then employed statistical modeling to discern whether these data were associated with current overdose deaths and could be used to predict future overdose deaths. Results from our study (n=47) suggests that a 25% increase in wastewater fentanyl levels predicted an increase in fatal fentanyl-related overdoses in the following week. Using SatScan software, five statistically significant areas of high overdose rates were discovered that were primarily located in census block groups with a median household income below $49k per year. The use of a spatial regression routine identified that median income level was associated with fentanyl-related overdose deaths. However, this relationship was non-stationary, meaning the influence varied by geographic location. These results suggest that wastewater-based surveillance can be used as an early warning tool to predict overdose deaths in a given region and that the use of spatial statistics identifies locations of interest that can be further targeted for public health interventions.

3219 Subchronic Systemic Alcohol Exposure Results in Ocular Toxicity

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A strong clinical association exists between acute and chronic alcohol consumption and incidence of ocular surface disease. The goal of this study was to investigate the toxicological and pathophysiological effects of alcohol consumption on the ocular surface. Mice in vivo (n=6L/6Rjir) were acclimated to ethanol (5% vol/vol) in the diet or remained on an isocaloric diet for 10 days. Corneal fluorescein staining was performed on day 10 as clinical readout for ocular surface damage and corneal tissue was collected and processed for histological analysis. Fluorescein staining was quantified, and tight junction integrity. This data provides the first preclinical evidence that dietary alcohol leads to signs of ocular toxicity manifesting clinically as ocular surface disease. In vitro, alcohol exposure caused activation of NF-κB-mediated oxidative stress pathways and disrupted epithelial barrier function.

3220 Network-Based Digital Phenotyping of Uterine Toxicology Screening in the Emergency Department

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Structured designs within the electronic health record (EHR) provide an incomplete picture of a patient’s presentation and clinical history. In addition, the reductionist nature of EHR-based data models lose important information that can lead to novel insights from the highly related and interconnected data that represent a patient’s health status. We developed a high-fidelity graph model to represent health status at the time of presentation to the emergency department that included diagnoses and symptoms extracted through natural language processing and joined these with urine drug screen results to create a network-based analysis of clinical toxidrome and comorbidities. These were further linked with the SNOMED ontology hierarchy to create a comprehensive knowledgebase of the clinical history for patients with and without a positive urine drug screen. We used retrospective data for all urine drug screening (UDS) and associated opiate-related confirmation testing by liquid chromatography and mass spectrometry (LCMS) performed at a single tertiary care, level I trauma center. Between January 2021 and November 2022, 34,724 urine specimens underwent UDS testing. Of those that screened positive and underwent confirmation testing (n=1522), 83% (n=1274) tested positive for one or more opioids. Taking into account all specimens by LCMS-based confirmation testing. Among the substances detected by LCMS, morphine (n=1048) and hydromorphone (n=507) were the most common substances detected. Multiple co-occurring substances were noted with the most common being morphine and hydromorphone (n=214), morphine and codeine (n=88), and morphine and hydromorphone (n=88). Knowledge graphs can be used to integrate data from diverse sources and create a more comprehensive model to represent a patient’s clinical condition and care trajectory. The work here demonstrates the validation of such a model by identifying known cooccurrences and possible interactions between clinical toxidromes. Furthermore, these models can also identify novel relationships and risk factors for presentation. Additional work is needed to develop standardized and generalizable models that can be implemented for computational approaches to rapid phenotype identification and risk prediction in the emergency department setting.

3221 Regulation of Hepatic Xenosensor Function by HNF4alpha

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Ligand-activated receptors, including Aryl hydrocarbon Receptor (Ahr), Constitutive Androstane Receptor (CAR), Pregnane X Receptor (PXR), and Peroxisome Proliferator-Activated Receptor-alpha (PPARα) function as sensors of xenobiotics in the liver. Hepatocyte nuclear factor 4alpha (HNF4α) is a highly conserved member of the liver receptor superfamily that is essential for liver function. Previous studies indicate a signaling crosstalk between HNF4α and these hepatic xenosensors. Here, we tested the hypothesis that the HNF4α function is essential for the activation of these major nuclear receptors. Wild-type (WT) and hepatocyte-specific HNF4α knockout (HNF4α-KO) mice were treated with either vehicle or mouse-specific activators of AHR (TCDD), CAR (WY-14643), PPARα (PCN), PXR target genes only in WT mice. It reduced proliferation in HNF4α-KO mice. It reduced proliferation in HNF4α-KO mice. TCD2 activated AHR in WT mice and HNF4α-KO mice, confirmed by increased transcriptional activation of its target genes. TCPOBOP (CAR agonist) significantly increased the LW/BW ratio in both WT and HNF4α-KO mice. Blood and liver were obtained 3-5 days after the injections and used to determine changes in liver weight to body weight ratio, liver injury, liver cell proliferation, and hepatic gene expression. TCD2 (AHR agonist) treatment did not affect the liver weight to body weight ratio (LW/BW) and serum ALT levels in the WT and HNF4α-KO mice. It reduced proliferation in HNF4α-KO mice. TCD2 activated AHR in WT mice and HNF4α-KO mice, confirmed by increased transcriptional activation of its target genes. TCPOBOP (CAR agonist) significantly increased the BW/LW ratio, serum ALT, proliferation, and mRNA expression of CAR target genes in WT mice, but not in HNF4α-KO mice. WY-14643 (PPARα agonist) significantly increased the LW/BW ratio in both WT and HNF4α-KO mice. PCN did not affect serum ALT levels in these mice. It increased proliferation only in HNF4α-KO mice. PCN significantly induced PXR target genes only in WT mice. The treatment of WT- YW-14643 (PPARα agonists) increased LW/BW ratio in HNF4α-KO mice. YW- 14643 did not change serum ALT levels significantly in either WT or HNF4α-KO mice. It upregulated PPARα target genes only in WT mice. Taken together, these data showed that activation of CAR, PXR, and PPARα but not that of AHR was disrupted in HNF4α-KO mice. These results demonstrate that HNF4α function is critical for the activation of toxicologically important hepatic xenosensors.

3222 Cinnabarinic Acid—Induced Aryl Hydrocarbon Receptor–Mediated Hepatoprotection against Nonalcoholic Fatty Liver Disease

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The Aryl hydrocarbon Receptor (AhR) is a ligand-activated-transcription factor known to regulate adaptive and toxic responses to a variety of chemical pollutants including the polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Upon activation by TCDD, the AhR translocates into the nucleus and binds to DNA at the Xenobiotic Response Element (XRE) in partnership with the Aryl hydrocarbon Receptor Nuclear Translocator (ARNT) to drive target gene expression, most notably Cyp1a1. Our recent studies identified stannocinacin 2 (St2c2) as a novel AHR target gene responsive to the endogenous AhR agonist cinnabarinic acid (CA). We further showed that CA-induced AHR-ST2C signaling pathway can protect hepatocytes against plethora of ER/oxidative stressors. In the current study, we examined the ability of CA to protect against palmitic and oleic acid-induced in vitro and obeseogenic diet-induced
in vivo models of non-alcoholic fatty liver disease (NAFLD). Our data demonstrates that CA treatment significantly attenuates hepatic steatosis, inflammation, liver injury and metabolic deterioration. CA regimen reduced hepatic lipid uptake and fatty acid synthesis by downregulating expression of genes involved in fatty acid uptake, and de novo lipogenesis. However, CA treatment failed to alleviate hepatic steatosis and triglyceride accumulation in hepatocyte specific AhR knock-out mice (AhR-floxed) but protected control (AhR-hKO) mice against obesogenic diet-induced weight gain and lipotoxicity. Additionally, the hepatocyte specific Stc2-knockout mouse showed exacerbated hepatosteatosis, hypertriglyceridermia and elevated profile of lipogenesis and inflammatory markers indicating plausible role of Stc2 in CA-mediated protection against NAFLD. In summary, this study describes a novel hepatoprotective role of AhR in response to CA treatment via activation of STC2 signaling. Finally, future characterization of AhR-STC2 pathway will be critical to highlight CA as a potential pre-clinical therapeutic against NAFLD. This work is supported by National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK122028 to AD-J).

The Regulation of Bile Acid Transporters by Dopamine Receptor D1 (DRD1) in Human Hepatoma Cells

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Bile salt export pump (BSEP) and Sodium-taurocholate co-transporting polypeptide (NTCP) are the primary bile acid efflux and uptake transporters, respectively, in the liver. Dopamine receptors, including DRD1 and DRD2 subfamily, play essential roles in many neurological processes as well as cell differentiation, growth, and metabolism. Functional differences unknown to date between these pathways regulate bile acid metabolism and homeostasis. In the present study, we determined the regulation of NTCP and BSEP expression by DRD1 in cultured human hepatoma cells. Our results showed that activation of DRD1 by SKF 81297 increased mRNA expression of BSEP, but not NTCP in both Hep3B and HepG2 human hepatoma cells. In contrast, inhibition of DRD1 by SCH 23390 decreased constitutive mRNA expression of BSEP and NTCP in HepG2 cells. In conclusion, the activity of DRD1 is required for the constitutive expression of both NTCP and BSEP and may contribute to the inductive expression of BSEP in human hepatoma cells.

Mechanisms Underlying Emergence of a Sub-G1 Population of Cells in a Mutant Xenopus laevis Line Lacking AHR1alpha


The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates toxicity of dioxin-like compounds. In the absence of xenobiotics, AhR also plays roles in development and physiology, including promoting cell cycle progression through the G1/S checkpoint. Unlike humans and rodents, the frog Xenopus laevis expresses 2 paralogous AhR proteins, AhR1α and AhR1β, the result of a genome duplication ~17-18 mya. We recently generated mutant lines lacking either AhR1α or AhR1β. Growth of each strain was measured using colony formation assays and the water-soluble tetrazolium (WST-8) method, each demonstrating that the XLK-WG cell line lacking a functional version of either AHR1α or AHR1β. Further, a discrete sub-G1 population (~20%) was unique to the ahrlα-/-. Cells in the sub-G1 population undergo pyroptosis, a distinct form of programmed cell death that resembles apoptosis in the breakdown of nuclear membrane and plasma membrane. Our results are nonetheless consistent with a unique mechanism, as yet undetermined. Our results are nonetheless consistent with a unique mechanism, as yet undetermined. This study contributes to the understanding of the distinct functions of AhR paralogs and how their individual activities and expression patterns affect interpretation of data from non-mammalian toxicological models. Grant support from NIEHS R05 ES011130 and NSF MRI 1725426.

Histopathological Characterization of Lung Injury Caused by Activation of the Aryl Hydrocarbon Receptor


Ligand-dependent activation of the aryl hydrocarbon receptor (AhR) can result in a variety of toxicities especially in the lung, liver, and immune system. AFPP464, a known AhR agonist and aminoflavone produg, induces liver injury in dogs and humans, but not in rodents. However, the pulmonary toxicities of AFPP464 are poorly characterized and clarifying the mechanism of species-specific differences in pulmonary toxicities is important. To do this, we verified AFPP464 toxicities in mice, a dog, and in human precision-cut lung slices (PCLS). To female C57BL/6J mice, AFPP464 (0.175, 35, 70 mg/kg) was administered intravenously once a day for 5 days. To a female beagle dog (34 months old), AFPP464 (11.4 mg/kg) was infused once a week for 2 weeks. Evaluations included, body weight, food intake, blood count, blood biochemistry, histopathological examination, and immuno-histochemical evaluation for yh2AX as a marker of DNA damage. Furthermore, human PCLS were cultured over 7 days in the presence of AFPP464 (0.1-10 μM), with measurements including cell viability, cytokine levels, and histopathological examination. In the mouse high-dose group, weight loss, liver toxicity and a decrease in the total number of white blood cells was observed, but lung abnormalities were absent. However, the dog exhibited shallow rapid breathing 4 days after initial administration. Due to deterioration in the dog's clinical condition, it was then sacrificed. Necropsy revealed pulmonary edema with regression failure of the lung lobe. Histopathological experiments presented pulmonary edema, eosinophilic material deposition on the interstitium, and regenerative hyperplasia of the bronchial and alveolar epithelium. The human PCLS assays revealed an AFPP464-induced dose-dependent cytotoxicity with an increase in TNFα and IL-1β secretion. AFPP464 is known to induce ROS generation. Therefore, pulmonary injury is likely to have occurred a few days after AFPP464 infusion due to ROS-mediated DNA damage via AhR activation. However, immunohistochemistry did not detect yH2AX-positive cells in the affected lungs. Our results suggest that lung injury may not be involved in AFPP464-induced lung injury. Further study is necessary to clarify the mechanism and species-specific differences in lung toxicity caused by tumor therapeutics.

Evaluation of Newly Developed 14 Human TK6–Derived Cell Lines That Individually Express a Human Cytochrome P450 for Toxicity Studies


In vitro genotoxicity testing has played a key role in identifying DNA-reactive chemicals for hazard identification. However, most mammalian cells used for in vitro genotoxicity testing lack effective metabolizing enzymes. We recently developed a battery of TK6-derived cell lines that individually express one of fourteen cytochrome P450s (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7). The increased gene and protein expressions of each CYP in the corresponding cell line were confirmed by using real-time PCR and Western blotting, while the parental TK6 cells and empty vector (EV) transduced cells had negligible CYP levels. In addition, the metabolic function of CYPs in the stably transduced TK6 cells were determined by mass spectrometry analysis using their corresponding substrate. To evaluate these cell lines for toxicity studies, we first selected two prototypical polycyclic aromatic hydrocarbon mutagens, 7,12-dimethylnaphthalene (DMNTa) and benzo(a)pyrene (B[a]P), that require metabolic activation to exert their genotoxicity. When compared to EV controls, DMBA-induced dose-dependent cytotoxicity, phosphorylation of histone H2A.X, and micronucleus (MN) formation were significantly increased in TK6 cells with CYP1A1, 1A2, 1B1, 2B6, and 2C9 expression, while B[a]P increased cytotoxicity, DNA damage, and chromosomal damage in TK6 cells expressing CYP1A1 and 1B1. Secondly, we tested three pyrazolinedione alkaldoids (PAs) - lascapine (the heliotrine-type of PA), riddeline (the retronecine-type of PA), and senkirkine (the otoinecine-type of PA) - to identify which CYP is responsible for their bioactivating and toxicity. Among the fourteen cell lines, cells expressing CYP3A4 showed significant increases in PA-induced cytotoxicity evidenced by decreased ATP production and cell viability, and increased caspase 3/7 activities. LC-MS/MS analysis revealed the formation of 1-hydroxymethyl-7-hydroxy-6,7-dihydropyrazoline (DHP), the main reactive metabolite of PAs, in CYP3A4-expressing TK6 cells. Three PAs induced concentration-dependent increases in 1%MN in three CYP3A variant-expressing TK6 cell lines (CYP3A4, 3A5, and 3A7). These results indicate that our TK6 cell system holds promise for toxicity screening of compounds requiring metabolic activation, identifying specific CYPs involved in bioactivation, and discriminating the genotoxic compounds that have different chemical structures.
Aflatoxin B1 (AFB1) is the most toxic, mutagenic, and carcinogenic aflatoxin that causes hepatocellular carcinoma (HCC), the fourth most common cause of cancer death in the world. AFB1 induces hepatocarcinogenesis through a genotoxic mechanism including metabolic activation to an epoxide, formation of the AFB1-DNA adducts, and mutagenesis of genes associated with HCC. The AFB1-DNA adducts are removed mainly by nucleotide excision repair. The knowledge of AFB1-DNA adducts formation and repair is basic to understanding the mechanisms of mutagenesis and carcinogenesis in the development of HCC. Although many DNA damage and repair factors have been identified, an open question to be answered is how these factors regulate and/or modulate the DNA damage formation and repair events. Identifying the precise locations where the dynamic DNA damage formation and repair events occur in the genome can provide insights into the complex relationships between carcinogen susceptibility, DNA repair efficiency, chromatin structure, spatial genome organization and mutagenesis. Recently, we developed a translesion eCKiCision Repair-seqencing (XRR-seq) method by using an appropriate translesion DNA synthesis polymerase and generated a genome-wide repair map of the cigarette carcinogen benz(a)pyrene. To better understand the hepatocarcinogenesis caused by AFB1, we adapted the XRR-seq method to analyze genome-wide nucleotide excision repair of AFB1-induced DNA damage at the level of 3D genome structure. We generated a single-nucleotide resolution repair map of AFB1-DNA adducts in the human genome and identified the unique dual incision pattern for AFB1-induced DNA damage. We examined the role of 3D genome structure in the repair of AFB1-induced DNA damage and found heterogeneous repair at different genome organization levels. The results will facilitate our understanding of aflatoxin-induced DNA damage and repair, mutagenesis, and carcinogenesis in human liver cancer.

Nitrosamine impurities have been identified in various drugs, leading to massive drug recalls. Although nitrosamines are considered a ‘cohort of concern’ because of their potential human health risks, most of this concern is based on rodent carcinogenicity and the Ames test data. Little is known on their genotoxicity in human-based systems. As such, we used human lymphoblastoid TK6 cells transduced with human cytochrome P450s (CYPs) to evaluate the genotoxicity of six nitrosamines (N-nitrosodimethylamine (NDEA), N-ethyl-N-nitrosourea (ENU), N-nitrosodiethylamine (NDEA), N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutanoic acid (NMBA), 2-propanamine (NEIPA), N-nitroso-N-methyl-4-aminobutanoic acid (NMBA), N-nitrosomethylphenylamine (NMPA), N-nitrosodimethylamine (NDIPA), and N-nitrosodibutylamine (NDBA)) that have been found or suspected to present in drug products. None of the nitrosamines induced any toxicity in wild-type TK6 cells without metabolic activation. On the other hand, 24-h treatments with NDEA, NEIPA, NMBA, and NDBA caused concentration-dependent increases in the phosphorylation of γH2AX in CYP2A6-expressing TK6 cells. NMPA is the most potent nitrosamine investigated, inducing a statistically significant increase in γH2AX at concentrations as low as 0.625 μM. Bioactivation of these four nitrosamines by CYP2A6 also caused increases in microneuclear frequency as well as G2/M phase cell cycle arrest. In addition, nuclear PCR product was upregulated in CYP2A6-expressing TK6 cells exposed to NDEA, NEIPA, and NMPA. Overall, the genotoxic potency of six nitrosamine impurities in our test system was NMPA > NDEA = NEIPA > NMBA > NDBA = NDIPA, although the genotoxicity of NDBA should be carefully re-evaluated due to its poor metabolism. We provided novel information on the genotoxicity of nitrosamines in human cells, complementing test results generated from bacteria mutation assays and partially addressing the issue of the relevance of nitrosamine impurities in our test system. Find up-to-date information at www.toxicology.org/2023.

Black cohosh extract (BCE) is one of the most popular botanical products for relieving menopausal symptoms. However, recent studies indicate that BCE is not only ineffective for menopausal therapy but also induces genotoxicity through an aneugenic mode of action (MoA). In this study, the cytotoxicity of five constituents of BCE was evaluated in human lymphoblastoid TK6 cells. Among the five constituents, actein (up to 50 μM) showed the highest cytotoxicity and was thus selected for further genotoxicity evaluations. Actein caused DNA damage proportionally to concentration as evidenced by the phosphorylation of the histone protein H2A.X (γH2AX) and resulted in chromosomal damage as measured by the increased percentage of micronuclei (MN%) in cells. In addition, actein activated DNA damage response (DDR) pathway through induction of p-ATM, p-Chk1, and p-Chk2, which subsequently induced cell cycle changes and apoptosis. Moreover, both BCE and actein increased intracellular reactive oxygen species (ROS) production, decreased glutathione levels, and activated the mitogen-activated protein kinases (MAPK) signaling pathway. N-acetylcysteine, a ROS scavenger, attenuated BCE- and actein-induced genotoxicity, apoptosis, and DNA damage. These findings indicate that BCE- and actein-induced genotoxicity is mediated, at least partially, through oxidative stress. Taken together, our data show that actein is likely one of the major contributors to BCE-induced genotoxicity.

Phenelmin Enhances UVB in Cyclobutane Pyrimidine Dimer Induction and Mutagenesis

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It is well-established that lightly pigmented Caucasians are much more sensitive than darkly pigmented Africans to sunlight-related non-melanoma skin cancer and melanoma and that sunlight induces many more photodermatologists in the former than in the latter. These results indicate that sunlight and the types and/or quantities of pigments play crucial roles in skin cancer and melanomagenesis. In this study, we used the immunochromatographical method with specific antibodies and T4 endonuclease V and UvrrABC nuclease incision method to determine the effect of two major human pigments: eumelanin (EUM) and phenelmin (PheM). UVB, the major sunlight UV spectrum that reaches the Earth’s surface, induced UV photodimer formation, the DNA damage effect of UVB irradiation. We found that UVB irradiation-induced cyclobutane pyrimidine dimer (CPD) induction but has no effect on <6-4>-pyrimidine-pyrimidone (6-4>PP) induction. In contrast, EUM reduces UVB-induced CPD and <6-4>PP induction. PheM also enhances UVB-induced mutagenesis, while EUM reduces UVB-induced mutagenesis. Mapping the CPD formation at the sequence level for the human B-RAF codon 600, the cutaneous melanoma mutation hotspot, we found that PheM enhances UVB induction of CPD formation at all pyrimidine-pyrimidione positions, with much more prominent effects on pyrimidine tracts, including the -TTTCA- sequence in the neighboring codon 600. These results indicate that PheM functions as a triplet sensitizer in enhancing UVB-induced CPD formation and reveal the possible underlying cause of the higher sensitivity of lightly pigmented Caucasians compared to darkly pigmented Africans in sunlight-related skin cancer.

A Feasibility Study to Evaluate Genotoxicity of Reference Combustible and Oral Tobacco Products Using In Vitro Regulatory and Mechanistic Genotoxicity Assays

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The use of in vitro mechanistic genotoxicity assays, following a positive response in standard regulatory in vitro genotoxicity assays, provides additional information on the mode of action of genotoxicity. In this feasibility study, we evaluated its potential application in toxicological assessment of tobacco products, including combustible reference cigarettes (3R4F and 1R6F) and reference oral tobacco products (LO x 200). The assay used yeast (Saccharomyces cerevisiae) for analyzing direct DNA-interacting mechanisms in response to CS, wherein both reference oral tobacco products were negative for genotoxicity. The 3R4F induced a 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the MultiFlow® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the ToxTracker® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the ToxTracker® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the ToxTracker® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the ToxTracker® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity. In the ToxTracker® assay, 3R4F induced 1.5 fold increase in the number of cells with micronuclei, suggesting presence of indirect mechanisms as well. Both reference oral tobacco products were negative for genotoxicity, while the reference oral tobacco products were negative for genotoxicity.
For more than forty years, anthracyclines doxorubicin and epirubicin have represented one of the most commonly used classes of drugs against metastatic breast cancer. Anthracyclines interact with DNA in a variety of complex manners. The major anthracycline anti-tumor function is thought to be accomplished by an inhibition of DNA Topoisomerase 2 activity. Studies in our lab have shown that anthracyclines are mutagenic and cytotoxic in bacteria (Salmonella typhimurium and Escherichia coli), and can induce intrachromosomal recombination events in the yeast Saccharomyces cerevisiae. The objective of this study is to accurately access the role of DNA type 2 Topoisomerases in the presence of anthracycline compounds. Wild type as well as gyrA, gyrB, parC, and parE mutant cells were exposed to doxorubicin or epirubicin. Cell viabilities were determined for all strains. Experiments indicate that anthracyclines have an effect on cell viabilities for parC alleles compared to wild type, and these results will be discussed regarding the role of bacterial topoisomerases in the presence of these anticancer drugs.

3232 Cytoxicity of Anthracyclines, DNA Topoisomerase 2 Inhibitors, in Escherichia coli, Salmonella typhimurium, and Saccharomyces cerevisiae

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3233 Mechanisms of Nitrosamine Mutagenicity and Their Relationship to Carcinogenicity


N-Nitrosamines are a class of mutagenic carcinogens considered to be cohort of concern chemicals because they are highly potent. The major mechanism of N-nitrosamine mutagenicity is related to the structural feature where an aliphatic alpha-CH-functionality next to the N-nitroso group leads to alpha-hydroxylation ultimately forming a highly reactive diazonium species which can then alkylate DNA. We set out to conduct a literature review to understand how other mechanisms of nitrosamine mutagenicity contribute/relate to the carcinogenic potency of N-nitrosamines. This involved searching for in vitro and in vivo mechanisms relating potency to chemical structure described in the literature as well as retrieving available references for all mutagenicity and carcinogenicity experimental data described in the CPDB, Leadscope, and Vitac (Lhasa Ltd) databases. From these literature reviews a number of mechanisms related to N-nitrosamine mutagenicity and carcinogenicity were identified. Brief summaries of each of these mechanisms of mutagenicity and how they relate to carcinogenicity potency are provided.

3234 Automated Analysis of Human Lymphocytes in the In Vitro Micronucleus Assay

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The purpose of this experiment was to evaluate the automated analysis of micronuclei in cultivated human peripheral blood lymphocytes (HPBLs) using the Amnis® ImageStream®X MKI imaging flow cytometer with a 96-well plate auto-sampler. Although micronuclei analysis is routinely automated by flow cytometry using various cell lines, HPBLs are still typically analyzed by the time-consuming method of manual microscopic examination of prepared slides. In addition, standard flow cytometry does not allow for analysis of binucleated cells, but rather provides an evaluation of free nuclei and micronuclei in suspension. The ImageStream® is an imaging flow cytometer that captures high resolution images of individual cells during acquisition. It then utilizes A.I. software to analyze the images to identify and quantify micronuclei. The Bscl2-GFP is a reporter program as a high-throughput, resource-efficient predictive model for identifying compounds with potential genotoxic hazard. The Bsc12-GFP is a reporter for pro-mutagenic DNA lesions and DNA replication stress while the Rtkn-GFP marker is a reporter for DNA double strand breaks. Extensive validation work has shown that activation of Bsc12-GFP and Rtkn-GFP biomarkers well correlate with a positive Ames test, respectively. In this study, correlation between Rtkn-GFP biomarker and in vitro micronucleus test using human peripheral blood lymphocytes (HPBL) was evaluated using five agrochemical compounds. The ToxTracker assay was conducted following the standard procedures (with or without phenobarbital/5-6 benzoflavone (PB/BNF)-induced rat liver S9 for a duration of 24-hours). The HPBL in vitro micronucleus testing was conducted with a short term (4-hour) PB/BNF S9-metabolically activated treatment and a continuous non-activated treatment for a duration of 24-hours. The ToxTracker assay has been specifically designed and validated with lower S9 concentrations (0.4% v/v) allowing for a continuous treatment, whereas in the HPBL has been specifically designed and validated with lower S9 concentrations (0.4% v/v) allowing for a continuous treatment, whereas in the HPBL this is accomplished by an inhibition of DNA Topoisomerase 2 activity. Studies in our lab have shown that anthracyclines are mutagenic and cytotoxic in bacteria (Salmonella typhimurium and Escherichia coli), and can induce intrachromosomal recombination events in the yeast Saccharomyces cerevisiae. The objective of this study is to accurately access the role of DNA type 2 Topoisomerases in the presence of anthracycline compounds. Wild type as well as gyrA, gyrB, parC, and parE mutant cells were exposed to doxorubicin or epirubicin. Cell viabilities were determined for all strains. Experiments indicate that anthracyclines have an effect on cell viabilities for parC alleles compared to wild type, and these results will be discussed regarding the role of bacterial topoisomerases in the presence of these anticancer drugs.

3235 ToxTracker and In Vitro Micronucleus: A Comparative Assessment

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Genotoxic studies are performed to support regulatory decision-making related to chemical safety and human health risk assessment. A minimum base set of genotoxic studies such as Ames test, in vitro micronucleus test, mammalian gene mutation assay (typically a mouse lymphoma assay or HPRT) and an in vivo micronucleus test are standard requirements for global acceptance. Such studies are expensive, time consuming, and use a combination in vitro and in vivo models to generate a cytotoxicity profile. Early detection of genotoxic chemicals can save time and may promote the development of sustainable compounds. The ToxTracker assay, developed by Toxys, provides a method for evaluation of the genotoxic properties of chemicals. Using six GFP-labeled mouse embryonic reporter cells lines, the ToxTracker assay can provide an assessment of direct and indirect genotoxic effects and provide insight into the genotoxic mode of action of a chemical. A modified screening version of ToxTracker based on two of the reporter cell lines, Bsc12 and Rtkn, has been implemented in early discovery programs as a high-throughput, resource-efficient predictive model for identifying compounds with potential genotoxic hazard. The Bsc12-GFP is a reporter for pro-mutagenic DNA lesions and DNA replication stress while the Rtkn-GFP marker is a reporter for DNA double strand breaks. Extensive validation work has shown that activation of Bsc12-GFP and Rtkn-GFP biomarkers well correlate with a positive Ames test, respectively. In this study, correlation between Rtkn-GFP biomarker and in vitro micronucleus test using human peripheral blood lymphocytes (HPBL) was evaluated using five agrochemical compounds. The ToxTracker assay was conducted following the standard procedures (with or without phenobarbital/5-6 benzoflavone (PB/BNF)-induced rat liver S9 for a duration of 24-hours). The HPBL in vitro micronucleus testing was conducted with a short term (4-hour) PB/BNF S9-metabolically activated treatment and a continuous non-activated treatment for a duration of 24-hours. The ToxTracker assay has been specifically designed and validated with lower S9 concentrations (0.4% v/v) allowing for a continuous treatment, whereas in the HPBL this is accomplished by an inhibition of DNA Topoisomerase 2 activity. Studies in our lab have shown that anthracyclines are mutagenic and cytotoxic in bacteria (Salmonella typhimurium and Escherichia coli), and can induce intrachromosomal recombination events in the yeast Saccharomyces cerevisiae. The objective of this study is to accurately access the role of DNA type 2 Topoisomerases in the presence of anthracycline compounds. Wild type as well as gyrA, gyrB, parC, and parE mutant cells were exposed to doxorubicin or epirubicin. Cell viabilities were determined for all strains. Experiments indicate that anthracyclines have an effect on cell viabilities for parC alleles compared to wild type, and these results will be discussed regarding the role of bacterial topoisomerases in the presence of these anticancer drugs.

3236 Assessment of the Mutagenic Potential of a Protoporphyrogen-XO oxidase Inhibitor Herbicide

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Protoporphyrinogen-oxidase inhibitors (PPOi) is a class of herbicides, which acts on both chlorophyll as well as heme synthesis. Thus, a toxicological characteristic of PPOi herbicides is the induction of anemia in mammals to varying degrees. A
series of PPOi candidates showed, despite their clear negative in vitro mutagenic profile, enhanced micronucleus formation in rodent bone marrow. Since enhanced erythropoiesis is a cause of micronuclei formation, a series of investigations were performed in order to determine whether the micronuclei finding is attributed to a mutagenic effect or linked to the observed regenerative anemia. A PPOi candidate with the characteristics as described above was tested in a 14-day micronucleus assay using the ToxTracker assay, and the 488 assay. The ToxTracker assay contained six different GFP-tagged reporter cell lines that together allow the accurate identification of specific cellular signaling pathways upon chemical exposure. ToxTracker has demonstrated a successful laboratory transfer in both somatic and germ cell models.

3237 In Silico and In Vitro Mutagenicity Evaluation of Estradiol Hemihydrate Impurity D: An Impurity in Estradiol

In recent times, regulatory agencies have been requesting in silico mutagenicity evaluation of known impurities which have been listed in pharmacopoeias for which mutagenicity data is not available. As a part of this exercise, an assessment of genotoxic potential of all known impurities of Estradiol hemihydrate was performed. The European Pharmacopoeia (E.P) provides acceptance criteria of not more than 0.3% for four impurities viz Impurity A, B, C and D of impurities A, B and C were predicted to be non-mutagenic based on DEREK and SARAH Nexus silico tools. However, Impurity D also called delta 9, 11 - Estradiol contained a misclassified feature. Although the in silico analysis predicted the structure to be negative for bacteriological mutagenesis, the misclassified structure in the structure provided reason to doubt the prediction. This misclassified feature in the structure was a hexahydrophenanthrene moiety which is mutagenic in the Salmonella typhimurium strains TA98 and TA100 in presence of metabolic activation. Structurally similar substances related to the misclassified feature retrieved from the DEREK database were positive for mutagenicity. Hence, an in vitro mutagenicity of Estradiol Impurity D was investigated using the bacterial reverse mutation test, on four histidine deficient mutant tester strains of Salmonella typhimurium and tryptophan deficient tester strain of Escherichia coli WP2uvrA (pKM101). Bacterial cultures were exposed to Estradiol Impurity D at concentration levels ranging from 1.5 to 5000 µg/ml. No increase in the number of revertant colonies was observed, in the absence and presence of the metabolic activation system in all tester strains. Based on the initial test outcome, a confirmatory test was performed at Estradiol Impurity D concentration levels ranging from 156.25 to 5000 µg/ml in the absence and presence of the metabolic activation. Estradiol Impurity D did not induce any significant increase in the number of revertant colonies, in trials with and without S9 mix, in any tester strain up to the highest tested dose of 5000 µg/ml. These results are in line with the negative prediction by the in silico quantitative structure-activity relationship (QSAR) tools for the Estradiol Impurity D and provide good correlation of in silico and in vitro data. This study data further supports the safety of estradiol drug formulations.

3238 Update on the Ongoing OECD Validation of the ToxTracker Assay for Genotoxic Mode-of-Action Assessment
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ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific signaling pathways upon chemical exposure. ToxTracker contains six different GFP-tagged reporter cell lines that together allow the accurate identification of genotoxic substances and discrimination between induction of DNA damage, oxidative stress and/or protein damage in a single test. More recently, the assay was extended to allow the discrimination between clastogenic and aneugenic activities. The ToxTracker is used at Gentronix in a large international inter-laboratory validation study, approved by the OECD. The goal of this prospective validation study is to explore the applicability of ToxTracker for regulatory applications, establish the transferability and reproducibility of the assay and to explore how it can be applied to improve the in vivo genotoxicity testing strategies. The validation has been conducted strictly following OECD guidance document 334. ToxTracker was transferred to seven laboratories. The validation labs were trained to perform the assay and tested a training set of compounds to show their proficiency to run ToxTracker. Next, the labs evaluated a selection of 64 coded, well-established genotoxic and non-genotoxic chemicals with each compound being tested in three labs independently. All the experimental work has been completed and data has been analyzed. The accuracy to predict genotoxicity, as well as the intra- and inter-laboratory reproducibility were determined. In this poster, we will give an overview how the ToxTracker validation was performed and the first results from this OECD validation.

3239 Laboratory Transfer and Proficiency Establishment of the OECD 488 Transgenic Rodent Somatic and Germ Cell Mutation Assay

Assessment of new drugs and chemicals for their mutagenic potential and risk to human health and the environment is a global regulatory requirement. OECD 488 transgenic rodent (TGR) mutation assays play a key role in determining the in vivo mutagenicity risk of substances that have positive in vitro mutagenicity data. The Big Blue® rat is listed as a suitable TGR model that allows investigation of in vivo mutation at the cII locus contained within the genome integrated lambda bacteriophage shuttle vector. Gentronix has conducted a laboratory transfer and proficiency assessment for detection of mutation frequency at the cII locus in duodenum, glandular stomach, liver, bone marrow, kidney and male seminiferous tubules of Big Blue® rats, with data compared with that of the previous laboratory. The exercise used frozen tissues banked during previously conducted Big Blue® rat studies, in which a 28-day exposure period was followed by either 3- or 28-day (somatic tissues) or 28 day (germ cells) fixation period for untreated/vehicle and N-Ethyl-N-nitrosourea (ENU) positive control treated animals. Genomic DNA was extracted from all tissues using the Agilent DNA RecoveryEase methodology, and then packaged using Agilent Trasnpack reagents to create infectious phage particles that were then expressed using the G1250 E. coli strain, plated on agar. The plates were incubated at 2 temperatures: 37°C, where both wildtype and mutated phage enter the lysogenic cycle (packaging efficiency; 24%), whereas all cII mutated phages can enter the lytic cycle and the mutant frequency (MF) was determined from the resulting phage plaques scored. Packaging efficiency was demonstrated across all tissue types with the mean number of phage screened from each DNA sample achieving >200,000 phage titre in both untreated/vehicle and ENU treated animals, in accordance with OECD 488 (2022). The ENU treatment produced a statistically significant increase in mutant frequencies over control for all tissues tested, with mean mutation frequency fold increases of: 18, glandular stomach; 16, duodenum; 7, liver; 16, bone marrow; 8, kidney, 8, male germ cells. Absolute mutant frequencies for all tissues in the untreated/vehicle control groups were consistent with the 95% control limits of historical data generated in this assay. Together these findings demonstrated the method was successful and the TGR assay is an effective and reliable in vivo genotoxicity test for the Big Blue® rat model. These data illustrate that Gentronix is proficient in the methods of transgene recovery from genomic DNA and in reproducing expected mutant frequencies for positive and negative controls in the Big Blue® TGR assay across both somatic and germ cell tissues.

3240 In Vitro and In Vivo Micronucleus Assessment of Trifluorooiodomethane (CF3I) following 28-Days Inhalation Exposure
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Trifluorooiodomethane (CF3I) is a colorless and odorless gas used primarily as a fire suppressant. In the EU, it has been classified - in accordance with the criteria under the EU Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures - as mutagen category 2 based on an outdated finding that a 28-days inhalation exposure in vivo finding following OECD 488 transgenic rodent (TGR) mutation assay was inconclusive in vivo findings with both somatic and germ cell tissues.

In an effort to replicate previously in vivo findings, a 28-days inhalation exposure to CF3I followed by micronucleus assessment was performed with Wistar rats following OECD guidelines 412 and 474. CF3I was administered as a gas to rats at target aerosol concentration of 2, 4, or 8% by nose-only inhalation for 4-weeks (5 days/week, 6 hours/day for 2 or 4%; 2 hours/day for 8%). There was no statistically significant increase in MNC observed in the group mean %MPCE (micronucleated polychromatic erythrocytes) of male or female rats compared with control. There was no statistically significant decrease in accuracy to predict genotoxicity, as well as the intra- and inter-laboratory reproducibility were determined. In this poster, we will give an overview how the ToxTracker validation was performed and the first results from this OECD validation.
3241 Naphthalene-DNA Adduct Formation Is Attenuated in Blood and Lungs of Cyp2bfgs-Null Mice Compared to Wild-Type Mice

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Naphthalene (NA), a persistent environmental contaminant, is a possible human carcinogen. The exact mechanisms of lung tumor formation in NA-exposed animals are still unknown. NA and its metabolite 1,2-naphthoquinone (1,2-NQ) form stable and/or depurinating adducts with DNA ex vivo in rodent and primate airway epithelants, and in mouse skin following topical exposure. The tissue origination of the adducts formed in vivo has yet to be elucidated. The ability of 1,2-NQ to form depurinating DNA adducts in vitro have previously been characterized by mass spectrometry. The goal of this study is to determine the involvement of P450-mediated NA bioactivation in DNA adduct formation. DNA adduct formation in the lung (the target tissue) and blood (an accessible tissue for biomonitoring) was analyzed in wild-type (WT) and Cyp2bfgs-null mice, the latter having reduced ability to activate NA. Initial studies were conducted to detect 1,2-NQ-DNA adducts in male and female mice injected intraperitoneally with NA at 200 or 300 mg/kg in corn oil. Depurinating adducts were isolated from whole blood and lung samples after solid-phase extraction (SPE). Stable adducts were isolated from DNA of white blood cell pellets and lung tissue, followed by SPE. Adducts were identified based on chromatographic retention time and mass spectral matching with synthetic standards and deuterated internal standards. A depurinating adduct, 1,2-dihydronaphthalene (DHN)-guanine, and a stable adduct, 1,2-DHN-deoxyguanosine, were detected in the blood and lungs of WT mice, but not Cyp2bfgs-null mice. Other NA-DNA adducts were identified. This result confirmed the involvement of the 1,2-NQ-DNA adducts formed via P450-mediated NA bioactivation. Further studies are underway to determine quantitative and qualitative relationships between adducts in the lung and blood as well as to gauge the feasibility of using blood NA-DNA adduct levels to predict adduct levels in lung. The outcome of this study will facilitate further investigations on the involvement of genotoxic mechanisms in NA-induced lung carcinogenesis and biomonitoring. Supported by NIEHS grants ES020867, ES007091, and ES034268.

3242 Micronucleated Reticulocytes and Pig-A Mutant Phenotype Erthyrocytes as Translational Biomarkers of Genotoxicity: A Case Study with Hydroxyurea

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Hydroxyurea is approved for treating children and adults with sickle cell anemia (SCA). Despite its proven efficacy, concerns remain about its mutagenic and carcinogenic potential that hamper its widespread use. This study assessed the utility of translating genotoxicity biomarkers from established preclinical models to humans. Crt/Cd(SD) rats were exposed to hydroxyurea via oral gavage for 28 consecutive days up to the maximum tolerated dose (125 mg/kg/day). Two peripheral blood-based endpoints of genotoxicity were studied at 2 or more time points: micronucleated reticulocytes (MN-RET) and Pig-a mutant phenotype reticulocytes and mutant erythrocytes (MUT RET and MUT RBC) as indicators of gene mutation. We performed proof-of-concept translational studies of these endpoints in testicular cancer patients undergoing standard-of-care chemotherapeutic treatments and demonstrated clear responses in both assays. This work was then extended to pediatric patients receiving hydroxyurea for treatment of SCA. One study used a cross-sectional design that compared 26 SCA patients receiving hydroxyurea to 13 SCA patients without exposure. A prospective study was also conducted with 10 SCA patients that compared pre-treatment blood samples with those obtained after 6 or 12 months of therapy. In the rat model, hydroxyurea induced MN-RET, but not MUT RET or MUT RBC: In SCA patients, hydroxyurea induced MN-RET in both the cross-sectional and prospective studies. However, no evidence of Pig-A gene mutation was found in hydroxyurea-treated children, despite the fact that the two assays use the same rapidly-drying, highly-exposed cell type. These results reinforce the complementary nature of MN-RET and Pig-A mutant erythrocytes and demonstrate the translational value of the biomarkers. The results also show that hydroxyurea was clastogenic but not mutagenic in young patients with SCA.

3243 Optimization of the In Vitro Alkaline Comet Assay with Liver Cells for Genotoxicity Testing of N-Nitrosamines


Potentially carcinogenic N-nitrosamines (NASs) are currently of high concern for pharmaceuticals, as NA impurities derived from active pharmaceutical ingredients have been found in certain products. One of the three Mutamind projects, funded by European Medicine Agency, aims at investigating the predictivity of the in vitro alkaline Comet assay (CA) for a carcinogenic outcome of NA exposure. In the project, three different liver cell models were used as test systems i.e., human hepatocellular carcinoma HepG2 cells and primary rat and human hepatocytes. As NASs need metabolic activation for DNA adducts formation and subsequent mutagenicity and carcinogenicity, the liver represents one major target organ for NASs. The objective of the present experiments was to optimize the standard alkaline CA protocol for genotoxicity screening in NASs and to exhibit some metabolic competence, were initially used as easy-to-handle human liver cell model. As a known carcinogen, N-nitrosodimethylamine (NDMA) at 0.1 - 20 mM served as NA and ethyl methanesulfonate (EMS) as direct clastogenic positive controls for optimization purposes. Cyclophosphamide monohydrate (CP) was, furthermore, used as metabolic activation control. HepG2 cells were pre- cultured for either 24 or 48 h and subsequently exposed to NDMA for 4, 6 or 24 h, with cytotoxicity determined by cell counting. A commercial 10-donor mixed batch of primary human hepatocytes (PHHs) served as normal, metabolic competent liver cell model. PHHs represent the gold standard for in vitro toxicity studies, due to its metabolic activity. For in vivo genotoxicity experiments, NA was treated 2 h after tailing and plating for 2 h with 0.1 - 10 mM NDMA, 0.5 µl/ml EMS or 1 mM CP. The used HepG2 batch was initially characterized regarding morphology, doubling time, and sensitivity towards NA-relevant solvents and standard positive controls i.e., EMS and CP. As HepG2 cells were not able to activate CP, S9-mix, an ex vivo metabolic system, was included in the optimized CA treatment protocol. As S9-mix, we used for HepG2 cells. However, experiments with 4 and 6 h of incubation with NDMA (1, 5, 10, and 20 mM) without S9-mix, demonstrated that HepG2 cells were able to directly activate NDMA in a concentration-dependent manner, with a comparable 3.1- (4 h) and 3.3-fold (6 h) increase in mean tail intensity (TI) at 20 mM NDMA, as compared to the respective negative controls. This effect was dependent on the pre-culture time (24 or 48 h), and both a longer incubation period of 24 h and the addition of S9-mix did not markedly alter the genotoxic effect of NDMA. At 20 mM NDMA, a slight NDMA-mediated decrease in cell count to 75% of the negative control was noted. In contrast, when incubating PHHs with NDMA, a significant, and clearly concentration-dependent increase in mean TI (1, 5, 10, and 20 mM) was evident for all concentrations used, starting already at 0.1 mM (2.4-fold increase) and reaching a 6.8-fold increase at 10 mM NDMA, whereas the increase amounted to only 2.5-fold in HepG2. In conclusion, the alkaline CA was able to clearly detect genotoxicity of NDMA using both HepG2 cells (even without S9-mix) and PHHs. However, since HepG2 cells were not able to metabolically activate CP, their overall metabolic competence seems limited and is currently under investigation. Thus, when using HepG2 cells, NASs with unknown genotoxicity/carcinogenicity should be tested ≥ S9-mix to avoid false-negative results, whereas PHHs seem to represent a highly sensitive, but also cost-intensive, cell model for genotoxicity screening of at least small NASs.

3244 Elucidating the Mode of Action of Genotoxic Substances Using a Combination of GFP Reporter Genes and Duplex Sequencing

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The standard genotoxicity testing strategy typically investigates induction of gene mutations, chromosomal aberrations, and numerical chromosome changes. ToxTracker is an in vitro mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways to identify direct DNA damage induction as well as indirect genotoxicity caused by oxidative stress and protein damage. The assay provides insight into the genotoxic mode of action (MOA) and can discriminate between clastogenic and aneugenic compounds. ToxTracker was shown to predict in vivo genotoxicity of compounds with a >90% sensitivity and specificity. The TwinStrand Duplex Sequencing mutagenesis assay uses a highly accurate sequencing technique that tracks both strands of the sequenced DNA molecules to limit sequencing errors. The mutagenesis assay can detect and characterize mutations induced upon chemical exposure and is supported with an easy-to-use bioinformatics pipeline. To combine the MOA information and accurate detection of gene mutations, we applied the TwinStrand Duplex Sequencing mutagenesis assay in the ToxTracker reporter cells to further unravel the MOA of genotoxic substances and determine their mutagenic potential. Providing a mutual fingerprint of compounds the MOA of compounds helps to further explore the MOA of genotoxic substances, thereby improving the in vitro genotoxicity prediction. In a pilot study, we have tested the genotoxic substances N-ethyl-N-nitrosourea, benz[a]pyrene, and potassium bromate in ToxTracker and determined their mutational fingerprint using duplex sequencing.
The Reconstructed Skin Micronucleus (RSMN) assay is an OECD accepted method demonstrated to have 80% accuracy, 75% sensitivity, and 84% specificity in predicting in vivo genotoxicity outcomes [Pfuhler et al. 2021]. This assay has been recommended as an alternative to animal testing to follow-up on skin relevant compounds found to be positive in standard in vitro clastogenicity/aneugenicity assays. In the context of dermal exposure, human skin specific metabolism has been shown to be correctly reflected in RS models, especially when using extended exposures (e.g. 72h RSMN assay). However, there may be scenarios where substances penetrate the skin and undergo further metabolism in the liver. We therefore have evaluated the ability of rat liver S9 to complement the current RSMN assay. Two approaches were examined to introduce rat liver S9 into the skin model via the media: 1) a short exposure (4h) with a higher concentration of rat liver S9 followed by a recovery period and 2) a continuous exposure with a low concentration of S9. Due to the known cytotoxicity of rat liver S9, cell health was monitored in the RSMN assay via the binucleation (BN) rate. To enable this, cytochalasin B was added to inhibit cytoplasmic division resulting in binucleated cells that have completed one mitosis. Decreases in percent BN can be used as an indicator of cytotoxicity and furthermore, the evaluation of micronuclei (MN) focuses on these cells that have divided once during the duration of the assay. The low-dose continuous S9 treatment (ranging from 0.0625-0.25% S9) resulted in a clear decrease in the BN rate, indicating increased cytotoxicity even at the lowest concentration tested. In contrast, the 4h S9 + recovery approach (ranging from 0.5-2.0% S9) did not affect the %BN nor the background MN rate when compared to the without S9 control. The 4h S9 + recovery approach using 0.5% S9 was then further evaluated for its ability to detect clastophosphamide (CP). This compound is known to require metabolic activation to show a genotoxic effect and was previously shown to give a weak positive response in the standard RSMN assay [without S9] [Kidd et al. 2019]. Multiple concentrations of CP, up to 3.07mg/ml were tested and statistically significant and dose dependent increases in MN frequency were seen (S9 control=0.53% MN, 3.07mg/ml CP=4.04% MN). These are promising results indicating that rat liver S9 can be incorporated into the RSMN assay enhancing its ability to detect compounds that may require liver-cytochrome P450 metabolism. Further work is ongoing to confirm this and optimize the exposure scenario. In summary, we have shown that incorporation of 0.5% rat liver S9 for 4h into the RSMN assay can improve the efficiency with which CP is detected after metabolic activation. This protocol is hoped to complement the current RSMN protocol in scenarios where liver metabolism is expected to be important. Pfuhler S, et al.: Validation of the 3D reconstructed human skin micronucleus assay: an animal-free alternative for following-up positive results from standard in vitro genotoxicity assays. Mutagenesis. 2021 Apr 28;36(1):1-17. doi: 10.1093/mutage/gea035. PMID: 33544138; PMCID: PMC8081377. Kidd D, et al.: The 3D reconstructed skin micronucleus assay: considerations for optimal protocol design. Mutagenesis. 2021 Apr 28;36(1):37-49. doi: 10.1093/mutage/gez037. PMID: 31793640.

In Vitro Micronucleus Scoring by Flow Cytometry Using TK6, CHO-K1, and HepG2 Cell Lines: A Look into Specificity

This laboratory has developed flow cytometric methods for the evaluation of micronuclei (MN) formation in cell lines, i.e. In Vitro MicroFlow. A large proportion of this work has been conducted with TK6 cells, and it has become clear that in some chemical spaces assay specificity is low, presumably due to the cells propensity to apoptose. To address this issue, alternative cell lines were explored. TK6, hamster-derived hepG2 cells and human-derived erythroleukaemia (K562) cells are following agents: methyl methanesulfonate (MMS), carbendazim (Carb) and camptothecin (Camptothecin) as well-established genotoxic agents, and carboxyl cyanide chlorophenylhydrazone (CCCP), tunicamycin (Tun), thapsigargin (Thap) as nongenotoxic cytotoxicants. Cells were harvested after 1.5-2.0 cell doublings and processed for flow cytometric analysis. In addition to MN prevalence, cytotoxicity was measured as relative increased nuclei counts (RINc). All compounds demonstrated dose-related increases in MN in TK6 cells at cytotoxicity limits that were consistent with current OECD 487 test guidelines. Examination of the same compounds in both CHO-K1 and HepG2 cells revealed significant elevations in MN following exposure to MMS, Carb and Campt. Conversely, no change from baseline were evident with the nongenotoxic compounds CCCP, Tun or Thap, even at the highest acceptable concentrations based on RINc. These data suggest that improvements to assay sensitivity may achieved through the choice of certain cell lines. Future work will examine additional chemicals, other treatment schedules, and use of exogenous metabolic activation.

Oxidative stress is linked to cancer development by the oxidation of DNA which results in mutations. DNA oxidations are repaired through the base excision repair pathway. Polymerase beta (polβ) is the enzyme in charge of filling the gap once the oxidised base is removed. Mutations in polβ can increase the mutational load, genomic instability, and cancer development. A large fraction of Germline genetic variants (GV) has been associated with cancer and interestingly, most of them are variants of uncertain significance (VUS), because of their low frequency, the sample size required, and the reduced statistical power limits studies in humans. VUS can account for a significant proportion of cancer susceptibility factors by acting dominantly or through the summation of the effects of a multiple low-frequency GV. DNA repair GV are associated with cancer and therapy response. Current genomic approaches to assess mutations and cancer risk are limited to somatic mutations and GV are overlooked, however, these variants may have a key mechanistic role in the carcinogenesis process. We have identified BER GV, in key enzymes that induce a carcinogenic phenotype when expressed in non-transformed cells. Our objective is to know the role of the polβ P242R GV in the carcinogenesis process. P242R variant results in a slow polymerase that accumulates DNA gaps, therefore, we hypothesized that under a highly oxidative environment, DNA damage will accumulate in P242R expressing phenotype, inducing genomic instability and cellular transformation. By using immortalized non-transformed cells, we observed chromosomal aberrations (fusions and fragments) and anchorage-independent growth in the absence of exogenous DNA damage. Interestingly, no increment in mutation frequency was observed indicating that this variant does not lead to a mutator phenotype. By treating cells with menadione to enhance reactive oxygen species, an increase in H2AX, a DNA damage marker, was observed, likely as a result of the slow gap-filling activity of P242R, which was not recovered after 6 hours. Furthermore, we created a mouse model carrying the P242R GV by CRISPR-Cas9 technology and homozgyous mutant mice showed an increase in the micronuclei frequency in peripheral blood, a marker for genomic instability. Our results suggest that over time, cells harboring this variant would incur more double-strand breaks than cells without P242R, leading to deletions and insertions if repaired by non-homologous end joining, gene fusions, or other types of genomic instability, which in turn could lead to cancer. Environmental exposures and/or diagnostic procedures over a P242R-carrying individual’s lifetime could serve to enhance the rate or levels of genomic instability and perhaps decrease the latency of cancer.

Effects of Clock Disruption and UVB Exposure on Circadian Behavior in SKH-1 Mice
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Over a quarter of the American population participates in night shift work, which entails activity during regular sleeping hours (between midnight and 5:00 a.m.). Our circadian clock entrains to daily environmental cues - such as sunlight - to align itself with the day-night cycle of the earth. This entrainment is disrupted by commitment to the night-active schedule, which is a common feature of modern society. Night shift work has further been classified as a Group 2A Carcinogen since 2007 and has been correlated with increased incidence of skin cancer (the most diagnosed type of cancer in the United States). Solar Ultraviolet-B (UVB) radiation is a significant source of exogeneous DNA damage in the skin, and night shift workers are particularly susceptible to UVB exposure because of their disturbed circadian rhythmicity in a manner unaffected by UVB exposure. As both circadian rhythmicity and UVB exposure influence circadian and UVB sensitivity, a P242R-carrying individual’s lifetime could serve to enhance the rate or levels of genomic instability and perhaps decrease the latency of cancer.

P242R GV in the carcinogenesis process. P242R variant results in a slow polymerase that accumulates DNA gaps, therefore, we hypothesized that under a highly oxidative environment, DNA damage will accumulate in P242R expressing phenotype, inducing genomic instability and cellular transformation. By using immortalized non-transformed cells, we observed chromosomal aberrations (fusions and fragments) and anchorage-independent growth in the absence of exogenous DNA damage. Interestingly, no increment in mutation frequency was observed indicating that this variant does not lead to a mutator phenotype. By treating cells with menadione to enhance reactive oxygen species, an increase in H2AX, a DNA damage marker, was observed, likely as a result of the slow gap-filling activity of P242R, which was not recovered after 6 hours. Furthermore, we created a mouse model carrying the P242R GV by CRISPR-Cas9 technology and homozgyous mutant mice showed an increase in the micronuclei frequency in peripheral blood, a marker for genomic instability. Our results suggest that over time, cells harboring this variant would incur more double-strand breaks than cells without P242R, leading to deletions and insertions if repaired by non-homologous end joining, gene fusions, or other types of genomic instability, which in turn could lead to cancer. Environmental exposures and/or diagnostic procedures over a P242R-carrying individual’s lifetime could serve to enhance the rate or levels of genomic instability and perhaps decrease the latency of cancer.
results indicate that conditions simulating night shift work may have toxicological implications regarding increased cancer risk. Future work will determine how these circadian conditions influence the initiation and progression of skin carcinogenesis.

3249 Evaluation of Cyclophosphamide at Various Dose Levels to 8-, 10-, and 12-Week-Old Female Sprague Dawley and Wistar Hans Rats
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While 2 consecutive oral doses of cyclophosphamide at 10 mg/kg/day as a positive control for the in vivo micronucleus test evaluated by flow cytometry have shown to be appropriate for Sprague Dawley males, excessive reticulocyte cytotoxicity has been observed in female Sprague Dawley and in both male and female Wistar Han rats. Differences between each strain have been apparent, with Wistar Han females positively responsive with a higher threshold for results, resulting in scoring less than 4000 reticulocytes, which is the minimum requirement according to the OECD 474 guideline for obtaining an accurate incidence of micronucleated immature reticulocytes. In addition, due to the increased addition of this assay to repeat dose toxicity studies, a difference in sensitivity to cyclophosphamide based on age has been observed. Therefore, this study was designed to determine the appropriate dose level that results in being able to score more than 4000 reticulocytes and also having a substantial increase of % micronucleated reticulocytes. Five female Sprague Dawley and Wistar Han rats per group were orally administered cyclophosphamide for 2 consecutive days at dose levels of 6, 8, or 10 mg/kg/day to induce micronucleus in peripheral blood reticulocytes. Three different ages (8, 10, and 12 weeks) were tested for each strain. Peripheral blood was collected from each animal between 36 and 48 hours following the last dose administration and evaluated via flow cytometry. Administration of cyclophosphamide at 10 mg/kg/day resulted in a decrease of total reticulocytes and % reticulocytes across both strains at all ages when compared to lower dose levels. For both strains, % reticulocytes decreased with increasing dose level across all ages. Overall % micronucleated reticulocytes were greater at 8 and 10 mg/kg/day when compared to the 6 mg/kg/day dose level. Results of this study suggest that regardless of age and strain, cyclophosphamide dose levels of 6 and 8 mg/kg/day to rats result in an appreciable number of total reticulocytes and % reticulocytes while also showing a substantial increase of % micronucleated reticulocytes. While a dose level of 10 mg/kg/day may be acceptable in terms of % micronucleated reticulocytes, the probability of being unable to score at least 4000 reticulocytes is greatly increased across both strain, which could result in an invalid study.

3250 Usefulness of 3D HepG2 Liver Spheroid Model for Genotoxicity Assessment of Chemicals and Pharmaceuticals
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Genotoxicity testing is an essential part of hazard and safety evaluation of chemicals and pharmaceuticals. Although two-dimensional (2D) cell culture has been traditionally used in genotoxicity testing, it generally lacks the in vivo-like morphological and physiological characteristics and could lead to misleading and non-predictive information of an in vivo response. In the last decade, the three-dimensional (3D) cell culture models have tested as a novel preclinical test system for safety assessment. The objective of this study was to provide a comparative evaluation of the genotoxicity potential of reference compounds in both traditional 2D cell culture system and 3D HepG2 liver spheroid model. The in vitro micronucleus assay was performed as per OECD 487 (2016) testing guideline with two test systems. Aflatoxin B1, a human hepatocarcinogen that requires metabolic activation and Mitomycin C, a direct acting genotoxic compound were evaluated in this study. The aflatoxin B1 was performed in HepG2 cells. The HepG2 liver spheroid model was prepared using the hanging drop method and 8 to 10 spheroids/concentration were treated. Aflatoxin B1 and Mitomycin C were tested at concentrations of 0.00625 μg/mL to 0.1 μg/mL and 0.025 μg/mL to 0.4 μg/mL, respectively. Treatment was performed for 24h followed by a recovery period of an additional 24h in presence of cytochalasin B. Cytokinesis-blocked proliferation index (CBI) was used as the cytotoxicity marker and percent micronucleus (%MN) frequency was evaluated as genotoxicity endpoints. These endpoints are commonly evaluated in the in vitro micronucleus assay for regulatory submission. The CBI ranged from 1.22 to 1.52 for 2D cultures and 1.19 to 1.47 for 3D HepG2 liver spheroid models. Although a concentration dependent and statistically significant (p<0.01) induction in MN frequency was observed in both test systems, the magnitude of MN induction was much higher and robust with the 3D HepG2 liver spheroid model. For both test compounds, up to a 5-fold increase in %MN over vehicle control was observed in the 2D culture system whereas up to a 10-fold induction was observed in 3D HepG2 liver spheroid models. The magnitude in the CBI in the spheroid model compared to the 2D test system was likely due to the relatively decreased availability of nutrients to the inner cells of spheroids. Similarly, a 2-fold higher induction of %MN in spheroid model compared to the 2D test system indicates inter-cellular interaction in spheroid model provides better metabolic environment to the cells. In conclusion, the preliminary data indicates 3D HepG2 liver spheroid model is a sensitive, easy-to-use, and comparative test system for genotoxicity evaluation of chemicals and pharmaceuticals. A comprehensive validation test is being planned with additional set of reference compounds for routine use of 3D HepG2 liver spheroid model in genotoxicity testing.

3251 Mammalian Cell Genetic Toxicology Screening Assays for Drug Discovery and Lead Compound Development
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Knowledge regarding the potential of new chemicals, food additives, and pharmaceuticals to cause the human genome and cause mutations (heritable changes in the cellular genome) remains critical to public health. Genotoxicity induced mutations are regarded as key events in the induction of cancer, birth defects, and neurodevelopmental diseases. The National Toxicology Program (NTP) required by regulatory agencies worldwide to initiate clinical trials and for registration of crop protectants, flavorings, and food additives includes bacterial and in vitro mammalian cell tests to assess genotoxicity and mutagenicity, and in vivo tests for genotoxicity; bone marrow MN assay and the Comet assay in liver. The in vitro chromosomal aberration and micronucleus (MN) tests done in rodent cells are p53 deficient cell lines have a higher false positive rate than genotoxicity assessments in p53 proficient human cells such as TK6 cells. Secondly, the currently used cell lines used in genetic toxicology and in high-throughput testing programs are deficient in xenobiotic metabolizing Phase I and Phase II enzymes and have deletions of toxicity biomarker liver cytochrome P450 (CYP2B1) incorporated into the regulatory genetic toxicology test battery used to provide biotransformation/bioactivation of xenobiotics with cells devoid of endogenous metabolism does not include Phase II conjugation enzymes, and false positives due to CYP450 bioactivation without Phase II detoxication can cause needless animal testing. We have developed non-animal toxicology testing battery that are rapid medium throughput low cost genotoxicity assays that can be implemented in pharmaceutical and lead compound to prevent failure in the regulatory genetic toxicology test battery. Flow-based MN assay in TK6 cells can be combined with the CometChip® assay to assess DNA damage, and expression profiling using TGx QX biomarker, to predict genotoxicity in in “screening” mode. More recently we have focused on the use of metabolically competent HepaRG™ cells as a genetic toxicology New Approach Methodology as a testing platform to replace/reduce in vivo genetic toxicology testing. HepaRG™ retain many of the characteristics of primary human hepatocytes, including morphology, expression of key xenobiotic metabolizing enzymes, and nuclear receptors (CAR/PXR/Arh/PPAR) that are absent in HepG2 cells. In HepaRG™ cells, we have qualified the MN, CometChip® assays combined with the TgX DDI biomarker to determine genotoxicity outcomes in a human metabolically competent cell line that can replace in vivo genetic toxicology testing in p53 deficient rodent cells using drug candidates that are associated with a high false positive rate. HepaRG™ cells can measure the same genetic toxicity endpoints that are measured in vivo (MN and Comet assay) as required by regulatory agencies. The approach of overlapping genetic toxicology endpoints in human TK6 cells and in HepaRG™ cells is a modern 21st century human-relevant non-animal genotoxicity testing paradigm aimed at reducing false positives and replace/reduce reliance on rodents by genotoxicity directly human relevant cell lines.

3252 Assessment of DNA Damage–Induced by 10 Nitrosamine Impurities Using 2D and 3D HepaRG Models
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Nitrosamine impurities, which are potent genotoxic agents, have been detected in certain human drug products and some are classified as probable or possible human carcinogens such as N-nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), and N-Nitrosodiisopropylamine (NDIPA), N-Nitrosomethylphenylamine (NMMP), N-Nitrosodimethylamine (NDMA). Since most nitrosamines require metabolic activation to exert their carcinogenic properties, the genotoxicity of ten nitrosamines tested in 2D and 3D HepaRG models. DNA damage was determined using the high-throughput CometChip assay with concurrent cytotoxicity assessment by the ATP assay. Following a 24-hour treatment, NDMA, NDEA, and NDMA induced positive responses in both 2D and 3D models, while negative responses were observed for N-nitrosodimethylamine and N-nitrosodimethylamine with methyl ester in both 2D and 3D models. Overall, 3D HepaRG spheroids, which express higher levels of CYP450 enzymes compared to 2D cultures, showed a higher sensitivity (80%) than 2D cultured cells (30%) in detecting DNA damage responses of the ten-nitrosamines. From the quantitative analysis, 3D HepaRG models provide more conservative estimates for benchmark dose values of nitrosamine impurities.
Molnupiravir (MOV) has received Emergency Use Authorization by the U.S. FDA for the treatment of COVID-19, which is caused by SARS-CoV-2 infection. MOV is a produg of the ribonucleoside analog, N-hydroxycytidine (NHC). Upon phosphor- ylation, NHC incorporates into nascent viral RNA during replication triggering “catastrophic” mutation of the viral genome. In nonclinical safety assessments, MOV and NHC were positive (i.e., mutagenic) in the Ames assay, and multiple other in vivo studies conducted in bacteriophages, bacteria, fungi, and mammalian cells have reported that NHC can induce DNA mutations. In this study, we evaluated the DNA mutagenic potential of NHC in mouse lymphoma L5178Y cells by PacBio HiFi sequencing and Illumina clone sequencing. The former can directly identify mutations from cell populations, while the latter requires the generation of single- cell clones. Both systems are capable of detecting mutations on whole genomes. NHC treatment of mouse lymphoma cells for 5 days at concentrations comparable to those observed in the plasma of humans who received clinical doses of MOV resulted in dose-dependent increases in genome-wide mutation frequencies, which were mainly driven by accumulations of A:T-G:C substitutions. The magnitude of these increases and types of mutations were similar in both sequencing systems. Our results demonstrate that NHC can induce DNA mutations in mouse lymphoma cells. We speculate that NHC, like many other ribonucleoside analogs, may be converted to its deoxy-ribonucleotide form, which could lead to its DNA incorpora- tion and the induction of DNA mutations.

Next generation sequencing can be used for direct detecting and characterizing mutations in various in vitro and in vivo models. We used high-fidelity whole-genome NGS method (HiFi Sequencing) for identifying mutations in the kidney, spleen, and liver of CD-1 mice exposed by gavage to a single 40 mg/kg dose of N-ethyl-N-nitrosourea (ENU) at 12 weeks of age. The tissues were harvested for mutation analyses 4 weeks post-exposure. HiFi Sequencing employs sequencing of tissue genomic DNA using Illumina short-read methodology (for building an averaged genome of the sample) and sequencing of the same DNA sample using PacBio highly accurate circular consensus sequencing (CCS) methodology (for identifying unique mutations present in the tissue sample). Up to 7-fold increase in the frequency of genomic DNA mutation was observed in ENU-treated mice, the highest increase was in the spleen and the lowest was in the kidney. Most frequent mutations in ENU-treated mice were T>A and T>C. HiFi Sequencing may comple- ment reporter gene-based mutation detection assays for detecting mutagenic- ity in tissues where performing assays based on endogenous reporter genes is technically impossible (e.g., Pig-a assay cannot be performed in solid tissues) or in mōdel systems with reporter transgenes (e.g., bacterial lacI, cII, or lacZ transgenes) are not available. HiFi Sequencing provides information on the spectra of mutations. Such information may be useful for characterizing genotoxicity in nonclinical safety assessments.

Many gene editing is performed with a CRISPR-Cas9 system and user-defined guide RNAs (gRNAs). Base editors represent improvements from the original gene editing systems and are composed of a nuclease-deficient Cas9 (dCas9) fused to a DNA-modifying enzyme, such as cytosine deaminase. This combination can achieve specific cytosine-to-thymine (C→T) point mutations at desired target sites (i.e., on-target mutations) with less DNA damage than traditional CRISPR- Cas9 systems. Base editors, however, are not perfect and can also generate undesired off-target mutations throughout the genome that are very difficult to detect. We and others have shown that PacBio HiFi sequencing can detect genome-wide mutations resulting from chemical mutagenesis at mutation frequencies ×10⁹ mutations/base pair. In this study, we evaluated whether PacBio HiFi sequencing can detect the on- and off-target effects of an inducible cytidine deaminase-dCas9 base editor targeting the LacZ gene of E. coli. Overnight (16h) induction of the system resulted in on-target mutations ranging from ~7% to ~68%, depending on the gRNA employed, and no on-target mutations were detected in controls that lacked the base editor. Under the same conditions, we observed a 3-fold increase in the frequency of off-target mutations compared to controls, irrespective of gRNA employed. These off-target events were composed of C→T substitutions widely distributed throughout the genome. Our results demonstrate that PacBio HiFi sequencing can be used to evaluate the on- and off-target effects of base editors on whole genomes.

Lumilin, the most common presumpstive test for blood at a crime scene, has multiple issues, such as false positive results with chemical agents, no luminescence due to “active oxygen” cleaning agents on bloodstains, and inability to penetrate textile materials. A combination of indolizine squaraine dye and ionic liquid (IL), or Dye Enhanced Textile Emission for Crime Tracking (DETECT), has shown potential to address these issues. In the presence of serum albumin, DETECT increases in fluorescence 100 fold. To ensure that the test used experiments and can be used with DETECT, biocompatibility of DNA with IL and dye was assessed by examin- ing the DNA’s structure and the effects of choline glycolate 1:1 IL and S03SQ dye with circular dichroism (CD). The distinctive peaks present in the CD spectrum with only DNA were also present in the CD spectrum of DNA with IL and the dye, which suggests there is limited structural damage to the DNA. Based on the results, DETECT is a promising candidate for potentially replacing lumilin while maintain- ing the viability of the DNA. Furthermore, ionic liquids in DETECT have the potential to protect DNA in the presence of gene-altering chemicals, such as lumilin.

Pregnancy is a critical window of vulnerability for the fetal brain. Exposure to environmental pollutants including flame retardants (FRs) has long been associ- ated with adverse neurodevelopmental outcomes. While the placenta performs a variety of functions to protect fetal development, disruption of placental activity has shown that up through mid-gestation the placenta provides the sole source of serotonin (5-HT), derived from maternal tryptophan, for the fetal brain and is essential for fetal forebrain development. Firemaster 550 (FM 550), a prevalent FR mixture, consisting of organophosphate (OPFR) and brominated (BFR) flame retardants, is used in various products including foam-based furniture and infant products such as strollers, mattresses, and nursing pillows. Evidence of endocrine disruption and neurotoxicity has raised concerns about the effects of FM 550 on neurodevelopment. Our lab has previously shown in rats that FM 550 has sex-spe- cific adverse effects on placental tryptophan metabolism and, serotoninergic fetal brain innervation, suggesting placental disruption of fetal development. It is unclear, however, which components of the mixture are driving the effects, and rodent work, albeit valuable, is animal intensive and cannot fully replicate the human conditions. Thus, here, we used human-derived trophoblast stem cells (TSCs) to determine which components within FM 550 contribute to disrupted serotonin biosynthesis in the placenta. The use of TSCs allows for the investigation of first trimester placental function and development in a simplified in vitro model. TSCs were exposed to FM 550 components for 48 h. Cell viability was assessed with resazurin assay. TSCs were also assessed for the impact of exposure on the gene expression of serotonin transporters and enzymes responsible for the biosynthesis and metabolism of serotonin using RT-qPCR and 5-HT secretion using ELISA. FM 550 components were cytotoxic in the 1 mM-10 μM range. There were limited effects on gene expression in TSCs exposed to OPFRs while the studies involving BFRs remain ongoing. Follow-up studies will be conducted using agonist and antagonists of 5-HT synthesis to pharmacologically block or rescue the observed effects. These studies both provide a novel understanding of serotonin biosynthesis in the first-tri- mesteral placenta and the impact environmental exposure could have on placental neuroendocrine function.

The brain accounts for 20-25% of the total energy consumption. Glucose is taken up by facilitated diffusion via glucose transporters (GLUTs) at the blood-brain barrier which is mainly mediated via GLUT1 encoded by the SLC2A1 gene. From the blood capillaries, astrocytes take up glucose and metabolize it to lactate which is then transported to neurons. GLUT1 deficiency syndrome (G1D) is a neurodegenera- tive disorder which is characterized by the early onset of seizures, gait disorders, and intellectual disability. Although glucose metabolism has been mostly investi- gated from the neuronal perspective but the fate of glucose metabolism at the neurovascular unit (NVU) is less explored. The aim of this study is to develop and

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characterize G10-astrocytes and neurons by gene editing using induced pluripotent stem cells (iPSCs). GLUT1 deficient induced pluripotent stem cells (G10-iPSCs) were developed by genetically editing IPS (IMR90)c4, resulting in a truncation of GLUT1 by its 4th transmembrane domain. Two viable clones were generated: C7 and E8 clones. Such iPSCs were differentiated into astrocytes and neurons using established protocols available in the literature. Cell phenotyping, glucose uptake, lactate release and glucose metabolism were assessed in the system. GLUT1 deficient-iPSCs (GLUT1DS-iPSCs) were viable and showed no significant differences in terms of phenotype or growth, compared to unedited iPSCs. Differentiated GLUT1DS astrocytes showed reduced GLUT1 expression, both at mRNA and protein level to the control however, expression of other GLUT transporters (GLUT3 and GLUT4) remains unchanged. GLUT1DS astrocytes showed reduced glucose uptake, increased lactate production and impaired glucose energy metabolism. Differentiated GLUT1DS neurons showed similar expression of GLUT3 and GLUT4 glucose transporters and glucose uptake but reduced glycolysis compared to the control. Our data suggest that GLUT1DS astrocytes display reduced GLUT1 expression and glucose uptake, increased lactate production. Given the cellular heterogeneity within the cells, the GLUT1DS in vitro osteogenic differentiation model based on human embryonic stem cells (hESCs). These chemicals, including 4 reference chemicals, were selected from the ToxCast I library to establish reliable parameters that would allow us to statistically categorizing chemicals into four risk levels (none, low, moderate, and high). Next, the transcriptional response of EBs to these 34 chemicals were examined by RNA sequencing. Forty-two genes, validated by qPCR, were selected as biomarkers to separate teratogenic and nonteratogenic chemicals. From the screening results tested with recursive feature elimination, the Random Forest-based classification model showed an 81% prediction accuracy in chemical risk level categorization, forming the second part of the prediction system. The third part of the system is based on chemical-elicited structural alterations in EBs, captured by high-content fluorescence screening and known effects on cell proliferation, cell cycle arrest, and SOX17 for endoderm). A highly accurate (82%) prediction model based on ResNET deep learning technology was obtained through training with 32760 EB images. To further validate the prediction accuracy and prove the practical value of this screening system, the teratogenicity of an additional 20 chemicals with limited toxicity information was assessed by this platform, and the results were consistent (80%) with previous studies. For example, tretinoin, was classified as a ‘high’ risk teratogen in humans by two subdisciplinary prediction models and showed a 9.1% toxicity similar with Ettreinitate, a confirmed teratogen from the same vitamin A derivative family. In contrast, Sucrose was classified as ‘nonteratogenic’ by all three subordinary models and showed a 51.9% similarity with Ascorbic acid in affecting EB morphology. These results suggest that our screening platform shows great promise in its ability to identify human developmental toxicants and help understand their etiology.

**3259 In Vitro Stem Cell Models to Predict Adverse Effects of Chemicals on Bone Development**

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About 3% of all birth defects have been linked to the prenatal exposure of pregnant women to environmental toxicants, which is roughly 3,600 babies per year. Musculoskeletal defects, which include malformations in facial and other bones, represent a large portion of these birth defects. Since many chemicals in our environment are untested for their safety, human exposure risk assessment is direly needed. Current risk assessment assays that are extrapolated to human exposure consist of in vivo rodent model systems, in vitro embryotoxicity assays, and in silico prediction programs. Yet, these methods remain in need of a higher predictivity rate on humans. In addition, while the above-mentioned methods are considered standard for screenings, they remain labor- and cost-intensive and require sacrificing pregnant animals to recover embryos. With the advent of human pluripotent stem cell models and their application to derive bone cells, it has now become possible to leverage these cells for musculoskeletal risk assessment associated with environmental exposure. Here, I present results from screening 60 chemicals using the induced pluripotent stem cells (iPSCs) differentiation model on the skeleton using an in vitro osteogenic differentiation model based on human embryonic stem cells (hESCs). These chemicals, including 4 reference chemicals, were selected from the ToxCast library to establish reliable parameters that would allow us to statistically group toxicants based on their embryotoxicity. Cells were concomitantly exposed to the selected chemicals during differentiation to osteoblasts to obtain half-maximal inhibitory concentrations based on two endpoints: cytotoxicity (MTT assay) and matrix calcification. The latter was used to indirectly quantify the amount of mature, functional bone-forming osteoblasts from a calcium assay, normalized to the protein content of the cultures. All half-maximal inhibitory concentrations were then related to a half-maximal inhibitory concentration obtained from exposing fully differentiated fibroblasts. This was done using three Fisher functions developed previously. Using this biostatistical model, 23 chemicals were correctly categorized based on their known in vivo embryotoxicity. A canonical plot was also able to categorize all chemicals based on their known in vivo embryotoxicity with the exception of two. Together, these hESCs-based endpoints will provide a valuable assay for the formation of bone-forming osteoblasts, or lack thereof, depending on chemical exposure going forward. In contrast, when the screen was repeated using mouse embryonic stem cells, nine chemicals were classified incorrectly. Among those chemicals were such that are often found in cosmetic or food products (ethylparaben, coumarin), clearly indicating that the human-cell-based screening assay is preferable to predict the risk associated with human exposure. Ultimately, this research will allow efficient screening of toxic chemicals and observe their effects on osteogenic differentiation, as the expected impact is to gain a better understanding of how toxicants affect embryonic bone development.

**3260 Combining Human Pluripotent Stem Cell–Based Platforms with Artificial Intelligence Technology to Screen Developmental Toxicants**

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Environmental factor-induced congenital disabilities raise survivors’ risk of lifelong disabilities and increase the economic burden on their families and society. Of the 80,000 registered chemicals in the United States, only small percentage have undergone safety testing. Therefore, a rapid and accurate method for predicting human developmental toxicants is strongly desired. Here, we combine out human pluripotent stem cell (hPSC)-based high-throughput platform with our artificial intelligence technology to screen for developmental toxicants. The use of hPSCs to form embryoid bodies (EBs) recapitulates many events in the early embryonic process. A toxicity prediction system comprised of three independent subdisciplinary models was created based on the viability change, transcriptional response, and morphological alteration of EBs. Thirty-four chemicals with confirmed teratogenicity in humans were used as standards. EBs were exposed to these chemicals for ten days. Twenty of the 34 chemicals impaired the viability of EBs in a dose-dependent manner up to 100µM. The first prediction model was built based on the dose-response curves and showed good accuracy (76%) in categorizing chemicals into four risk levels (none, low, moderate, and high). The second prediction model was based on the ToxCast I library to establish reliable parameters that would allow us to statistically categorizing chemicals into four risk levels (none, low, moderate, and high). Next, the transcriptional response of EBs to these 34 chemicals were examined by RNA sequencing. Forty-two genes, validated by qPCR, were selected as biomarkers to separate teratogenic and nonteratogenic chemicals. From the screening results tested with recursive feature elimination, the Random Forest-based classification model showed an 81% prediction accuracy in chemical risk level categorization, forming the second part of the prediction system. The third part of the system is based on chemical-elicited structural alterations in EBs, captured by high-content fluorescence screening and known effects on cell proliferation, cell cycle arrest, and SOX17 for endoderm). A highly accurate (82%) prediction model based on ResNET deep learning technology was obtained through training with 32760 EB images. To further validate the prediction accuracy and prove the practical value of this screening system, the teratogenicity of an additional 20 chemicals with limited toxicity information was assessed by this platform, and the results were consistent (80%) with previous studies. For example, tretinoin, was classified as a ‘high’ risk teratogen in humans by two subdisciplinary prediction models and showed a 9.1% toxicity similar with Ettreinitate, a confirmed teratogen from the same vitamin A derivative family. In contrast, Sucrose was classified as ‘nonteratogenic’ by all three subordinary models and showed a 51.9% similarity with Ascorbic acid in affecting EB morphology. These results suggest that our screening platform shows great promise in its ability to identify human developmental toxicants and help understand their etiology.

**3261 Concentration-Response Transcriptomic Changes Induced by Cannabidiol in Hepatocytes Derived from Human Induced Pluripotent Stem Cells**


Cannabidiol (CBD) is a non-intoxicating cannabionoid found in the Cannabis sativa L. plant and is claimed to have many purported health benefits. However, available preclinical modeling of CBD may lack in accurately characterizing concentration effects on the skeleton using an in vitro osteogenic differentiation model based on human embryonic stem cells (hESCs). These chemicals, including 4 reference chemicals, were selected from the ToxCast library to establish reliable parameters that would allow us to statistically group toxicants based on their embryotoxicity. Cells were concomitantly exposed to the selected chemicals during differentiation to osteoblasts to obtain half-maximal inhibitory concentrations based on two endpoints: cytotoxicity (MTT assay) and matrix calcification. The latter was used to indirectly quantify the amount of mature, functional bone-forming osteoblasts from a calcium assay, normalized to the protein content of the cultures. All half-maximal inhibitory concentrations were then related to a half-maximal inhibitory concentration obtained from exposing fully differentiated fibroblasts. This was done using three Fisher functions developed previously. Using this biostatistical model, 23 chemicals were correctly categorized based on their known in vivo embryotoxicity. A canonical plot was also able to categorize all chemicals based on their known in vivo embryotoxicity with the exception of two. Together, these hESCs-based endpoints will provide a valuable assay for the formation of bone-forming osteoblasts, or lack thereof, depending on chemical exposure going forward. In contrast, when the screen was repeated using mouse embryonic stem cells, nine chemicals were classified incorrectly. Among those chemicals were such that are often found in cosmetic or food products (ethylparaben, coumarin), clearly indicating that the human-cell-based screening assay is preferable to predict the risk associated with human exposure. Ultimately, this research will allow efficient screening of toxic chemicals and observe their effects on osteogenic differentiation, as the expected impact is to gain a better understanding of how toxicants affect embryonic bone development.

**3262 A Sensitive and Specific Human Primary Stem Cell-Based In Vitro Assay for Predicting the Gastrointestinal Toxicity Risk of Therapeutic Agents**


Gastrointestinal (GI) toxicities are the most common drug-induced adverse events in human clinical trials. Pharmaceuticals that cause GI toxicity often affect the proliferative stem and progenitor cell populations responsible for maintaining the cellular composition, self-renewing capacity, and barrier function of the intestinal epithelium. This disruption leads to impaired GI barrier integrity, culminating in a

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broad range of clinical symptoms, including diarrhea, inflammation, and increased risk of infection. Typical preclinical animal models, such as rats and mice, are inadequate predictors of GI toxicity. Typically, such liabilities are not discovered until the later stages in pre-clinical development or after the drug is released to the market. Furthermore, existing cell-based model systems, such as Caco-2 cells, are of limited predictive value as they are cancer-derived, unnatural, epithelial. As such, there is an unmet need for a model capable of assessing GI toxicity in early drug development that can directly access GI safety as a metric for compound optimization and lead selection. Ideally, such a model would (1) closely reprise natural human GI physiology and cellular composition; and (2) enables reproducible, robust, and reliable assessments of drug effects on intestinal cell proliferation and barrier function. To this end, Altis has developed an assay to assess GI toxicity potential using the RepliGut® Planar platform, which consists of a primary human stem cell-derived epithelium in a 96-well Transwell® format. Quantitative dose-response curves were generated for test articles utilizing EDU incorporation and transepithelial electrical resistance (TEER), providing quantitative readouts for changes in proliferative and non-proliferative cell populations and barrier function, respectively. A set of benchmark drugs with known clinical incidence of diarrhea were tested over a 6-log dose range to create competent dose curves, to enable modeling of the TC50. For drugs with known clinical GI toxicity, including Bortezomib, Colchicine, and Afatinib, the in vitro TC50 values for TEER/barrier integrity were within 10-fold of the known human plasma Cmax. Furthermore, Bortezomib was equally toxic to all cell populations, while values for TEER/barrier integrity were within 10-fold of the known human plasma Cmax. Additionally, the model was able to accurately predict GI toxicity in a broad range of clinical symptoms, including diarrhea, inflammation, and increased risk of infection.

The protein levels of Perilipin, PPARγ and Stearoyl-CoA Desaturase 1 (SCD1) was measured. The proliferation and differentiation potential was accelerated in arsenic exposed AdSCs. Insulin stimulated glucose uptake (ISGU), adipokines and differentiation and adipogenesis were examined. The prolif-

eration and differentiation potential was accelerated in arsenic exposed AdSCs.

**Clinical GI toxicity, including Bortezomib, Colchicine, and Afatinib, the RepliGut® Planar platform, which consists of a primary human stem cell-derived epithelium in a 96-well Transwell® format. Quantitative dose-response curves were generated for test articles utilizing EDU incorporation and transepithelial electrical resistance (TEER), providing quantitative readouts for changes in proliferative and non-proliferative cell populations and barrier function, respectively. A set of benchmark drugs with known clinical incidence of diarrhea were tested over a 6-log dose range to create competent dose curves, to enable modeling of the TC50. For drugs with known clinical GI toxicity, including Bortezomib, Colchicine, and Afatinib, the in vitro TC50 values for TEER/barrier integrity were within 10-fold of the known human plasma Cmax. Furthermore, Bortezomib was equally toxic to all cell populations, while values for TEER/barrier integrity were within 10-fold of the known human plasma Cmax. Additionally, the model was able to accurately predict GI toxicity in a broad range of clinical symptoms, including diarrhea, inflammation, and increased risk of infection.**

**Development of in vitro assays for early detection of liabilities to chemical adversity is crucial for the prediction of liver toxicity. Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) are an attractive in vitro model to study mechanism-based xenobiotic toxicity. We set out to build a panel of fluorescent hiPSC reporters, suitable for high-content-screening of cellular stress response activation, upon compound exposure. We established a pipeline for efficient, validated reporter generation and reporter functional characterization upon differentiation to relevant lineages, including HLCs. Here, we present the generation and application of fluorescent hiPSC reporter lines for sulfiredoxin-1 (SRXN1) and pirin (PIR), direct downstream targets of Nuclear factor-erythroid-2-related factor 2 (Nrf2), which plays a central role in the regulation of antioxidative, general stress response. Oxidative stress model (DEM), sulfonaphtho and nitrofuratantrant was monitored using live-cell confocal imaging of hiPSC reporter lines upon differentiation into HLCs. Endogenous levels of eGFP-tagged oxidative stress biomarkers accumulated in the cytoplasm of HLCs over 24 hours window, indicating activation of the cellular adaptive machin-

eries.**

**Cytochrome P450 1B1 (CYP1B1) is an enzyme responsible for the metabolism of a broad range of xenobiotic and endogenous substrates including those vital to the development of the eye. Moreover, CYP1B1 mutations have been implicated in the onset of primary congenital glaucoma (PCG). PCG alters the vasculature of the ocular tissues and trabecular meshwork leading to poor aqueous flow. The resulting increase in intraocular pressure damages the optic nerve causing vision loss. Further research examining CYP1B1’s mechanism of action in PCG is required to explore novel therapeutic strategies. Previously a CYP1B1 knockout (KO) mouse retinal endothelial cell (REC) model demonstrated defective capillary branch formation and altered metabolism. Capillary formation was resuscuable in the presence of conditioned culture media from wild-type cells suggesting a soluble CYP1B1 metabolite may regulate REC cell differentiation. In the present study CYP1B1’s role in PCG was explored in vivo using CYP1B1-KO zebrafish by performing behavioral assays and assayed attempts in mass spectrometry-based metabolomics. KO zebrafish showed a significant differential response when compared to their wild-type counterparts in several behavior assays performed at larval and adult life stages. During the larval photo-motion response (LPR) assay KO zebrafish displayed elevated movement in response to both light and dark stimuli. In vision-driven adult behavioral assays, KO zebrafish displayed differential responses to videos of both schooling zebrafish and a predator. In addition, untargeted metabolomics analysis of whole larval zebrafish revealed significant differences in nucleoside and amino acid compounds among others. Pending further validation, the CYP1B1-KO zebrafish model may be a tool to explore novel therapeutic strategies to treat PCG. This research was supported by the National Eye Institute, National Institutes of Health under Award Number P30 E030287 and the Agriculture Research Foundation. The content is solely the responsibility of the authors and does not necessitate the official views of the National Institutes of Health.**

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**Utilization of Free Fatty Acids**

**Altered DNA Methylation Promotes Persistent IGF2 Expression in Adipose-Derived Stem Cells Leading to Early Onset of Metabolic Syndrome in Pernatally Arsenic-Exposed Mice**

K. Koshita, A. Chauhan, and V. Srivastava. CSIR-Indian Institute of Toxicology Research, Lucknow, India. Sponsor: N. Tewari-Singh.

Metabolic Syndrome (MetS) is defined as the cluster of multiple pathological conditions including central obesity, glucose intolerance, hypertriglyceridemia, reduced serum HDL-C levels and high blood pressure which increases the risk of cardiovascular diseases, chronic kidney disease and type 2 diabetes. Adipose dysfunction plays major role in progression of MetS, particularly upon exposure to any endocrine disrupting chemicals including arsenic. In our previous animal experiments, perinatal exposure to arsenic (0.04mg/kg) resulted in increased level of MetS in male progeny along with adipose dysfunction. The role of arsenic in gestational conditioning of adipose-derived mesenchymal stem cells (AdSCs) and its contribution to MetS progression needs to be assessed. The AdSCs were isolated from epidymid white adipose tissue (EAT) of males, perinatally exposed to arsenic, to determine the effects of arsenic on growth, differentiation and metabolism. As EAT is a dynamic organ, relatively normal tissue with high differentiation potential. Insulin stimulated glucose uptake (ISGU), adipokines and underlying cellular pathways involved in adipogenesis were examined. The prolif-

eration and differentiation potential was accelerated in arsenic exposed AdSCs. The protein levels of Perilipin, PPARy and Stearyl-CoA Desaturase 1 (SCD1) was significantly increased.** Utilization of Free Fatty Acids**

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up and fluorescent in dead/dying cells. At the highest dose, cells expressing MT+ER CYP2E1 were more sensitive to lethality (75% cell death), followed by ER-CYP2E1 (52% cell death). MT-CYP2E1 and HepG2 cells had similar cytotoxicity (~40%). Oleic acid did not cause significant cytotoxicity on its own, but when co-exposed with palmitic acid, it ameliorated the toxicity in all cell lines. Next, we studied linoleic acid, which was cytotoxic in all CYP2E1-expressing cell lines, potentially due to epoxide formation. To examine the double bond oxidation, we used Nile Red staining and found that when treated with oleic acid or a mixture of palmitic and oleic acids, lipid accumulation was dramatically increased in MT+ER and ER-CYP2E1 expressing cells. In follow-up experiments, we measured mitochondrial respiration in cells with and without oleic acid supplementation for five days. We found that HepG2 cells and MT-CYP2E1 cells have an oleic acid-induced increase in respiration that is blocked completely by expression of ER-CYP2E1 in the other lines. In ongoing experiments, we are investigating gene expression in FFA-treated cells to understand various cellular responses such as ER stress, lipid peroxidation, dysregulated lipid metabolism and synthesis, triglyceride synthesis, antioxidant pathways, and oxidative stress. If CYP2E1 is mediating initiation and/or progression of NAFLD, it represents a potential therapeutic opportunity and thus understanding this role more clearly is of vital importance.

### 3267 Study of the Roles of Cytochrome P450 (CYPs) in the Metabolism and Cytotoxicity of Perhexiline

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Perhexiline is a coronary vasodilator for angina treatment, and it enjoyed worldwide success before reports of severe side effects including hepatotoxicity, caused its withdrawal from most of the markets. In the clinical setting, cytochrome P450 (CYP) 2D6 is considered as a possible risk factor for the adverse effects of perhexiline. Some patients have polymorphisms in CYP2D6 genes that resulted in the absence or inactivation of CYP2D6 in their liver and, thus, express acetylated perhexiline or reduced amounts of its metabolites was detected in their plasma when taking the drug. However, information is sparse regarding the precise roles of CYP-mediated metabolism in toxicity or detoxification of perhexiline, especially no data are available in the intact cells. Using our previously established HepG2 cell lines that individually express 14 CYPs and human liver microsomes, we identified that CYP2D6 plays a major role in the hydroxylation of perhexiline. We also determined that CYP1A2, 2C19, and 3A4 contribute to the metabolism of perhexiline in intact cells and in human liver microsomes. In addition, we determined the formation of six metabolites of perhexiline in cDNA-expressed CYP bactosomes. The toxic effect of perhexiline was reduced in CYP2D6-overexpressing HepG2 cells at the concentrations of 2.5-10 μM for 24 h as measured with ATP assay and LDH release. Overexpression of CYP3A4 showed marginal protective effect against perhexilinemediated cytotoxicity. Overexpression of CYP1A2 and 2C19 played less role in perhexilinemediated cytotoxicity. These findings suggest that CYP2D6-mediated metabolization protects the cells from perhexiline-induced cytotoxicity and support the clinical observation that CYP2D6 poor metabolizers may have higher risk for perhexiline-induced hepatotoxicity.

### 3268 Maternal Exposure to Polycyclic Aromatic Hydrocarbons Causes Lung Dysbiosis in Mice Lacking the Cyp1a2 Gene: A Potential Protective Role

D. Narke, G. Xia, W. Jiang, and B. Moorthy. Baylor College of Medicine, Houston, TX.

Polycyclic Aromatic Hydrocarbons (PAHs) are complex mixtures of chemicals that are found in automobile exhaust, industrial effluents, and superfund materials. Maternal exposure to PAHs is known to exacerbate lung injury in neonates through the activation of Cyp1a1/1b1 enzymes. The Cyp1a2 enzyme, predominantly expressed in the liver, is known to be protective against PAH-induced lung injury. However, little is known about the role of prenatal exposure on lung development and normal microbiome and the role of the Cyp1a2 enzyme in modulating these microbial communities. We hypothesize that the Cyp1a2 enzyme protects against prenatal PAH exposure-induced lung injury by maintaining eubiosis. Timed pregnant wild-type (WT) (C57BL/6J) and Cyp1a2-null mice were orally administrated a PAH mixture of benz[a]pyrene (BP) and benzo[b]fluoranthene (BbF) (7.5mg/kg each) or the vehicle corn oil (CO) once daily on gestational days 16-19. The offspring were exposed to room air for 14 days after birth. Mice were sacrificed on postnatal day (PND) 15 and microbiome analysis was performed on the lung and intestinal contents using 16S rRNA gene sequencing. Prenatal PAH exposure altered lung beta diversity and relative abundance of commensal bacteria in Cyp1a2-null mice but not in the WT mice. However, prenatal PAH exposure showed no significant change in the intestinal beta diversity and relative abundance of commensal bacteria in Cyp1a2-null and WT mice. Our study suggests that prenatal PAH exposure may cause dysbiosis in the lung microbiome in the absence of the Cyp1a2 gene hinting towards a potential protective role.

### 3269 Functional Characterization of Allelic Variations of Human Cytochrome P450 2C (V181I, I244V, I331T, and L361F)


Cytochrome P450 2C is responsible for the metabolism of various clinical drugs as well as arachidonic acid. Therefore, its allelic variations can significantly influence the metabolic outcomes. In this study, we characterized the functional effects of four nonsynonymous single nucleotide polymorphisms from P450 2C15*,16, 17, 18 alleles, which were recently found. Recombinant allelic variant enzymes of V181I, I244V, I331T and L361F were successfully expressed in Escherichia coli and purified. Steady state kinetic analysis in pacitaxel 6-hydroxylation showed the significant differences of enzymatic activity in V181I, I244V, and L361F. The calculated catalytic efficiency of catalytic efficiency (kcat/Km) displayed 5 – 25% of that of wild-type enzyme. This reduction of activities was due to both decrease kcat values and increased Km values of variants. P450 2C30 catalyzes the epoxidation of arachidonic acid mainly to produce 11,12-epoxy-arachidonic acid (EET) and 14,15-EET. Three variants (V181I, I244V, and L361F) showed the significant reduction of epoxidation catalysis. Especially, L361F variant yielded only 4 – 5% of wild-type catalytic efficiency in u9- and u6-epoxidation reactions. These reductions were mainly due to reduction of kcat values of L361F variants. Binding titration analysis of pacitaxel and arachidonic acid showed that the binding affinities of all variants had similar values of the wild-type (10-14 μM for pacitaxel and 20-49 μM for arachidonic acid). The substrate docking study using the crystal structure of P450 2C8 suggested that the variation of L361F induced incorrect orientation of pacitaxel in the active site therefore the 6'CO of pacitaxel was moved away from the productive catalysis. This study implicated that individuals carrying these P450 2C allelic variations are likely to have the altered metabolism of clinical medicines and production of fatty acid derived signal molecules.

### 3270 Novel QSAR Models for Prediction of Reversible and Time-Dependent Inhibition of Cytochrome P450 Enzymes

S. Faramarzi, Y. Yang, A. Bassani, K. Crossi, G. Myatt, D. A. Volpe, and L. Stavitskaya. US FDA, Silver Spring, MD; and *Instem, Staffordshire, United Kingdom.

The FDA’s in vitro drug-drug interaction (DDI) guidance [https://www.fda.gov/media/134582/download] states that in vivo DDIs caused by metabolites may be possible even if in vitro studies suggest that the parent drug alone will not inhibit any major cytochrome P450 enzymes (CYPs). Furthermore, the guidance recommends that sponsors evaluate metabolites in vitro for their inhibitory effects on CYPs. Specifically, an in vitro CYP inhibition study is recommended if the metabolite is less polar than the parent drug and the area under the plasma concentration-time curve (AUC) of a metabolite is ≥ 25% of AUC of the parent, or if the metabolite is more polar than the parent drug and the AUC of metabolite is ≥ AUC of the parent drug. In addition, a lower cut-off value for the metabolite-to-parent AUC ratio was also considered if a metabolite contains a structural alert for potential mechanism-based inhibition (MBI) of CYPs, since such inhibition carries a higher risk of causing DDI due to their prolonged inhibition effect. To facilitate identification of structural alerts, an extensive literature search was performed, in a recent study, and alerts for MBI of CYPs were collected. Furthermore, in the present study, five quantitative structure-activity relationship (QSAR) models were developed to predict not only time-dependent inhibition of CYP 3A4, an enzyme that metabolizes approximately 50% of all marketed drugs, but also reversible inhibition of 3A4, 2C9, 2C19 and 2D6. The non-proprietary training database for the QSAR models was harvested from FDA drug approval packages and published literature from sources such as Binding Database, Google Scholar, PubMed, and the US Patent database, to give a total of 10,286 chemical structures. The cross-validation performance statistics for the new CYP models range from 69% to 79% in sensitivity and 73% to 91% negative predictivity. Additionally, the performance of the newly developed models was assessed using external validation sets. Overall performance statistics showed up to 60% in sensitivity and up to 80% in negative predictivity. The newly developed models will provide a faster and more effective evaluation of potential drug-drug interaction caused by metabolites.

### 3271 Mechanistic Insights from Profiling Chemical-Mediated Transcription Factor Transactivation with the Integration of Cytochrome P450 Metabolism


Profiling chemical effects on transcription factor activity is an important new approach methodology (NAM) that can help characterize the mechanisms by which chemicals may perturb the human transcriptome and biological systems. The Attagene cis-FACTORIAL™ assay uses a reporter system to detect activity of 46 transcription factors that provides a quantitative assessment of chemical effects. A new version of this assay, CYP-FACTORIAL™, includes addition of nine key cytochrome P450 (CYP450) enzymes to enable the evaluation of chemical effects on transcription
factor activity with and without CYP-mediated phase 1 metabolism. This supports a better understanding of whether CYP-mediated metabolism results in an altered bioactivity profile. The current study examined 24 expert-selected chemicals across four test concentrations in the cis-FACtORIAL and CYP-FACtORIAL assay formats. Results for this proof-of-concept study suggest that the transcription factors showing the greatest difference in response with CYP450 metabolism are those activated downstream (AHR), aryl hydrocarbon receptor (AhR), and oxidative stress response (nuclear factor erythroid 2-related factor 2 or NRF2) pathways. Comparisons of study chemical profiles to those of reference chemicals identified a highly conserved PAH toxicity signature involving activation of AhR, NRF2, and ER. Interestingly, a profile in which ER and AhR are activated but NRF2 is not activated correlated to non-toxic compounds, suggesting the possibility of using differences between signatures to predict toxic outcomes. Between profiling approaches and the integration of metabolism into a multiplexed in vitro assay system, this assay platform provides insight into chemically induced bioactivity and thus facilitates the development of mechanistically based, human-relevant NAMs. This project was funded with federal funds from NIEHS, NIH under Contract No. HHSN27201500010C.

2374 Crystal Structure of Racemic Bucetin, N-(4-Ethoxyphenyl)-3-Hydroxybutanamide: Significance to Mechanisms of Toxicity of Phenacetin

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1Southern University and A&M College, Baton Rouge, LA; and 2Louisiana State University, Baton Rouge, LA.

Bucetin [N-(4-ethoxyphenyl)-3-hydroxybutanamide] is an analgesic and antipyretic that is similar in structure to phenacetin [N-(4-ethoxyphenyl)acetamide]. Once thought to be a better substitute for phenacetin, bucetin was introduced into the markets in Germany but was soon withdrawn from use due to renal toxicity. The renal toxicity of bucetin, renal papillary necrosis, is similar in nature to that induced by phenacetin but is somewhat less pronounced, presumably due to a difference in the rate of deacetylation by microsomal enzymes leading to the formation of 4-ethoxyaniline. Thus, the renal papillary necrosis by phenacetin and bucetin appears to be a manifestation of the formation of 4-ethoxyaniline and the subsequent inhibitory action of this putative metabolite (or its hydroxylated and/or autoxidation products, N-(4-ethoxyphenyl)hydroxylamine and 1-ethoxy-4-nitrosobenzene) on PGE2 synthesis and the possible reduction of COX-2 expression. However, unlike the case with phenacetin, there is limited or no information on chlorinated, nitrated, or other metabolites of bucetin likely to be formed in vivo through cellular oxidants, namely, peryoxinitrite/peroxynitrite acid and hypochlorite/hypochlorous acid.

To address this and to better understand the mechanisms of toxicity of bucetin, we determined the crystal structure of racemic bucetin analyzed using a Bruker Kappa APEX-II DUO diffractometer. Single crystals of bucetin were prepared by slow cooling of a nearly saturated solution of bucetin in boiling deionized water (resistance: 18.2 MΩ/cm). The crystal bucetin crystallizes in monoclinic space group P21/c with Z=4. Refinement using low temperature (100 K) X-ray diffraction data yielded R=0.045 for 2139 reflections and 153 parameters. The molecule is in an extended conformation, with 170.14(15)° C-C-C torsional angle in the ethoxy group and torsion angles C-N-C-C=177.2(16)°, C-N-C-C=170.08(15)°, and C-C-C-C=177.2(15)° in the butanamide chain. The OH group donates an intermolecular hydrogen bond to amide carbonyl oxygen and accepts an intermolecular hydrogen bond from an additional OH group. The geometries of these hydrogen bonds are 2.7268(17) Å for OH...O (at 1-x, 1-y, 2-z) and 2.8611(19) Å for NH...O (at x, 1/2-y, z-1/2). The former forms 12-membered dimeric rings about inversion centers, and the latter form chains in the [0 0 1] direction. The overall hydrogen-bonded network is two-dimensional, with no propagation in the [1 0 0] direction. Given the current understanding that deacetylation is the key pathway to dealing with toxic doses of EOGC in mice, the formation of a Bruker Kappa APEX-II DUO diffractometer. Single crystals of bucetin were prepared by slow cooling of a nearly saturated solution of bucetin in boiling deionized water (resistance: 18.2 MΩ/cm). The crystal bucetin crystallizes in monoclinic space group P21/c with Z=4. Refinement using low temperature (100 K) X-ray diffraction data yielded R=0.045 for 2139 reflections and 153 parameters. The molecule is in an extended conformation, with 170.14(15)° C-C-C torsional angle in the ethoxy group and torsion angles C-N-C-C=177.2(16)°, C-N-C-C=170.08(15)°, and C-C-C-C=177.2(15)° in the butanamide chain. The OH group donates an intermolecular hydrogen bond to amide carbonyl oxygen and accepts an intermolecular hydrogen bond from an additional OH group. The geometries of these hydrogen bonds are 2.7268(17) Å for OH...O (at 1-x, 1-y, 2-z) and 2.8611(19) Å for NH...O (at x, 1/2-y, z-1/2). The former forms 12-membered dimeric rings about inversion centers, and the latter form chains in the [0 0 1] direction. The overall hydrogen-bonded network is two-dimensional, with no propagation in the [1 0 0] direction. Given the current understanding that deacetylation is the key pathway to dealing with toxic doses of EOGC in mice, the fact that the acyl group in bucetin (3-hydroxybutyryl) is much larger in size compared to the acetyl group in 4-alkoxycacetanilides and has a chiral center, the information on bucetin crystal structure presented here may help in the development of more accurate models of toxicity effects of 4-alkoxycacetanilides.

2375 Twinned Crystal Structure of N-(4-Methoxy-3-Nitrophenyl) Acetamide: A Putative Non-enzymatic Oxidation Product of N-(4-Methoxyphenyl) Acetamide with Implications to Mechanisms of Toxicity of 4-Alkoxycacetanilides

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Belonging to the class of 4-alkoxycacetanilides (4-AAs), phenacetin [N-(4-ethoxyphenyl)acetamide] is the first synthetic fever reducer and non-opioid analgesic to go to the markets worldwide as early as the 1980s. While the analgesic effects of 4-AAs are thought to be due to their actions on the sensory tracts of the spinal cord, the antipyretic effects arise as a result of their actions on the brain where the temperature set point is lowered. In vivo, 4-AAs most undergo oxidative dealkylation to give N-(4-hydroxyphenyl)acetamide, the clinically relevant analgesic, while a small portion may undergo deacylation, producing carcinogenic, kidney-damaging 4-alkoxyanilines. There has been extensive information on phase I and phase II biotransformation of 4-AAs, but little is known about their biotransformation by non-enzymatic mechanisms including the formation of peroxynitrite and hypochlorous acid (NO)-derived oxidants (NOS, NO2, CO2, and ONOOH). Previous studies from our laboratory and elsewhere have shown, for instance, that N-(4-hydroxyphenyl)acetamide forms nitrated products along with varying amounts of dimers when reacted with NOS under physiologically relevant conditions. We reason that similar...
products (or their positional isomers) may be formed in the reactions of 4-AAs with NO2. In order to understand these reaction products and to shed light on molecular targets, we have synthesized N-(4-methoxy-3-nitrophenyl)acetamide by acetylation of 4-methoxy-3-nitroaniline using acetic anhydride and purified by recrystallization twice from water. Crystals of N-(4-methoxy-3-nitrophenyl)acetamide grown from an aqueous solution were analyzed ion using Bruker Apex II Duo diffractometer. It was found that N-(4-methoxy-3-nitrophenyl)acetamide crystallizes in the monoclinic space group P2₁/n with Z = 4 with a disordered nitro group in twinned crystals. Refinement using low temperature (90K) X-ray diffraction data yielded R=0.059 for 1675 reflections and 160 parameters. Both the methoxy group and the acetamide groups are nearly coplanar with the phenyl ring, with respective torsion angles 0.0(4)° for C=C-C-O and 4.9(4)° about the C-N bond to the ring. The C-C=N torsion angle is also slightly different from zero, 0.2(4)°. Overall, the 12 atom methoxyphenylacetamide group is coplanar to a mean deviation 0.04 Å. The nitro group is twisted out of this plane by about 30°, disordered into two orientations with opposite sense of twist. The dihedral angle between the two disordered C-NO2 planes is 59.2°. The N-H group donates intermolecular hydrogen bonds with N...O distances 3.122 (4) Å to nitro oxygen at x-1/2, 1/2-y, z-1/2, forming chains in the [0 1 0] direction. Interestingly, the amide carbonyl oxygen atom is not involved in hydrogen bonding. Combined with the recent revelations of mechanisms of action of N-(4-hydroxyphenyl)acetamide through indirect activation of CB1 receptors by hydrogen bonding. G. Xia, W. Jiang, C. Chu, G. Gastelum, L. Wang, D. Narke, N. Pultini, and B. Moorthy, Baylor College of Medicine, Houston, TX.

Polycyclic aromatic hydrocarbons (PAHs), a group of chemicals containing 2 or more fused benzene rings but no heteroatoms, are reported to increase the risk of lung cancer in humans via inhalation exposure. After entry into the lung, cytochrome P450 (CYP) 1A1/2 and 1B1 will be induced by PAHs and aid the bio- transformation of PAH-DNA adducts. Previously, our laboratory reported that Cyp1a1/-/- mice were less susceptible to PAH-induced pulmonary carcinogenesis, while Cyp1a2/-/- mice were more susceptible. However, what role of CYP1B1 in PAH induced lung carcinogenesis, and how CYP1B1 interacts with CYP1A1/2 in this program is largely unknown. Here, we tested the hypothesis that mice lacking the CYP1B1 gene will display altered susceptibility to PAH-induced pulmonary carcinogenesis. Wild-type (WT), Cyp1a1/1a2-/- (2KO), Cyp1a1/1a2/1b1-/- (3KO) and Cyp1b1-/- (1KO) male and female mice were treated with 3-methylcholanthrene (MC) and Benzo[a]pyrene (BP) for cancer initiation and tumor formation studies. In WT mice, CYP1A1, 1A2, 1B1 expression was induced by both acute MC and BP treatments. In 1KO, 2KO, and 3KO mice, there is a compensating compensatory pattern regarding CYP1A1, 1A2, 1B1 expression profiles and ethoxyresorufin O-deethylase/methoxyresorufin O-demethylase enzymatic activities. Unexpected, a significant sex differences were found in MC treated, but not BP treated mice in the chronic tumorigenesis study. Tumor multiplicity and incidence study followed a specific but convincing trend to show that compared with WT, tumor counts were significantly increased in 2KO, with a substantial decrease in 1KO while 3KO had the lowest incidences in both male and female mice. Collectively, deleting Cyp1b1 is protective in PAHs induced lung carcinogenesis. Future study will focus on the measurements of DNA adducts and DNA repair genes/pathways in dissection Cyp1b1’s susceptibility to PAHs-induced pulmonary carcinogenesis.

3276 The Gut Microbiome Drives Methylmercury Demethylation and Elimination

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Methylmercury (MeHg) is a potent neurotoxicant found in most fish and seafood. The level of exposure to MeHg following ingestion of contaminated food and subsequent toxicity are directly related to the kinetics of MeHg elimination from the body. Importantly, there is a large degree of inter-individual variability in MeHg elimination and the factors driving this outcome are poorly understood. Several lines of evidence suggest that MeHg demethylation by gut microbes is a rate limiting step in elimination, but the microorganisms responsible for this process in the human gut microbiome are currently unknown. Here, we examined the relationship between MeHg elimination, demethylation activity, and gut microbiome diversity. Total Hg elimination rates were quantified from fecal samples collected from 27 human volunteers following a controlled feeding protocol with tuna. Stool samples from each participant were sequenced and metagenomic analyses were conducted to test for correlations between fast elimination and microbial taxa and/or function (genes/protein clusters). Stool was also cultured in vitro to assess MeHg demethylation potential. Elimination half-life (t1/2) ranged from 30 to 89 days across participants. MeHg demethylation activity in stool cultures correlated with the overall elimination rate observed in participants. However, bioinformatic analyses indicated that microbial diversity was not strongly correlated with MeHg elimination. Microbiome species were identified from metagenomic assembled genomes (MAGs) and the abundance of several sources of secondary data, secondary sources, etc. and 5 references contained both health effects and supporting data. High-level data extraction was conducted on the 33 references that included human or animal health effects data. All human data and 54% of animal studies focused on the inhalation route; 46% of animal studies focused on the oral route. The most-studied endpoints included body weight, hepatic, neurological, and cancer effects. While evidence from human studies is mixed, findings from animal studies are consistent with the existing toxicological profile for MeCl (ATSDR 2000), indicating that the nervous system, liver, and kidney are potential toxicity targets of MeCl. Additional studies in animals also reported carcinogenic effects. All data were exported into Tableau Public to create an interactive literature flow diagram (https://public.tableau.com/app/profile/eha.tableau.team/viz/LitFlowDiagram-MethylmercuryChloride2022/Dashboard) and interactive human health database inventories (https://public.tableau.com/app/profile/eha.tableau.team/viz/DRAFTSEMDataVisualizationForMethylChloride/HealthEffectsOverview). Based on the full-text review conducted during the SEM, very few of the studies identified during the updated literature search address the data needs identified in the current toxicological profile for MeCl (ATSDR 2000), and none of the identified studies are expected to impact the existing inhalation or oral MRLs. 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.

3277 Modulation of Polycyclic Aromatic Hydrocarbon (PAH)–Mediated Pulmonary Carcinogenesis in Mice Lacking Genes for Cyp1a1/1a2, Cyp1a1/1a2/1b1, or Cyp1b1: Role of CYP1B1 in Carcinogenesis

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The Agency for Toxic Substances and Disease Registry (ATSDR) developed a systematic evidence map (SEM) approach to determine if recently published studies would contribute new and essential information to existing toxicological profiles, or support development of new profiles. The SEM approach includes: 1) Literature searches to identify relevant studies published since the most recent toxicological profile; 2) Screening of literature search results using methods consistent with principles of systematic review to determine if identified studies meet the Populations, Exposures, Comparators, and Outcomes (PECO) inclusion criteria designed to capture literature relevant to assessing human health; 3) Preparation of interactive literature inventory figures to provide an overview of the new evidence that meets PECO criteria, and 4) High-level data review and extraction of studies identified during the updated literature search to determine if any could potentially address key data needs or impact existing minimal risk levels (MRLs) for the compound of interest. The methylene chloride (MeCl) profile, published in 2000, was selected as a case-study for application of the SEM approach. Literature searches from all bibliographic databases yielded 9,005 unique references to be screened in DistillerSR by two independent reviewers. Title-abstract screening identified 328 items that met PECO criteria, these items plus 14 citations identified during review of grey literature outside DistillerSR underwent full-text review. Of these, 257 references were identified as PECO-relevant: 33 references included health effects data, 218 references contained other supporting data (e.g., toxicokinetics, mechanistic data, etc.), and 5 references contained both health effects and supporting data. High-level data extraction was conducted on the 33 references that included human or animal health effects data. All human data and 54% of animal studies focused on the inhalation route; 46% of animal studies focused on the oral route. The most-studied endpoints included body weight, hepatic, neurological, and cancer effects. While evidence from human studies is mixed, findings from animal studies are consistent with the existing toxicological profile for MeCl (ATSDR 2000), indicating that the nervous system, liver, and kidney are potential toxicity targets of MeCl. Additional studies in animals also reported carcinogenic effects. All data were exported into Tableau Public to create an interactive literature flow diagram (https://public.tableau.com/app/profile/eha.tableau.team/viz/LitFlowDiagram-MethylmercuryChloride2022/Dashboard) and interactive human health database inventories (https://public.tableau.com/app/profile/eha.tableau.team/viz/DRAFTSEMDataVisualizationForMethylChloride/HealthEffectsOverview). Based on the full-text review conducted during the SEM, very few of the studies identified during the updated literature search address the data needs identified in the current toxicological profile for MeCl (ATSDR 2000), and none of the identified studies are expected to impact the existing inhalation or oral MRLs. 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.
While ample research exists on the effects of single metal exposures during pregnancy on the mother and child, there is a paucity of information on the effects of exposures to metal combinations. This study aims to identify and characterize prevalent combinations of cadmium (Cd), mercury (Hg), and lead (Pb) in pregnant women who participated in the National Health and Nutrition Examination Survey (NHANES) in the United States. A total of 10,152 women aged 20-44 were selected from the NHANES 1999-2018. The blood levels of Cd, Hg, and Pb were dichotomized as “high” and “low,” using the median values as cut points. The prevalence of the three metals’ unique combinations (singular, binary, tertiary) was calculated, where each metal concentration was at a “high” level. The prevalence of each unique metal combination was examined using multinomial logistic regression. Among the pregnant women (n=1,297), singular Hg was most prevalent (19.2%), followed by singular Cd (14.7%), tertiary combination Cd/Hg/Pb (11.0%), binary combinations Cd/Pb (9.8%), Hg/Pb (9.2%), Cd/Hg (7.8%), and singular Pb (5.5%). After adjusting for potential confounders (age, race/ethnicity, poverty income ratio, and serum cotinine), the odds of having the Cd/Hg/Pb (adjOR=0.49: p<0.001) and Cd/Pb (adjOR=0.68: p<0.0364) combinations in blood levels were significantly lower in pregnant women than in non-pregnant women. The odds of having singular Pb blood levels were significantly lower in the 1st & 2nd trimesters (n=563) (adjOR=0.31: p<0.0001); whereas the odds were higher in the 3rd trimester (n=366) (adjOR=1.23: p<0.0471) in non-pregnant women. The odds of having singular Pb blood levels were compared between trimesters, the odds were significantly higher in the 3rd trimester than in the 1st & 2nd trimesters (adjOR=4.00: p<0.0004). Further research is warranted to understand the relationship between metal combinations exposure during pregnancy and possible adverse birth outcomes. This study used a novel approach to determine the prevalence of specific combinations of metals in pregnant women and non-pregnant women. This project uses a library of over 4,000 unique chemicals that are part of the ToxCast Program to establish a CDS database for their assessment. Chemicals were distinguished into 13 classifications: industrial chemicals, color dyes, cosmetic ingredients, disinfection-by-products, flame retardants, food additives, natural xtocs, pesticides, per-/polyfluoroalkyl substances, pharmaceuticals, plastics, polycyclic aromatic hydrocarbons, and surfactants. Each chemical is prepared at 10μM in 50:50 water/methanol and analyzed via IMS-MS using direct flow injection. All compounds are analyzed using electrospray ionization (ESI) in positive and negative ion modes, as well as atmospheric pressure photoionization (APPI) in positive mode. Agilent IMS-MS Browser software is used to derive CCS values for chemicals ionizable by each ion mode and the analytical signal for each analyte is manually verified to ensure confidence in CCS values. We found that across all ionization modes, over 50% of the tested compounds are detectable and have acceptable instrumental reproducibility of CCS values within +/-1% A2. Depending on the ionization mode used, it was necessary to conduct suspect screening of these substances. Overall, the database developed herein is critical for high-throughput suspect screening for contaminants in environmental samples to inform rapid exposure and risk assessment.

The structure and function of the nervous system gradually deteriorate in neurodegenerative disorders like Alzheimer’s disease (AD). Studies have been done on the relationship between environmental toxicants and both animals and humans. At the community or individual level, knowledge of the interactions between environmental exposure, non-chemical stressors, cumulative effects and AD is limited. In this scoping review, we aim to identify potential underlying mechanisms and pathways related to AD that are potentially impacted by both chemical and non-chemical stressors and their mixtures. To identify environmental chemicals, social determinants of health, and AD, keywords were searched from 2000 to the present in PubMed, national chemical libraries, and other pertinent sources. 26 manuscripts were retained for full-text evaluation. Information from these manuscripts reported an association and possible link to AD. The hypothalamic-pituitary-adrenal axis (HPA axis) is linked to metals along with greater amyloid-β deposits in the hippocampus region. Epidemiological studies have linked socioeconomic factors, such as access to healthcare, food, education, geographic location, and income, to an increased risk of AD mainly because of chronic stress. These non-chemical stressors may affect biological pathways of inflammation, oxidative stress, and HPA activation. Thus, by altering the function of the HPA axis, chemical and non-chemical stressors can work in conjunction to increase overall oxidative stress. This could lead to an increase in the production of free radicals, causing molecular and cellular damage and the proliferation of pathological traits that are frequently linked to AD. The potential combined molecular pathways of metals (single and mixtures) and non-chemical stressors implicated in the etiology of AD were emphasized by this scoping research review. In conclusion, these results can be extended further and used to assess communities dwelling in areas susceptible to cumulative elevated levels of environmental contaminants along with greater exposure to chronic nonchemical stressors, which are a result of their lifestyles and environment. Future studies that comprehensively examine environmental exposures and their biological, social, and psychological effects on the etiology of AD and other neurodegenerative disorders might be beneficial. The findings and conclusions in this abstract 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.

To fulfill the promise of reducing reliance on mammalian in vivo laboratory animal studies, new approach methods (NAMs), including human cell-based in vitro assays, need to provide an actionable basis for derivation of toxicity values and regulatory decision-making. However, existing evaluations of their consistency have had mixed results, with in vitro-to-in vivo extrapolation (IVIVE) appearing protective, but poorly correlated, with in vivo points of departure (PODs). In this study we hypothesized that improved concordance can be achieved by reducing the heterogeneity of in vitro models, including human cells, and recognizing the importance of using PODs, and address- ing species differences in toxicokinetics (TK). For in vivo PODs, we targeted ~40 ATSDR Substance Priority List chemicals with regulatory toxicity values, deriving Bayesian benchmark dose (BMD) based PODs where possible. For in vitro PODs, we compared the results from using active concentration 50% (AC50) values from ToxCast with using PODs from a compendium of human cell-based models, including several derived from induced pluripotent stem cells (iPSCs). Additionally, a combination of human and animal TK data, in vitro TK assays, and default allometric body weight-scaling approaches were utilized to convert between in vivo and in vitro PODs. We found that utilizing BMDs and addressing species differences significantly improved concordance, with greater correlation of in vitro PODs and human toxic equivalency ratios closer to 1. Additionally, among individual NAMs, population-based hiPSC-derived cardiomyocytes had the best concordance. Overall, we demonstrated an approach that increases the confidence on using NAMs, especially those based on human cells, to predict in vivo toxicity values, and provides a foundation for future risk assessment and decision making based on NAMs.

Reviews of environmental chemicals such as Organohalogen Flame Retardants (OFRs), are essential for identifying hazards to the health of humans and wildlife. Systematic reviews are the gold standard methodology to summarize and critically assess existing evidence on potential health risks associated with an environmental exposure. These methods provide a rigorous framework designed to minimize
bias and maximize transparency. However, the rigor and comprehensiveness of the systematic review approach is time-consuming, resource intensive and generally designed to address focused questions. Systematic evidence maps (SEM) and scoping reviews utilize the rigor of the systematic review methodology to identify trends in a broader evidence base, including evidence clusters and evidence gaps, and can support multiple decision-making scenarios. Scoping reviews and systematic evidence maps (SEM) share issues, but questions that often require the survey of relatively broad topics to determine the extent of the evidence. SEM can identify research opportunities, for instance what areas need studies for verification, mechanism or to strengthen existing findings. SEM can also identify areas that could be candidates for meaningful systematic review evaluation to support public health decision making and regulation. Many steps of the systematic review process have the potential to utilize semi-automated approaches. In fact, considerable progress has been made to reduce the screening burden and the recent development of Dextr supports semi-automated data extraction of environmental health science literature. There are many tools that are designed to support systematic review methods. This work outlines a potential workflow designed to approach automated approaches across the systematic review process - which includes scoping and evidence mapping - for environmental health evaluations. This approach is designed to leverage available tools, data structures and interoperability to support efficient information retrieval from scientific literature and minimize the redundancy of tasks. This poster demonstrates a workflow that leverages informatics approaches for systematic reviews in toxicology with a case study evaluating the toxicity of OGRs to demonstrate the integration, connection, and user verification of the various tools throughout the systematic review process.

3284 Evaluating the Relative Responsiveness of Health Effects to Exposure to Polychlorinated Biphenyl (PCB) Mixtures in Mammalian Model Systems

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Polychlorinated biphenyls (PCBs) are a group of chemicals that occur in the environment as mixtures of individual congeners. Despite an extensive literature documenting adverse health effects from exposure to these mixtures, the relative sensitivity of various health effects to PCB exposure, especially at low, environmentally relevant levels, remains unclear. We used systematic review methods to compile dose-response information for noncancer health effects across a wide range of PCB mixtures. We identified 526 references that included health effect data for select nonhuman mammalian species (rat, mouse, monkey, mink) of any life stage. Only 12 PCB mixture containing four or more congeners. For each study, we identified and extracted lowest observed adverse effect levels (LOAELs) and compared the responsiveness of health effects to various PCB mixtures using three dose metrics: administered doses, estimated internal doses, and human equivalent doses. Given the lipophilicity and biopersistence of PCBs, internal tissue levels commonly increase with repeated dosing. We addressed this by employing pharmacokinetic models that account for bioaccumulation, which is not reflected in comparisons based on administered dose alone. As such, we found that the apparent sensitivity of specific health effects depends, to some extent, on the dose metrics considered. Taken together with factors such as the strength of evidence available to support dose-response relationships, conclusions of systematic review approaches to PCB exposure and health effects and the relative biological significance of each effect, identification of the effects most sensitive to PCB exposure could provide a basis for prioritizing outcomes for human health risk assessment. Disclaimer: The views expressed in this abstract are those of the authors, and do not necessarily represent the views or opinions of the US EPA.

3285 Derivation of ATSDR's Provisional Minimal Risk Levels for Vinyl Chloride

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A minimal risk level (MRL) is an estimate of daily exposure to a hazardous substance that is likely to be without appreciable risk of adverse health effects over a specified route and duration of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used by the Agency for Toxic Substances and Disease Registry (ATSDR) health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. Vinyl chloride is a volatile compound used almost exclusively by the plastics industry to produce polyvinyl chloride (PVC) and several copolymers in the United States. Anthropogenic sources are responsible for all of the vinyl chloride found in the environment. Therefore, the most likely route of exposure for the general population is inhalation in either the workplace or near emission sources. The most sensitive health effects following vinyl chloride exposure appear in the hepatic, neurological, immunological, and developmental systems. Limitations in the research database precluded the generation of a comprehensive database of in vivo data for select nonhuman mammalian species (rat, mouse, monkey, mink) of any life stage. Only 12 PCB mixture containing four or more congeners. For each study, we identified and extracted lowest observed adverse effect levels (LOAELs) and compared the responsiveness of health effects to various PCB mixtures using three dose metrics: administered doses, estimated internal doses, and human equivalent doses. Given the lipophilicity and biopersistence of PCBs, internal tissue levels commonly increase with repeated dosing. We addressed this by employing pharmacokinetic models that account for bioaccumulation, which is not reflected in comparisons based on administered dose alone. As such, we found that the apparent sensitivity of specific health effects depends, to some extent, on the dose metrics considered. Taken together with factors such as the strength of evidence available to support dose-response relationships, conclusions of systematic review approaches to PCB exposure and health effects and the relative biological significance of each effect, identification of the effects most sensitive to PCB exposure could provide a basis for prioritizing outcomes for human health risk assessment. Disclaimer: The views expressed in this abstract are those of the authors, and do not necessarily represent the views or opinions of the US EPA.

3286 Cardiovascular and Respiratory Effects of Inorganic Mercury Salts: A Systematic Review and Analysis

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A systematic evidence map was developed to inform problem formulation for an IRIS assessment of oral exposures to inorganic mercury salts, including mercuric chloride, mercuric sulfide, and mercurous chloride. Population, Exposure, Comparator, and Outcomes (PECO) criteria were developed to focus the research question(s) and inclusion criteria. Systematic review methods were used to conduct a comprehensive literature search, study screening (title/abstract and full text), study evaluation, and data extraction from the studies meeting the PECO criteria. In addition, studies containing potentially relevant supplemental material were tracked and categorized. All relevant health outcomes were assessed; however, this presentation is focused on cardiometabolic and respiratory effects of exposure to inorganic mercury salts. Study evaluation and data extraction were conducted using the Evidence Assessment Collaborative (E2C2). A systematic evidence map provides a visual tool, and visualized using both HAWC and Tableau. Study confidence ratings (high, medium, low, uninformative) were given based on evaluation of several domains such as exposure measurement, chemical administration and characterization, confounding factors, observational bias, sensitivity, etc. No studies were identified that evaluated the cardiovascular and respiratory effects of oral exposure to inorganic mercury salts in humans. Several animal studies (subchronic, chronic, reproductive, and developmental) in male and female rats, mice, and goats evaluated cardiometabolic effects following oral exposure to mercuric chloride; however, animal studies investigating these health effects were not identified for other inorganic mercury compounds. This systematic evidence map contributes to the understanding of the limited evidence on cardiovascular and respiratory outcomes available for mercuric chloride and helps to inform the usability of these data in the hazard identification and dose response analysis to be conducted in the forthcoming IRIS assessment of inorganic mercury salts. Discussion: In vivo data for select nonhuman mammalian species (rat, mouse, monkey, mink) of any life stage, exposed to PCB mixtures containing four or more congeners. For each study, we identified and extracted lowest observed adverse effect levels (LOAELs) and compared the responsiveness of health effects to various PCB mixtures using three dose metrics: administered doses, estimated internal doses, and human equivalent doses. Given the lipophilicity and biopersistence of PCBs, internal tissue levels commonly increase with repeated dosing. We addressed this by employing pharmacokinetic models that account for bioaccumulation, which is not reflected in comparisons based on administered dose alone. As such, we found that the apparent sensitivity of specific health effects depends, to some extent, on the dose metrics considered. Taken together with factors such as the strength of evidence available to support dose-response relationships, conclusions of systematic review approaches to PCB exposure and health effects and the relative biological significance of each effect, identification of the effects most sensitive to PCB exposure could provide a basis for prioritizing outcomes for human health risk assessment. Disclaimer: The views expressed in this abstract are those of the authors, and do not necessarily represent the views or opinions of the US EPA.

3287 Guidance Development for Safety Assessment of Food Contact Material Produced from Recycled PET Plastic in Thailand

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Plastics are one of the burden on the garbage management around the world. In order to reduce the waste and to promote circular economy, the use of recycling of plastic should be widely promoted. However, the safety of recycling plastic, particularly PET (rPET) for food packaging is of much concern. The aims of the study were to develop guidance for safety assessment of food contact material made from rPET for the Thai-FDA approval. The safety assessment guidelines of rPET for food contact material made from rPET for the Thai-FDA approval. The safety assessment guidelines of rPET for food contact material made from rPET.
EU were reviewed. Based on the data available in Thailand, Consumption Factor (CF) and food type distribution factor (fT) should be used to estimate the exposure of migrated substances for risk assessment if the packaging factors derived from data in country in order to be able to use, more convincingly, the USFDA’s model. To derive the CF value of IPET, the weight ratio of the daily diet expected to contact a PET packaging to the weight of all food packaged was alternatively determined.

The results from Euromonitor market data and consumption survey showed PET has the CF level of 0.314 and 0.3894, respectively. A CF of 0.0974 rather than 0.3894 derived from consumption survey can be used in estimation exposure since up to 25% of IPET will be used in finish article. Accordingly, the CF of 0.002% and fT could be used to calculate the maximum contaminant concentration of IPET to be at the level of 210 microgram contaminant/kg packaging material by assuming that an individual consumes 3 kg of food per day, and a PET density of 1.31 g/cm³ with thickness of 0.29 mm. For considering the efficiency of PET recycling, the surrogate contaminants testing should be included by using the simulation of various chemical contaminants (a volatile nonpolar organic; a volatile polar organic; a nonvolatile nonpolar organic; and nonvolatile polar organic).

The survey data showed the contamination from consumer misuse may be due to all types of substances such as household cleaners (9.5%); pesticides (1.9%); excreta (1.6%); car maintenance products and gasoline (1.2%); and bath products (0.7%). Another factor needed is the migration testing that represents the concentration of a food contact substance which expected to migrate to food in the daily diet by measuring the levels in food simulants. Finally, if the proposed recycling process cannot be shown to remove contaminations to meet the maximum acceptable contaminant levels under the proposed use, then additional factors or limitations on use may be justified.

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3288 Using Existing Assessments for Problem Formulation in the IRIS Program: A Case Example for the Reassessment of Uranium-Induced Toxicity


The IRIS Program is currently undertaking a reassessment of the noncancer health effects of uranium via oral exposure. The literature review and scientific analysis considered toxicokinetic and toxicodynamic data and biological and behavioral endpoints. The IRIS assessment. The ATSDR derived Minimal Risk Levels (MRLs) for oral exposure to uranium compounds are based on uranium-induced renal, developmental, and reproductive toxicity. The IRIS assessment will examine whether newly available data indicate a need to revise the conclusions for these and consider other potential mode of action (MoA) pathways. A systematic review and synthesis of such evidence based from 2011 to early 2022 was generated to explore newly available data. Epidemiological and toxicokinetic endpoints were screened according to Populations, Exposure, Comparators and Outcomes (PECO) criteria and analyzed to determine whether newly available data indicate a need to update exposure estimations and toxicity values for principal health outcomes from the 2013 ATSDR Toxicological Profile. PECO-relevant studies were evaluated to determine whether they had a “clear study limitation” which would reduce the confidence level in the study for use in an IRIS assessment. Any study not having a clear limitation that met PECO criteria was considered “possibly impactful.” The analysis of whether newly identified studies appeared to alter 2013 ATSDR conclusions (or identified new outcomes to be evaluated) is based on expert judgement by the reviewers. For identified outcomes with new data, the IRIS Program will conduct evidence synthesis across the new studies and the studies cited in ATSDR 2013. This approach leverages existing assessments to develop a literature search strategy and analysis that is focused on outcomes for which there is new relevant evidence that can inform hazard evaluation and dose-response. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

3289 Development of a Database to Derive Inhalation TTC Values for Airborne Compounds

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The threshold of toxicological concern (TTC) concept establishes values below which exposure to a specific compound is low to negligible risk to human health. Several authors have shown that the oral TTC concept, specifically the classification of non-DNA-reactive compounds using the Cramer decision tree, does not lead to robust thresholds for inhalable compounds. Therefore, the inhalation TTC (inhTTC) project aims to define appropriate threshold values for airborne compounds based on structural and physicochemical considerations. For this purpose, an extensive curated TTC database is being constructed comprising high-quality repeated-dose inhalation exposure studies from three databases including RepDose (719 studies), Research Institute for Fragrance Materials (348 studies), and ToxVal (5576 studies). The objective is to present the criteria developed for evaluating the quality of the in vivo inhalation exposure studies for finalizing the database. Approximately 1800 potential preclinical inhalation studies with repeated exposure were first identified from the ToxVal database. Subsequently, studies were reviewed using defined inclusion criteria: repeated-dose studies of a minimum study duration of 28 days in rats, mice or dogs and performed after 1970. These studies were evaluated concerning study conduct and resulting outcomes. Exclusion criteria were also considered and included limited examination scope, such as single dose testing, overall poor documentation, or missing examinations (e.g., haematology or clinical biochemistry). Of the 1800 studies, 1100 met inclusion criteria and can be used to derive the Adult Inhalation TTC (inhTTC) value (inhalTTC). The results from Euromonitor market data and consumption survey showed PET has the CF level of 0.314 and 0.3894, respectively. A CF of 0.0974 rather than 0.3894 derived from consumption survey can be used in estimation exposure since up to 25% of IPET will be used in finish article. Accordingly, the CF of 0.002% and fT could be used to calculate the maximum contaminant concentration of IPET to be at the level of 210 microgram contaminant/kg packaging material by assuming that an individual consumes 3 kg of food per day, and a PET density of 1.31 g/cm³ with thickness of 0.29 mm. For considering the efficiency of PET recycling, the surrogate contaminants testing should be included by using the simulation of various chemical contaminants (a volatile nonpolar organic; a volatile polar organic; a nonvolatile nonpolar organic; and nonvolatile polar organic).

The survey data showed the contamination from consumer misuse may be due to all types of substances such as household cleaners (9.5%); pesticides (1.9%); excreta (1.6%); car maintenance products and gasoline (1.2%); and bath products (0.7%). Another factor needed is the migration testing that represents the concentration of a food contact substance which expected to migrate to food in the daily diet by measuring the levels in food simulants. Finally, if the proposed recycling process cannot be shown to remove contaminations to meet the maximum acceptable contaminant levels under the proposed use, then additional factors or limitations on use may be justified.

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3290 Pathology Risk Profile: Separating Toxicology Findings from Background

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Data analytics in toxicology has largely been hindered by inconsistencies in vocabulary and data structures. The introduction of Standard of Exchange for Nonclinical Data (SEND) controlled terminology (CT) to preclinical toxicity data provides a framework for more robust and accurate data analyses. Because historical studies prior to the SEND framework differed in terminology and structure, we developed a Python code to apply SEND CT to historical data. This has enabled large-scale cross-study statistical analyses of all our preclinical toxicology studies. A major hurdle in interpreting preclinical toxicology studies is differentiating background control findings from treatment and vehicle related effects. Leveraging the wealth of historical data, we developed a tool to elucidate findings that exceed background control incidences. The first strategy calculates odds ratios of histopathology results in treatment vs. control. Odds ratios are also employed to delve into how study duration impacts toxicity. The second approach calculates estimated population incidences to separate background findings from treatment-related effects and to design appropriately powered studies to detect findings of interest. We have created an intuitive visualization framework for population incidences to facilitate cross-study, study design simulation, and treatment vs. vehicle comparisons for new toxicology studies. Furthermore, our novel tool enables study results to be published in context of historical findings for the same species, study duration, and gender to identify findings diverging from historical controls.

3291 Implementation of the Key Characteristics to Optimize Mechanistic Evidence Extraction and Organization for Ethylbenzene

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Since the last U.S. Environmental Protection Agency Integrated Risk Information System (IRIS) Toxicological Review of Ethylbenzene (EB) in 1987, hundreds of new mechanistic studies have been published on EB. Mechanistic studies report measurements related to health outcomes that inform biological or chemical events associated with phenotypic effects in both mammalian and non-mammalian model systems. They use a variety of model systems, including in vitro, in vivo, ex vivo and in silico models. Although these studies have the potential to inform mode(s) of action and support risk assessment decisions through providing evidence for chemical-induced health hazards, identification, organization, and synthesis of such a diverse evidence base can be challenging when many studies are identified. A literature search was performed to identify all the available studies up to the date of the search (January 2022). The literature search identified 375 mechanistic EB studies. Our goal was to employ a method that would efficiently organize and extract the available mechanistic evidence associated with EB-induced human health outcomes. Studies were categorized by health outcome and details were extracted based on study type (e.g., human, animal, in vitro, in silico, or other), human relevance, and study design. Because the "Key Characteristics (KC)" approach provides a systematic method for identifying, organizing, and synthesizing mechanistic evidence, health outcomes were further categorized or mapped in DistillerSR to specific published KCs (e.g., male and female reproductive
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Liver weight changes in chemically exposed animals have long been recognized as sensitive endpoints in toxicological studies. However, their use for the derivation of toxicity values depends on an understanding of biologically relevant levels of absolute liver weight (ALW) and relative liver weight (RLW). Spontaneous positive liver weight changes (RLW) have been identified in rats and mice over the point of departure (POD). To improve this understanding, ALW and RLW values and associated histopathologic findings were extracted from the Chemical Effects in Biological Systems (CEBS) database (National Toxicology Program) for subchronic rodent toxicity studies. Statistically significant ALW and RLW changes at the lowest exposures were determined and analyzed based on their association with histopathologic liver changes. ALW and RLW were found to display positive and negative changes. Positive ALW changes associated with histopathologic changes in rats and mice ranged from 8.4% to 45.5% (median: 17.3%), and the difference between species was not statistically significant (Mann-Whitney two-tailed p < 0.05). Positive ALW changes not associated with histopathologic changes ranged from 6.3% to 31.5% (median 13.8%), and the difference between mice and rats was not significant. Histopathologic changes most frequently reported for ALWs ranging from 11.3% to -26.2% were most frequently associated with inflammatory changes, hepatocyte diaphragmatic nodules, and centrilobular hypertrophy combined with glycogen depletion. Only increased RLWs were associated with histopathologic changes for both rodent species. The RLW changes ranged from 5.8% to 65.7% (median: 14.3%), and the differences between species were not statistically significant. These results indicate biologically relevant ALW and RLW changes that can be used to inform the selection of benchmark responses (BMR) for dose-response modeling of liver weight data. Disclaimer: The views expressed in this document are those of the authors and do not necessarily reflect the views or policies of the US EPA.

There is a growing concern regarding the presence of contaminants or hidden and potentially harmful chemicals in dietary supplements. Recent studies have identified detectable levels of persistent organic pollutants (POPs) in dietary supplements and foodstuffs. The analysis reveals inconsistencies between in vitro and in vivo outcomes which requires further investigations before any conclusion on the human in vivo DNT potential can be drawn. Causal factors could be due to diverging specificity or sensitivity of human and rodent neurosystems, or in vitro test concentrations which do not represent the rodent in vivo situation. In order to focus on the major toxicokinetic factors that could account for the difference, we used an approach to organize and extract mechanistic data that may be employed for use in other chemical risk evaluations. The resulting mechanistic evidence map may help inform human health effects associated with EB exposures and facilitate the identification of data gaps in the literature, and assist in identifying studies that inform biological plausibility and MOA(s). Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

A clear causal link between exposure and developmental neurotoxic effects has been demonstrated for few chemicals and is currently being investigated by several research activities. For agrochemicals, the developmental neurotoxicity assessment is triggered if indications from neurotoxicity or other studies indicate potential developmental neurotoxicity. The data are usually generated in vivo studies, necessary and required by regulatory authorities to assess the safe use. Study designs usually follow published OECD test guidelines (OECD Test No. 426 and 443 with cohorts 2A or 2B). These studies are complex, use a high number of rodent animals and encompass many endpoints and observations. The relatively low number of studies over the last decades, the heterogeneity of test designs and dependence on expert knowledge for the data interpretation creates uncertainties which however could be addressed by methodological improvements and globally harmonization of data descriptions and assessments. Nevertheless, the in vivo data provide valuable information from physiologically intact mammalian models and is considered the most relevant variability-towardxicological variation for estimating in vivo toxicokinetic and toxicodynamic considerations command to limit vertebrate testing, particularly if it is to get only a first indication of the hazard profile or to screen a large number of chemicals for which few data exist. Therefore, the Organisation for Economic Co-Operation and Development (OECD) has established a working group that drafted a guidance on the Evaluation of Data from a Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (IVB) that was built from several human in vitro test methods developed by the intensive work of several academia groups and applied to 119 chemicals (Masjosthumann et al, 2020, EFSAs External Scientific Report). In this project, we collected and compared this and other publicly available DNT human in vitro data for several agrochemicals with available in vivo data from rodent studies. The analysis reveals inconsistencies between in vitro and in vivo outcomes which requires further investigations before any conclusion on the human in vivo DNT potential in an individual substance assessment should be drawn. Causal factors could be due to diverging specificity or sensitivity of human and rodent neurosystems, or in vitro test concentrations which do not represent the rodent in vivo situation. In order to focus on the major toxicokinetic factors that could account for the difference, we used an approach to organize and extract mechanistic data that may be employed for use in other chemical risk evaluations. The resulting mechanistic evidence map may help inform human health effects associated with EB exposures and facilitate the identification of data gaps in the literature, and assist in identifying studies that inform biological plausibility and MOA(s). Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.

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p-isopropyltoluene), sec-butyl groups were also accepted, because they have active benzyl positions analogous to isopropyl groups. The carbon atoms at the benzyl positions are either primary (methyl) or tertiary (isopropyl or sec-butyl), because radicals can form at these positions. Analogues that can form similar radicals at a benzyl position were included, since radical formation may be part of the biological activity of p-isopropyltoluene. The benzyl positions are sites for metabolic transformations, and the substitution patterns at the benzyl position can influence the metabolites formed. Toluene, ethylbenzene and ethyl-substituted benzenes were excluded as analogues due to expected differences in their metabolic pathways relative to p-isopropyltoluene, specifically greater metabolism by ring oxidation pathways and formation of more epoxides and phenols. In contrast, the primary metabolic pathway for p-isopropyltoluene involves extensive oxidation of the methyl substituent and isopropyl side chain. Ring oxidation products are not formed in large quantities. Xylenes were not identified as candidate analogues using the structural similarity tools. Based on expert judgment, they were added as potential analogues considering their similarity to p-isopropyltoluene in terms of both structure (simple aromatic ring with up to two methyl or isopropyl substituents) and metabolism (primary pathway is oxidation of the methyl substituents). The refined list of candidate analogues included 10 substances, two with inhalation toxicity values: isopropylbenzene and xylene (mixed isomers). Comparisons of limited available toxicity data for p-isopropyltoluene with the two candidate analogues were performed to understand potential toxicodynamic similarities/differences among this group of chemicals and evaluate the suitability of analogues for read-across. This case study demonstrates the importance of expert judgment, both in narrowing the list of candidates from computational tools to those most likely to produce similar outcomes to the target chemical, and in supplementing that list to include other relevant candidate analogues.

3298 Challenges to Identifying Candidate Analogue Compounds in Read-Across Assessment for Compounds with Simple Molecular Structures: Case Study with Dimethyl Sulfide

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Read-across assessments of compounds lacking adequate data begin with identification of appropriate chemical analogues. The standard approach for the identification of candidate analogues developed by Wang et al. (2012) utilizes automated tools (e.g., ChemiDPlus, CompTox Chemicals Dashboard, OECD QSAR Toolbox) to generate an initial list of candidate analogues based on structural similarity scores. However, these tools are not well-suited to identify analogues for compounds with very simple molecular structures. We present a case study utilizing an expanded approach to identify candidate analogues for a compound with a simple structure, dimethyl sulfide (DMS). Using the standard automated tools, a total of 44 unique candidate analogues for DMS were identified. Their similarity scores to DMS (1,3-dithiane; 1,1bis(methylthio)ethane) met the following structural criteria for a suitable analogue of DMS developed by a chemist with expertise in read-across: 1) Exclude radicals, nucleonides, and deuterated compounds, 2) Exclude compounds with elements other than carbon, hydrogen, and sulfur, and 3) Exclude compounds with molecular weight greater than twice the molecular weight of DMS. Because neither of the two potential analogues identified using structural similarity scores has an inhalation toxicity value, a strategy was developed to identify additional candidate structural analogues for DMS. U.S. EPA, ATSDR, and Cal/EPA toxicity value databases were searched for the terms “sulfide” and “mercaptan,” applying the same exclusion criteria used for automated tools. Potential analogues identified by this method were hydrogen sulfide, carbon disulfide, and carbonyl sulfide. Carbon disulfide and carbonyl sulfide were not considered suitable because they are sulfur analogues of carbon dioxide and differ from DMS in that they have sulfur double-bonded to carbon. Both compounds are reactive and undergo metabolic transformations that are not relevant to DMS. Hydrogen sulfide was not a suitable candidate structural analogue of DMS and has an inhalation toxicity value. Hydrogen sulfide, however, lacks the methyl groups of the DMS molecule. To provide additional comparative toxicokinetic and toxicodynamic information pertaining to the influence of the methyl groups, two additional structural analogues were selected: "comparative" candidate structural analogues, even though they do not have inhalation toxicity values: methyl mercaptan (which has one methyl group on the sulfur) and dimethyl sulfide (two methyl groups and two sulfur atoms). Dimethyl sulfide (DMSO), the primary metabolite of DMS, was also included as another "comparative" candidate analogue with two methyl groups. Inclusion of the "comparative" analogues informs risk management by providing supporting evidence for selection of hydrogen sulfide as an appropriate analogue for DMS. The critical effect for hydrogen sulfide, the only candidate analogue with an inhalation toxicity value, is nasal lesions (basal cell hyperplasia, olfactory neuronal loss). Among the "comparative" candidate analogues lacking toxicity values for DMS (DMSO, dimethyl disulfide) also produced nasal lesions following repeated inhalation exposure, suggesting that nasal tissues may be a sensitive target for this group of compounds. Nasal cavity toxicity data were not located for methyl mercaptan, but pulmonary effects were observed, reinforcing the suggestion that respiratory tissues may be a sensitive target for these chemicals. The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the US EPA.
and neurotoxicity. Worker studies involve adult individuals where they are exposed to unknown amounts of suspected ototoxic substances along with other substances in a may or may not be ototoxic. Ethylbenzene (a volatile organic compound, VOC) and cadmium (a metal) have been found to target the outer hair cells of the Organ of Corti. Study data for these substances individually are rich. Exposure to both could potentiate this ototoxic effect. In addition to cadmium, cigarettes contain nicotine, which is also ototoxic to hair cells—suggesting possible potentiation for ethylbenzene-exposed smokers. Many VOCs, like N-hexane, have exposure studies that are not designed for discerning between ototoxic or neurologic effects. Styrene has been widely found to cause hearing loss because of structural modification of the inner ear membrane. In contrast, no study data point to the pathway of damage. Further, a repeated dose inhalation study for hexachloroethane indicated a NOEL for exposures lasting 15-364 days. Using this risk-based screening assessment framework, when temporal variability is incorporated into a screening assessment, risk estimates are more valid and likely lower than when default methods are applied. That when temporal variability is incorporated into a screening assessment, risk estimates are more valid and likely lower than when default methods are applied.

The present work, we combine these methods in an iterative decision tree with clear concise steps that guide one through the process using ammonia case as an example, which demonstrated that mice and rat studies can be used together very effectively. The first step in the meta-analysis is a test for homogeneity of the data. Then upon the result of the first step, the analysis branches into a categori- cal regression analysis subtree and distribution of effects subtree (that includes random-effects branches). If meta-analysis is applied to more than two combined studies and they are heterogeneous, the procedure may require iterative repetition after leave-one-study-out dataset reduction, as illustrated by the ammonia example, which demonstrated that mice and rat studies can be used together effectively. Branching of the binary decision tree is determined by statistical tests at the nodes. These include a goodness-of-fit test for parallel slope planes, Cochran’s Q test on statistics. In summary, the proposed decision tree provides straightforward methodology of conducting a meta-analysis for the TLE of airborne agents and enhances scientific understanding of concentration-dura- tion relationship of short-term inhalation exposures. This decision tree is expected to help to increase precision and scientific rigor to public health assessments of adverse health effects caused by short-term exposures to hazardous substances that, when applied to airborne toxicants, also require iterative repetition after leave-one-study-out dataset reduction, as illustrated by the ammonia example.

While health risk assessment frameworks have existed for almost four decades, most are based on relatively simple paradigms in which exposures are assumed to not vary substantially over the lifecycle of the industry being assessed. Unconventional oil and gas development (UOGD) offers a clear example of a situation that involves multiple phases with different exposure intensities that may vary from hour to hour, day to day, and year to year. Because of the varying durations of the phases and varying intensities of emissions, it is essential to consider whether exposures are acute, sub-chronic or chronic, and identify the proper health risk benchmark. For example, applying health risk benchmarks for chronic health risks to the brief preproduction phases of UOGD would produce a demonstrably overestimated risk for the population. We provide here a framework to help characterize risk that accounts for temporal variability and changes in the concentration and composition of emissions over time and distance from the source. Using benzene, a chemical constituent that potentially poses both noncancer and cancer risk, and publicly available 45-minute duration ambient air concentration measurements taken by the Colorado Department of Public Health and Environment (CDPHE) near an UOGD operation located close to the Bella Romero Academy 4-8 school campus in Greeley, Colorado, we developed a risk assessment framework. When individual benzene concentration measurements were compared to a 9 ppb “acute” duration risk benchmarks meant to be applied for durations of 1-14 days (Agency for Toxic Substances and Disease Registry (ATSDR) acute-duration risk benchmarks exceeded the acute duration MRL once, with other measures approaching this value. However, when the multiple 45-minute measurements were combined to calculate daily average benzene concentrations to compare with the acute duration MRL, these daily average concentrations were well below 9 ppb. Similarly, CDPHE compared pre-pro- duction phase average concentrations to the ATSDR chronic duration MRL of 3 ppb. However, CDPHE found no evidence of chronic health risk.

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damage to the ileum including ulceration and inflammation of villi adjacent to a thinned submucosa. A significant decrease in thickness of the muscularis externa was also observed in both ileum and colon. This was associated with shortening and fragmentation of elastin fibers in the submucosa. Goblet cells were larger and more acidic in the ileum of NM exposed rats when compared to control. Expression of mucin-2, a gel-forming glycoprotein associated with intestinal protection and integrity, was upregulated in the villi and crypts of NM-exposed rats. NM exposure to bile acids, which are known to exert hormone-like functions via activation of nuclear and membrane-bound receptors, can modulate intestinal integrity, and immunity. NM was found to markedly reduce total serum bile acids (65.73 ±15.65 µM/L and 19.19±3.51 µM/L for control and NM treated rats, respectively). These data demonstrate that NM causes damage to the ileum, villi and crypts. Combined with alterations in bile acid homeostasis, this likely impacts normal intestinal function. Further studies to determine the mechanism of vesicant induced damage to the gut are essential for the development of effective countermeasures against GI toxicity. Supported by NIH U54AR055073, ES020721 and ES005022.

3303 Long-Term Effects of Sulfur Mustard following Whole-Body Exposure of Rats

Exposure to sulfur mustard (SM) may be devastating causing severe multi-organ injuries that take very long time to heal and often result in long-lasting pathologies. SM was used in many conflicts around the world including recently in Syria. The high incidence of pathologies that were observed has led to the need for studying SM-induced long-term effects. The current study aimed to assess the multi-organ late pathologies induced by whole body exposure to SM vapor in order to identify potential treatment strategies. Groups of rats were exposed to SM vapor in a whole-body exposure system. Air containing SM flowed through the chamber containing rats and the chamber was continuously monitored using FTIR. Clinical evaluation including body weight and clinical severity score, blood counts, monitoring of respiratory function and histological evaluation were performed during six months following exposure. All rats developed typical SM acute intoxication symptoms such as excessive rhinorhea, ocular inflammation, ronchi and wheezing, breathing difficulties and weight loss. There was a 50% reduction in spleen weight within one week of exposure and a significant transient decrease in the number of lymphocytes in the blood indicating possible involvement of the immune system. Following the peak of the acute injury, there was a gradual healing process and within 4-5 weeks rats returned to baseline weight and most of the clinical symptoms were resolved. Two months following the exposure, reduced body weight and reduced breath rate compared to the control rats up to six months as well as long-lasting corneal and pulmonary damage. The long-term pathologies following whole body exposure, that were demonstrated, for the first time in an animal model, will be the targets for future research aiming at identifying potential treatments to prevent or ameliorate these pathologies.

3304 Role of the Farnesoid X Receptor (FXR) in Regulating Macrophage Cholesterol Homeostasis following Pulmonary Exposure of Rats to Nitrogen Mustard Injury
J. A. Meshanin, B. Sun, K. Vayas, G. Guo, A. J. Gow, J. D. Laskin, and D. L. Laskin, Rutgers, The State University of New Jersey, Piscataway, NJ.

Nitrogen mustard (NM; bis(2-chloroethyl)methylamine) is a cytotoxic vesicant known to cause pulmonary injury that can progress to fibrosis. This is associated with an accumulation of inflammatory macrophages in the lung, which have been implicated in NM toxicity. Farnesoid X Receptor (FXR) is a nuclear receptor important in bile acid and lipid homeostasis. It has been identified as a major regulator of cholesterol metabolism, which plays a central role in the development of profibrotic foam macrophage formation and dyslipidemia. To explore mechanisms underlying this response, we assessed macrophage mRNA expression of the lipid uptake receptor CD36 and the cholesterol synthesis protein SREBP-2. NM upregulated gene expression for both of these proteins. NM also caused an increase in total and free intracellular cholesterol in lung macrophages, and an increase in cholesterol efflux. Treatment of rats with OCA mitigated the effects of NM on macrophage foam cell formation; this was associated with decreases in CD36 and SREBP-2 and normalization of cholesterol levels and cholesterol efflux. This was linked to reduced fibrosis in the lung. These findings demonstrate that FXR plays a role in preventing profibrotic foam cell formation following NM exposure by limiting cholesterol accumulation in lung macrophages. These data suggest that targeting FXR for activation may represent an effective therapeutic approach for preventing the development of pulmonary fibrosis after exposure to mustard vesicants. Supported by NIH grants AR055073, ES005022, and T32ES007148.

3305 Nitrogen Mustard Impairs Bioenergetics and Airway Function in Precision-Cut Lung Slices
A. Bellomo, J. Herbert, M. J. Kudlik, A. J. Gow, J. D. Laskin, and D. L. Laskin, Rutgers, The State University of New Jersey, Piscataway, NJ.

Nitrogen mustard (NM, mechloroethamine hydrochloride) is a cytotoxic alkylating agent that causes acute lung injury which progresses to chronic disease. Injury is characterized by DNA damage, oxidative stress, and inflammation. Previous studies have demonstrated that inflammatory macrophages contribute to NM-induced lung injury, however, little is known about the role of resident lung cells in initiating the pathogenesis. In the present study, isolated lung macrophages (PCLS) were used to investigate functional and metabolic responses in resident pulmonary cells following exposure to NM. PCLS are viable slices of lung tissue, which preserve lung architecture, maintain function, and can be directly exposed to toxicants. To prepare PCLS, C57BL/6 mice were euthanized and intratracheally instilled with low-melting point agarose. Lungs were isolated and lobes cut into 300 µm slices using a tissue slicer. One day later, PCLS were exposed to NM (50 µM) or CTL (culture medium) for 1 h. Airway contractility and cellular bioenergetics were measured 1 d later. Airway contractility was assessed in cross-sectional airways incubated with increasing doses of the bronchoconstrictor, methacholine (MCh), by video-microscopy. NM blunted MCh-induced airway contraction by 18 ± 2.6% and 25 ± 3.1% at the higher doses of MCh (10 4 and 10 5 M, respectively). We next utilized an Agilent Seahorse to investigate the effects of NM on glycolytic function (extracellular acidification rate, ECAR) following sequential treatment of PCLS with glucose, oligomycin and 2-deoxyglucose and mitochondrial respiration (oxygen consumption rate; OCR) after treatment with the mitochondrial uncoupler, FCCP and the antioxidants, SOD and catalase. No glycolytic function and mitochondrial respiration were suppressed following NM exposure. Thus, basal glycolytic activity and maximal glycolytic activity were reduced, along with basal respiration and maximal respiration. These results demonstrate impaired pulmonary function and bioenergetics in resident lung cells after NM exposure. This may contribute to inflammatory cell accumulation in the lung and the development of long-term pathology. Supported by NIH Grants AR055073 and ES005022.

3306 Precision-Cut Lung Slices in Acetylcholinesterase Reactivator Research: Comparison of Colorimetrically Determined AChE Activity and Airway Responsiveness
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The recent use of organophosphorus nerve agents (OPNA) highlights the need for effective countermeasures. OPNA exposure renders acetylcholinesterase (AChE) inactive, resulting in an accumulation of acetylcholine (ACH). The subsequent cholinergic crisis results in bronchoconstriction due to overstimulation of muscarinic receptors. Precision-cut lung slices (PCLS) are a suitable test system to investigate airway responsiveness and AChE activity after OPNA exposure ex vivo. Furthermore, PCLS help to reduce the numbers of animal experiments and promote the 3R concept. The current study investigated AChE activities in OPNA-exposed and reactivator-treated PCLS and correlates the AChE activity to the respective airway responsiveness. PCLS were prepared from lungs of male Wistar rats. After five washing steps, PCLS were stored in an incubator overnight and used the next day. AChE activity in intact PCLS was determined by a modified colorimetric Ellman assay. For this purpose, four PCLS were pinned together in the middle of a well and were then exposed to cyclosarin (GF), sarin (GB), or VX (all 1 µM) for 10 minutes. To ensure a poison-free environment a phosphotriesterase was added after OPNA exposure. As reactivators Hi-6, oxiboxide (OBI) (both 30 µM), or NOX-6 (100 µM), an experimental non-oxime reactivator, were studied. Ellman reagent and acetylthiocholine (ATCh) were added to analyze AChE reactivation for 30 minutes at 37°C. The inhibition and reactivation rates were calculated and related to the AChE base activity. Airway area changes were observed by video microscopy with an inverted microscope every 5 minutes in an experimental setup comparable to the AChE activity measurements. The initial airway area (IAA) was set as 100%, then compared to the airway area (AA) after exposure to OPNA and treatment. Despite low activity of AChE in PCLS, AChE activity could successfully be determined for their responses. PCLS were exposed to four intact PCLS. Exposure of PCLS to GB, VX, or GF caused an almost complete inhibition of the AChE activity (≤ 12±2%) and a very similar massive decrease of the airway areas (≤ 13±4%). The AChE reactivation after GB exposure was highest with OBI (83±6%) and Hi-6 (64±1%). For VX-inhibited AChE an experimental non-oxime reactivator (NOX-6) was investigated as well, due to previous promising human in vitro data (de Koning et al. DOI: 10.1016/j.jemch.2018.08.016). Reaction data were as follows: OBI 71% > NOX-6 53±4% ≥ Hi-6 51±1 suggesting that NOX-6 reactivation data was comparable to the reactivation of VX-inhibited AChE by Hi-6. AChE activity was lowest for GF-inhibited AChE reactivated with OBI (13±3%) and only slightly higher with Hi-6.
investigated the role of SP-D in Cl₂ lung. Surfactant protein D (SP-D) is a pulmonary collectin that functions to post H₂S comprehensive untargeted metabolic profiling at 5 min, 24 h, 72 h, and 28-day injury and mortality of this highly toxic gas. Supported by NIH Grants U54AR055073 of Cl₂ injury, oxidative stress and inflammation. Exposure of WT mice to Cl₂ analysis of H₂S exposure using GC-QTOF, HILIC-MS/MS, and CSH-MS/MS. Pathway Chlorine (Cl₂) gas is a cytotoxic pulmonary irritant that causes acute damage to the lung. Surfactant protein D (SP-D) is a pulmonary collectin, that functions to dampen macrophage inflammatory responses to lung injury. In these studies, we investigated the role of SP-D in Cl₂-induced lung injury and inflammation. Male WT and SP-D knockout (SP-D⁻/⁻) mice were exposed to air or Cl₂ (300 ppm, 40 min) in a whole-body exposure chamber. Bronchoalveolar lavage (BAL), alveolar macrophages, and lung tissue were collected 24 h later and analyzed for markers of injury, oxidative stress and inflammation. Exposure of WT mice to Cl₂ resulted in upregulation of heme oxygenase (HO)-1 and catalase, markers of oxidative stress. This was associated with lung inflammation as evidenced by increases in numbers of BAL cells and levels of fibrinogen, soluble receptor for glycation end product (sRAGE) and matrix metalloproteinase (MMP)-9 in BAL. Lung macrophage mRNA expression of IL-1B and the neutrophilic chemokine CCL3 were also increased, along with numbers of macrophages expressing cyclooxygenase (COX)-2. Whereas loss of SP-D had no effect on Cl₂-induced oxidative stress, BAL cells and levels of fibrinogen, sRAGE, and MMP-9, along with CD11b⁺ inflammatory cells in lung tissue, increased. BAL levels of IgM, a marker of alveolar endothelial barrier dysfunction also increased after exposure of SP-D⁻/⁻ mice to Cl₂. Conversely, IL-1B and CCL3 mRNA expression decreased. These changes were linked to a significant reduction in arginase positive anti-inflammatory macrophages in the lung. These data demonstrate that SP-D plays a central role in regulating Cl₂-induced inflammatory response and injury. Mechanistic understanding of the pulmonary toxicity of Cl₂ will be important in identifying efficacious approaches for mitigating morbidity and mortality of this highly toxic gas. Supported by NIH Grants U54AR055073 and P30ES005022.

3307 Role of Surfactant Protein D in Chlorine-Induced Lung Injury and Inflammation R. Malavigna, R. C. Rancourt, E. Abramova, K. Vayas, A. J. Gow, J. D. Laskin, and D. L. Laskin. Rutgers, The State University of New Jersey, Piscataway, NJ.

Chlorine (Cl₂) is a cytotoxic pulmonary irritant that causes acute damage to the lung. Surfactant protein D (SP-D) is a pulmonary collectin, that functions to dampen macrophage inflammatory responses to lung injury. In these studies, we investigated the role of SP-D in Cl₂-induced lung injury and inflammation. Male WT and SP-D knockout (SP-D⁻/⁻) mice were exposed to air or Cl₂ (300 ppm, 40 min) in a whole-body exposure chamber. Bronchoalveolar lavage (BAL), alveolar macrophages, and lung tissue were collected 24 h later and analyzed for markers of injury, oxidative stress and inflammation. Exposure of WT mice to Cl₂ resulted in upregulation of heme oxygenase (HO)-1 and catalase, markers of oxidative stress. This was associated with lung inflammation as evidenced by increases in numbers of BAL cells and levels of fibrinogen, soluble receptor for glycation end product (sRAGE) and matrix metalloproteinase (MMP)-9 in BAL. Lung macrophage mRNA expression of IL-1B and the neutrophilic chemokine CCL3 were also increased, along with numbers of macrophages expressing cyclooxygenase (COX)-2. Whereas loss of SP-D had no effect on Cl₂-induced oxidative stress, BAL cells and levels of fibrinogen, sRAGE, and MMP-9, along with CD11b⁺ inflammatory cells in lung tissue, increased. BAL levels of IgM, a marker of alveolar endothelial barrier dysfunction also increased after exposure of SP-D⁻/⁻ mice to Cl₂. Conversely, IL-1B and CCL3 mRNA expression decreased. These changes were linked to a significant reduction in arginase positive anti-inflammatory macrophages in the lung. These data demonstrate that SP-D plays a central role in regulating Cl₂-induced inflammatory response and injury. Mechanistic understanding of the pulmonary toxicity of Cl₂ will be important in identifying efficacious approaches for mitigating morbidity and mortality of this highly toxic gas. Supported by NIH Grants U54AR055073 and P30ES005022.


Hydrogen sulfide (H₂S) is a potent toxic gas, and humans are typically exposed by inhalation. It is a mitochondrial toxin known to inhibit cytochrome c oxidase. The lungs, heart, and brain are its primary target organs. In the lungs acute H₂S exposure induces pulmonary edema and acute respiratory distress though the underlying mechanisms are not well known. We hypothesized that H₂S causes lung injury by altering lung metabolism and that this alteration will be reflected in changes in lung and serum metabolome. To test this hypothesis, C57BL/6J mice were exposed once to 2 or 20 ppm H₂S for 60 min) in unanesthetized adult C57BL/6J mice caused extensive airway injury and epithelial sloughing. Chlorine exposure caused basal cell shedding in the airway lumen and decreased airway basal cell TF expression. TF expression and activity were increased in the BALF after chlorine exposure, further suggesting loss from the trachea. To understand the role of TF we utilized low-TF expressing mice. Low TF-expressing mice had fragile airway epithelium from increased basal cells in the BALF. Respiratory mechanics analysis of low TF expressing mice demonstrated altered PV loops and increased overall resistance. Low TF-expressing mice were found to be susceptible to chlorine-induced airway injury and mortality. These mice had increased systemic hemorrhage after chlorine inhalation, and rotational thromboelastometry (ROTEM, FIBTEM) measurements revealed significant coagulopathy after chlorine exposure. Recombinant TF (rTF) supplementation in the lungs improved BALF return and reduced loss of BCs from the airways. The rTF-treated mice had reduced airway obstruction and improved tissue oxygenation after chlorine inhalation as observed by pulse oximetry. Therefore, TF expression in BCs is required to maintain airway epithelial integrity and survivability after inhalation of toxic halogen gases.

3310 Model Development of HD-Induced Lung Toxidrome in Swine Model H. Mayfield¹, P. Anantharaman¹, A. Hunziger¹, K. Forsee¹, E. Peters¹, T. O'Neill¹, T. Hendry-Hoyer, V. Berbarie¹, and B. J. Day². ¹MRIGlobal, Kansas City, MO; ²University of Colorado Anschutz Medical Campus, Aurora, CO; and ³National Jewish Hospital, Denver, CO.

Sulfur M mustard (HD) is a vesicating chemical agent created during World War I that is still stored by many countries for use in chemical warfare. Inhalation of HD at high concentrations causes devastating pulmonary injury leading to the formation of airway obstructing fibrin casts, often leading to respiratory failure and death. No therapeutic currently exists to treat the effects of HD injury. In an effort to close this gap, MRIGlobal has developed an intratracheal HD vapor exposure model in Yorkshire pigs. Because of the similar size and physiology of their lungs to humans, swine have been increasingly used as a model for studying human respiratory disease, including the effects caused by HD inhalation. The goal of this study was to identify the HD dose that caused extensive lung injury in Yorkshire pigs and produced 70%-100% lethality over 48h. This was accomplished by using an up-down dose range finding study. In order to cause extensive lung injury, the dose needed to cause fibrin cast formation and severe clinical respiratory signs. Following exposure to HD, animals were monitored continuously for clinical signs and vital signs and once terminal, bronchoalveolar lavage fluid was collected from the right middle lobe to measure protein in the fluid which is indicative of damage. Following lavage, the rest of the lungs were fixed and dissected to identify cast formation. This effort resulted in a model for HD vapor exposure in Yorkshire pigs that reproducibly causes exposed animals to succumb to an HD challenge of 60 mg/kg within 24 hours. Development of this model is essential to the success of future studies aimed at testing the efficacy of medical countermeasures against HD-induced pulmonary toxicity.


Sulfur mustard (SM) is a potential warfare agent which has been used in the past. Inhalation of SM causes acute lung injury and systemic injury leading to mortality and morbidity. Mechanisms by which this injury progress is still not very clear. Previously, we demonstrated that inhalated exposure of rats to 2 chloroethyl ethyl...
sulfide (CEES), an analog of SM, caused the release of extracellular nucleic acids in the blood and airway lumens, leading to increased inflammation, barrier dysfunction, and hypercoagulation. Growing evidence suggests that several such factors and damage-associated molecular patterns are carried as cargo in exosomes a type of extracellular vesicles (EV). The significance of EVs lies in their ability to transfer such key mediators or cargo (e.g., DNA, proteins, lipids, mRNAs, and miRNAs) to other cells, thereby influencing recipient cell function. The present study was undertaken to characterize the proteomic content of exosomes during the progression of disease following CEES exposure. Rats were exposed to CEES (10% in ethanol) or ethanol alone via nose-only aerosol inhalation. Animals were sacrificed after 10 h, and 24 h, and blood was collected. EVs were isolated from platelet-poor plasma (PPP) using size exclusion chromatography and analyzed using NanoSight, cryo-electron microscopy to confirm identity. The EVs were further verified by exosome markers: CD63 and TSG101 by western blots. There was a significant increase in EVs in the CEES-exposed animals compared to the control group. This increase persisted at a later time of the injury. EVs from PPP of CEES-exposed animals, which were added to airway epithelial cells, increased mRNA levels of IL-1α, IL-6, CCL-2, CXCL-1, and TNF-α in a dose-dependent manner. EVs from CEES-exposed animals also increased inflammatory cytokines in endothelial and alveolar epithelial cells. Proteomic profiling of exosomes was also carried out using mass spectrometry and analyzed using Glucore and Ingenuity pathway analysis (Qiagen). These results indicate that EVs derived from CEES-exposed animals are pathogenic and that targeting them could potentially limit injury.

3312 Lung Toxicity from Vescicating and Nettle Agent Phospho Oxime in Rodents


Phospho Oxime (CX; dichloroforomoxime), an urticant categorized with vesicating agents, is of special interest as a chemical threat agent due to its high penetrative property through clothing and immediate toxic effects. CX exposure affects the eyes, skin, and lungs causing immediate irritation, injury, and systemic toxic effects. Previous studies have shown that CX exposure causes instant upper respiratory irritation and sinus pain at low doses. Higher doses of CX exposure could result in pulmonary edema, dyspnea, fibrosis, necrotizing bronchiolitis, thrombosis of pulmonary venules, and mortality. However, the molecular mechanisms of CX toxicity are understood hampering the development of targeted treatments. In order to understand mechanism of injury of CX, we have studied two injury models: dermal CX exposures to SKH-1 hairless mice (10 ul CX exposure at 0.5 or 1.0 min timepoints using two 12 mm vapor caps on the dorsal skin at MIRGlobal), and direct inhalation of CX aerosol exposures to Male Sprague Dawley rats (1.0 or 2.5mg/min/m3 CX exposure at 10-30 min timepoints at MRIGlobal). SKH-1 mice were euthanized at different time intervals after CX exposure and lung tissue was harvested and fixed for histopathological analysis. Male Sprague Dawley rats were euthanized at different time intervals after CX exposure and bronchoalveolar lavage (BAL) fluid was aspirated for collection. We have shown that dermal CX exposure in SKH-1 mice causes acute skin lesions and reduction in physiological parameters such as body weight, temperature. Mortality occurred at higher exposure duration. We have also shown that dermal CX exposure causes aggregation of RBCs in alveolar capillaries of the lungs. Histopathological analyses of the mouse lung tissue showed acute hemorrhage and tissue loss that appeared to progress over time after CX exposure. Extensive coagulation and loss of parenchyma was observed in lung tissues from mice at 8h post 1.0 min CX exposure. Thrombosis in lungs was further confirmed by increased mRNA expression of proinflammatory cytokines TNF-α, IL-6, and IL-18. In the rat model, differential counting of the BAL fluid using Giemsa staining showed hemorrhaging and increased levels of neutrophils, indicating inflammation. qPCR analysis on BAL fluid showed increased mRNA expression of IL-1β upon exposure to 2.5g/min/m3 CX compared to controls. Histopathological analysis of rat lung tissue showed that CX induced necrotizing bronchiolitis with intramural edema and hemorrhaging in submucosa. A marked exfoliation of airway epithelial cells and mixed inflammatory cell infiltration was also observed upon exposure to 2.5g/min/m3 CX. Toluidine blue staining showed an increased degranulation of mast cells in the lung tissue after CX exposure. Apoptotic cell death was evident by TUNEL staining was observed upon CX inhalation exposure in the lung tissue. Together these results indicate a role of inflammation, inflammatory cytokines, and mast cells in CX-induced lung toxicity.

3313 Adaptive T Cell Responses in Constrictive Bronchiolitis Mediated by a Single Vescicant Exposure


Background and Aim: Arsenicals are a class of ‘war-threat’ agents known as vesicants. Stockpiles of weaponized arsenicals-based vesicants developed for World War II still exist. Exposure to these vesicants may occur accidently or by deliberate release. The exposure to the skin causes severe local blistering and inflammation in dinte organs, such as the lung, causing injury and respiratory failure. There are few reports of deleterious systemic effects after exposure and progress to multi-organ failure and death. However, there are no animal models to study these possible effects. This study aimed to develop and characterize an animal model to study pulmonary injury after single cutaneous exposure. We have induced lung injury in mice. Methods: The delayed effect of single cutaneous exposure to various arsenicals on lungs was studied at 20 weeks post-exposure. For the study of chronic lung injury induced by vesicant injury by lewisite resulted in airway constriction. Respiratory system mechanics following increasing methacholine challenge in arsenical exposed mice were performed using FlexiVent, with signatures of activated adaptive T-cell response. Results: Cutaneous lewisite burn causes delayed effects by adaptive T-cell activation in the lung and the development of constrictive bronchiolitis.

3314 Phosphorylation and Subcellular Location of NMDA and GABA Receptor Subunits following Sarin Surrogate Exposure and Novel Oxime Therapy

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Nerve agents are proposed to induced seizures that are initially governed by hyperstimulation of the glutamatergic system followed by hyperstimulation of the glutamatergic system, which is the main excitatory system in the brain. Hyperstimulation of the glutamatergic system, solely attributed to N-methyl-D-aspartate (NMDA) receptors, leads to excitotoxicity. Excitotoxicity has been shown to be the basis of long-term cognitive and behavioral impairments seen in the surviving victims of the sarin Tokyo subway attack. In addition, there is evidence of reduced or inactive a-amino butyric acid (GABA) receptor activity following cholinergic-induced status epilepticus. This imbalance between excitatory and inhibitory systems allows for OP-induced seizures to become self-sustaining and non-respon-sive to traditional anti-convulsants. Because phosphorylation is a known post-translational mechanism of signal transduction, cell surface membrane expression and neurotransmission processes, we investigated the phosphorylation and subcellular location of important NMDA and GABA receptor subunits in critical brain regions of seizure initiation (piriform cortex and hippocampus) using an atropinized rat model. In this study, rats were challenged with a sarin surrogate nitrophenyl isopropyl methylphosphonate (NIMP, 0.325 mg/kg, s.c.) and patented novel oxime therapy (146 µmol/kg, i.m.) (US Patent 9,227,937) and then euthanized 3 hours post-NIMP challenge. Results using western blot techniques suggested that NIMP increased phosphorylation of NMDA receptor subunit NR1 but decreased GABA (A) receptor β (2+X 3) subunit in membrane fractions. Rats challenged with NIMP and given novel oxime therapy resulted in opposite effects when compared to NIMP only challenged rats. This suggested both NIMP and novel oxime therapy could be modulating phosphorylation. In addition to our novel oximes ability to enter the brain, reducing time to cessation of seizure like-activities, and attenuating OP-induced neuropathology, influencing phosphorylation of excitatory and inhibitory receptor subunits could be an additional neuroprotective mechanism. Supported by NIH U01NS107127 and U01NS122355.

3315 A Comparison of Pharmacokinetic Parameters between the FDA-Approved Treatment for Organophosphate Exposure, 2-PAM, and a Novel Substituted Phenoxylpyridinium Oxime

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Organophosphates are highly toxic acetylcholinesterase inhibitors that have the ability to be engineered as dangerous agents posing a global threat to our livelihood and well-being. The current approved treatment after exposure to these agents is the acetylcholinesterase reactivator, pralidoxime iodide (2-PAM), and the muscle relaxant neostigmine. However, 2-PAM does not restore brain function as it is minimally able to cross the blood brain barrier (<10%). Our laboratory has developed, and patented, novel substituted phenoxylpyridinium oximes (US patent 9,277,937) that have displayed improved efficacy for survival of lethal doses of organophosphate exposure in laboratory rats in vivo and reactivation of inhibited acetylcholinesterase across the blood brain barrier. The lower efficacy of 2-PAM may be associated with its pharmacokinetic properties and rapid clearance as compared to our novel oximes. The established half-life and T1/2 of 2-PAM are approximately 2.9 hours and 0.63 hours, respectively, in humans, and approximately 0.7 hours and 0.27 hours, respectively, in rats (as indicated in the literature). Our lead novel oxime (Oxime 20) has displayed a half-life and T1/2 of approximately 10 hours and 0.08 hours, respectively, in rats using our new delivery vehicle (90% water/10% benzyl alcohol). Furthermore, our laboratory has begun pharmacokinetic studies of Oxime 20 in Gottingen minipigs and thus far have displayed a half-life and T1/2 of approximately 6 hours and 0.08 hours, respectively. The faster T1/2 and longer half-life of our lead oxime may contribute to its survival efficacy in vivo in laboratory rats both by itself or in combination with 2-PAM. Therefore, our lead novel oxime has promise as a new and potentially improved treatment regimen...
The main action mechanism of organophosphorus compounds (OP) is the inhibition of acetylcholinesterase (AChE) that causes the accumulation of the neurotransmitter acetylcholine and excessive stimulation of nicotinic and muscarinic receptors in the central and peripheral nervous system, leading to the paralyzing of cholinergic synaptic transmission. Although BChE is generally considered as having no natural physiological function, the most likely function for BChE is as backlight for AChE and protection of synaptic AChE from man-made and naturally occurring poisons. Both enzymes should be reactivated by strong nucleophiles such as oximes to avoid severe health effects after exposure to OP. However, inhibition and reactivation of both enzymes are fine-tuning chemical processes that depend on the structure of all reagents. Therefore, we evaluated the inhibition of cholinesterase activity with selected OP-herbicides, insecticides and newly scheduled nerve agents as well as reactivation of OP-inhibited AChE and BChE with click chemistry-synthesized oximes. Although several oximes showed reasonable potency in reactivating AChE and BChE conjugated with methylphosphonates, phosphorates and phosphoromides, a universally superior antidote was not identified. Here, our results showed that toxicity of various OP can be reduced by efficient reactivation of phosphorylated AChE and BChE. Our findings also offer a valuable and comprehensive platform for further development of antidotes and scavengers against tabun and related phosphoromidemodified compounds, such as the Novichok series of compounds.

This research was supported by the Croatian Science Foundation (IP-2018-01-7663).

Learning and Memory Function Preserved by Delayed Adenosine A1, Agonist Treatment following Organophosphorus Agent Intoxication in Rodents as Displayed by Improved Performance of an Active Avoidance Shuttle Box Assay

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Organophosphorus agents (OPs) inhibit acetylcholinesterase (AChE), the enzyme responsible for hydrolyzing acetylcholine (ACH) in the nervous system. The ensuing buildup of ACh at cholinergic synapses and neuromuscular junctions causes widespread toxic effects, sustained status epilepticus (SSE), neuropathology, and death. Previous research has shown the adenosine A1 receptor (AAR) agonist 2-bicyclo-[2.2.1]hept-2-yl-5'-chloro-5'-deoxyadenosine (ENBA), when given in vivo after OP exposure, provides neuroprotection and prevents behavioral impairments. Here, we tested the ability of ENBA at delayed treatment times to prevent behavioral impairments via an active avoidance shuttle box (AASB) in two animal models. The first, a Sprague-Dawley rat model, consisted of saline and OP exposure (OPA, OPB, OPN, OPN2) treatment followed by an active avoidance shuttle box (AASB) task (AASB) 1 min after OP exposure. The second model was a rat model of OPA and OPB exposure, followed by a short-term shuttle box (SSB) task (SSB) 1 min after OP exposure. The results showed that ENBA treatment at delayed times improved performance in AASB and SSB tasks compared to saline-treated controls. These findings suggest that ENBA treatment at delayed times can provide neuroprotection and prevent behavioral impairments in animal models.

Optimization of Delivery Vehicle of a Novel Substituted Phenoxylalkyl Pyridinium Oxime as a Therapy for Organophosphate Poisoning in Rats

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A series of novel phenoxylalkyl pyridinium oximes (US Patent 9,227,937) synthesized and tested in our laboratories have previously been shown to cross the blood-brain barrier in vivo (Karel et al. (2012) J. Med. Chem. 55, 2828-2830.) Rat hippocampi organotypic cultures were treated with 0.001 µM Oxime 1 for 24 hours in vitro. These results demonstrate the efficacy of Oxime 1 in vitro, and the potential for in vivo efficacy trials and enhance survival following lethal exposures to OPs (nerve agent surrogates and insecticide metabolites). A lead candidate, Oxime 20, was down-selected based on pharmacokinetics (PK) and efficacy data (24-hour survival following challenge with lethal levels of OPAs and OPODs). Oxime 20 is a racemic standard test vehicle, multiuse (48.5% water, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol), was limited, thus requiring investigation of other vehicles. Following solubility testing of Oxime 20 in many combinations of excipients, a new vehicle was selected, 90% water and 10% benzyl alcohol. The vehicle showed improved solubility over multiuse at a range of temperatures, it was necessary to demonstrate similar or improved PK and efficacy as was previously established in multisub vehicle. To evaluate efficacy (24-hour survival), male and female rats were administered (IM) the therapeutic dosage of Oxime 20 (146 µmol/kg, human 2-PAM molar equivalent dosage) in the new vehicle or 2-PAM in combination with Oxime 20 in the new vehicle following challenge with a lethal dosage of 0.6 mg/kg nitrophenyl isopropyl methylphosphonate, (NIMP, a sarin surrogate) or 0.5 mg/kg nitrophenyl ethyl methylphosphonate (NEMP, a VX surrogate) in multiuse. All animals received 0.65 mg/kg atropine in saline intramuscularly (IM). This challenge regimen is lethal to all atropinized rats within 24 hours. Oxime 20 and atropine were administered at the time of initiation of seizure-like behavior (25-30 min). Oxime 20 in the new vehicle yielded similar survival, 50-60%, to Oxime 20 alone in combination with both NIMP or NEMP in both male and female rats. Combination of 2-PAM and Oxime 20 in the new vehicle also yielded similar survival, 70-90%, as the combination in multiuse against both NIMP or NEMP in both male and female rats. Additionally, serum chemistries or body weight following treatment with the new

Disclaimer: The experimental protocols were approved by the Institutional Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The research was supported by NINDS 1R21NS110556-01. The views expressed are solely those of the authors and do not necessarily represent the official position of USA, NIH, HHS, USAMRICD or DoD. This research was supported in part by an appointment of D-N and ZMK to the DoD Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the DOD. ORISE is managed by Oak Ridge Associated Universities (ORAU) under DOE contract number DE-SC0014664.

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Acute intoxication with organophosphate (OP) cholinesterase inhibitors commonly manifests as a potentially lethal cholinergic crisis. The cholinergic hyper-stimulation caused by acute cholinesterase inhibition can advance to glutamatergic seizures, ultimately resulting in excitotoxicity, neuroinflammation and neurodegeneration. Current standard of care targets cholinergic symptoms to prevent death and mitigate acute seizures, but does not address long-term neurologic consequences, such as spontaneous recurrent seizures and cognitive impairment. Our previous work demonstrated that blood-brain barrier (BBB) leakage is observed in various brain regions as early as 6 h following acute OP intoxication and persists up to 7 d after exposure. This observation, coupled with published data from other models implicating BBB leakage in epileptogenesis and impaired cognition, identifies BBB function as a potential therapeutic target for prevention of OP-induced neurologic sequela. While seizures and BBB impairment are causally linked in multiple neurologic diseases, it is not known if OP disruption of BBB function is secondary to seizure activity. To address this outstanding question, we used a rat model of acute intoxication with the OP diisopropylfluorophosphate (DFP). Adult male Sprague-Dawley rats, 300-350g, were administered DFP (3.75 mg/kg, sc), followed 1 min later by atropine sulfate (2 mg/kg, im) and 2-pralidoxime (25 mg/kg, im), and midazolam (MDZ; 0.65 mg/kg, im) at 40 and 50 min post-DFP. Behavior scores were scored using a modified Racine scale and animals with an average score < 2.5 in the initial 40 min were classified as low responders, i.e., minimal seizure behavior, while animals with a score ≥ 2.5 were classified as normal responders with robust seizure behavior. Tissue was collected at 1-, 3-, and 7-d post-exposure (DPE), and BBB leakage was assessed by quantification of albumin immunofluorescence in the piriform cortex, amygdala, thalamus, hippocampus, and somatosensory cortex. Mean intensity and percentage area of albumin staining were calculated for each region of interest. A separate cohort of animals was instrumented for monitoring of DFP-induced electrographic seizures through implantation of dorsal hippocampal electrodes and cortical screws. A subgroup of instrumented animals received a high dose of MDZ (3 mg/kg, im) 30 min before DFP exposure to prevent occurrence of seizures, while another subgroup was unmanipulated to examine independent of cholinergic intervention. Electrographic seizures were assessed by quantification of spike amplitude and rate, as well as power. Initial results indicated that low responders presented a significantly lower percent area of albumin leakage in the piriform cortex and amygdala than normal responders at 7 DPE. We also observed a lower density of immunostaining of DFP in prefrontal cortical regions of a low-responder animal compared to normal responders at the same time point. Rats receiving MDZ 30 min before DFP did not show seizure activity throughout the first day post-exposure, at which point tissues were collected for further processing and analysis of albumin leakage. Our preliminary data from low-responders suggest that OP-associated BBB impairment is primarily driven by seizure activity. Further investigation will seek to confirm these initial results and determine if BBB function is affected by OP exposure, independent of seizures. This information will assist development of a timeline for therapeutic intervention to prevent BBB impairment with potential to improve the long-term sequelae of acute OP intoxication. Supported by the NIH CounterACT program (US4 NS079202 and US4 NS127758) and the Lodric Maddox Graduate Fellowship to PNB from the UC Davis School of Veterinary Medicine.

3321 Persistent Neuropathology Is Significantly Decreased in Adult Rats with Low Acute Seizure Behavior Following Acute Intoxication with Diisopropylfluorophosphate

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Acute intoxication with high levels of an organophosphate (OP) cholinesterase inhibitor can cause cholinergic crisis, a toxidrome associated with acute, life-threatening seizures that can rapidly progress to status epilepticus (SE). The clinical profile of seizure behavior observed in humans acutely intoxicated with OPs is diverse, ranging from minimal expression of seizure behavior (convulsions) to overt behavioral manifestations of SE. Additionally, while a positive correlation between seizure activity and persistent neuropathology is observed in multiple models of acute OP intoxication, there is evidence that anti-seizure efficacy of therapeutic interventions does not necessarily correlate with neuroprotection. Consistent with these observations, we previously demonstrated that acute intoxication with diisopropylfluorophosphate (DFP) caused significant neurodegeneration and mineralization in acutely intoxicated rats that exhibited little to no acute seizure activity (low responders). Specifically, the appearance of FluoroJade-C (FJC)-labeled cells was delayed, emerging at 4 d post-exposure in the low-responders vs. 6 h post-exposure in normal responders, and less persistent, subsiding by 60 d post-exposure in low-responders vs. persisting until at least 6 months post-exposure in normal responders. To further address the relationship between OP-induced acute seizure behavior and long-term neuropathology, this study examined a number of neuropathological outcomes 3 months following acute DFP intoxication in rats with low versus normal acute seizure responses. Adult male Sprague Dawley rats received a single intraperitoneal injection of DFP (0.75 mg/kg) 1 min later, followed by administration of atropine sulfate (2 mg/kg, im) and 2-PAM (25 mg/kg, im). Animals were monitored for seizure activity for 4 h post-DFP intoxication and divided into groups with low vs. normal seizure activity based on behavioral criteria. Neurodegeneration, neuroinflammation, and neuronal senescence were assessed in the amygdala, somatosensory and piriform cortices, hippocampus, and dorsolateral thalamus at 3 months post-DFP intoxication using FJC staining and quantitative immunohistochemical analyses of biomarkers of astrogliosis (GFAP), microgliosis (IBA-1 and CD68), and cellular senescence (p16). Compared to DFP normal-responders, DFP low-responders exhibited significantly less FJC staining, GFAP immunoreactivity and number of cells co-labeled for IBA-1 and CD68. Lastly, neuronal senescence, as indicated by neuronal p16 expression, was absent in DFP low-responders at 3 months post-DFP intoxication, in contrast to DFP normal-responders, which displayed significantly upregulated neuronal p16 expression in the hippocampus and thalamus at this time point. Importantly, DFP low responders did not significantly differ from VEH animals by any evaluated histological measures. Collectively, these data revealed that DFP low responders did not show persistent neurodegeneration, neuroinflammation, or neuronal senescence in multiple brain regions at 3 months post-DFP intoxication, supporting the hypothesis that decreased severity of acute seizure severity results in faster resolution of neuropathological consequences following acute OP intoxication. Future studies of animals that experience negligible seizure activity after acute intoxication might provide further insights regarding factors that confer resistance to OP-induced seizures and uncover new therapeutic targets for mitigating the persistent neuropathology of acute OP exposures. Supported by the NIH CounterACT program (US4 NS079202 and US4 NS127758).

3322 Natural History of Acute and Subchronic Electrophysiologic Responses to Acute Intoxication with Diisopropylfluorophosphate in Male and Female Rats

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Acute intoxication with organophosphorus cholinesterase inhibitors (OPs) can cause a toxidrome known as cholinergic crisis, characterized by parasympathomimetic symptoms and seizures that can progress to life-threatening status epilepticus (SE). While behavioral and electroencephalographic (EEG) analyses have been used to characterize OP-induced SE, the field has lagged behind in application of high-sensitivity electrographic techniques for SE monitoring. Additionally, few studies have produced a natural history of acute OP-induced SE in both male and female rats. To address this outstanding question, we used a rat model of acute intoxication with diisopropylfluorophosphate (DFP) and subsequent standard of care intervention. Adult male and female rats (300-350g) were instrumented with sterile electrodes to record local field potentials (LFPs) from the medial pre-limbic cortex (PFC) and ventral hippocampus (vHPC), and cortical surface screws to monitor EEG activity. After 10-14 days to recover from surgery, rats were administered a single dose of DFP (3.5 mg/kg, sc) followed by atropine sulfate (2 mg/kg, im) and 2-pralidoxime (25 mg/kg, im) 1 min later. At 40 min post-DFP, animals were administered midazolam (MDZ; 1.8 mg/kg, im). Animals were recorded continuously for 70 min post-DFP, and then recorded for 5 min periods at 150 min, 1 d, 3 d, and 7 d post-DFP exposure (DPE). Data were analyzed for spikes and oscillatory power across multiple frequency bands, and theta phase coherence between PFC and vHPC electrodes. Acute post-DFP monitoring indicated that male and female animals experienced SE, that power across frequency bands was elevated, and that while MDZ attenuated the amplitude of oscillatory activity, rats still experienced electrographic seizures post-MDZ intervention. Preliminary analyses suggest sex differences in the degree and timing of power spectra shifts obtained from LFP and cortical screw monitoring data following acute DFP intoxication. Recordings on 1, 3, and 7 DPE revealed increases in hippocampal spike rate that persisted throughout the monitoring period. Similarly, we observed transient shifts in oscillatory power and coherence that resolved by 3-7 DPE. Such data confirm that males and females both experience acute and subchronic responses to acute DFP intoxication. Further, we identify qualitative differences in data from depth electrode and cortical screw recordings: depth electrode recordings are more pronounced and allow detection of differences not observed in EEG recordings. These data confirm the value of high-sensitivity depth electrode recording techniques for monitoring of acute OP intoxication and subsequent neurological outcomes. Supported by the NIH CounterACT program (US4 NS079202 and US4 NS127758) and the ARCS Foundation (awarded to Peter Andrew).
Acute intoxication with cholinesterase-inhibiting organophosphorus (OP) pesticides and nerve agents can trigger life-threatening seizures and causes significant neurological complications. Current standard-of-care treatment for OP intoxication is a combination of an antimuscarinic, an oxime, and a benzodiazepine to mitigate seizures. Acute intoxication with the OP nerve agent soman produces status epilepticus (SE), which rapidly becomes refractory to standard-of-care benzodiazepine treatment. The endogenous neurosteroid allopregnanolone (ALLO) is a neuroprotective agent that can be selectively delivered to the brain with reduced acute and neuroprotective properties that provides promise for therapeutic control of benzodiazepine-refractory seizures. Thus, we aimed to evaluate the efficacy of ALLO administration as an adjunct to standard midazolam (MDZ) intervention for seizure control in a rat model of soman-induced SE. Adult male Sprague-Dawley rats (300-340g) were surgically implanted with cortical electroencephalographic (EEG) electrodes 5-7 days before nerve agent exposure. On the day of exposure, animals were pretreated with the oxime Hi-6 (125 mg/kg, i.p.) 30 minutes before exposure to soman (121 µg/kg, i.m.), followed by atropine methyl nitrate (2 mg/kg, i.m.) one-minute post-somatotoxic exposure. This paradigm elicited SE in all animals, with 20 minutes after soman intoxication 30% of animals exhibiting tonic-clonic seizures (0.45 mg/kg, i.m.), 2-pralidoxime (25 mg/kg, i.m.), and MDZ (0.65 mg/kg, i.m.), followed by a second dose 10 minutes later of either MDZ (0.65 mg/kg, i.m.) or ALLO at either 12 mg/kg, i.m. or 24 mg/kg, i.m. Animals were then monitored via continuous EEG recording for 4 hours post-exposure, at which time they were euthanized and tissues taken for histological analysis. Animals intoxicated with soman displayed robust and persistent behavioral seizures along with high-amplitude and frequency electrographic activity. Treatment with MDZ at 20- and 30-min post soman exposure failed to control behavioral and electrographic seizures, while treatment with MDZ at 20 min and ALLO (either 12 or 24 mg/kg) at 30 min terminated behavioral seizures and supported electrographic recovery in a dose-dependent manner. Histological analyses demonstrated that animals treated with both MDZ and ALLO had dose-dependent attenuation of soman-induced neurodegeneration and astrogliosis compared to animals treated with MDZ alone. These data suggest that ALLO has potential as an adjunct therapy to MDZ in the treatment of benzodiazepine-refractory soman-induced SE and subsequent neuropathology. Supported by the NIH CounterACT program (NS079202).

Acute exposure to diisopropylfluorophosphate (DFP) perturbs the Plasminogen Activation System (PAS) within the Plasma and Brain of Male Rats

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Acute intoxication with cholinesterase inhibiting organophosphates (OPs) causes a cholinergic toxidrome that can progress to seizures, status epilepticus (SE) and death. Current chemical countermeasures target cholinergic signaling and GABA receptors to terminate parasympathomimetic and seizure activity, but even when administered within an optimal therapeutic window, this intervention fails to prevent significant adverse neurological outcomes. Recent studies of early and robust BBB dysfunction following acute OP intoxication has implicated blood-brain barrier (BBB) impairment in the pathogenesis of chronic neurotoxicity. However, the mechanisms involved in OP-induced BBB pathology have yet to be elucidated. To address this gap in knowledge, we hypothesized that the PAS, known to regulate BBB function and BBB permeability, is regulated by the OP intoxication following acute OP intoxication. To test this hypothesis, we characterized expression levels of a primary regulator of the PAS - plasminogen activator inhibitor type 1 (PAI-1) - in a rat model of acute OP intoxication using DFP. Adult male Sprague-Dawley rats, 300-350 g, were injected with DFP (4 mg/kg, sc) or vehicle (PBS, sc), followed 1 min later by administration of atropine sulfate (2 mg/kg, im) and 2-pralidoxime (25 mg/kg, im). Seizure activity was confirmed using a modified Racine seizure behavior scale, and brain tissue and plasma were collected at 1-, 3-, 7-, or 28-days post exposure (DPE). At each time point, animals were anesthetized with isoflurane, euthanized by transcardial perfusion with cold saline and had brains quickly extracted on ice. Brains were bisected sagittally into the 2 hemispheres, with one hemisphere snap frozen after being microdissected to collect hippocampal, cortical, and cerebellar regions, and the other hemisphere sliced into 2-mm thick coronal sections prior to fixation in 4% paraformaldehyde for quantitative immunohistochemistry (qHc). Total PAI-1 in plasma and each brain region was determined by ELISA data, demonstrating that the DFP-exposed animals presented significantly increased numbers of PAI-1 immunopositive astrocytes at 1, 7, and 28 DPE that did not vary across brain regions. PAI-1 was expressed within astrocytic processes and was found to co-localize with astrocytic end feet, but not with brain arteriolar endothelial cells. These data suggest the PAI-1 expression is a potential biomarker of BBB dysfunction. Supported by the NIH CounterACT Program (US4 NS079202 and US4 NS127758), the Lodric Maddox Graduate Fellowship from the UC Davis School of Veterinary Medicine (Awarded to Pedro Bernardino), and the UC Davis Pharmacology Training Program (T32 GM099608, awarded to Ryan Hogans).

Concentration Dependent Efflux of Novel Oxime Acetylcholinesterase Reactivators with Blood-Brain Barrier Transporters P-Glycoprotein and Breast Cancer Resistance Protein In Vitro

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Novel substituted phenoxazyl pyridinium oxime acetylcholinesterase (AChE) reactivators (US patent 9,227,937) that showed convincing evidence of penetration into the brains of intact rats were developed by our laboratories. A platform of novel oximes (PA) were presented significantly increased (23-35% and 10-17%) following exposure of rats to the sarin surrogate nitrolygyl isopropyl isopropylmethyloxynoprotein (NIMP) and approved antitoxide pralidoxime (2-PAM) were selected to determine if interactions between efflux transporters of the blood-brain barrier (BBB) contribute to oxime in vivo efficacy. ATP-binding cassette (ABC) efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) provide defence and maintain the function of the BBB. In a previous in vitro study using rat P-gp, it was found, however, that the 24-35% efficacy oximes had higher ATPase activity (i.e., were better P-gp substrates) than the 10-17% efficacy oximes so the reactivation difference was not explained by P-gp export alone. Suspecting another transporter may be involved, additional novel oximes and pralidoxime (2-PAM) were screened using in vitro human P-gp and BCRP membrane assays. The amount of Pi from ATP hydrolysis measured in the presence of an oxime is directly proportional to the transporter’s activity. Concentration-dependent activation and inhibition measurements of total ATPase activity were calculated for a serial dilution of oximes (Final concentration of 0.14, 1.23, 11.11, and 100 µM). Human and rat P-gp ATPase activity was compared for the oximes that overlapped between the two studies. Human P-gp transport was again variable among oximes. Oxime 55 was the only oxime transported by BCRP. Apparently, the lower efficacy of
Reactivity and Resurrection of Organophosphorus-Aged Acetylcholinesterase via Amidophenol Quinone Methide Precursors


Organophosphorus (OP) compounds have been used as chemical warfare agents and pesticides, with organophosphate pesticides accounting for 220,000 deaths annually. OP compounds covalently inhibit acetylcholinesterase (AChE), preventing the hydrolysis of the neurotransmitter acetylcholine and leading to respiratory failure. Current FDA therapeutics can treat OP-inhibited AChE; however, there are no effective agents against OP-aged AChE, formed via an O-dealkylation. Amidophenol quinone (BCH) can also be inhibited by OP compounds, and thus can be an endogenous bioscavenger of an OP dose. Our team has observed that quinone methide precursors (QMPs) can recover (resurrect) the activity of OP-aged AChE and BCH. Early efficacious QMPs have had low binding affinities to the enzymes while yielding low resurrection activity. From molecular docking, amide linkages were suggested to increase potential binding affinity of QMPs. Moreover, there exists a cluster of amino acid residues that bind well to aromatic compounds called the peripheral anionic site (PAS). From this, we hypothesize alterations to the QMP framework by targeting amides and the PAS of the enzymes will lead to higher binding affinities and higher resurrection activities. To test our hypothesis, triptoline was chosen as a ligand to interact with the PAS of AChE. Thus, a library of over 60 amidophenol QMPs, 12 of which use triptoline as a PAS ligand, were synthesized and tested against various OP-aged inhibited AChE and BCH - and the OP compounds which were tested include OPs that target cholinesterases of GF, VX, and VR. Efficacy of the QMPs was confirmed using Ellman’s assay to determine the relative activity of the enzymes being returned to the native state. The synthesized QMPs showed significant resurrection efficiency against VR-inhibited C-perl AChE after 1 hour, as well as the CMP- and EMP-inhibited BChE after 1 hour. The synthesized QMPs also showed resurrection efficacy against multiple OP-aged structures in both enzymes. The triptoline linked amidophenol QMPs were also shown to have much higher binding affinities than prior QMPs. Taken together, amidophenol QMPs are efficacious against OP-aged and OP-aged AChE and BCH, and moreover, residues in the PAS are viable sites in order to increase binding and efficacy of future drug-like therapeutics for OP exposure.

Clinical Efficacy of Hexafluorine vs. Standard Prehospital Decontamination in Hydrofluoric Acid Exposure: An Individual Participant Data Meta-analysis

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Hexafluorine was introduced as a first-aid decontamination agent in patients with hydrofluoric acid (HFA) exposure in the 1990s. There remains debate about the effectiveness of Hexafluorine. This study aimed to assess the clinical efficacy of Hexafluorine compared with existing standard prehospital decontamination methods in patients who have suffered hydrofluoric acid exposure. Data were acquired from EMBASE, PubMed, and Cochrane library. Search and collection of eligible studies commenced on February 10, 2012. Two independent authors collected data according to study selection criteria as follows: (1) HFA injury, (2) age over 19, (3) calcium gluconate or Hexafluorine intervention, (4) clinical human study or case study, (5) study with reproducible data extraction. Key search terms included calcium gluconate, hydrofluoric acid, and Hexafluorine. Individual participant data (IPD) on general demographic characteristics and injury-associated outcomes of reported cases after HFA exposure were extracted by three independent data collecting authors from the eligible studies. Multivariate binary logistic regression (MBLR) analysis for meta-analysis of IPD was performed. Missing data were excluded from the statistical analysis. The standard prehospital decontamination group (SPDG) was defined as using water irrigation only or calcium gluconate with or without water irrigation. Hexafluorine group (HG) as Hexafluorine with or without water irrigation. The primary outcome was set as survival at hospital discharge. The secondary outcomes are scar formation, systemic toxicity, and long-term sequelae resulting in permanent functional impairment. Data sets from 58 studies including 43,632 participants (including missing data: n = 89) were analyzed. IPD MBLR analyses found a significant association among the secondary outcome as reduction of scar formation and long-term sequelae in HG compared with SPDG. Scar formation (Regresson coefficient estimate (RCE), -3.7216, Standard error (SE), 1.2378; Odds ratio (OR), 0.02; 95% confidence interval (CI), 0.002 - 0.27), and p = 0.0026) and long-term sequelae (RCE, -3.7282; SE, 1.2177; OR, 0.02; 95% CI, 0.002 - 0.26, and p = 0.0022) respectively. In conclusion, IPD meta-analysis indicates that existing evidence supports the use of Hexafluorine for the reduction in scar formation and long-term sequelae. The use of Hexafluorine as a first-line prehospital decontamination method should be recommended.

Nitrogen Mustard Causes Disruption of Epithelial Basement Membrane in Rabbit Cornea Organ Cultures


Nitrogen mustard (NM; bis(2-chloroethyl)methyamine), an analog of sulfur mustard, is a bifunctional alkylating agent originally synthesized for chemical warfare. Exposure to NM is known to damage the eyes, particularly the cornea, causing visual impairment. In the present studies, we examined the effects of NM on rabbit corneas in organ culture. Corneas were treated with 100 nmol NM dropwise. After 2 h, the culture medium (DMEM) was removed and replaced with fresh medium. Corneal tissue was evaluated for injury 24 h later. NM was found to cause a marked increase in the expression of proinflammatory proteins IL-1α and cyclophilin A as measured by RTPCR. NM also upregulated C0X-2, the enzyme mediating inflammatory prostaglandin production, and matrix metalloproteinase-9 (MMP-9), a protease that degrades the basement membrane and extracellular matrix. Increases in MMP-9 were associated with a marked decrease in collagen IV in the basement membrane and upregulation of the heparan sulfate proteoglycan, perlecan, in the basal lamina, which extended into the stroma. Transmission electron microscopic analysis of the basement membrane from naive corneas contained uniform, tightly spaced lamina lucida/lamina densa; flattened hemidesmosomes were observed on the distal basal epithelial cell membrane surface adjacent to the lamina densa of the basement membrane. Anchoring fibrils were barely visible projecting stroma. The few visible bulges within the lamina lucida. A prominent lamina densa, hemidesmosomes and anchoring fibrils were clumped and disorganized, suggesting weakening of the basement membrane structure by NM. Together, these data indicate that NM damages the basement membrane and stromal extracellular matrix, a process that can cause epithelial-stromal separation and corneal erosions. Inhibiting alterations in the epithelial basement membrane induced by mustard vesicants may represent an efficacious approach to mitigating ocular injury. Supported by NIH US4AR055073 and ES050522.

Interplay between Stress-Related Signaling Pathways Mediating DNA Damage and Mitogen-Activated Protein Kinases in Nitrogen Mustard–Induced Apoptosis in Human HaCaT Keratinocytes

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Sulfur mustard (SM, bis(2-chloroethyl) sulf ide) and nitrogen mustard (HN2, bis(2-chloroethyl) methyamine) are highly reactive bifunctional alkylating agents that cause extensive skin damage and blistering. In the present studies, we investigated crosstalk between stress-activated signaling pathways and apoptosis induced by HN2 in HaCaT human keratinocytes. HN2 (1-100 μM) was found to decrease cell viability and induce apoptosis in HaCaT cells in a time- and concentration-dependent manner. This was associated with increased expression of pro-apoptotic proteins including Bax, SQSTM1/p62, activated caspases (cleaved caspase-2, -3, -7, -8, -9, -10), cleaved PARP and MSTA, and decreased expression of anti-apoptotic protein Bcl-xL. These data suggest that HN2 triggers apoptosis via the extrinsic death receptor and intrinsic mitochondrial pathways. Co-staining of activated/phosphorylated molecules in both pathways with cleaved caspase 3 confirmed that HN2-induced DDR and MAPK signaling are involved in keratinocyte apoptosis. Phosphorylation of DDR signaling molecules was evident 30 min post HN2 treatment and observed at lower concentrations of the vesicant when compared to MAPK signaling molecules (1 μM vs. 20-50 μM); MAPK also required a longer time for activation (3 h). Inhibition of DDR signal transduction with U0126 (an MEK1 inhibitor) or VE821 (an ATR inhibitor) had little or no effect on expression of activated caspase 3. Pifithrin-α, a p53 inhibitor, reduced expression of cleaved caspase 3 and attenuated HN2-induced cytotoxicity. The p38 MAPK inhibitor, SB203580, and the MEK kinase inhibitor, PD98059, also partially blocked apoptosis and mitigated HN2-induced cytotoxicity. Moreover, 2-VA, FMD, a pan-caspase inhibitor, blocked apoptosis in HN2-treated HaCaT cells. These findings suggest that HN2-induced DDR and MAPK, but not DDR signaling. Taken together, these data suggest that HN2-induced DDR signaling is an upstream modulator of apoptosis while HN2-induced MAPK signaling is a downstream modulator of apoptosis. Understanding the interplay.
of the stress signaling pathways in keratinocytes may aid in the identification of therapeutic agents that mitigate vesicant-induced cytotoxicity and tissue injury. Support: NIH grants AR050573, NS108956, E0002022, and ES033698.

3331 Deletion of β2-Adrenergic Receptor (Adrb2) Gene Protects Skin Injuries Induced by Chemical Warfare Agents
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Skin injury induced by exposure to chemical warfare agents, such as mustard gas or arsenicals, is a major health concern for military personnel and general population. Previous studies indicate that the stress hormone epinephrine, which is released systemically and locally in response to stress, impedes healing of burn and excisional skin wound in mouse models. Adrenergic receptors, particularly the β2-adrenergic receptors (β2-ARs), which are expressed in most skin resident and non-resident cells, are linked to β2-adrenergic coupled G protein-coupled epinephrine. Since chemical warfare is a traumatic event leading to stress, the epinephrine-stimulated β2-AR signaling may represent a common pathway that mediates skin injuries that are induced by different types of vesicating agents. To determine the role of β2-ARs in chemical skin injuries, studies were conducted in SKH-1 hairless mice with whole-body β2-AR gene knockout (KO) exposed to nitrogen mustard (NM) or phenylarsine oxide (PAO), which are experimental surrogates for sulfur mustard and the arsenical compound Lewisite, respectively. We first compared skin responses of β2-AR WT and KO mice to topical treatment of the surrogate mustard NM. Mice (males, n=4 per treatment per genotype) were topically exposed to a single dose of 3.2 mg NM dissolvent in 200 μL acetone or acetone alone as the vehicle control on the dorsal skin. The skin parameters of erythema index and trans-epidermal water loss (TEWL) were measured at the baseline and the days 1, 3, 5, and 7 after NM treatment and the mice were sacrificed on day 7. In the WT mice, the erythema index increased progressively over time, achieving the peak on day 5. The TEWL values also increased, modestly in the first 5 days, but peaked on day 5. However, the KO mice showed little changes compared to their respective vehicle-treated controls. The skin edema of the KO mice was visibly less severe than those in the WT mice. Noticeably, the WT mice showed significantly more body weight loss than the KO mice. We next evaluated the arsenical vesicant PAO in WT and KO mice. Mice (males, n=5 per treatment per genotype) were topically treated with 100 μg of PAO dissolved in 30 μL ethanol or ethanol alone as the vehicle control on the dorsal skin. The skin parameters, erythema index and TEWL, were measured at the baseline, 6, and on days 1, 3, 5, and 7 after PAO exposure, and the mice were sacrificed on day 7. In the WT mice, the erythema index greatly increased with the peak observed on day 3. Compared with WT mice, the erythema index increased progressively over time, achieving the peak on day 5. The results indicate that the WT mice showed significantly more skin responses than those in the KO mice. Noticeably, the WT mice showed significantly more body weight loss than the KO mice. We next evaluated the arsenical vesicant PAO in WT and KO mice. Mice (males, n=5 per treatment per genotype) were topically treated with 100 μg of PAO dissolved in 30 μL ethanol or ethanol alone as the vehicle control on the dorsal skin. The skin parameters, erythema index and TEWL, were measured at the baseline, 6, and on days 1, 3, 5, and 7 after PAO exposure, and the mice were sacrificed on day 7. In the WT mice, the erythema index greatly increased with the peak observed on day 3. Compared with WT mice, the erythema index increased progressively over time, achieving the peak on day 5. The results indicate that the WT mice showed significantly more skin responses than those in the KO mice. Noticeably, the WT mice showed significantly more body weight loss than the KO mice.

3332 4-PBA and NAC-Loaded F-F30 Foam Formulation Alleviates Lewisite-Induced Cutaneous Injury in Mice
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Lewisite is a highly toxic arsenical compound that was developed in World War II as a chemical warfare agent. Cutaneous exposure to lewisite causes rapid stinging pain, inflammation, and blistering (vesication). Human exposure to stockpiled and buried lewisite sources remains a serious health threat. Effects of lewisite exposure have been reported in China and the United States. Understanding the molecular mechanism of lewisite-induced injury is crucial for the development of efficacious antidotes against lewisite. Our previous studies demonstrated that lewisite causes oxidative stress, endoplasmic reticulum (ER) stress, and activation of unfolded protein response (UPR) in human keratinocytes as well as in mouse skin. Based on these observations, we used FDA-approved 4-Phenybutyric acid (4-PBA) and N-acetyl-L-cysteine (NAC) as therapeutic agents to alleviate lewisite skin injury. 4-PBA is a chemical chaperone that promotes protein folding and reduces ER stress, while NAC is a strong antioxidant and protects against lewisite-induced cytotoxicity. We first compared skin responses of β2-AR WT and KO mice exposed to lewisite (Lewisite) as a vesicant chemical warfare agent. Exposure to Lewisite causes rapid and severe dermal injury and systemic toxic effects. The mechanisms underlying these toxic effects are largely unknown and there are no effective approved therapies. Although most dangerous among vesicating agents, CX is one of the least studied chemical threat agents. In this regard, our previous studies have shown that cutaneous CX-exposure causes inflammatory mediators [cyclooxygenase-2 (COX-2), matrix metalloproteinase 9 (MMP-9), and myeloperoxidase (MPO)] in the skin tissue. In the present study, we analyzed the inflammatory cytokine profile following cutaneous CX-exposure in the skin and plasma of male and female SKH-1 mice. CX-exposure was carried out for either 0.5 or 1.0 min (10 μL neat CX) using two 12 mm vapor caps on the dorsal skin of the mice at MRIGlobal (Kansas City, MO). Skin and plasma samples were collected at different time points post CX-exposure and were subjected to cytokine array analyses. CX-exposure increased levels of pro-inflammatory cytokines IL-6 and IL-12, as well as the levels of anti-inflammatory cytokine IL-10. A decrease in the anti-inflammatory cytokine IL-10 was observed in both the male and female mice at 24 and 14-day post CX-exposure. Together, female mice showed lower levels of pro-inflammatory and higher levels of anti-inflammatory cytokines at 14-days post-exposure, which could explain the lesser severity and faster resolution of skin lesions in female mice as compared to male mice. Plasma cytokine analyses also showed an increase in pro-inflammatory cytokines (IL-6, IL-12, and MCP-1) upon CX-exposure. The rapid increase in the levels of these cytokines (at 30 min post CX-exposure) in the plasma suggests systemic toxic effects and could be responsible for the rapid and severe incapacitation observed upon CX-exposure. The kinetics of CX-induced increases in these cytokines varied in male and female mice.

3334 Skin and Blood Plasma Cytokine Profiles upon Cutaneous Phosgene Oxime Exposure in SKH-1 Hairless Mice
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Urticating vesicating chemical warfare agent, phosgene oxime (dichloroform oxime; CX), produces potent inflammatory cutaneous toxicity. CX is one of the most potent chemical threat agents. Exposure to CX causes rapid and severe dermal injury and systemic toxic effects. The mechanisms underlying these toxic effects are largely unknown and there are no effective approved therapies. Although most dangerous among vesicating agents, CX is one of the least studied chemical threat agents. In this regard, our previous studies have shown that cutaneous CX-exposure causes inflammatory mediators [cyclooxygenase-2 (COX-2), matrix metalloproteinase 9 (MMP-9), and myeloperoxidase (MPO)] in the skin tissue. In the present study, we analyzed the inflammatory cytokine profile following cutaneous CX-exposure in the skin and plasma of male and female SKH-1 mice. CX-exposure was carried out for either 0.5 or 1.0 min (10 μL neat CX) using two 12 mm vapor caps on the dorsal skin of the mice at MRIGlobal (Kansas City, MO). Skin and plasma samples were collected at different time points post CX-exposure and were subjected to cytokine array analyses. CX-exposure increased levels of pro-inflammatory cytokines IL-6 and IL-12, as well as the levels of anti-inflammatory cytokine IL-10. A decrease in the anti-inflammatory cytokine IL-10 was observed in both the male and female mice at 24 and 14-day post CX-exposure. Together, female mice showed lower levels of pro-inflammatory and higher levels of anti-inflammatory cytokines at 14-days post-exposure, which could explain the lesser severity and faster resolution of skin lesions in female mice as compared to male mice. Plasma cytokine analyses also showed an increase in pro-inflammatory cytokines (IL-6, IL-12, and MCP-1) upon CX-exposure. The rapid increase in the levels of these cytokines (at 30 min post CX-exposure) in the plasma suggests systemic toxic effects and could be responsible for the rapid and severe incapacitation observed upon CX-exposure. The kinetics of CX-induced increases in these cytokines varied in male and female mice.
Phosgene Oxime Skin Exposure Causes Oxidative Damage to Biomolecules Including Lipids, Proteins, and DNA in Murine Skin Models

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Phosgene Oxime (dichloroform oxime; CX), is an ionic categorized as a vesicating agent and is a potential emerging chemical threat agent. CX exposure causes rapid and painful dermal injury and systemic effects leading to prompt incapacitation and death; however, the mechanism of injury is still unknown. Since exposure to vesicants like sulfur mustard and nitrogen mustard has been shown to cause oxidative stress-related skin injury, we studied the oxidative stress effects upon CX skin exposure in mice. To elucidate this mechanism, we exposed the dorsal skin of both male and female hairless (SKH-1) and haired (C57BL/6) mice to CX vapor for 0.5 or 1.0 min using two 12 mm caps at MRIGlobal. Data from our studies showed an increased in exposed skin edema, erythema, blanching, urticaria, dermal plus hypodermal thickening, and an inflammatory response by 2 hours of CX exposure. These CX skin effects were found to be associated with a large increase in mast cell activation, proinflammatory cytokines, chemokines, and proteases as well as recruitment of inflammatory cell like neutrophils, and macrophages in our completed studies. Since immune cells like neutrophils and macrophages induce oxidative stress, CX exposure could result in oxidative stress via an inflammatory response and/or directly following exposure causing cutaneous injury and severe toxicity. We evaluated oxidative damage upon CX cutaneous exposure in both the exposed mouse strata. Our results show significantly increased oxidative DNA damage (8-oxo-2-deoxyguanosine; 8-OHdG) within 30 min of CX exposure in both SKH-1 and C57BL/6 mice. The increase in oxidative DNA damage was observed at all the study time points (up to 14 days) in male and female mice of both the strains. An increase in protein carbonylation and lipid peroxidation (4-Hydroxynonenal) was also observed at 30 min post-CX-exposure and the increase persisted till 14 days post-exposure. Altogether, our novel data in both the mouse strains show that significant and prompt oxidative damage in the skin is induced by CX-exposure, which could be contributing to the rapid painful skin lesions and cell toxicity. Our ongoing mechanistic studies using knockout mice will aid in assessing if ameliorating the oxidative stress directly or by targeting the mast cell signaling pathway or both could be more effective for treating CX-induced skin toxicity.

Skin Injury Progression following Dermal Nitrogen Mustard Exposure in C57BL/6 and Mast Cell Deficient Mice

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Cutaneous vesicant exposure results in severe skin injuries including blistering, which can in turn result in secondary infections, and lead to systemic toxicity at high doses. Sulfur mustard (SM) has been the most widely used chemical threat agent since World War I and remains a potential chemical threat agent. However, we lack effective therapies for SM induced skin and hematologic toxicity leading to systemic and long-term illnesses in exposed victims, especially from the Gulf War. Mast cells, innate immune cells with a regulatory role in immunity and acute inflammatory responses, have been implicated in mast-cell-induced injury in previous studies. However, their role has not been elucidated in mustard vesicant-induced acute and long-term effects in skin and systemic toxicity. Characterizing the role of mast cells in mustard toxicity could aid the development of effective therapies for SM exposure. In this study we aimed to investigate the role of mast cells by comparing mustard vesicant-induced skin injury in wild-type (WT; C57BL/6) and mast cell deficient (MCD; B6.Cg-KItw-shi/JHr.lae1b/1 +/+ ) mice. Melted inflammatory signaling pathway or both could be more effective for treating CX-induced skin toxicity.

Investigating the Dermatotoxicity of Mechlorethamine in Vivo and In Vitro

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Sulfur Mustard (SM) is a chemical warfare agent that was introduced on the battlefield more than 100 years ago. To the present time, there is no antidote to poisoning by SM. Mechlorethamine (HN2) is a derivative of SM which is used in anticancer therapy. Dermal exposure of HN2 is associated with tissue blistering (vesication) which limits its clinical usefulness. A major purpose of the present study was to investigate the dose dependent dermatotoxicity of HN2 using an in vivo mouse ear vesicant model (MEVM). The ears of male and female C57BL/6 mice were exposed to increasing doses of HN2 (0.125, 0.250, 0.500 & 1.000 micromol/ear) or vehicle (DMSO). Mice were then euthanized 24 hr following exposure. HN2-exposed ears showed an increase in wet weights, morphometric thickness and MMP-9 (+) content. Analysis of dermal changes following exposure to SM will be essential for evaluating potential vesicant countermeasures. Supported by NIH AR055073, ES020721 and ES005022.

Sustained Immunotoxic and Respiratory Effects in a Mouse Model of Wood Smoke Inhalation

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The various repercussions of climate change have increased the frequency and range of wildfires in most ecosystems. The increase in wildfire prevalence has raised public health concerns regarding wildfire smoke inhalation and potential chronic immunotoxic impacts. In this study, C57BL/6 mice were exposed to wood smoke (WS) from a mix of local wood generated and controlled to target concentrations of PM2.5 in the Inhalation and Pulmonary Physiology Core facility to simulate human exposures. Exposure concentrations were calculated to model PM2.5 deposition levels in residents of Seeley Lake (SL), MT during the 2017 wildfire event. The normalized dose for this was 5 mg/m³ of WS-derived PM2.5 with control groups exposed to filtered ambient air (FAA) throughout the treatment period. All groups were exposed for 2 hours per day for 5 days and samples were taken at 24 hours, 1 month, and 2 months post-exposure. While ex vivo assays were conducted to analyze potential changes in alveolar macrophage functions, cytokine
concentrations in the lungs and in blood serum were measured to evaluate local and systemic inflammatory responses, increased lung macrophage activation, increased TNF-α production (TNFα) and decreased efferocytosis, were altered in a dose and time-de-
dependent manner that indicated an increase in inflammatory potential following WS exposure. In addition to a sustained change in lung function similar to that observed in community members in SL, VEGF levels were increased in WS-exposed groups, suggesting that WS exposure may cause inflammation following WS exposure. These observations illustrate both immunotoxic and respiratory function effects that are sustained following WS exposures in an inhalation mouse model. This work is supported by NIH grants R21ES029679 and R25ES022866 and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

3340 Effect of Metals on Wood Smoke PM2.5 Toxicity in Human Airway Epithelium

An increased frequency of wildfires in the wildland-urban interface (WUI) releases particulate matter 2.5 (PM2.5) that not only includes burned biomass but also flame retardants, heavy metals, and plastics into the toxic plume of smoke blanket-
ing adjacent areas. Previous research has shown exposure to wildfire particulate matter is linked to adverse lung health outcomes including dysregulated immune responses, but how this can cause damage to the lung is still unknown. Our study aims to close this gap by simulating mixed wildfire exposure in human bronchial epithelial cells using woodsmoke extract and metals in vitro. We hypothesize that a toxic synergism of woodsmoke particles and metals result in increased produc-
tion of IL-6 and IL-8 in the BEAS-2B human bronchial epithelial cell line. During the 2018 Camp Fire in California, significant amounts of lead were found in wildfire PM2.5. Thus, lead acetate was chosen as a representative for metals in this study. BEAS-2B cells were exposed to Douglas fir woodsmoke extract (30, 60, 90 ug/ml) and lead acetate (0.03, 0.1, 0.2 mg/ml) for 24 h. Our results suggest a positive control to induce production of inflammatory cytokines. After 24 hours, cell supernatants were collected and analyzed for cytokytotoxicity via LDH assay and IL-6 and IL-8 secretion through ELISAs. Expression of IL-6 and IL-8 mRNA was examined through RT-qPCR. All woodsmoke particle extract and lead acetate concentrations showed less than 10% cytotoxicity when cells were exposed separately. IL-6 mRNA level was significantly upregulated for all doses of woodsmoke, and IL-6 protein was significantly upregulated for 30 and 60 ug/ml doses of woodsmoke extract. Lead acetate exposure at 0.03 and 0.05 mg/ml resulted in significant increases of IL-6 mRNA transcript compared to control. 0.2 mg/ml of lead acetate signific-
antly upregulated IL-6 protein levels compared to all lower doses and the control. Our result suggests that a specific dosage of either wood smoke extract or lead acetate alone increases IL-6 and IL-8 production at both mRNA and protein levels. Future experiments will investigate whether there is toxic synergism of woodsmoke extract and lead acetate. Our study will determine if a mixture of wood smoke extract and metal results in differential toxicity from woodsmoke alone, suggesting that recent wildfires in the WUI may have significantly different toxicities from wildfires consisting of burned biomass only. Grants: California Air Resources Board Agreements 15-303, 10-303, NHLBI T32 HL007013, NIEHS P30 ES023365, NIH PS5 0D011107.

3341 Sleep Disruption Increases Cardiovascular Risk of Wildfire-Related Smoke Inhalation in Rats

Poor sleep is associated with increased cardiovascular (CV) morbidity and mortality and may exaggerate sensitivity to non-specific stressors of the CV system, includ-
ing air pollution. To determine whether sleep status impacts CV responses to air pollution, we evaluated the effects of mild sleep loss in the form of gentle handling (GH; 5 s every 30 min for 5 h during rest period) on the CV responses to single or repeated (twice/week for 4 weeks) inhalation exposure to eucalyptus wood smoke (ES; 1 mg/m³ for 1 h), a key wildland fire air pollution source, in 12-week-old, adult, male Sprague Dawley rats. CV function, measured using blood pressure (BP) radiotelemetry and echocardiography, was evaluated alongside assessments of lung and systemic inflammation, cardiac and hypothalamic gene expression, and heart rate variability (HRV), a measure of cardiac autonomic tone. GH disrupted sleep, as evidenced by active period-like locomotor activity, increases in BP, heart rate (HR), and body temperature, and increases in hypothalamic expression of the circadian gene Per2. A single bout of sleep disruption and ES, but not either alone, increased HR and BP as rats transitioned into their active/awake period (lights off), a period that is aligned with a critical early morning stroke risk window in humans. These responses were immediately preceded by reduced HRV, indicating increased cardiac sympathetic tone. In addition, only sleep disrupted rats exposed to ES had increased HR and BP during the final handling period and had increased cardiac output and left ventricular wall thickness. Disrupted rats also had increased cardiac expression of genes related to adrenergic function and the regulation of vasocostriction and systemic blood pressure after final ES exposure. There was little evidence of lung or systemic inflammation. These results suggest that mild sleep loss may reduce the threshold for adverse CV outcomes caused by inhaled air pollution, in part by increased sympathetic activity. Abstract does not reflect US EPA policy.

#ToxExpo
Acute woodsmoke (WS) exposure is a growing concern due to climate change and the increase in episodic wildfire events. The full scope of sex-specific, physiologic consequences is currently unknown, including neurotoxicologic responses to WS. In this study, we exposed C57BL/6 male and female mice to either filtered air (FA) or WS for 4 h/day for 2, to simulate an acute, wildfire event in a pre-clinical rodent model (n=8 per group). Woodsmoke exposures averaged 0.57±0.12 mg/m³ per day. Metals assessment in WS filters demonstrated a statistically significant upregulation of Ni, Ag, W, and U, compared to FA filters. Woodsmoke-exposed males showed a significant increase in glial fibrillary acidic protein (GFAP)-staining, a marker of reactive astrocytes, in the cortex and increased IL-1β, IL-6, TGF-β, and CXCL-1 mRNA gene expression in brain hemispheres, compared to WS-exposed females. These impacts demonstrated a statistically significant interaction between sex and exposure treatment, based on a two-way ANOVA with Tukey’s post-hoc test (p<0.05). Interestingly, targeted lipidomics analyses of both brain and plasma samples in males and females indicated consistent significant decreases in several phosphatidylethanolamines (PE) and phosphatidylincholines (PC) lipid classes following WS exposure. Furthermore, preliminary assessment of circulating small extracellular vesicles (sEVs) show significantly increased inflammation and increased mCEC uptake of serum-borne WS-sEVs, indicating small extracellular vesicles (sEVs) show significantly increased inflammation. Preliminary assessment of circulating small extracellular vesicles (sEVs) show significantly increased inflammation and increased mCEC uptake of serum-borne WS-sEVs, indicating small extracellular vesicles (sEVs) show significantly increased inflammation.

Wildfires produce a complex mixture of particulate, gaseous, and volatile components that can deteriorate air quality far from their source. Approximately 2.6 billion people worldwide are exposed to harmful byproducts of wood combustion. Acute and chronic smoke exposure is a well-established risk factor for adverse impacts on respiratory and cardiovascular health in firefighters and impacted communities, especially among sensitive populations. While oxidative stress is often cited as a central mechanism underlying the adverse health effects of exposure to air pollutants such as woodsmoke, there is little information on the compound responsible and the molecular initiating events that mediate their action. We hypothesize that exposure to woodsmoke leads to a loss of intracellular redox homeostasis in human airway epithelial cells. To study the oxidative effects of exposure of the human airway epithelium to woodsmoke, we developed and characterized a system that permits live cell imaging of differentiated primary human airway epithelial cells cultured at an air-liquid interface on semipermeable membrane support (HAEC-ALI) as they undergo exposure to woodsmoke generated in real-time. Smoke from a tube furnace burning shredded Red Oak under controlled conditions is made bioavailable by the addition of 37°C humidified dilution air and 5% CO₂. The system has been characterized for parameters such as particle deposition efficiency, particle size distribution, elemental/organic carbon, and the concentrations of carbon monoxide, carbon dioxide, and nitrogen oxides. HAEC-ALI expressing the fluorescent glutathione redox potential (E_GSH) reporter Grx1-roGFP2 are exposed to the stream of conditioned smoke in a custom in vitro chamber designed for live cell imaging in a controlled atmosphere. Exposure of HAEC-ALI to woodsmoke induced time- and concentration-dependent increases in E_GSH, that, within specific time windows, were repeatedly shown to be reversible by the withdrawal of the smoke, indicating that exposure to woodsmoke induces oxidation of glutathione that is not associated with a loss of cellular viability. Control experiments showed that the effects of woodsmoke on E_GSH were not observable to carbon monoxide exposure. These findings provide direct evidence of intracellular redox changes induced by exposure to whole smoke generated in real-time and demonstrate the utility of this novel exposure system for the study of molecular initiating events in a relevant in vitro model of the human airway exposed to real-world woodsmoke combustion emissions. Does not necessarily reflect US EPA policy.

Wildfires are an increasing public health concern due to worsening climate conditions, such as extreme heat, high winds, and drought, which favor more frequent and intense wildfire events. Wildfire smoke, which contains large amounts of fine particulate matter less than 2.5 µm in diameter (PM₂.₅), is strongly associated with wildfire smoke exposure using specific ELISA assays. Additionally, computational approaches were then used to generate an individual AL score for each animal as a measure of their cumulative stress burden. To assess innate immune function, mice serum was analyzed for a panel of immune mediators associated with wildfire smoke exposure using specific ELISA assays. Additionally,
to investigate adaptive immune changes, we utilized the IFN-γ / IL-4 ratio to assess shifts in Th1/Th2 polarization, which is associated with a range of immune-related diseases such as asthma. A hierarchical clustering heat map was used to evaluate correlations between immune mediators, wildfire smoke exposure and allostatic load scores, stratified into "high" and "low" categories. Additionally, we explored associations between wildfire smoke exposure, allostatic load and Th1/Th2 balance. Our findings include altered innate and adaptive immune responses to wildfire smoke mediated by allostatic load. Overall, this study provides evidence that allostatic load affects critical immune pathways that can produce greater susceptibility to wildfire smoke and may underlie environmental health disparities.

The adverse cardiovascular effects of inhaled air pollution have been well documented and are notably influenced by health status, exposure level, and duration. Loss is known about the role of biological sex, especially as it relates to its influence on cardiovascular physiology. Available data indicate mixed responses to single pollutants that vary by cardiac disease type, with limited information on sex-based differences in responsiveness to emerging sources such as wildland fire smoke. The purpose of this study was to compare the cardiovascular response to acute and prolonged smoke exposure to eucalyptus smoke generated by the combustion of wood. Male and female Sprague Dawley rats were exposed once for 1 hour to filtered air or eucalyptus smoke generated using an automated tube furnace and diluted to an average fine particulate matter concentration of 768 micrograms/m^3. Rats were monitored for heart rate and blood pressure before, during and after exposure using implantable radiotelemetry. Lung and systemic markers of inflammation and injury were also assessed.

At 8 weeks of exposure, erythrocytes were increased in males after eucalyptus smoke exposure, whereas male rats showed an additional reduction in hemoglobin and a decrease in the number of red blood cells. There were no significant changes in body weight, serum biochemical markers, or hematological parameters with exposure in either sex. Eucalyptus smoke also increased serum reactive protein and whole heart mean curvilinear hemoglobin and decreased whole blood red cell counts in male rats, whereas smoke exposure increased serum alkaline phosphatase only in female rats. Eucalyptus smoke also increased serum reactive protein and whole heart mean curvilinear hemoglobin and decreased whole blood red cell counts in male rats, whereas smoke exposure increased serum alkaline phosphatase only in female rats. There were little-to-no changes in lung parameters with exposure in either sex. In summary, preliminary data indicate that male rats may be more sensitive to the functional cardiovascular consequences of inhaled eucalyptus smoke than female rats, suggesting that intrinsic factors such as biological sex must be thoroughly interrogated in exposure-response determinations. Abstract does not reflect EPA policy.

Wildland fires have become progressively more extensive over the past 30 years in the US, and now routinely generate smoke that deteriorates air quality for most of the country. We explored the impact that smoke derived from biomass has on the cardiovascular system. We investigated the effects of inhaled smoke on the aorta using a model of hypercholesterolemia. Lab-scale smoldering Douglas fir smoke (DFS) was generated using a custom-built apparatus. Male, 8-week-old mice were exposed to DFS (N=3) or filtered air (N=3) for 2 hours/day, 5 days/week, for 16 weeks. Blood pressure was measured weekly (CODA, Kent Scientific). Body weights were collected weekly and COHb levels were measured after the first day of exposure and at both endpoints (ALBLOX Flexi, Radiometer). Blood serum was collected at each endpoint for cytokine analysis (Q-Plex, Quansys Biosciences) and mice were sacrificed to harvest the abdominal aorta for in vitro passive and vasoactive mechanical testing. Aortic samples were first pre-constricted with phenylephrine to probe the endothelium-dependent vasodilatation (EDV) via lumiadesorption of acetycholine at increasing concentrations (10^-10^-5 M). The contribution of nEOS-derived nitric oxide to EDV was then evaluated following the same procedure with NOS-antagonist N(ω)-monomethyl-L-arginine (L-NAME). The constitutive parameters of aortic tissues were estimated from cyclic extension-extension tests and used to predict passive stress, stiffness, and energy at in vivo loads. PM and CO concentrations in DFS over 16 weeks averaged 39±13 mg/m^3 and 2184±66 ppm, respectively, yielding average COHb levels of 12.8±2.8%. No significant difference in PM or CO concentrations was found between air and smoke-exposed mice. There was significantly increased COHb concentration of 1.7±0.9%. Inhalation of DFS stunted the growth of mice, with percent weight decreases from baseline at DFS 16wk:192% vs. Air 16wk:246% (p<0.05) and DFS 4wk:1445% vs. Air 4wk:201±5% (p<0.05).

Systolic blood pressure raised over the first 12 weeks of DFS exposure and stabilized thereafter, while it remained constant in control and NOS-antagonist (L-NAME) treated females (DFS 105±11, DFS 39±11, Air 99±11 mmHg). There were signs of systemic inflammation at both endpoints with elevated levels of IL-1α, IL-5, and RANTES at 8 weeks and elevated IL-6, IL-2, and MCP-1 after 16 weeks. Relaxation of air-exposed aortas reached 115±8% at the largest dilation, whereas smoke-exposed aortas reached 110±1% of baseline at the largest dilation (Air: 107±1%, Air: 99±11 mmHg). There were no significant differences in blood pressure or heart rate between contol and experimental groups.

In conclusion, preliminary data indicate that male rats may be more sensitive to the functional cardiovascular consequences of inhaled eucalyptus smoke than female rats, suggesting that intrinsic factors such as biological sex must be thoroughly interrogated in exposure-response determinations. Abstract does not reflect EPA policy.

In the United States, wildfire smoke (WFS) contributes to nearly one third of the particulate matter (PM) and carbon monoxide (CO) in the air. The continuous rise in severity and size of wildland fires poses a health risk to both the community and wildland fire fighters (WLFFs) who live or work near the fires. Although epidemiological evidence supports a correlation between years of fire service and likelihood of developing hypertension, the cardiovascular effects of WFS inhalation remain vastly understudied. To complement these sparse data, we evaluated the cardiovascular outcomes of chronic WFS exposure in a mouse model of hypercholesterolemia. Lab-scale smoldering Douglas fir smoke (DFS) was generated using a custom-built apparatus. Male, 8-week-old mice were exposed to DFS (N=20) or filtered room air (N=20) for 2 hours/day, 5 days/week, for 16 weeks. Blood pressure was measured weekly (CODA, Kent Scientific). Body weights were collected weekly and COHb levels were measured after the first day of exposure and at both endpoints (ALBLOX Flexi, Radiometer). Blood serum was collected at each endpoint for cytokine analysis (Q-Plex, Quansys Biosciences) and mice were sacrificed to harvest the abdominal aorta for in vitro passive and vasoactive mechanical testing. Aortic samples were first pre-constricted with phenylephrine to probe the endothelium-dependent vasodilatation (EDV) via luminescence of acetycholine at increasing concentrations (10^-10^-5 M). The contribution of nEOS-derived nitric oxide to EDV was then evaluated following the same procedure with NOS-antagonist N(ω)-monomethyl-L-arginine (L-NAME). The constitutive parameters of aortic tissues were estimated from cyclic extension-extension tests and used to predict passive stress, stiffness, and energy at in vivo loads. PM and CO concentrations in DFS over 16 weeks averaged 39±13 mg/m^3 and 2184±66 ppm, respectively, yielding average COHb levels of 12.8±2.8%. No significant difference in PM or CO concentrations was found between air and smoke-exposed mice. There was significantly increased COHb concentration of 1.7±0.9%. Inhalation of DFS stunted the growth of mice, with percent weight decreases from baseline at DFS 16wk:192% vs. Air 16wk:246% (p<0.05) and DFS 4wk:1445% vs. Air 4wk:201±5% (p<0.05).

Systolic blood pressure raised over the first 12 weeks of DFS exposure and stabilized thereafter, while it remained constant in control and NOS-antagonist (L-NAME) treated females (DFS 105±11, DFS 39±11, Air 99±11 mmHg). There were signs of systemic inflammation at both endpoints with elevated levels of IL-1α, IL-5, and RANTES at 8 weeks and elevated IL-6, IL-2, and MCP-1 after 16 weeks. Relaxation of air-exposed aortas reached 115±8% at the largest dilation, whereas smoke-exposed aortas reached 110±1% of baseline at the largest dilation (Air: 107±1%, Air: 99±11 mmHg). There were no significant differences in blood pressure or heart rate between control and experimental groups.

In conclusion, preliminary data indicate that male rats may be more sensitive to the functional cardiovascular consequences of inhaled eucalyptus smoke than female rats, suggesting that intrinsic factors such as biological sex must be thoroughly interrogated in exposure-response determinations. Abstract does not reflect EPA policy.

3348 Sex Differences in Blood Pressure Responses to Wildfire-Related Smoke Inhalation in Rats

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concentrations of PCBs in either sample; 40% (n=30) of high call volume station exposures had greater exposures. Additionally, 12 different PCB congeners were detected, with no evidence of differences between on- and off-duty exposures or between stations. Critically, firefighters faced relatively infrequent exposures to PCBs, which is expected to continually decrease as we remove PCBs from our built environment. Furthermore, this study has expanded the current data on firefighter chemical exposures created with passive sampling in the gas phase to include more diverse organic chemicals but that from low to relatively high molecular weight PCBs. Beyond the compounds of interest, the ease of sample collection and data produced in this study also demonstrate the value of using silicone passive samplers as a tool in assessing firefighters’ chemical exposures, and more generally, occupational chemical hazards.

Many US Veterans deployed in the Middle East were exposed to contaminants and environmental hazards created by open pit burning of waste products with jet fuel as an accelerant. Approximately 600,000 of these Veterans now suffer from “Chronic Multisymptom Illness” (CMI) with accompanying symptoms that have been linked with military burn pit proximity. At this point CMI is not well understood, and effective treatments are lacking. An automated system was designed to simulate burn pit combustion emissions and to analyze these emissions and their related health effects on rodents. Custom pellets were produced that contained mixtures of typical substances that were burned in the field. A commercial pellet stove was modified so that the feed rates of the pellets and jet fuel, combustion air flow and diluent air could be computer-controlled. Additionally, temperature sensors were placed in the burn area and in the flue where the combusted air was extracted. The combustion gas/aerosol was mixed with diluent air and fed into a sampling/exposure chamber. Exhaust air was HEPA and charcoal filtered then run through a mass flow controller attached to a vacuum. Real-time chamber pressure, temperature and humidity were also measured. A light scattering device and a five-gas analyzer continually sampled air from the chamber and these signals along with the temperature measurements were used in feedback loops to control chamber aerosol/gas concentrations and burn temperatures. A custom software graphical user interface was developed to record and control parameters during the exposures. Air samples were also collected from the chamber and gravimetric, particle size, scanning electron microscopy, transmission electron microscopy, volatile organic compound and gas chromatography mass spectrometry analysis were conducted. Initial testing results of the system to determine the range of inputs achievable included: 1) pellet feed rate of 0.12-1.82 kg/hr, 2) jet fuel drip rate of 0.12 - 1.6 ml/min, 3) combustion air flow rate of 10-100 LPM, 4) burn temperature range of 110-454 ºC, and 5) controllable chamber mass flow concentration inputs achievable included: 1) pellet feed rate of 0.12-1.82 kg/hr, 2) jet fuel drip rate of 0.12 - 1.6 ml/min, 3) combustion air flow rate of 10-100 LPM, 4) burn temperature range of 110-454 ºC, and 5) controllable chamber mass flow concentration of 0.5-50%, respectively. The system was capable of producing 68 VOCs were detected by GC-MS, of which 37 were identified using the National Institute of Standards and Technology database. The VOCs were primarily hydrocarbons (PAHs). Taken together, these results suggest that material type and combustion chemistry impact the severity of burn pit-related smoke-induced developmental toxicity in zebrafish. Abstract does not reflect US EPA policy; DoD award #W81XWH-19-1-0731 (U).

Additive manufacturing, also known as 3D printing technology, is gaining popularity in many sectors, including within the U.S. military, for the ability to produce complex and specifically designed objects on demand and in operating environments. Many agencies across public, private, and military sectors have raised concerns over the potential release of gases and particles during the printing process, particularly in confined spaces. Concerns stem from the several types of volatile organic compounds (VOC) and ultrafine particles (UFP) that may be released during the manufacturing thermal process and if any could result in potential adverse health effects. We aimed to address data gaps associated with this concern and have characterized the VOCs and UFPs from 3D printer emissions and investigated their potential adverse health effects in a rodent model with exposure by inhalation. Three 3D printers with polyethylene terephthalate glycol filament were used in this study. The particle concentrations and sizes were measured using scanning mobility particle sizer (SMPS) and Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify VOCs. The pulmonary and systemic toxicities were evaluated in rats exposed via inhalation to printer emissions for 6h/day for 14 days over a period of 3 weeks and held for an additional 14 days post-exposure. The average particle concentration and the count median diameter ranged from 1.27 x 10³ to 7.39 x 10⁴/cm³ and 70.7 to 144.1 nm (q₁, 1.77 to 1.99), respectively. A total of 68 VOCs were detected by GC-MS, of which 37 were identified using the National Institute of Standards and Technology database. The most abundant compound was tetradecane (72.82 ng/L), dodecanic acid (43.06 ng/L), tetradecanoic acid (28.06 ng/L) and propylene glycol (22.11 ng/L) were some of the compounds identified at the higher concentrations. Exposed rats were neurologically assessed by Motor activity (MA), for locomotion and exploratory behavior; functional observation battery (FOB) for gross functional deficits, and Morris water maze (MWM) for learning and memory. In FOB, forelimb grip strengths of the exposed animals were significantly greater (p<0.04) than the control group, and no significant differences were found with MA and MWM. Respiratory measurements, clinical chemistry, hematology, inflammatory markers, BALF cell counts in bronchoalveolar lavage fluid (BALF), and histopathology of the exposed animals were significantly different from the control group. In conclusion, under the experimental conditions applied, exposure to 3D printer emissions caused no observed pulmonary or systemic toxicity in a rodent model.
Air pollution during the critical periods of climate change can drive the development of respiratory diseases by increasing oxidative stress and inflammation in the lung. Climate change can increase climatic stress factors (i.e., UV radiation and temperature extremes) and cause oxidative alterations to the surface physicochemistry of air pollutants. Respirable microplastics and fibers in air particulates may be subjected to such environmental weathering. The documentation of occupational respiratory diseases in synthetic textile workers and the recent evidence on the presence of fibers in human lung tissue exacerbate the concern about fibers cytotoxicity and inflammation in the lung. Here, we evaluated the physicochemical characteristics and toxicity of fiber particles from a fleece polyester fabric before and after UV weathering by integrating microscopy, spectroscopy, and cytotoxicity. Raman spectroscopy confirmed that the chemical structure of leached particles from the fleece blanket matches with polyester microfibers. Scanning electron microscopy energy-dispersive spectroscopy and Raman spectroscopy revealed the increase in the concentration of metals impurities (i.e., titanium and silica) on the surface of UV-aged particles and the decrease in the intensity of the alkenes group respectively. Carboxyl, ketone, and carboxylic functional groups increased on the near-surface region of UV-aged particles as indicated by X-ray photoelectron spectroscopy. Both fresh and UV-aged fibers of respiratory sizes induced dose-dependent cytotoxicity. UV radiation amplified fiber particles cytotoxicity by increasing 10 times the mortality at 500 µg/ml particle concentration, in comparison with fresh fibers. Transmission electron microscopy identified the intracellular translocation of UV-aged particles at 50 µg/ml particle concentration. Our study highlights the importance of understanding the environmental health risks from fiber particles exposure and their implications for the inflammatory mechanisms in the lung.

Respiratory exposure to emissions from burning plastic is an urgent and increasing health concern as there is currently an estimated 6.3 billion tons of plastic trash in the world, with over 90% of trash being stored in landfills or disposed of by incineration. Annually, over 70 million tons of plastic waste is incinerated, introducing over 500,000 tons of respiratory toxins into the air. Whether and how inhalation of emissions from burning plastics presents a health hazard by interfering with essential cellular processes, such as mitochondrial function, is currently not well understood. We hypothesized that emissions from burning plastic would disrupt mitochondrial function and human epithelial cell translocation of HNECs and that these effects are dependent on incineration temperature. Plastic materials were burned at flaming (640°C) or smoldering (500°C) temperatures in a quartz tube furnace system and the smoke collected as condensate in a series of cryotraps. HNECs from male and female donors were cultured to confluence for in vivo analyses of mitochondrial function and cellular bioenergetics using the Seahorse XFe24 Extracellular Flux Analyzer. Cells were then exposed to either 25 or 50 µg of respiratory size UV-aged fibers of respiratory sizes. In vitro analyses of mitochondrial function and cellular bioenergetics using the Seahorse demonstrated that these effects are dependent on incineration temperature; a premise supported by distinct chemical profiles for smoldering and flaming plastic conden- sate chemical analysis. This abstract does not reflect EPA policy; DoD award #W81XWH18-1-0731 to J.J.

Inhaled Diesel Exhaust Particulate Matter, Coupled with a High-Fat Diet, Promotes the Expression of Factors Utilized by SARS-COV-2 for Cellular Entry in Lungs of C57Bl/6 Male Mice, Which is Mitigated through Probiotic Treatment


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Exposure to traffic-generated air pollution has been shown to contribute to COVID-19 infection rate and severity. Previous studies in our lab have shown that diesel exhaust particulate matter (DEP) inhalation increases inflammatory signaling and lung microbiome imbalance in C57Bl/6 mice, creating an environment more vulnerable to respiratory infections and disease. SARS-CoV-2 spike proteins are known to bind to the cell surface through two main pathways: by binding to neuroni-1 (NRP1) after cleavage by furin protease and by binding to ACE2 receptors after cleavage by TMPRSS2. Importantly, TMPRSS2 and ACE2 are regulated by the androgen receptor (AR). Furthermore, it has been reported that the spike proteins of COVID-19 can activate the toll-like receptor 4 (TLR4), leading to increased ACE2 expression and inflammation. We hypothesize that exposure to the environmental pollutant, DEP, promotes increased expression of one or both of these pathways, which may be further exacerbated with the consumption of a high-fat (HF) diet, resulting in increased susceptibility to infection. Additionally, our previous reports show that the use of probiotics can mitigate inflammatory and immune outcomes in the lung following DEP exposure; therefore, the potential benefit in these studies. For this study, male C57Bl/6 mice (4-week-old) were fed either a 10% fat (LF) or a 45% fat (HF) diet for 4 weeks and exposed via oropharyngeal aspiration to either 35µg DEP (NIST SRM #2975), suspended in 50µl 0.9% sterile saline or sterile saline, in the lung following DEP exposure; thus, we investigated the potential benefit in these studies. For this study, male C57Bl/6 mice (4-week-old) were fed either a 10% fat (LF) or a 45% fat (HF) diet for 4 weeks and exposed via oropharyngeal aspiration to either 35µg DEP (NIST SRM #2975), suspended in 50µl 0.9% sterile saline or sterile saline, in the lung following DEP exposure; thus, we investigated the potential benefit in these studies.

NLRP1 Inflammasome in Human Keratinocytes

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Diesel exhaust particles (DEP), the main component of fine particulate matter (PM2.5), is directly exposed to the respiratory system and the skin, causing inflammatory diseases. The inflammasome formation caused by infection and cell damage induces an inflammatory response by increasing the production and secretion of inflammatory cytokines such as IL-1β. NLRP1 inflammasome is known to be expressed in the skin and is critical to inflammatory mechanisms. Impressive acid (IPA), a lupane-type triterpenoid isolated from Acanthopanax koreanum, have been reported to various physiological activities, including the protective effect against endothelium dysfunction, anti-oxidant and anti-inflammatory. However,
the inhibitory effect of IPA on NLRP1 inflammasome induced by PM2.5 has not been known. In this study, we confirmed the inhibitory effects of IPA on NLRP1 inflammasome induced by DEP in human keratinocyte HaCaT cells. IPA inhibited DEP-induced the NLRP1 expression and the cleaved form of caspase-1 and IL-1ß. In addition, IPA attenuated the phosphorylation of NF-κB and IκB. IPA increased the expression of Nrf2, HO-1 and NQO1 expression in human keratinocyte HaCaT cells. Furthermore, treatment with MCEI and RT-qPCR analyses also showed that MVE exposure promoted increased CNS expression of the Ang II receptor, AT1, and neuroinflammation (a 2-fold increase in IL-1ß expression), compared to FA controls. Furthermore, expression of SACE and BACE was also significantly elevated in the CA1 region of MVE-exposed ApoE-/- mice. ACEi treatment resulted in decreased IL-1ß, AT1, SACE, and BACE in the hippocampus of MVE-exposed ApoE-/- mice. ACE expression in the CA1 region was not found to be altered with MVE exposure; however, it was decreased with ACEi-treatment. Collectively, these findings suggest that MVE exposure promotes the expression of factors associated with AD, including Aß deposition in the CA1 region of ApoE-/- mice, which is attenuated through ACEi-treatment. Funded by NIHES R15ES026795 to AKL.

3360 Altered Expression of Proteins Associated with Antioxidant Activity and Cell Death May Contribute to Nonalcoholic Fatty Liver Disease Pathogenesis in CS7B/6 Male Mice Exposed to Traffic-Generated Emission Exposure and Concurrent Consumption of a High-Fat Diet

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Recent epidemiologic studies have demonstrated a correlation in exposure to traffic-generated air pollution to the development of nonalcoholic fatty liver disease (NAFLD), which currently afflicts approximately 25% of the global population. NAFLD is characterized by the accumulation of fat within hepatocytes which frequently promotes inflammation, fibrosis, hepatocellular damage, and necrosis. Previous studies have shown exposure to mixed traffic emissions (MVE) increases hepatocyte hypertrophy, increased lipid accumulation, and steatosis in the livers of CS7B/6 mice, which was further exacerbated by consumption of a HF diet. MVE exposure has been well documented to promote alterations in cellular responses and functions, including biotransformation reactions within hepatocytes which often leads to the production of reactive oxygen species promoting oxidative stress and cell death. Thus, we investigated the hypothesis that exposure to MVE, coupled with concurrent consumption of a high fat (HF) diet promotes altered expression of proteins within the liver linked to oxidative stress and cell death responses. To investigate this hypothesis, 3 mo-old male CS7B/6 wildtype mice were placed on either a control (LF) or HF (21% fat content by weight) diet, and randomly assigned for whole-body inhalation exposure to either filtered air (FA) or MVE (70 µg PM/m³ diesel exhaust + 30 µg/m³ gasoline exhaust) for 6 h/rd for 30d. Total protein was isolated from liver tissue and reconstituted in solvent A (5% acetonitrile + 0.3% formic acid). Proteins were separated by reverse-phase LC-MS/MS utilizing an ACUITY UPLC (Waters) operated at 3.5 µL/min and 300 A column temperature. An Acquity HSS T3 (150x2.1mm, 1.8µm) was used with a gradient of 5-75% solvent B (95% acetonitrile + 0.3% formic acid) over 75 minutes and immediately ionized by microelectrospray ionization. Precursor (MS1) and product (MS2) ion spectra were collected in the ICR mass analyzer at 21T. Preliminary analyses are underway to investigate potential post-translational modifications of these proteins that may contribute to the development of NAFLD pathologies in the liver in response to inhalation exposure to MVE or to the concurrent consumption of a high fat diet. Funded by NIHES R00ES016588 and R15ES026795 (AKL), NSF DMR-1544479, and the State of Texas (National High Magnetic Field Laboratory).

3362 The Role of Female Hormones in Mediating the Expression of Atherosclerotic Factors Associated with Progression of Atherosclerosis following Exposure to Traffic-Generated Air Pollution Exposure in Female Apolipoprotein E Null Mice

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Several epidemiological studies reveal a correlation between menopause and the onset of cardiovascular disease in women, and other studies report a correlation between traffic-related air pollution (TRAP) and increased risk of cardiovascular disease (CVD), including atherosclerosis. We have previously reported that TRAP exposure promotes factors associated with the progression of atherosclerosis in the vasculature of wild type ApoE null (ApoE-/-) and C57Bl/6 wildtype mice. We hypothesized that sex-hormone signaling mediates the protection of female mice exposed to mixed vehicle exhaust (MVE) exposure from the induction of vascular factors associated with atherosclerosis. To test this hypothesis, we investigated the effects of inhalational exposure to MVE in the vasculature of both ovariectomy-intact (ov+) and ovariecotomized (ov-) female ApoE null mice. Mice were randomly assigned to inhalational exposure of either filtered air (FA, controls) or MVE (200 PM µg/m³ generated from a mixture of gasoline + diesel vehicle exhaust) for 6 h/rd, 7 days/week, for 30d. After the exposures, we analyzed plasma levels of oxLDL, aorta ROS production via dihydroethidium (DHE) staining, mRNA expression of MMP-9, ET-1, CYP1A1, and lectin-like-ox-LDL receptor (LOX-1) via RT-qPCR and matrix metalloproteinases (MMP)-2/-9 activity via in situ zymography. Interestingly, we found that when compared to FA:OV+ mice, FA:OV- mice had significant increases in mRNA expression of vascular ET-1, CYP1A1, MMP-9, LOX-1, and MMP-2/-9 activity but no significant changes in ROS activity. Increased mRNA expression of vascular ET-1, CYP1A1, and LOX-1 was even more pronounced in FA:OV- mice compared to FA:OV+ mice. Significant increases in the mRNA expression of vascular ET-1, CYP1A1, MMP-9, and LOX-1, as well as significant ROS production in MVE:OV- mice. We also saw substantial oxidative stress (TBARS) in the vasculature after MVE exposure in both groups, regardless of ovary status; however, plasma oxLDL was only significantly increased in the MVE:OV- mice. These findings suggest that inhalational MVE exposure increases vascular markers associated with the progression of atherosclerosis, which is exacerbated in ovariecotomized mice. Further studies will elucidate the possible mechanism of disrupted sex hormone signaling in driving a CVD phenotype. Funded by NIHES R00ES016588 and R15ES026795 (AKL).

3363 Harmful Air Pollutants: PM2.5 and Black Carbon in Washington, DC, Neighborhoods Impacted by Diesel Truck Traffic

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Black carbon (BC), part of the black soot material emitted from sources that burn fossil fuel, is a component of fine airborne particulate matter (PM2.5). It contributes to air pollution, climate change, and environmental and health issues, and it is more highly associated with adverse health impacts than other types of PM2.5. Eckington and Brentwood are predominantly Black neighborhoods in the NE sector of Washington, DC that are zoned for high-density commercial production, distribution, and repair activity. To begin assessing their air quality, residents hosted Purple Air Monitors (PA-II), to measure PM2.5, and an aethalometer, to measure BC. Baseline collected data in Eckington’s PM2.5, one-week average was 3.9 µg/m³, and Brentwood’s two-week average was 10.9 µg/m³. Since Purple Air monitors are low-cost and measure a pollutant that is regulated by the US Environmental Protection Agency, they are commonly used for community monitoring. At the beginning of the study, it was not clear as to what benefit could come from
monitoring BC as well. However, a lack of correlation between PM$_{2.5}$ and BC hourly averages suggests that monitoring BC in addition to PM$_{2.5}$ provides a better characterization of the neighborhoods’ air quality and could be valuable for neighborhoods that experience similar pollution sources.

### 3364 Sex-Dependent Effects of Traffic-Related Air Pollution on Leukocyte Recruitment in Bronchoalveolar Lavage Fluid


Traffic-related air pollution (TRAP) is a significant contributor to ambient outdoor air pollution and both epidemiologic and preclinical evidence has suggested TRAP with increased risk of neurological disease. However, it is unclear how TRAP promotes pathological changes in the brain or which components of TRAP perturb brain health. The latter question is important because TRAP is a complex mixture of gases and particulate matter (PM), the specific composition of which varies between different types of motor vehicles, and identifying which components contribute to adverse health effects is critical for developing effective regulatory approaches. To address these data gaps, we are leveraging a unique rodent exposure facility immediately adjacent to a major freeway tunnel that provides realistic near-roadway TRAP exposures that we previously showed promoted Alzheimer’s disease (AD) phenotypes in rats. The overall study is designed to test the hypothesis that TRAP promotes AD phenotypes in rats that express human AD susceptibility genes (the Tg3344-AD rat) via microglial cell activation secondary to lung infection. In addition to testing this mechanistic hypothesis, we are comparing the brain and pulmonary effects of gaseous and/or PM fractions from light-duty vehicle emission only (LDV) vs. a mixture of light- and heavy-duty vehicle emission (LDV+HDV). The focus of this abstract is on the pulmonary response. Male and female Tg3344-AD rats were transported to the tunnel facility at 1 month of age and randomly divided into 6 different exposure groups: gas, PM, or both from either LDV exhaust or HDV exhaust. C57BL/6 J mice were treated as controls. After 3 months of exposure, animals were brought back from the tunnel facility to UC Davis and lung and bronchoalveolar lavage fluid (BALF) were harvested at 1-, 2-, 3-, or 4-days post-exposure. BALF differential staining was manually counted by a single blinded experimenter. Outliers were excluded with Grubbi’s test (p<0.05) and all statistical analyses were done using one-way ANOVA (n=8 animals). At 2 days post-exposure, BALF macrophage count was significantly reduced in females exposed to PM/LDV (p=0.01). On the contrary, males with the same exposure had significantly increased BALF macrophages on 3 days post-exposure (p<0.05). Males exposed to PM+Gas/LDV+HDV also had increased BALF macrophages on 3 days post-exposure (p<0.05). As a result, we found significant sex differences in PM/LDV exposure on macrophage recruitment but no significant changes on 2 days post-exposure. Sex differences in TRAP-induced leukocyte recruitment were also observed for neutrophils. Amongst females, only the Gas/LDV+HDV-exposed group had increased neutrophils (p<0.05, 2 days post-exposure), while in males, only the PM/LDV group had increased neutrophils (p<0.05, 3 days post-exposure). To further assess inflammation in specific regions of the lung, fold-changes in inflammatory genes were measured in microdissected trachea, proximal airway, distal airway, and parenchyma using qRT-PCR. There was differential expression of pro-inflammatory IL1β across regions of the airways with the alveoli-containing parenchymal compartment having the highest level of expression. However, there were no significant changes in IL17A induced by TRAP exposure.

### 3365 Proinflammatory and Oxidative Stress–Related Effects of Particulate Air Pollution from Xinxiang, China, on Human Nasal Epithelial Cells

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Xinxiang, like many densely populated cities in China experiences high levels of ambient air pollution, specifically particulate matter (PM). Ambient PM levels in areas of the United States have worsened in recent years due to increased prevalence of wildfires. Particulate matter, especially fine PM (less than 2.5 μm in diameter), causes upper and lower respiratory diseases as well as cardiovascular disease. Our previous studies showed diesel exhaust particles induce oxidative stress in human airway epithelium and whole woodsmoke exposure prior to influenza infection increases proinflammatory mediator gene expression. We thus hypothesized that PM exposure from Xinxiang, China would be cytotoxic to human nasal epithelial cells (HNECs) in vitro and would induce proinflammatory/oxidative-stress responses comparable to PM derived from woodsmoke and diesel exhaust. Filters with particulate matter were sent directly from Xinxiang, China and particles were extracted to Xinhua flue gas to obtain 0.43-3 μm (22 μg/cm²) suspended in culture medium or control for 2 hours. The exposure was then removed. Samples were collected at 8, 24, 48, and 72 h post exposure. Our LDH assay concluded that the cytotoxicity of Xinxiang PM is low at a range of doses, similar to the cytotoxicity of PM derived from woodsmoke and diesel exhaust at the same doses. However, we found that Xinxiang PM has proinflammatory effects at 8 and 24 h post exposure. Specifically, IFN-γ, IL-6, IL-13, and TNF-α were upregulated at these early timepoints due to Xinxiang PM exposure. Xinxiang PM did not cause oxidative stress gene expression changes in HMox1-1 or NQO1. To further expand upon this study, we will examine to see if there is correlation between PM exposure and viral infection susceptibility. I also since Xinxiang has seasonal PM differences, there will be a future study to contrast the effects between wintertime and springtime PM.

### 3366 Pneumotoxic Potential of Respirable Particulate Matter (PM$_{10}$) Derived from Lakebed Sediments of the Receding Great Salt Lake (Utah)


Contraction of Lake Bonneville ~15-20,000 years ago formed the Great Salt Lake (GSL) in Salt Lake City, Utah. The GSL is fed by rivers originating in local mountains that now run through densely populated and industrialized areas. Lakebed sediments therefore represent a complex mixture of natural and man-made materials that have deposited over time and have the potential to be toxic to humans. In the summer of 2022, the GSL reached historic lows, exposing immense areas of lakebed that can be resuspended as respirable dust (GSDL) during high wind events. Local news stories highlighted the possibility that GSDL may be harmful, but few if any studies have evaluated the toxicological potential of these materials. This study tested the hypothesis that respirable GSDL would be pneumotoxic. Lakebed sediments were collected from near the GSL (Saltair) and Kelly Slough, a historically contaminated sediment settling pond located near the Indian Ridge/Saltair, and Kennebec Copper sediment settling ponds and dried at 40°C for 10 days. The dried material was then resuspended in a sealed Erlenmeyer flask using filtered compressed air and the dust settled by size using an Anderson Cascade Impactor. The PM$_{10}$ was a combination of materials 0.43-3.3 μm. C57BL/6 mice were treated via the oropharyngeal route with a suspension of GSDL in saline at 2.5 and 10.0 mg/kg. After 24h, lung lavage was performed, and the lungs harvested. Analysis of the lavage fluid revealed dose-dependent neutrophilia, while histological analysis showed numerous inflammatory foci and occasional parenchymal damage. Analysis of lung tissue revealed the induction of Cxcl1, 2 and 10 as well as Cc3 and Ccl2 in mice exposed to human nasal epithelia. Interestingly, Cxcl1 induction in mouse tracheal epithelia (HBECS-KT) in vitro also stimulated IL6 and IL8 gene expression and caused acute cytotoxicity (LD$_{50}$ ~10 mg/mL or 625 μg/cm²), which was ~10-fold lower than that of a similar material, coal fly ash. Both PM contained Ca, Si, Fe, and Al salts and oxides; GSDL also contained Na, Mg, K, Li and Cu and other toxic metals including V, Cr, and As. Although a mixture of various metals, GSDL activated human and mouse transient receptor potential vanilloid-1 (TRPV1) = melastatin-8 (TRPM8) > ankyrin-1 (TRPA1); ion channels that mediate pulmonary inflammation and injury triggered by common combustion-derived PM, and for which polymorphic variants associate with variable asthma symptom control among children residing in the Salt Lake City area. These data suggest that GSDL may pose a risk to human health via multiple mechanisms. Support: ES017431 and ES020715.

### 3367 Species Differences in TRPM8 Affect Pulmonary Sensitivity to Chemical and Particulate Matter Agonists

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Exposure to air pollution is often unavoidable and mechanisms leading to short- and long-term adverse effects are not fully understood. Particulate matter (PM) in air pollution has many origins, including coal fly ash (CFA), diesel exhaust, cigarette smoke, calcium oxide, and wood smoke are examples of PM. Activation of transient receptor potential channels (TRP) is one mechanism by which PM can trigger adverse effects in the lungs, including asthma exacerbation. Notably, TRP1, TRPV1 and TRPM8 have been linked to the initiation of inflammation and cell injury. Initial findings using human airway epithelial cells have shown that activation of TRPM8 by CFA and CaO nanoparticles triggers cytokine gene expression and secretion, which was not replicated in mice. We hypothesized that species-specific differences in TRPM8 activation by PM may underlie this difference. Alignment of human and mouse TRPM8 revealed three differences in the amino acid sequence of the putative pore-loop domain. Mouse and human TRPM8 plasmids, and mutants thereof, were prepared and transiently transfected into GaMP6-overexpressing HEK-293 cells; calcium flux assays were then performed. Mutation of mouse Trpm8 to incorporate the corresponding human residues conferred sensitivity to TRPM8 activation by PM but may alter this difference. Alignment of human and mouse TRPM8 revealed three differences in the amino acid sequence of the putative pore-loop domain. Mouse and human TRPM8 plasmids, and mutants thereof, were prepared and transiently transfected into GaMP6-overexpressing HEK-293 cells; calcium flux assays were then performed. Mutation of mouse Trpm8 to incorporate the corresponding human residues conferred sensitivity to TRPM8 activation by PM but may alter this difference. Alignment of human and mouse TRPM8 revealed three differences in the amino acid sequence of the putative pore-loop domain. Mouse and human TRPM8 plasmids, and mutants thereof, were prepared and transiently transfected into GaMP6-overexpressing HEK-293 cells; calcium flux assays were then performed. Mutation of mouse Trpm8 to incorporate the corresponding human residues conferred sensitivity to TRPM8 activation by PM but may alter this difference. Alignment of human and mouse TRPM8 revealed three differences in the amino acid sequence of the putative pore-loop domain.
complemented the in vivo data and suggested a role for CFA-sensitive TRPV1 in regulating responses to CFA in vivo. Identification of specific PM-sensing sites on TRP channels furthered our understanding of mechanisms by which TRPs are activated by PM, revealing potential limitations in standard animal models for assessing roles of TRP channels in particle toxicity and translation of findings to humans, as well as advance our understanding of mechanisms by which PM promote lung inflammation, injury, and disease. Support: ES017431, ES027015.

3368 Computational Analysis of PM$_{2.5}$ and Its Effects on COVID-19


Richmond, California has been affected by a disproportionately high rate of COVID-19. This can be potentially attributed to the Chevron Richmond Refinery, which releases large amounts of Particulate Matter 2.5 (PM$_{2.5}$) into the air, as well as to the higher rate of asthma that affects the city which has been debatable to be a risk factor for COVID-19. The goal of this project was to perform a computational analysis of PM$_{2.5}$ and its impact on exacerbating asthma and COVID-19. Potential connections between the inflammatory signaling of asthma and COVID-19 were investigated. Online databases were used to analyze the gene interactions of PM$_{2.5}$ and to build a chemical-gene-disease pathway. PM$_{2.5}$ upregulates Toll-like receptor 4 (TLR4), which increases expression of the angiogenesis converting enzyme-2 (ACE2) receptor and mediates SARS-CoV-2 entry into the cell, as well as activating inflammatory signaling. It was found that PM$_{2.5}$ increases the expression of Interleukin-13, Interleukin-4, and Interleukin-18, which have crucial roles in both asthma and COVID-19 pathogenesis. IL-13 induces the expression of Hyaluronan Synthase 1 (Has1), which produces hyaluronan (HA), a cellular matrix component that builds up in asthma-associated inflammatory lesions and is found at higher levels in severe COVID-19 patients. In addition, Vascular Cell Adhesion Molecule 1 (VCAM-1) and Intercellular Cell Adhesion Molecule 1 (ICAM-1) were identified as being upregulated by PM$_{2.5}$. These mediate transendothelial leukocyte migration, which can lead to asthma complications and COVID-related mortality. Overall, this project helped identify key connections between asthma and COVID-19 and the impact that PM$_{2.5}$ exposure has on the contraction of COVID-19 in asthmatic individuals.

3369 Arsenic (III Oxidation State) Methyltransferase is an Important Mediator of Arsenite-Induced Hematotoxicity in Male Mice

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Human exposures to environmental metals such as arsenic are a worldwide public health concern. Chronic exposures to arsenic are linked to many health effects, including anemia. Epidemiological studies in populations chronically exposed to arsenic have shown that methylation capacity, mediated by the arsenic (III oxidation state) methyltransferase (AsSMTP) enzyme, is associated with elevated disease risk. Arsenic metabolism has an important role in detoxification, but it also generates bioactive intermediates with toxicity that may be greater than the parent inorganic arsenicals. Many studies suggest that the metabolites generated through the biotransformation of arsenite (AsIII), including monomethylarsonic acid (MMAIII), may be the primary arsenicals responsible for toxicity in vivo; however, few studies have been performed to directly evaluate this. In the present study, we used male AsSMTP-knockout (KO) and wildtype, C57BL/6.J, mice to evaluate the role of arsenic biotransformation in the development of anemia following drinking water exposures to AsIII. We found that exposure to 1 mg/L (ppm) AsIII for 60 days resulted in the significant reduction of red blood cell counts, hematocrit, and hemoglobin levels in the blood of wildtype, but not AsSMTP-KO mice. In support, we also observed significantly elevated levels of hemoglobin in the serum of wildtype mice. Collectively, results from this study suggest that the process of arsenic biotransformation may have a critical role in mediating the hematotoxicity of arsenic. This work was supported by the National Institutes of Environmental Health Sciences (NIEHS) Grant Number R01 ES032969, R01 ES035969-03 S1, National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) Grant Number 1R16GM146669-01, P20 GM130422, Institutional Development Award (IDEA) P20 GM103451, NIEHS and UNM METALS Superfund Research Program Grant Number P42 ES025589; UNM Center for Metals in Biology and Medicine (CMBM) through NIH, and the National Science Foundation Louis Stokes Alliance for Minority Participation, Undergraduate Research Scholars Program.

3370 Longitudinal Study of Carcinogenic Metals in Navajo Children from the Navajo Birth Cohort Study

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There are over 500 abandoned uranium mines across the Navajo Nation, exposing communities to environmental metals known to act as carcinogens. Exposure to carcinogens at early-life stages has been shown to increase the likelihood of developing cancer later on. Children are at a developmental stage in life where they may be more susceptible to the current state to effects later in life. The objectives were to identify and analyzed metal exposures in longitudinal samples from 556 children enrolled in the Navajo Birth Cohort Study (NBCS). Concentrations of uranium (U), total arsenic (UTAS), dimethylarsinic acid (DMAA), monomethylarsinic acid (MMAA), and arsenic III acid (UASS) were assessed longitudinally between birth to 5 years of age. The goals of this study were to: (i) determine if metal levels in children to the national average found in adults, (ii) identify the changes in metal concentrations overtime, (iii) identify the overall distribution of metal concentration, and (iv) determine patterns of exposure (i.e. chronic or acute) in longitudinal samples. Findings demonstrate early life exposures to metals known to contribute to cancer incidence. Overall trends show increasing metal concentrations of uranium, arsenic, dimethylarsinic acid, monomethylarsinic acid, and arsenic III acid in children over the time period tested. It is especially concerning that the measured metal concentrations are at or approaching average adult concentrations as reported by the National Health and Examination Survey (NHANES). This work was supported by the National Institutes of Environmental Health Sciences (NIEHS) Grant Number 1R16GM146669-01, P20 GM130422, Institutional Development Award (IDEA) P20 GM103451, NIEHS and UNM METALS Superfund Research Program Grant Number P42 ES025589; UNM Center for Metals in Biology and Medicine (CMBM) through NIH, and the National Science Foundation Louis Stokes Alliance for Minority Participation, Undergraduate Research Scholars Program.

3371 Novel Discovery of the Choroid Plexus (CP)—Subventricular Zone (SVZ) Regulatory Axis: Evidence from Small-Sized Extracellular Vesicles Released from the CP to Altered Adult Neurogenesis in SVZ and Implications in Manganese-Induced Nonmotor Syndromes

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The choroid plexus (CP) in brain ventricles secretes cerebrospinal fluid (CSF) that contains health- and growth-promoting factors. Immediately adjacent to the CP is the subventricular zone (SVZ), the largest adult neural progenitor region that harbors neural stem cell (NSCs) and supplies newborn neurons to the olfactory bulb (OB) for normal olfaction. However, the relationship between the CP and SVZ adult neurogenesis remained elusive. Exposure to manganese (Mn) is known to cause anosmia/hyposmia (lost/decreased sense of smell) in the early stage of exposure. To examine this in these mice three months after the initial viral infection. Results revealed that infection with AAV5-shRNA(Smpd3) significantly downregulated the proliferating NSCs in the SVZ by 19.4% (p<0.05). Three months after ICV injection, mice infected with AAV5-shRNA(Smpd3) displayed an increased latency in finding the hidden food pellet (food-finding test), in comparison to AAV5-shRNA(Ctrl), mice infected with AAV5-shRNA(Ctrl). Consequently, infection with AAV5-shRNA(Smpd3) significantly downregulated the proliferating NSCs in the SVZ by 19.4% (p<0.05). Three months after ICV injection, mice infected with AAV5-shRNA(Smpd3) decreased neuronal differentiation in an established SVZ neurosphere model by 44.2% in the AAV5-shRNA(Smpd3) group compared with AAV5-shRNA(Ctrl). Conversely, infection with AAV5-shRNA(Ctrl) increased the newborn neurons in the OB by 28.9%, whereas Mn-seEVs decreased it by 18.3%, compared with PBS-injected controls (p<0.05), indicating a likely presence of a sEV-driven CP-SVZ regulatory axis that modulates the olfaction. To better understand this putative axis, we used adenov-associated virus serotype 5 (AAV5), a viral vector with specific tropism towards choroidal epithelial cells, to knockdown sphingomyelin phosphodiesterase 3 (SMPD3) that catalyzes the cellular process critical to sEV biogenesis and release. One month after ICV injection, mice infected with AAV5-shRNA(Smpd3) had a significantly decreased SMPD3 protein level in CP and, importantly, a reduced sEV concentration in the CSF by 76.6%, compared with AAV5-shRNA(Ctrl). Consequently, infection with AAV5-shRNA(Smpd3) significantly downregulated the proliferating NSCs in the SVZ by 19.4% (p<0.05). Three months after ICV injection, the newborn neurons in the OB were decreased by 44.2% in the AAV5-shRNA(Smpd3) group compared with AAV5-shRNA(Ctrl). A battery of neurobehavioral tests was further conducted to assess the olfaction in these mice three months after the initial viral infection. Results revealed that in comparison to AAV5-shRNA(Ctrl), mice infected with AAV5-shRNA(Smpd3) displayed an increased latency in finding the hidden food pellet (food-finding test), reduced sensitivity towards female urine (olfactory attractant test), and decreased avoidance behavior in response to 2-methylbutyric acid (olfactory avoidance test). Collectively, these observations provide first-hand evidence to support the novel concept of the CP-SVZ regulatory (CSR) axis, which, through the signals carried by
3372 Soluble Mercury Exposure Risk of Tibetan Medicine Zuotai Containing HgS and Its Compounding Preparations

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Mercuric sulfide (HgS) is used throughout history by many ethnic medicine systems. Zuotai is a classic Tibetan medicine containing HgS, which is believed to have the effects of reducing toxicity and enhancing efficacy. Mercury toxicity is closely related to its chemical form and bioaccessibility. So far, the risk assessment of Zuotai and its compounding preparations containing HgS is expressed as total mercury. However, HgS is a typical insoluble covalent sulfide. Therefore, it is unreasonable to take the total mercury of HgS as soluble inorganic mercury. The present research is aimed to establish an in vitro biomimetic approach to evaluate the real Hg exposure risk of Zuotai and its compounding preparations. Our previous research found the mercury leaching ratio of Zuotai in artificial gastric juice and artificial intestinal juice was about 276.1, so the contribution of artificial intestinal juice to the bioaccessibility of soluble mercury in Zuotai can be ignored. Based on this, we determined the leaching mercury of Zuotai and its compounding preparations in artificial gastric juice, then converted them into the exposure dose and the daily total exposure amount of soluble mercury for adults. We found that the soluble mercury exposure doses of 17 batches of Zuotai and 13 batches of its compounding preparations at clinical doses range from 0.0009 - 1.1181 μg/kg bw, and the average is 0.1240 ± 0.2065 μg/kg bw, less than the RFD value (0.3 μg/kg bw, EPA, US) of soluble inorganic mercury. Only 2 batches of Zuotai (0.3618 and 1.1180 μg/kg bw) exceed the toxic dose limit. The possible total exposure amounts of soluble mercury from these Zuotai and its compounding preparations at clinical doses range from 0.2297 - 31.9037 μg/day, and the mean value is 4.4743 ± 6.4020 μg/day, less than the permitted daily exposure (PDE) value (30 μg/day, International Council for Harmonisation of Requirements for Pharmaceuticals for Human Use) of soluble inorganic mercury. Only 31 batch of Zuotai (31.9037 μg/day) exceeds this PDE. The above shows Zuotai and its compounding preparations probably don’t cause mercury-derived risk when taken at clinical doses, but the quality of Zuotai needs to be strictly controlled to reduce batch-to-batch variability. And the research provides a new strategy to assess the real exposure risk of soluble mercury for traditional medicines containing HgS.

3373 Urinary Arsenic, Polycyclic Aromatic Hydrocarbons, and Metal Exposure and Risk of Stroke

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Exposure to chemicals or metals from various environmental and occupational settings has been associated with adverse health conditions such as cardiovascular diseases, pulmonary diseases, and cancers. Exposure to environmental chemicals can occur from contaminated food, water, inhalation (lung), and absorption (skin). Stroke is one of the primary causes of morbidity and mortality worldwide. In addition, Stroke is leading cause of death and long-term disability in the United States. Limited studies are conducted to assess the impact of polycyclic aromatic hydrocarbons, arsenic, and other metals exposure and their association with the risk of stroke. This study aimed to assess seven species of urinary arsenic (arsenous acid, arsenic acid, arsenobetaine, arsenocholine, dimethylarsinic acid, monomethylarsonic acid, and total arsenic), seven types of polycyclic aromatic hydrocarbons (PAHs), compounds (1-hydroxypyrene, 3-hydroxypyrene, 4-hydroxypyrene, 2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, 1-hydroxypyrene, 2-3-hydroxyphenanthrene) and 14 types of urinary metals (cadmium, barium, cobalt, molybdenum, mercury, cesium, manganese, antimony, lead, tin, strontium, tungsten, thallium, and uranium) and their association with stroke. The study aimed to assess the seven species of urinary arsenic, seven types of polycyclic aromatic hydrocarbons, and 14 types of urinary metals. Our study demonstrates that chronic As exposure associated with increased odds of stroke. Among metals, the third OR: 2.327, 95% CI: 0.961-5.632] and fourth [OR: 2.844, 95% CI: 0.947, 8.543] quantiles of urinary manganese showed a positive correlation with increased odds of stroke. The study observed that urinary PAHs and manganese are significantly associated with stroke. Future studies in humans is suggested to support or refute this finding.

3374 Chronic Arsenic Exposure Upregulates the Expression of Basal Transcriptional Factors and Increases Invasiveness of the Nonmuscle Invasive Papillary Bladder Cancer Line RT4


The bladder is a target organ for inorganic arsenic, a carcinogenic and common non-papillary contaminant found in soil and water. Urothelial carcinoma (UC) is the most common type of bladder cancer (BC) that develops into papillary or non-papillary tumors. Papillary tumors are mostly non-muscle invasive (NMIBC), easier treated, and have a better prognosis. Urothelial carcinoma can be molecularly sub-typed as luminal or basal, with papillary tumors generally falling into the luminal category and basal tumors exclusively forming muscle invasive urothelial carcinomas (MiUC). It is unclear why some UCs develop more aggressive basal phenotypes. We hypothesized that chronic arsenic exposure is a papillary luminal bladder cancer would lead to the development of basal characteristics and increase in invasiveness. We treated the human papillary bladder cancer cell line RT4 with 1 μM arsenite (As3+) for 20 passages. Throughout the study, key luminal and basal markers were monitored in the exposed and parent lines. At passage twenty, the cells were injected into athymic mice to evaluate tumor histology and measure protein markers using immunohistochemistry. Our data indicates that chronic As3+-treatment altered cellular morphology and decreased several luminal markers in cell culture. The histology of the tumors generated from this treatment showed a shift toward the basal phenotype similar to the parent (non-treated) however, they appeared to be more invasive in the liver and displayed elevated levels of several basal markers. Our study demonstrates that chronic As3+ exposure is able to convert a non-invasive papillary bladder cancer to an invasive form that acquires some basal characteristics.

3375 Prenatal Exposure to Metals Alters the Inflammatory Proteome in a Sex-Dependent Manner in the Placentas of Children Born Extremely Preterm

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Prenatal exposure to toxic metals is associated with altered placental function and adverse health outcomes, both acute (e.g., preterm birth and low birthweight) and long-term (e.g., altered neurodevelopment). It is also well established that associations between toxicant exposures and adverse pregnancy outcomes such as preterm birth are sex-specific. Although the placenta serves as a physical barrier between maternal and fetal tissues, it is imperfect, such that toxic metals pass from mother to fetus. While the underlying molecular mechanisms linking in utero toxic metal exposures with later-in-life health remain unclear, placental inflammation is posited as a potential driver. Here, we sought to understand whether in utero metal exposures are associated with alterations in the placental proteome, hypothesizing that metals levels would be positively associated with the altered expression of inflammation-associated pathways and that sex-specific patterns would be observed. Within the Extremely Low Gestational Aged Newborns (ELGAN) cohort (n=230) placental and umbilical cord tissue samples were collected at birth for male (n=131) and female (n=99) infants. Arsenic (As3+), cadmium (Cd), and lead (Pb), selenium (Se), and manganese (Mn) concentrations as the independent variables. Models were adjusted for infant sex, maternal pre-pregnancy body mass index, maternal socioeconomic status, and maternal cigarette smoking during pregnancy. In the non-sex-stratified analysis, 22 metals associated proteins (MAPs) displayed significant altered expression in the placenta in relation to cord tissue metal concentrations as the independent variables. Models were adjusted for infant sex, maternal pre-pregnancy body mass index, maternal socioeconomic status, and maternal cigarette smoking during pregnancy. In the non-sex-stratified analysis, 22 metal associated proteins (MAPs) displayed significant altered expression in the placenta in relation to cord tissue metal concentrations. When stratifying by sex, 90 MAPs were differentially expressed in female placentas, and 42 MAPs were differentially expressed in male placentas. Notably, many of these MAPs are known to be involved in inflammatory-related processes, highlighting the linkage between prenatal metals exposure and an altered placental proteome, with implications for altering the trajectory of proper fetal development.
Arsenic Disrupts Extracellular Vesicle–Mediated Signaling in Regenerating Muscle Cells

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Sensorimotor impairments and muscle atrophy related to chronic inorganic arsenic exposure affect over 10 million individuals worldwide. Despite the central role of muscle health to systemic metabolism and overall health, muscle-specific effects of arsenic exposure are a relatively understudied area. Reduced skeletal muscle regenerative capacity is a hallmark of arsenic exposure studies in mice, however, mechanisms remain elusive. The role of intercellular communication via extracellular vesicles (EVs) during regeneration has become increasingly evident but studying cell-specific arsenic effects on muscle cell paracrine signaling is made challenging by the complexity of the muscle environment. Therefore, we developed and employed a novel 3D muscle construct to investigate the hypothesis that arsenic can disrupt cell-autonomous paracrine communication in regenerating muscle cells. These constructs exposed to arsenic then injured with cardiotoxin recapitulated in vivo structural and functional impairments during muscle regeneration, attesting to the validity of the model. We observed similar regenerative deficits when constructs were treated with arsenic-conditioned media and arsenic-conditioned EVs (rather than arsenic itself), lending to support our hypothesis. Mass spectrometry of arsenic-conditioned media identified 153 differentially abundant secreted proteins, which were related to several pathways integral to MPC differentiation. Collectively, these results demonstrate the important role of cell-autonomous arsenic effects in disrupting EV-mediated intercellular communication during muscle regeneration. They demonstrate the further need for investigating the impact of ARP in these outcomes and their importance in the context of arsenic impacts in the full regenerative niche. Supported by NIH Grant R01ES033519.

Arsenic Exposure Induces Glucose Intolerance in Mice via Gut Microbiome–Bile Acid–FXR Signaling Pathway

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Arsenic (As) is a widespread toxic metalloid in environment and contributes to a number of human diseases, including diabetes. The association between inorganic As and diabetes has been established in numerous epidemiological studies. Gut microbiome plays a fundamental role in human health by regulating metabolic, and thus arsenic has become a major target for therapeutic intervention. Studies have suggested the role of the gut microbiome and Farnesoid X receptor (FXR) in the pathogenesis and prevention of diabetes in both animals and humans. However, how the inorganic As (iAs) altered gut microbiome affects diabetes risk remains to be determined. Here, we exposed 1 ppm and 50 ppm As to C57BL/6 mice and FXR-/ mice for 6 months and investigated whether As exposure induces glucose intolerance and whether arsenic exposure affects the bile acid homeostasis. Mice were fasted for 14 h overnight, orally administered 2 g/kg of body weight glucose and measured glucose levels before and 15, 30, 60, 90, and 120 min after glucose administration. We found that As treatment can significantly increase body weight and contiguity, and target for therapeutic interventions. In this study, we used a novel 3D muscle construct to investigate the hypothesis that arsenic disrupts cell-autonomous paracrine communication in regenerating muscle cells. These constructs exposed to arsenic then injured with cardiotoxin recapitulated in vivo structural and functional impairments during muscle regeneration, attesting to the validity of the model. We observed similar regenerative deficits when constructs were treated with arsenic-conditioned media and arsenic-conditioned EVs (rather than arsenic itself), lending to support our hypothesis. Mass spectrometry of arsenic-conditioned media identified 153 differentially abundant secreted proteins, which were related to several pathways integral to MPC differentiation. Collectively, these results demonstrate the important role of cell-autonomous arsenic effects in disrupting EV-mediated intercellular communication during muscle regeneration. They demonstrate the further need for investigating the impact of ARP in these outcomes and their importance in the context of arsenic impacts in the full regenerative niche. Supported by NIH Grant R01ES033519.

Inorganic arsenic (iAs) is a class I human carcinogen. Approximately 225 million people, including >2 million in the U.S., are exposed to high iAs concentrations (>10 μg/l) by drinking contaminated water. Skin is a major target organ of iAs, and chronic iAs exposure results in hallmark skin lesions including iAs-induced skin cancer. iAs is also well-recognized as a clastogen; iAs exposure is associated with increased iAs DNA double-strand breaks (DSBs) accumulated in mouse populations and in vitro. Structural chromosomal instability (CIN), characterized by DSBs, is a hallmark of cancer. Since iAs does not directly interact with DNA, it is likely that other mechanisms, such as inhibition of DSB repair and signaling, may be responsible for iAs-induced CIN. Our hypothesis is that chronic iAs exposure results in decreased ATM pathway activation in keratinocytes. Studies in cultured human lung cells show particulate matter exposure increased DSBs and decreased phosphorylated ATM and CHEK2 occurred concomitantly in both human keratinocyte cell lines chronically exposed to iAs. Significantly increased DSBs and decreased phosphorylated ATM and CHEK2 occurred concomitantly in both human keratinocyte cell lines chronically exposed to iAs indicating that chronic iAs exposure significantly reduced ATM pathway activation in keratinocytes. Chronic iAs exposure results in hallmark skin lesions including iAs-induced skin cancer. iAs is also well-recognized as a clastogen; iAs exposure is associated with increased iAs DNA double-strand breaks (DSBs) accumulated in mouse populations and in vitro. Structural chromosomal instability (CIN), characterized by DSBs, is a hallmark of cancer. Since iAs does not directly interact with DNA, it is likely that other mechanisms, such as inhibition of DSB repair and signaling, may be responsible for iAs-induced CIN. 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alone by oophorogynal aspiration. There were two timepoints: a single dose for 24 hours and a second dose once per month for 3 days. Cr was found in every rat lung lobule with more Cr found in the right lung. We found both DNA double strand breaks and HR repair increased in a concentration-dependent manner in rat lungs after 24-hour Cr(VI) exposure. However, after 90 days of exposure, we found DNA double strand breaks increased, but HR repair decreased. Notably, these effects were distinct in bronchioles and more muted in alveoli. We also considered these outcomes in Cr(VI)-associated human lung tumors, and found DNA double strand breaks increased and RAD51 levels decreased in lung tumor tissue compared to adjacent normal lung tissue. Thus, Cr(VI)-induced DNA double strand break and HR repair inhibition translates from cells to experimental animals, normal human lung tissue, and Cr(VI)-associated human lung tumors. Our findings establish a mechanism for Cr(VI)-induced DNA double strand break and HR repair inhibition in Cr(VI) carcinogenesis. This work was supported by the National Institute of Environmental Health Sciences [R35ES032876 and ES016893 to JPW] and the University of Louisville School of Medicine Basic Grants Program (SSW).

Find up-to-date information at [3382 Cytotoxicity of Simulated Gastric Leachates of 3D Printer Metal-Containing Filaments in Rat and Human Intestinal Models](#)

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Agricultural products such as soils, fertilizers, soil conditioners, and pest control agents may contain varying levels of metals. This assessment was conducted to quantify potential occupational exposures to metals (e.g. arsenic, cadmium, chromium, lead) from 3D printer metal-fill objects in a commercial greenhouse setting. The exposures were compared to established or derived Safe Harbor Levels to determine product warning requirements for California Proposition 65 regulations. An exposure analysis was conducted for a variety of metals based on the intensity, frequency, and duration of typical exposures associated with use of such products. For a given metal, various exposure routes (oral, inhalation, dermal) were determined based upon product formulation (solid vs. liquid) and intended use. Exposure scenarios considered in the assessment were based upon potential direct contact with solid or liquid product, contact with diluted or dissolved liquid product, and contact with soil to which product was applied. For screening-level purposes, bioavailability and form of each metal in the products were generally not considered. Where appropriate, exposure estimates were based upon parameters described by USEPA human health risk assessment guidelines for heavy metals in contaminated soils and agricultural fertilizers, CalEPA regulations, and USEPA Exposure Factors Handbook. Liquid fertilizer products were associated with highest occupational metal exposures followed by soils and dry fertilizer products, which were greater than pest control products, with soil conditioners showing the least contribution to exposure. The oral route was the highest contributor to exposure followed by inhalation and dermal routes for all metals, with the exception of arsenic. As an example, assuming that a given product contained cadmium at a concentration of 10 mg/kg, estimated daily exposure from a given product ranged from 0.2 to 0.6 µg/day. Meanwhile, product-specific estimated exposures to arsenic were generally higher, ranging from 0.4 to 4.8 µg/day, based on higher contributions from dermal exposure due to greater skin absorption of arsenic compared to other metals. Based on the specific routes of exposure for each product, it is possible that metal concentrations were not consistent with Proposition 65 Safe Harbor Levels and warning requirements. For cadmium, product-specific maximum allowable concentrations were 118 mg/kg for soils and dry fertilizers, 79 mg/kg for liquid fertilizers, 77 mg/kg for pest control agents, and 782 mg/kg for soil conditioners. The approaches of this screening-level assessment are adaptable to a variety of product types and exposure scenarios. The results can be used for estimating occupational and consumer exposures in dermal compliance with relevant guidelines or regulatory standards, set product specifications, identify sources of exposure control, or further assessment if required.

Steel-fill leachates consisted primarily of iron. Subsequent studies focused on the copper containing object. A 4-hr exposure to CuSO₄ (25-1000 µg/ml), resulted in concentration-dependent decreases in rat cell viability. Addition of the copper chelator, bathocuproine disulphonate (1mM), to CuSO₄ (250 µg/ml) and copper-fill leachate restored rat cell viability to levels comparable to untreated cells, suggesting that decreased viability was a result of copper ion exposure. Glutathione levels in rat cells decreased following a 4-hr exposure to copper-fill leachate, suggesting the formation of reactive oxygen species. Hydrogen peroxide levels increased in rat cells exposed for 4 hr to copper-fill leachate. Overall, our data indicate that metals released from the simulated gastric acid exposure of print objects using metal-fill filaments, especially copper, are toxic to rat and human intestinal cells in a concentration- and time-dependent manner. Metals ions, particularly copper, released from metal-fill printer objects by this exposure may form reactive oxygen species leading to cytotoxicity. This abstract does not represent US EPA policy.

**3383 Alterations in Mitochondrial Lead (Pb²⁺) Uptake with Co-exposures to a Mitochondrial Uncoupler and a Mitochondrial Calcium Unipporter (MCU) Inhibitor**

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Lead (Pb²⁺) is a metal to which many people are exposed, and which causes developmental toxicity. The mitochondrial calcium uniporter (MCU) imports calcium ions, and it is believed that this complex is also responsible for the influx of lead into the mitochondria, via molecular mimicry. Since our environment contains mixtures of toxic agents, it is important to consider multi-chemical exposures. In this study, HepG2 cells were exposed to lead alone and in mixtures with other metals, including chromium, lead, among others) from use of such products in a commercial greenhouse setting. The exposures were compared to established or derived Safe Harbor Levels to determine product warning requirements for California Proposition 65 regulations. An exposure analysis was conducted for a variety of metals based on the intensity, frequency, and duration of typical exposures associated with use of such products. For a given metal, various exposure routes (oral, inhalation, dermal) were determined based upon product formulation (solid vs. liquid) and intended use. Exposure scenarios considered in the assessment were based upon potential direct contact with solid or liquid product, contact with diluted or dissolved liquid product, and contact with soil to which product was applied. For screening-level purposes, bioavailability and form of each metal in the products were generally not considered. Where appropriate, exposure estimates were based upon parameters described by USEPA human health risk assessment guidelines for heavy metals in contaminated soils and agricultural fertilizers, CalEPA regulations, and USEPA Exposure Factors Handbook. Liquid fertilizer products were associated with highest occupational metal exposures followed by soils and dry fertilizer products, which were greater than pest control products, with soil conditioners showing the least contribution to exposure. The oral route was the highest contributor to exposure followed by inhalation and dermal routes for all metals, with the exception of arsenic. As an example, assuming that a given product contained cadmium at a concentration of 10 mg/kg, estimated daily exposure from a given product ranged from 0.2 to 0.6 µg/day. Meanwhile, product-specific estimated exposures to arsenic were generally higher, ranging from 0.4 to 4.8 µg/day, based on higher contributions from dermal exposure due to greater skin absorption of arsenic compared to other metals. Based on the specific routes of exposure for each product, it is possible that metal concentrations were not consistent with Proposition 65 Safe Harbor Levels and warning requirements. For cadmium, product-specific maximum allowable concentrations were 118 mg/kg for soils and dry fertilizers, 79 mg/kg for liquid fertilizers, 77 mg/kg for pest control agents, and 782 mg/kg for soil conditioners. The approaches of this screening-level assessment are adaptable to a variety of product types and exposure scenarios. The results can be used for estimating occupational and consumer exposures in dermal compliance with relevant guidelines or regulatory standards, set product specifications, identify sources of exposure control, or further assessment if required.

Although arsenic alone induces cancers in the lungs, bladder, kidney, and skin, it is also a potent co-carcinogen. Studies suggest arsenic enhances UV skin cancer, but the mechanisms are not fully understood. One proposed mechanism is inhibition of the nucleotide excision repair (NER) pathway, which is responsible for repairing cyclobutane-pyrimidine dimers (CPDs), a type of UVR DNA damage that results in mutations found in UV skin cancers. Carcinogenic mechanisms can be explored using mutational signatures analysis, a novel whole genome sequencing approach that associates mutation patterns with specific molecular mechanisms. UVR mutational signatures are well defined, but no studies have used mutational signatures to investigate co-carcinogenesis. In this study two models, human skin cells and SKH-1 mice, were co-exposed to arsenic and UVR to investigate arsenic-altered mutation patterns of UVR exposure. Arsenic alone did not induce mutations, but significantly increased UVR mutations and altered the spectra of select UVR mutational signatures. The proposed UVR indel mutational signature, ID13, is only found in a subset of skin cancers. As the first study to investigate co-carcinogenesis we found ID13 in arsenic-UVR co-exposed groups, but not UVR alone groups indicating ID13 may be unique to arsenic-UVR co-exposure and may serve as a biomarker of arsenic and UVR co-exposure. These findings show arsenic alters select mutational processes of UVR carcinogenesis and demonstrates mutational signatures is a novel tool to investigate metal carcinogenesis.
Meconium serves as a repository of in-utero exposures, as it forms in the fetal gut starting the 12th gestational week and is not passed until birth. The influence of metal mixtures on endogenous metabolites in meconium remains unknown. In this study, we analyzed metal signatures within meconium that might influence the early metabolic landscape reflective of fetal life exposures. A cross-sectional pilot study was conducted on 20 first-pass meconium samples collected from random healthy newborns delivered at Oklahoma Children’s hospital. Collection, transport, and storage of all samples were performed consistently. Samples were digested using the nitric acid/ peroxide method in a microwave digestor and 25 metals were measured by inductive coupled plasma mass spectrometry using ICP-TQe. Metabolites including bile acids, amino acids, polyamines, and energy metabolites from the TCA cycle were measured by liquid chromatography-mass spectrometry using TSQ Quantis. Results showed a significant association between metals and metabolites using a multivariate regression method such as sparse partial least squares regression (sPLS) at correlation threshold ≥ 0.49 and P < 0.05 Metals and metabolites such as bile acid, amino acid, and polyamines clustered into 4 specific groups. Cluster 1 comprised the nutritional metal magnesium that was associated with amino acids such as tryptophan and phenylalanine, both of which form important components of neurotransmitters. Clusters 2 and 3 included metals associated with bile acids indicative of cholesterol elimination. While cobalt was associated with bile acid glycoconjugated acid in cluster 2, cluster 3 included both toxic (cadmium and antimony) and nutritional metals (molybdenum and selenium) that were associated with multiple conjugated bile acids tauroconjugated acid, taurourourourdexhydroxylic, glycocholic and taurocholic acid. Cluster 4 showed copper and nickel associated with polyamine metabolism including urea, and creatinine, which are protein breakdown products. In conclusion, implications for risk assessment could be determined using meconium as a source of in-utero exposures. Metal composition of meconium may further inform on causative agents in meconium-associated complications in neonates such as meconium aspiration syndrome, asthma, and brain injury.
Ocupational exposure to beryllium can cause Chronic Beryllium Disease (CBD) and cancer. Workers involved in abrasive blasting and support personnel are exposed to beryllium through inhalation, dermal contact, and inadvertent ingestion when using abrasives that contain beryllium in the slag. The 2017 Beryllium in Construction Standard included dermal contact and airborne exposure above the action level as a trigger for the implementation of occupational risk management strategies to prevent adverse health outcomes. However, OSHA’s revised final rule in 2020 excluded dermal contact as a trigger without providing exposure studies justifying its exclusion. The purpose of this pilot study was to measure beryllium in the air, on workers’ skin, worksite surfaces, and workers’ respirators to gain insight into the exposures experienced by abrasive blasters and support personal during abrasive blasting with slag abrasives; and, to help develop improved risk management strategies for this at-risk population. Exposures were assessed for two days in August 2022 during abrasive blasting structural steel with coal slag blast media in an abrasive blast yard. Four personal breathing zone air samples, 24-skin wipe samples, 12-surface wipe samples and four bulk samples were collected and analyzed for total beryllium via EPA method SW846 3050B/6010D. We measured 2.0 parts per million of beryllium in the bulk blasting media; however, no detectable beryllium transfer was measured to skin and surfaces indicating minimal risk to workers. Additional exposure assessments using much lower detection limits are needed to confirm no beryllium exposure risk.

Discovery of Phytochelatins in Human Urine and Their Impact on Cadmium Distribution and Excretion

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Phytochelatins [PyC; (γEC)k] are metal binding peptides formed from glutathione. They are used by plants and some invertebrates to transport and sequester heavy metals, notably cadmium (Cd). PyCs are present in nearly all plant material and present in nearly all human diets, but their route of excretion is unknown. Individuals with higher consumption of plant-derived foods have shown decreased Cd burden, suggesting that PyCs derived from the diet could alter Cd uptake and excretion. The aims of this study were to determine the metabolic fate of dietary PyCs and to determine their impact on Cd uptake and excretion. High-resolution metabolomics using LC-MS and ICP-MS-based Cd quantitation was performed on urine samples from a subset of healthy adults from Emory/Georgia Tech Predictive Health Institute’s Center for Health Discovery and Well-Being cohort. Urine was searched for PyCs and matches confirmed using MS/MS and an authentic chemical standard. PyC standards were incubated with gamma-glutamyltransferase (GGT) and carboxypeptidase A1 (CPA) to demonstrate mammalian metabolism of PyCs, and human urine metabolomics data was searched for the enzyme-derived metabolites. PyCs, PyC metabolites, and Cd were tested for correlations using Spearman’s rank correlation. The impact of PyCs on Cd reuptake was studied further using chickens. PyC treatments inhibited Cd reuptake and increased the amount of Cd excreted in the feces. We conclude that PyCs are absorbed from the diet and metabolized. Correlation of urinary PyC and urinary Cd suggests that dietary intake of PyCs influences uptake and/or excretion of Cd, and results from cell and animal experiments show that PyCs both protect against Cd deposition into bodily tissues and discourage reuptake of Cd from the urine. These results suggest that the capacity of PyCs for heavy metal interactions is not lost after absorption from the diet. PyCs may serve as a diet-derived source of protection against dietary heavy metal exposures.

Hexavalent Chromium Induces Homologous Recombination Repair Failure in Human Cells but Not in Alligator Cells

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Hexavalent chromium [Cr(VI)] is a metal known to cause human lung cancer however its carcinogenic mechanism is uncertain. Due to its heavy industrial use, Cr(VI) is prevalent in the air and water, causing exposure risks to humans and wildlife. Alligator skin samples show high levels of Cr(VI) accumulation, but alligators have never been known to develop cancer. Our study examines the mechanism of Cr(VI) carcinogenesis in human and alligator lung cells using our One Environmental Health philosophy. Cr(VI) is known to induce chromosome instability (CIN), but the mechanism for how it induces CIN is poorly understood. We focused on hexavalent chromium as a representative metal as it is a known human lung carcinogen that induces DNA double stranded breaks (DSBs). We show that Cr(VI) induces small DSBs resulting in CIN, an early event in lung cancer. The homologous recombination (HR) repair pathway plays a key role in preventing CIN by repairing DNA double strand breaks. Cr(VI) targets RAD51, a key effector protein within the HR pathway, and prevents its loading onto a nucleoprotein filament, but how it prevents this loading is unknown. Five classical RAD51 paralogues help orchestrate RAD51 loading and function through two distinct protein complexes: the BCDX2 complex, which consists of RAD51B, RAD51C, RAD51D and XRCC2 and the CX3 complex, which consists of RAD51C and XRCC3. The effects of Cr(VI) on these paralogs are poorly understood and largely uninvestigated. Thus, we hypothesize Cr(VI) disrupts RAD51 paralogues function leading to disruption of the HR repair in human lung cancer cells. We measured the effects of Cr(VI) on RAD51 and XRCC3 to represent the BCDX2 and CX3 complexes, respectively. Cells were exposed to acute (24 h) and prolonged (120 h) Cr(VI) treatments at 0.1, 0.2, and 0.3 μg/cm², using immunofluorescent foci formation for DNA repair, western blot for protein amounts, and qRT-PCR for mRNA levels. In human lung cells, 24 and 120 h exposure to Cr(VI) induced decreased levels of RAD51 foci formation, protein amounts, and mRNA levels, implicating this protein is potentially the primary target in contributing to the loss of RAD51 filament formation and failure of the HR repair response. However, we found Cr(VI) minimally affected XRCC3 of the CX3 complex further suggesting RAD51D as a part of the BCDX2 complex may be the key initial target in Cr(VI)-reduced RAD51 function and HR repair inhibition. In contrast, alligator lung cancer cells showed elevated RAD51 foci formation indicating HR repair is active after 24 and 120 h exposure. Completion of this study will identify key mechanisms for how hexavalent chromium causes CIN and lead to new ways to avoid or treat lung cancer. Supported by NIH grants R01ES016993 and R35ES032876 (J.P.W.) and T2ZE0011564 (A.W. and J.P.W.)
Arsenic is a toxicant that is ingested through drinking water and food, exposing nearly 140 million people in fifty countries to levels above the acceptable guideline concentration of 10 ppb. Studies have shown that arsenic affects stem cells, but the mechanisms by which arsenic alters differentiation and formation of adult cells in the small intestine is not understood. Adult Sox9<sup>fl/fl</sup>-EGFP mice were exposed to 0, 33, and 100 ppb sodium arsenite in their drinking water for 13 weeks, and sections of duodenum were examined for changes. Flow cytometric analysis indicated that arsenic exposure significantly decreased overall Sox9 expression in the small intestine, with dose-responsive reductions seen in Sox9-Lox tandem amplifying cells and Sox9-SubLo intestine stem cells. Control of intestinal cell differentiation relies on signaling from stromal cells in the villi and crypt. Telocytes produce bone morphological proteins (BMPs) that maintain a region of differentiation amplifying cells and Sox9-SubLo intestine stem cells. Control of intestinal cell differentiation relies on signaling from stromal cells in the villi and crypt. Telocytes produce bone morphological proteins (BMPs) that maintain a region of differentiation amplifying cells and Sox9-SubLo intestine stem cells. The telocyte marker Gli1 was downregulated by 1.4- and 2.3-fold in the 33 and 100 ppb exposure groups, respectively, while the trophocyte marker Grem1 was upregulated by 1.3 and 2.3-fold. Examining protein levels by IHC revealed that the expression of Pdgfra-Lo (trophocyte marker) was significantly upregulated by 3.4-fold in males from both exposure groups and downregulated by 8.3- and 10-fold in females. These alterations suggest that chronic arsenic exposure impairs stromal cell signaling in the small intestine, potentially leading to changes in cell differentiation.

### 3394 As3MT-Dependent Proteome Changes in Mouse Embryonic Fibroblasts

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The public health concern posed by arsenic toxicity is underscored by the pernicious effects of chronic arsenic exposure: cardiovascular disease, neurodevelopmental disorders, cancer, and diabetes. The primary metabolic pathway of arsenic is its methylation by arsenic (3) methyltransferase (As3MT). As3MT has only one known function—arsenic methylation—which serves to increase the rate of whole-body arsenic clearance. Fitting with this framework, As3MT knockout mice exhibit impaired arsenic methylation and slower whole-body arsenic clearance. Under basal conditions, As3MT<sup>-/-</sup> cells curiously exhibit a stress response and divide at a lower rate. Together with the arsenic-independent association of the As3MT locus with ischaemic heart disease and schizophrenia among humans, these observations give rise to the hypothesis that As3MT possesses at least one alternate function. Thus, we aimed to explore possible alternate functions of As3MT by studying differences in cell functions associated with loss of As3MT expression and successful recombination with arsenic-decreased changes. We compared basal and 1.0 μM inorganic arsenic-induced protein expression changes in wild type versus As3MT<sup>-/-</sup> mouse embryonic fibroblasts (MEFs) after 24 hours using liquid chromatography mass spectrometry. Preliminary analyses indicate broad differences in the protein expression of As3MT<sup>-/-</sup> MEFs with respect to cell adhesion, cytoskeleton and trafficking pathways—the pathways most affected by pathway enrichment analysis. The mass spectrometry data and immunoblot validation suggested that the As3MT<sup>-/-</sup> fibroblasts show pronounced downregulation of vimentin and β-catenin. Further validation will be performed to assess protein expression in liver homogenates of wild type and As3MT<sup>-/-</sup> mice. These data suggest that As3MT may have a physiological function beyond arsenic methylation.

### 3395 Characterization of an Arsenic 3 Methyltransferase<sup>fixed</sup>Mouse Model

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Arsenic 3 methyltransferase (As3MT) is the key enzyme in arsenic biotransformation, where it methylates arsenic, the only known target, using Sadenosylmethionine as a cofactor. Polymorphisms in human As3MT are linked to changes in enzyme efficiency and pathological outcomes. Mice lacking As3MT are significantly compromised in arsenic methylation. However, the relative contribution of tissue-specific As3MT expression to arsenic methylation and toxicity is unknown, although liver As3MT is considered the most important contributor. Here, we describe the generation of As3MT<sup>fl/fl</sup> mice. LoxP sites were inserted into the introns upstream of exon 3 and downstream of exon S. Successful recombination generates the same exon deletion as in the previously described As3MT knock-out mice, where the methyltransferase domain is excised. We have shown that in vitro, Cre recombinase addition results in successful recombination of DNA from mice containing both LoxP sites. Further, breeding of As3MT<sup>fl/+</sup><sup>s</sup> with albumin-Cre containing mice results in recombination in the liver, but not in tail or lung DNA. Mice are viable and born at similar expected ratios. As3MT<sup>fl/+</sup> mice grow similar to controls and are alive after 5 weeks of 200 ppb sodium arsenite exposure given in the drinking water. We will present data showing histology of liver and other tissues, as well as arsenic methylation profiles in mice and in both male and female mice. These mice will be important tools in dissecting the role of tissue-specific As3MT in arsenic toxicity, as well as potentially identifying alternative As3MT functions.

### 3396 Elevation of MEG3 Gene Expression by Isorohapontigenin Suppresses Migration and Invasion of Cr(VI)-Transformed Cells

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Chronic exposure of human bronchial epithelial BEAS-2B cells to hexavalent chromium (Cr(VI)) causes malignant cell transformation. These transformed cells exhibit increases in migration and invasion, accompanying by increased Slug and reduced E-Cadherin, two markers of epithelial-mesenchymal transition (EMT). Maternally expressed gene 3 (MEG3), a long noncoding RNA, functions as a tumor suppressor. The results from the present study showed that MEG3 was lost in Cr(VI)-transformed cells. Here, we report a precancer developmentally downregulated protein 9 (NEDD9), a multidomain scaffolding protein, was upregulated and β-catenin was activated in Cr(VI)-transformed cells. Overexpression of MEG3 decreased NEDD9 expression and β-catenin activation, resulting in inhibition of invasion and migration of those Cr(VI)-transformed cells. Isorohapontigenin (ISO), a phytolipophenol, is a derivative stilbene from Chinese herb gnetum cleistostachyum. Our study showed that ISO treatment in Cr(VI)-transformed cells decreased migration and invasion. Similar results were observed in human lung cancer cell lines A549, H239, and H1299. Further study indicated that treatment of Cr(VI)-transformed cells with ISO elevated MEG3 expression level. ISO treatment also increased E-Cadherin and reduced expressions of both Slug and NEDD9. This treatment reduced expression of SRY (sex determining region Y)-box 2 (SOX2), a transcription factor and essential for maintenance of stem cell self-renewal, which was increased in Cr(VI)-transformed cells. DNA methyltransferases (DNMTs) turn off MEG3 gene expression through hypermethylation in its promoter. Our results showed that ISO reduced expressions of DNMT3b and DNMT1, two members of DNMTs. The present study demonstrated that ISO increased MEG3 gene expression through reduced levels of DNMT3b and DNMT1, resulting in inhibition of β-catenin activation, reduction of NEDD9 expression, and downregulation of EMT, leading to suppression of invasion and migration of Cr(VI)-transformed cells. This study implicates that ISO is a potent chemotherapeutical agent for clinical application.

### 3397 Effects of Molybdenum Exposure on Zebrafish Development

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Molybdenum (Mo) is an essential trace element for human health due to its many metabolic roles in the molybdenum cofactor complex. The molybdenum cofactor complex is essential for catalyzing a diverse array of metabolic enzymes. Well water in southwest Wisconsin contains Mo levels notably higher than the Environmental Protection Agency’s (EPA) lifetime health advisory level of 40 μg/L, with one in five wells exceeding 90 μg/L. These high Mo concentrations are caused by the unique geological composition of the region. The biological consequences of variations in Mo exposure are not fully understood and have not been characterized in the context of early development, though limited studies have reported subtle differences in development and health safety regulations. Qualitative measures of each phenotype were taken from 3 biological replicate experiments. Quantitative measurements of phenotypes were made using Noldus DanioScope image analysis software. The given phenotypes appear to indicate the impacts of molybdenum on early development using zebrafish. Zebrafish embryos were exposed to different concentrations of molybdenum during development (Vehicle, 1 μg/L, 3 μg/L, 10 μg/L, 30 μg/L). The initial exposure occurred at 5 hours post fertilization (hpf) and solutions were subsequently refreshed daily. These zebrafish were screened annually decreasing brightfield microscopy for various developmental morphological phenotypes at 24 hpf, 48 hpf, and 72 hpf. These phenotypes include survival, head edema, yolk sac edema, pericardial edema, and spinal curvature. Qualitative measures of each phenotype were taken from 3 biological replicate experiments. Quantitative measurements of phenotypes were made using Noldus DanioScope image analysis software. The given phenotypes appear to indicate dose-dependent responses to Mo that vary by phenotype. The most frequent and severe phenotypes appeared in 30 μg/L and the dose-dependent relationship continues until 3 μg/L. These findings can inform further research on the biological influence and toxicity of Mo and can assist with informing various environmental and health safety regulations.

### 3398 Low-Dose Cadmium Exposure Results in the Release of Proinflammatory Extracellular Vesicles That Induce Chronic Inflammation and Vascular Dysfunction in Neighboring Healthy Cells

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Cadmium (Cd) is a toxic heavy metal, ubiquitous in the environmental from both natural (i.e., volcanos) and anthropogenic sources, such as industrial activity. Cd is present in the ground water, air and soil, posing a serious environmental health concern due to its long half-life in humans after consumption (20-30
arsenic (As) and cadmium (Cd) increase the risk of cardiovascular diseases, including atherosclerosis. Atherosclerosis is a vascular disease characterized by the accumulation of fibro-fatty plaques within arteries. Both metals individually induce pro-atherosclerotic activities, however their combinatorial effect remains unknown. Here, we hypothesized that co-exposure to both metals will further increase atherosclerosis beyond the individual metals. To test this hypothesis, we utilized a combination of in vitro and in vivo approaches to study effects of exposure to mixtures of low-dose As and Cd to investigate mechanistic mechanisms of combined atherosclerosis.

First, we investigated early pro-atherogenic changes in two key cell types found at atherosclerotic plaques: macrophages and endothelial cells. These assays include the generation of reactive oxygen species, markers of endothelial death, and lipid accumulation assay. We used a range of metal concentrations that reflect the blood levels identified in humans and/or previously utilized in vitro. We cultured RAW 264.7 murine macrophages with metal mixtures for 1 hour and assessed the superoxide production by high content imaging. Then, we measured vascular cell adhesion molecule-1 (VCAM-1) expression in murine C166 endothelial cells after 4- and 24-hour metal exposure. No significant changes were observed in the reactive oxygen species level in RAW 264.7 cells and in the VCAM-1 expression in C166 cells after 24-hour metal exposure.

Concentrations and proportions of iAs and its mono- and di-methylated metabolites were determined in urine and livers using hydride generation-cryotrapping-ICP-MS. Kruskal-Wallis test with Dunn’s multiple comparisons post-test and Welch unequal variance t-test were used to evaluate differences in measures of iAs, AsMAs, and DMAs. Total arsenic levels (i.e., sum of iAs, AsMAs, and DMAs) were determined in urine and livers using hydride generation-cryotrapping-ICP-MS. Kruskal-Wallis test with Dunn’s multiple comparisons post-test and Welch unequal variance t-test were used to evaluate differences in measures of iAs, AsMAs, and DMAs. Total arsenic levels (i.e., sum of iAs, AsMAs, and DMAs) were determined in urine and livers using hydride generation-cryotrapping-ICP-MS. Kruskal-Wallis test with Dunn’s multiple comparisons post-test and Welch unequal variance t-test were used to evaluate differences in measures of iAs, AsMAs, and DMAs. Total arsenic levels (i.e., sum of iAs, AsMAs, and DMAs) were determined in urine and livers using hydride generation-cryotrapping-ICP-MS. Kruskal-Wallis test with Dunn’s multiple comparisons post-test and Welch unequal variance t-test were used to evaluate differences in measures of iAs, AsMAs, and DMAs.

Evidence from population studies suggests that dietary folate intake may stimulate iAs metabolism and decrease iAs toxicity. The goal of the present study was to determine folate intake affects iAs metabolism in mice and if the impact of folate on iAs metabolism is more pronounced in mice expressing human AS3MT. Male and female wild-type (WT) C57BL/6 mice and C57BL/6 iAs expressing the human BORCS7/AS3MT locus (Hs) were fed folate deficient (FD, 0 mg folic acid /kg), folate adequate (FA, 2 mg folic acid /kg), or folate supplemented (FS, 10 mg folic acid /kg) diets for 6 weeks, followed by exposure to 0 ppb (control) or 400 ppb iAs (arsinite) in drinking water for 5 weeks, while on the same types of diet. Plasma folate levels were measured before and after the exposure using Folate AccuBind ELISA Kits. Concentrations and proportions of iAs and its mono- and di-methylated metabolites (MAS and DMAs) were determined in urine and livers using hydride generation-cryotrapping-ICP-MS. Kruskal-Wallis test with Dunn’s multiple comparisons post-test and Welch unequal variance t-test were used to evaluate differences in measures of folate and arsenic species between the treatment groups. Plasma folate levels differed significantly among mice fed different types of diet regardless of mouse strain, sex, or iAs exposure. The pattern of iAs metabolism in Hs mice differed from that in WT mice and was characterized by lower proportions of DMAs in urine and higher retention of iAs and MAS in livers. Total arsenic levels (i.e., sum of iAs, MAS, and DMAs) were generally higher in livers of female Hs mice as compared to Hs males. Total arsenic levels in urine of Hs male and female mice exposed to iAs correlated positively with the folate intake and the plasma folate levels, which is consistent with our hypothesis. Folate intake had no effect on proportions of iAs, MAS, and DMAs in urine of Hs or WT mice. Similarly, no statistically significant differences were found in total arsenic levels or in proportions of arsenic species in livers of Hs or WT mice fed different types of diet. This is in contrast with results of human studies that linked increased in folate intake to an increased secretion.
of total arsenic and/or higher proportions of DMAs in urines. Thus, iAs metabolism in Hs mice appears to be less sensitive to folate intake than iAs metabolism in WT mice.

### 4302 Exposure to Mercury in Wild Birds from Forests Near a Gold Mining Area in Colombia


Artisanal and small-scale gold mining is the activity with the highest mercury (Hg) emission to the environment. This heavy metal is a hazardous environmental pollutant, causing damage to biota. In Colombia, a biodiversity rich country, the numbers of species of birds (>1900), is one of the largest in the world. The aim of this work was to evaluate the levels of total Hg (T-Hg) in feathers of wild birds from the San Martin de Loba Mining District (SMDL), Bolivar. Specimens were safely captured with mist nets in forest areas located near mining sites in SMDL, and also from Mahates and Pueblo Bello, these last sites without mining activity. Morphometric parameters were measured, and chest feathers collected before releasing each specimen, from a total of 121 birds. The feathers were washed and dried and T-Hg analysis was performed using a direct mercury analyzer. The mean concentration of T-Hg in feathers from SMDL (n=52), Mahates (n=40), and Pueblo Bello (n=21), were 2.1±0.41, 0.7±0.07, and 0.41±0.19 µg/g, respectively. Total Hg levels varied according to bird diet, with the highest concentration found in piscivores (2.25±0.7 µg/g), followed by insectivores (1.6±0.28 µg/g), nectarivores (0.73±0.28 µg/g), frugivores (0.34±0.6 µg/g) and granivores (0.26±0.06 µg/g). The species that showed the highest levels of T-Hg was Campylopterus greucus (insectivore) with an average of 11.5±0.85 µg/g, while Leptotila sp. (granivore) presented the lowest (0.03±0.01 µg/g). These results suggest mercury in birds depends on diet, with gold mining being a pivotal factor on Hg accumulation in these animals. Biodiversity protection requires the abolishment of Hg use as well as new mining alternatives for gold exploration in this region, as previously described in the Minahasa Convention, Univ.Cartagena (Support to Research Groups and Doctoral Programs, 2020-2022, Grant 155/2019), MinCiencias (Young Talent 826/2020 and Doctoral formation, 647/2014).

### 4303 Dietary Folate Supplementation Enhances Effects of Inorganic Arsenic Exposure on Gene Transcription in Oocytes of BORCS7/AS3MT Humanized Mice


Previous study from the Styblo lab has suggested that preconception exposure to inorganic arsenic (iAs) may contribute to development of diabetic phenotypes in mouse offspring by altering transcriptomic profiles in parental germ cells. Arsenic methyltransferase (AS3MT) methylates iAs using methyl groups from the folate-dependent one-carbon metabolism. Evidence from population studies has shown that dietary folate intake modifies iAs metabolism and toxicity. We hypothesized that folate intake would alter effects of iAs exposure on transcriptome of oocytes by modifying iAs metabolism, and that the impact of folate would be more pronounced in oocytes of iAs-exposed mice fed FS diet with the same folate intake identified 7 differentially expressed genes in FA, 6 genes in FD, and 1528 genes in FS group. The same comparisons among the unexposed mice found 6, 4, and 15 differentially expressed genes, respectively. Thus, iAs exposure had a major effect on oocyte transcriptome of Hs mice, but only in presence of FS diet. Only minor effects were found in oocytes of WT mice regardless of folate intake. This data suggests an interaction between iAs exposure and folate intake in Hs, but not WT mice.

### 3404 Attenuation of Gut Dysbiosis by Naringenin against Cobalt Toxicity in Rats


Gut microbiota plays an important role in detoxifying ingested heavy metals which is essential to intestinal homeostasis. Gastrointestinal exposure to heavy metals such as cobalt chloride (CoCl2) can destabilize the balance of the gut microbial community and cause systemic metabolic stress. Cobalt has been documented to be selectively absorbed at high doses, it elicits gastroprotective activities at lower doses (25mg/kg and 62mg/kg) however, 300, 600, and 650mg/kg CoCl2 have been documented to be toxic to the cardiac and renal system. Naringenin (Nar) chelates heavy metals to form flavonoid-metal complexes thus reducing toxicity. However, the activities of these complex on gut microbiota is vague. This study investigates the activities of cobalt and co-administration with naringenin on the intestinal microbiota. Fifty male Wistar rats were grouped into 10: animals treated with CoCl2 (25, 62, 150, and 300mg/kg), with or without Nar (50mg/kg), another group received Naringenin (50mg/kg) alone, Control group (received neither CoCl2 nor Nar). By the eighth day, excised cleaned intestinal tissue was assayed for biochemical activity, and microbial studies were carried out on collected fecal samples per group using 16S DNA sequence analysis amplified by PCR. Data were analyzed using Two-way Analysis of Variance (ANOVA), p ≤ 0.05 was significant. CoCl2 significantly increased intestinal mucin levels (150mg/kg > 300mg/kg > 62mg/kg > 25mg/kg) compared with control or Nar alone groups. Co-administration with Nar significantly reduced mucin content in CoCl2 (150mg/kg) but increased in other CoCl2 groups (300mg/kg > 25mg/kg > 62mg/kg) compared with CoCl2 groups. CoCl2 (150mg/kg > 25mg/kg > 300mg/kg > 62mg/kg) and CoCl2 (62mg/kg > 300mg/kg > 25mg/kg > 62mg/kg) significantly increased Lactobacillus casei and Pediococcus damnosus counts respectively. Only Nar increased Enterococcus faecalis compared with all other groups. Cobalt increased the growth of lactobacillus and decreased the growth of probiotics (Lactobacillus casei and Pediococcus damnosus) probably through enhanced intestinal mucin levels while co-administration with Naringenin attenuated the growth of Pseudomonas aeruginosa, thus ameliorating intestinal dysbiosis.

### 3404 Synergistic Effect of Chronic Low-Dose Exposures of Cadmium (CLEC) and Hyperglycemia on Insulin Resistance in an In Vitro Hepatic Cell Model

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Hepatocellular carcinoma (HCC) is the sixth commonest cancer and third leading cause of cancer-related mortality worldwide. The incidence of HCC is rising rapidly here in the US, particularly among Native American and Hispanic populations of the southwestern US for unknown reasons. HCC has strong risk association links with obesity, uncontrolled diabetes, and the consumption of alcoholic drinks. Cobalt (Co) is a toxic heavy metal with documented to be toxic to the cardiac and renal system. Naringenin (Nar) chelates heavy metals to form flavonoid-metal complexes thus reducing toxicity. This study investigates the activities of cobalt and co-administration with naringenin on the intestinal microbiota. Fifty male Wistar rats were grouped into 10: animals treated with CoCl2 (25, 62, 150, and 300mg/kg), with or without Nar (50mg/kg), another group received Naringenin (50mg/kg) alone, Control group (received neither CoCl2 nor Nar). By the eighth day, excised cleaned intestinal tissue was assayed for biochemical activity, and microbial studies were carried out on collected fecal samples per group using 16S DNA sequence analysis amplified by PCR. Data were analyzed using Two-way Analysis of Variance (ANOVA), p ≤ 0.05 was significant. CoCl2 significantly increased intestinal mucin levels (150mg/kg > 300mg/kg > 62mg/kg > 25mg/kg) compared with control or Nar alone groups. Co-administration with Nar significantly reduced mucin content in CoCl2 (150mg/kg) but increased in other CoCl2 groups (300mg/kg > 25mg/kg > 62mg/kg) compared with CoCl2 groups. CoCl2 (150mg/kg > 25mg/kg > 300mg/kg > 62mg/kg) and CoCl2 (62mg/kg > 300mg/kg > 25mg/kg > 62mg/kg) significantly increased Lactobacillus casei and Pediococcus damnosus counts respectively. Only Nar increased Enterococcus faecalis compared with all other groups. Cobalt increased the growth of lactobacillus and decreased the growth of probiotics (Lactobacillus casei and Pediococcus damnosus) probably through enhanced intestinal mucin levels while co-administration with Naringenin attenuated the growth of Pseudomonas aeruginosa, thus ameliorating intestinal dysbiosis.
production, an increased glucose uptake of 2-NBDG fluorescent analog and dysreg-ulated pAKT pathway signaling in the circulating cells grown under hyperglycemic conditions (15mM) with cadmium chloride (1 uM > 200 nM). In summary, our invitro CLEC exposure paradigm shows specific long-term effects of Cd on dysregulated glucose metabolism in HepG2 and HHU-7 cells. Physiologically relevant CLEC exposures used in our study suggest a non-trivial impact of altered insulin-PI3K-Akt signaling as a key mechanism by which Cd leads to increased insulin resistance. Long-term, low-dose effects of Cd on chronic liver dysfunction have not been studied in detail thus far. Our invitro CLEC exposure paradigm lays the foundation for future studies using animal models to improve our understanding of the role of Cd exposure on metabolic bioenergetic and signaling pathway dysfunction.

3407 Investigating the Environmental Risk Factors of Diabetes Epidemic with a Focus on Heavy Metal Exposure

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Diabetes, especially type 2 diabetes (T2D), has become a major public health problem in the United States (U.S.) and worldwide in recent decades due to its rising prevalence. In the U.S., according to CDC’s latest report, over 37 million Americans had diabetes and 96 million adults had pre-diabetes as of 2022. Kentucky ranks among the states with the highest adult diabetes rates and environmental pollution is a major health concern in the state. While a growing number of studies have explored the effects of environmental risk factors on diabetes, inconsistent results have been reported with respect to the effects of exposure to toxic heavy metals on diabetes. In light of the pervasiveness of diabetes and the complexity of its environ-mental risk factors, additional studies are needed to better understand the role of diabetes and its associations with exposures to toxic heavy metals. This study attempted to investigate the geographical inequalities in diabetes and its environ-mental risk factors including exposure to arsenic, cadmium, lead, and mercury using innovative spatial statistical approaches. The data for diabetes used in our analysis were obtained from the Centers for Disease Control and Prevention (CDC’s) national Behavioral Risk Factor Surveillance System (BRFSS) survey in 2019. The primary data on ambient concentration of heavy metals were extracted from Environmental Protection Agency (EPA)’s newly released 2018 Air Toxics Screening Assessment database. Both diabetes and metal exposures were aggregated to the geographic level. First, we used geographic information systems (GIS) to examine the spatial patterns of diabetes across Kentucky’s census tracts and then used multiple regression analyses to investigate the associations between diabetes and the selected four types of heavy metals while accounting for other sociodemographic confounding factors. We found that diabetes prevalence has a remarkable geographical pattern; the distribution of diabetes prevalence is 2.0-25.6%. Geographically contiguous census tracts that had significantly higher prevalence rates than the state average (i.e., hot spots), were mainly observed in the eastern Appalachian Mountain region, part of the “diabetes belt” in the eastern U.S. prior studies have identified. Moreover, hot spots of neighborhoods were also noticeable in urban communities (e.g., Louisville) that are disproportionately represented by low-income and minority populations. The results of regression models indicated that arsenic (b = 6563.794, p = 0.045), cadmium (12276.087, p = 0.040), lead (r = 69.981, p = 0.007), and mercury (329.015, p = 0.572) all showed negative associations with diabetes prevalence, which contradicted our hypotheses about the most deleterious effects of heavy metals. Building upon the findings advanced by environmental health research, this article investigated the associations of diabetes and environmental risk factors including exposure to toxic heavy metals. Applying innovative geospatial techniques to the analysis of statewide data for diabetes and heavy metal exposures permits us to gain new insight into the spatial heterogene-ity of diabetes in relation to environmental risk factors. Findings of this study could contribute to the establishment of evidence for risk assessment and inform public health policies and community intervention strategies to save lives and cut the skyrocketing costs due to diabetes. The ecological nature of the aggregate data for both diabetes and heavy metal exposure limited the applicability of this study, which can be improved using individual level data for diabetes and more direct measures of mental exposure in future research.

3408 Composite Nanobulges for Anticoronaviral Treatment of Air Filters

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Coronavirus disease (COVID-19) is still ongoing despite the vaccination of a considerable number of people worldwide. The filtration of indoor air has been proven to be an effective intervention to suppress the spread of COVID-19 because humans spend approximately 90% of their time indoors. The deposition of the contagious viruses on air filter surfaces has led to the adoption of antiviral coatings to reduce the spread of viral infections from this type of fomite. This has further caused issues regarding the deterioration of the antiviral performance during filter operation as well as the transportation of toxic metalloids or viable viruses from the discarded filters. The development of a plug-in safe-by-design antiviral coating is required to regenerate antiviral functions period-ically and inactivate the infectiousness conveniently of the viruses collected before discarding the used filters. Although installing ultraviolet radiation devices near the

filter unit was proposed as a rapidly implementable alternative to suppressing this problem completely, there are problems of limited radiation coverage for deep bed and cartridge filters as well as the photodegradation of filter materials. Another issue faced when applying exothermic material coatings on filters includes the need for temperature control, flame retardancy, and biosafety. To resolve these difficulties, this study developed a compact system of in-flight nanobulge manufac-ture to contaminate nanobulges in-place on the filter surfaces by simply plugging in a system to inactivate coronaviruses collected on the surfaces rapidly while retain-ing biosafety, thereby increasing the effectiveness of the air filtration in preventing the transmission of coronaviruses. A single-pass aero-blowing and -sintering Fe containing ultraviolet particles (<5 nm) on SiO2 nanobeans (ca. 130 nm) enabled the formation of surface roughness for their effective interaction with coronavirus spikes to generate reactive oxygen species and Fenton-mediated hydroxyl radicals, resulting in comparable antiviral activity to similar-sized individual Zn, Ag, or Cu particles despite the significantly smaller fraction of the metallic component.

3409 Mentholated Fourth-Generation E-cig Aerosols Induce Ventricular Arrhythmias and Early Repolarization Defects in Mice

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Millions of young adults and adolescents in the United States use e-cigarettes (e-cigs). We recently found that acute exposure of mice to menthol-flavored aerosols of e-cigarettes robustly induced heart arrhythmia which altered early repolarization on the electrocardiogram (ECG). In this study, we investigated the cardiac impacts of various e-cig aerosols generated by a contem-porary 4th generation device and discerned their mediation by nicotine and/or flavors. Male C57BL/6J mice (n=4) were exposed to air or e-cig (JUUL) aerosols using personal air samplers. The aerosol sampling time was 3.5 hours, with fifteen 9-minute puff sessions (18 puffs per session, 270 puffs total). In addition to vehicle-derived aerosol, mice were also exposed to nicotine-containing aerosols from PG:VG, JUUL Menthol, and JUUL Virginia Tobacco e-liquids (all 5% nicotine benzoate). ECG signals were acquired continuously throughout exposure via radio telemetry technology and analyzed using software for changes in morphology and occurrence of arrhythmias. Only exposure to JUUL Menthol aerosol induced S wave depression and J wave elevation (both p<0.05 vs. Air), indicating changes in repolarization consistent with our prior findings with 1st generation devices. JUUL Menthol aerosol was also the only to robustly increase ventricular premature beat (VPB) frequency (7.68 times, p<0.05). VPB frequency correlated with the percent of nicotine in the aerosol (R²=0.88, p<0.05). Liquid mass spectrometry revealed that all nicotine-containing aerosols (PG:VG+nicotine, JUUL Menthol, and JUUL Virginia Tobacco) similarly increased urinary total nicotine equivalents. Thus, differences in nicotine intake may not underlie the arrhythmogenicity of e-cig aerosols. Overall, the unique impacts of JUUL menthol aerosol on electrophysiology suggest that changes in early repolarization may underlie the arrhythmogenic effects of mentholated e-cig aerosols. Our findings warrant future studies to determine the causality between menthol and ventricular arrhythmias, and to systematically assess in humans the cardiac risks of e-cig flavors.

3410 Elucidating the Role of Inducible Nitric Oxide Synthase in E-cigarette Toxicity Using Precision-Cut Lung Slices

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Although e-cigarette (e-cig) use has continuously increased, little is known about its acute toxicity. Using the ex vivo Precision Cut Lung Slice (PCLS) model we previously showed that menthol-flavored e-cig condensate exposure increases mitochondrial dysfunction and oxidative stress while decreasing lung function. Inducible nitric oxide synthase (iNOS) is a key enzyme mediating nitric oxide production in macrophages; it is also known to drive proinflammatory signaling. In the present study, we analyzed the role of NO in e-cig toxicity. E-cig condensates were collected by aerosolizing e-liquids containing vehicle, nicotine only, and nicotine+menthol. Condensate exposure doses were normalized to glycerol equivalents. Thus, differences in nicotine intake may not underlie the arrhythmo-genicity of e-cig aerosols. Overall, the unique impacts of JUUL menthol aerosol on electrophysiology suggest that changes in early repolarization may underlie the arrhythmogenic effects of mentholated e-cig aerosols. Our findings warrant future studies to determine the causality between menthol and ventricular arrhythmias, and to systematically assess in humans the cardiac risks of e-cig flavors.
presence of iNOS has a protective role in e-cig toxicity by reducing the toxic effects on ciliary function and pulmonary cells. Further studies will reveal the mechanisms of iNOS depletion in PCLS on oxidative stress, airway contractility, and nitrite/nitrate metabolism and will thus contribute to elucidating the mechanism behind e-cig toxicity in the lung. NIHES050522.

### 3.4.11 Testing the Toxicity and Virus Infectivity of Tobacco-Flavored E-cigs in Human Bronchial Epithelial Cells

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E-cigarette use has become prevalent recently amongst youths and adolescents alike. While the health impacts of cigarette smoke and increased susceptibilities of smokers towards viral infections is well known, there are contradictory evidences with regards to the toxicity and health impacts of e-cig use in humans. Considering this, we studied the toxicity and viral infectivity of differently flavored e-cigs on human bronchial (BEAS2B and 16-HBE) epithelial cells. To test this, 16-HBE and BEAS2B cells were grown in 6-well plates and exposed to varying doses (3 or 5 sessions of 30 sec puff duration with 10 sec interval, for a total of 10 min) of tobacco flavored (Virginia tobacco (JUUL): 3% and 5% nicotine; JUNO: 48mg/mL nicotine) e-cig aerosols. Cell media and cell pellets were collected to perform ELISA and other assays in the future. Since, ACE2 (SARS-CoV-2 receptor) and Furin (associated protein) have been known to be upregulated amongst smokers making them more susceptible to a potential COVID-19 infection, we tested the activities of ACE2 and Furin in the cell lysates from e-cig aerosol treated human bronchial epithelial cells. Our results indicate, a dose-dependent differential response in the cytokine (IL-6 and IL-10) and co-stimulatory molecule (CD80 and CD86) expression and exposing human lung epithelial cells (BEAS2B and 16-HBE) to e-cig aerosols from both the brands (Virginia Tobacco (JUUL) and JUNO Tobacco) of vape pods. Interestingly, no change in the ACE2 activity was observed in the cell lysates from human bronchial epithelial cells exposed to varying doses and nicotine content of tobacco flavored (Virginia Tobacco) vape pod. We further observed a dose-dependent increase in the expression of iNOS in the lung epithelial cells, with exposing BEAS2B cells to varying doses of e-cig aerosols from JUNO tobacco. Furin levels were found to increase in cells exposed to varying doses of aerosols from vape pods, but none of these changes were significant. Future work will test the SARS-CoV2 infectivity of e-cig aerosol treated human lung and bronchial epithelial cells to better understand the mechanism of viral infectivity to lung cells amongst e-cig users. Supported by WNY Center for Research on Flavored Tobacco Products (CroFT) # U54CA228110, and CroFT pilot award (GK).

### 3.4.12 Evaluation of Respiratory Tract Organ Toxicity and Carcinogenicity of E-vapor Aerosols in an 18-Month Inhalation Study in AJ Mice

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There is limited toxicological information on the long-term inhalation exposures of e-vapor aerosols containing various flavors, humectants, and nicotine. Following Organisation for Economic Co-operation and Development (OECD) Test Guideline 453, AJ mice were used to evaluate respiratory tract toxicity as well as lung tumor incidence and multiplicity upon life-time exposure to cigarette smoke (CS) or to aerosols from a prototype e-liquid formulation containing 38 selected flavors. Mice were exposed via whole-body inhalation for 6 h/day for 5 days/week for up to 18 months to aerosols from pro-e cigarette aerosols (PG/VG, nicotine (N, 2% [w/w]); PG/VG/N with flavors (F) at low, medium, and high concentrations (1.2 to 16.8% [w/w]; PG/VG/F-High; or CS from the 3R4F reference cigarette). Histopathological evaluation, lung function and morphometric measurements were the key endpoints to evaluate respiratory tract toxicity and carcinogenicity. Plasma nicotine and nicotine were measured to confirm the systemic exposure. Exposure to e-vapor aerosols resulted in minimal or no changes in respiratory rate, lung function, lung inflammatory and emphysema parameters, as well as lung tumor incidence and multiplicity compared to the Sham group. In contrast, exposure to CS suppressed the respiratory rate, altered lung function, led to pulmonary inflammation and emphysemaous changes, and— at terminal dissection—increased lung tumor incidence and multiplicity compared to the Sham group. In addition, histopathological evaluation of the respiratory tract showed severe changes and papilloma development in the larynx. In contrast, laryngeal histopathological changes were only observed in the high flavor e-vapor groups and showed significantly lower severity and incidence compared to the CS groups. Nasal and pulmonary changes when present in the e-vapor groups also showed lower severity and incidence compared to the CS groups. In summary, chronic exposure to e-vapor aerosols under the tested conditions showed consistently reduced toxicity and carcinogenicity responses in the respiratory tract compared to CS, supporting its potential role in tobacco harm reduction.

### 3.4.13 Variances in Vaping Behavior Contribute to Adverse Periodontal Health Effects

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The electronic nicotine delivery systems (ENDS) market has shown remarkable growth and design evolution aimed at enhancing vaping experience and consumer preferences. The continual shift in ENDS technology has outpaced safety assessments, creating a significant challenge when developing regulatory guidance necessary for protecting public health. Considering the mounting concerns over potential adverse respiratory effects, a knowledge gap pertaining to how vaping behavior may contribute to poor oral health continues to grow. Several studies have associated negative oral health effects due to ENDS usage, yet there is lack of clinical studies that fully characterize how variances in vaping behavior contribute to oral health. Cigarette smoke (CS) exposure modulates both lung and periodontal inflammation—increased lung tumor incidence and multiplicity compared to the Sham group. The increasing puff volumes and flow rates caused linear increases in total deposited particulates, aldehydes, and volatile organic compounds, such as formaldehyde and ethyl benzene. Likewise, levels of toxic metals including chromium and zinc were also elevated due to variances in puff volume and flow rate. Our community periodontal index of treatment needs (CPITN) assessment determined higher levels of calculus, gingival bleeding and pocket depth in current exclusive ENDS users compared to the never-established tobacco users (p<0.05). Evaluation of participant saliva showed differential metabolite and cytokine profiles between never-established tobacco users and current exclusive ENDS users with 917 metabolites increased and 511 decreased (p<0.05) in ENDS users. When saliva metabolite data were stratified into exposure mediator groups (low, medium, and high), exposure-dependent alterations were identified in saliva metabolites. Specifically, as exposure increased from low to high, 258 metabolites were increased while 541 were decreased in exposure-dependent manners. For example, 1-methyladenosine, a proposed marker of cancer progression, and nicotine were increased while energy metabolism markers (coenzyme A, carnitine, and others) were decreased. Pro-inflammatory cytokines including IL-6, IL-8, TNF-α, and IL-1β were significantly increased in ENDS users in comparison to controls. This work demonstrates the potential for vaping to cause oral injury and inflammation while highlighting the importance of addressing consumer behaviors in exposure assessments, which may drive pathological changes leading to periodontal disease.
flavor induced significant immunosuppression, while Brand A tobacco elicited anergic immune response. Overall, menthol flavor caused an allergic inflammatory response with increased RANTES levels in BALF compared to tobacco flavor with marginal strain dependence. Genotoxicity markers were p21, H2A.X, MDM2, and ATR, significantly increased by both brands, including PG/VG, in lung tissue. These inflammatory and genotoxicity responses-associated with destabilized NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes and transient receptor potential ankyrin1 (TRPA1) by menthol flavor. The presence of nicotine decreased surfactant protein D (SP-D) and elevated plasminogen activator inhibitor 1 (PAI-1) menthol and tobacco flavors, respectively, suggesting acute lung injury. Integration of inflammatory and metabolic pathway gene expression analysis showed immunometabolic regulation in T-cells. Cellular signaling protein kinases, p38 and p70S6K, were elevated by all aerosols. Proteomics analysis suggested that suppressed immunity or the allergic immune response may be due to metabolic reprogramming of T-lymphocytes via PI3K, Akt, and mTOR signaling. This study provides insights into the comparative toxicological effects of the same flavor by various brands and the need to conduct these toxicological parameters and report the pharmacological effects by cigarette products. This study was supported by K99ES033835 and USCA228110.

3415 Comparative Repeated Exposure Studies of Extracts from Combustible Cigarette Smoke and Heated Tobacco Vapor Using In Vitro Organotypic Cultures of Bronchial Epithelial Cells

Organotypic three-dimensional (3D) models are widely used for drug screening and toxicological assessment because they resemble human tissues. Air Liquid Interface (ALI) cultures of airway epithelial cells are a 3D model that reproduces the airway epithelium structure and function with a long shelf life. Therefore, it is expected that these models will help investigate the cumulative effects of repeated exposure to stimuli. The cumulative effect of cigarette smoke (CS) is considered a cause of chronic diseases including chronic obstructive pulmonary disease. Recently, novel tobacco products such as heated tobacco products have emerged in the market. We previously reported that exposure to DT3.0a, a novel heated tobacco product, vapor extract (Aerogel Collected Mass, ACM) caused less acute toxicity compared with CS. However, the potential effects of chronic exposure to DT3.0a ACM are unclear. In this study, we assessed the long-term biological effects of repeat exposure of 3D human bronchial epithelial cells (HBEC) to DT3.0a ACM and compared it with the total particulate matter of cigarette smoke (TPM). 3D cultured HBEC were exposed to TPM (5, 25, or 100 µg/mL) or ACM (25, 100, 500, or 2000 µg/mL) three times a week for 6 weeks continuously or intermittently. In the continuous exposure experiments, cells were exposed to TPM or ACM throughout the experimental period whereas intermittent exposure experiments consisted of 4-hour exposure periods followed by a 44-hour recovery period with TPM- or ACM-free medium. The cumulative effects of repeated exposure to stimuli at higher than 12.5 µg/mL of CSE exposure reduced goblet cell hyperplasia. Therefore, this suggested that suppressed immunity or the allergic immune response may be due to metabolic reprogramming of T-lymphocytes via PI3K, Akt, and mTOR signaling.

3416 RNA-Seq Analysis of Macrophage-Mediated Endothelial Dysfunction in Vasculature-on-a-Chip

Endothelial dysfunction is one of the primary triggers of atherosclerosis, an increasing cause of worldwide deaths. Impaired barrier integrity, upregulation of adhesion molecules, and monocyte recruit to the endothelial surface are found in dysfunctions of the endothelium and considered indicators of early-stage atherosclerosis. Oxidative stress and inflammation resulting from various lifestyle habits, such as an unhealthy diet and smoking, are known mediators of endothelial dysfunction. Cigarette smoke (CS) is known to contain thousands of chemicals, a part of them are considered harmful and potentially harmful constituents (HPHCs). However, vascular cells are not directly exposed to CS, because the respiratory tissues play a critical role as the first line of defense against inhaled chemicals. Vascular cells would rather interact with the exposed cells in the respiratory tissues. We hypothesize that immune cells such as tissue resident macrophages have important roles in eliciting CS-inducible oxidative stress and inflammation in the vasculature. We previously reported that the culture supernatant of macrophages exposed to CS induced expression and secretion of various cytokines, growth factors, and chemokines. Here, we evaluated the propiery heated tobacco product, Direct-Heating Tobacco System Platform 3 Generation 3 Version A (DT3.0a), because we previously reported that multiple toxicological endpoints including oxidative stress were less in the airway cells by exposure to DT3.0a vapor than CS, thus it is expected also less biological impact in vascular cells. Ingenuity pathway analysis (IPA) revealed that differentially expressed genes (DEGs) in indirectly exposed cells were significantly enriched for the “atherosclerosis signaling” pathway. However, DEGs in response to direct exposure were related to inactivation of various canonical pathways in IPA, suggesting direct exposure of CS to the vascular-on-a-chip model is unsuitable for the detection of endothelial activation. Indirect exposure was predicted to accelerate pro-inflammatory responses of macrophages associated with the “macrophage classical activation” pathway in IPA, which can exacerbate macrophage-mediated inflammation in the vasculature. DT3.0a showed similar effects for both direct and indirect exposure, although a much higher concentration was needed to produce these effects, as expected. Thus, we demonstrated the effectiveness of macrophage-mediated exposure to assess the potential risk of endothelial dysfunction using in vitro testing. Recapitulation of late-stage atherosclerosis, such as plaque formation, with this in vitro system is a natural next step.

3417 Cigarette Smoke Extract Enhances IL-13-Induced Goblet Cell Hyperplasia In Vitro

The overproduction of mucus produced by goblet cells is an important pathophysiological characteristic of chronic obstructive pulmonary disease (COPD). Interleukin (IL)-13 is mainly produced by immune cells and is a key inducer of goblet cell hyperplasia in the human airways. In our previous studies, goblet cells were increased when three-dimensional cultured normal human bronchial epithelial (3D-NBHE) cells were exposed to IL-13. Cigarette smoke is a risk factor for COPD; however, cigarette smoke extract (CSE) did not induce goblet cell hyperplasia in 3D-NBHE cells. To investigate the influence of CSE on goblet cell hyperplasia, 3D-NBHE cells were exposed to CSE with IL-13 for 2 weeks in this study. We evaluated goblet cell hyperplasia by measuring the area of mucus that was periodic acid-Schiff staining positive. The concentrations of CSE used were from 0 to 50 µg/mL and the concentrations of IL-13 were from 0 to 1 ng/mL in the medium of the culture supernatant. We found that the area of mucus increased relative to the IL-13 concentration and was not increased by CSE as previously observed. Interestingly, goblet cell hyperplasia was enhanced by co-exposure to IL-13 and CSE. The concentration of CSE that maximized the area of mucus was 12.5 µg/mL and concentrations higher than 12.5 µg/mL of CSE exposure reduced goblet cell hyperplasia. Morphological changes of 3D-NBHE cells were observed after CSE exposure, which might explain why goblet cell numbers did not increase with higher CSE concentrations that might have been cytotoxic. To elucidate the mechanism of the synergy between CSE and IL-13, expressions of the IL-13 receptors, IL-13Rα1 and IL-4R, were measured immuno-histologically by measuring the intensity of immunofluorescence staining using antibodies for each IL-13 receptor. Although exposure to IL-13 only did not increase the expressions of IL-13Rα1 and IL-4R in 3D-NBHE cells, co-exposure to IL-13 and CSE increased the expressions of both IL-13 receptors regardless of the IL-13 concentration. The expressions of IL-13 receptors were increased in a CSE concentration-dependent manner. Therefore, this suggested that CSE increased the IL-13 receptor expression, and as a result, enhanced goblet cell hyperplasia by IL-13. Previous studies reported that goblet cell hyperplasia depended on the amount of IL-13 and/or other cytokines produced by immune cells. It was also reported that IL-13 production by immune cells was induced by cytokines including IL-33 released from stimulated epithelial cells. Based on these findings, the co-culture of epithelial cells and immune cells should be appropriate to assess goblet cell hyperplasia in vitro. However, co-culture with different types of cells is not always possible and optimization of the culture conditions requires considerable time because cells have different optimal culture conditions. In this study, we found that CSE increased the sensitivity of epithelial cells to IL-13, which might be involved in the mechanism of goblet cell hyperplasia. In addition, the IL-13 experiments used here can be used as a simple method to test goblet cell hyperplasia induced by cigarette smoke and other environmental materials.
Electronic nicotine dispensing systems (ENDS), also known as electronic cigarettes (EC), are marketed as a safer and less harmful alternative for traditional tobacco cigarettes. The use of EC is common in young adults with limited knowledge about the detrimental effects on lung pathologies, particularly during adolescence. Tobacco cigarettes are known to cause pulmonary inflammation, impairing lung structure and function, and reducing lung function. However, EC use is rising among young adults, and little is known about its effects on lung health.

High levels of nicotine and toxicants are released during the use of EC, leading to local lung inflammation. Previous studies have suggested that EC exposure increases human distal lung viral infection. Up-regulation of TRAIL release by EC during IAV infection may serve as a mechanism in determining the severity of viral infection. Maintaining an appropriate level of TRAIL in human lungs exposed to EC would be important to control viral infection.

Cannabis is mainly consumed by smoking joints, exposing users to high concentrations of various toxicants. Users are increasingly interested in potential safer inhalation alternative methods to consume cannabis, including cannabis vaporizers and electronic non-nicotine delivery systems (ENNDS) using e-liquid containing cannabinoids, in order to reduce their exposure to toxicants. However, very few studies investigated the toxicological profiles of these alternatives in laboratory conditions and compared them to joints. Therefore, this study aimed to characterize the toxicity of cannabis aerosols emitted by vaporizers and ENNDS in comparison to cannabis smoke (without tobacco). Emissions were generated using a smoking machine and the concentrations of 91 compounds in emissions were quantified. Three cannabis vaporizers, three ENNDS, and three different e-liquids containing cannabinoids were tested. The three vaporizers consisted of one convection-based device and two devices using a combination of convection and conduction, including a medical-grade device. The following six chemical groups were quantified in each cannabis product tested: aldehydes, volatile organic compounds (VOCs), phenolic compounds, poly cyclic aromatic hydrocarbons (PAHs), aromatic amines, and heavy metals. In addition, cannabinoids were quantified in each tobacco products and in emissions to compare the efficiency of Δ9-THC delivery for each device. A significant reduction of the toxicant concentrations were observed in emissions of cannabis vaporizers and ENNDS compared to joints. However, few irritants and carcinogens are still released, and high aldehyde concentrations were observed for one of the three tested e-liquids, due to an overheating of the ENNDS resistance. No differences in toxicant concentrations were observed between the two heater systems (i.e., convection-based device or hybrid system), and the emissions of the medical device were comparable to the two other vaporizers in terms of toxicants. The efficiency of Δ9-THC delivery was the highest in emissions of ENNDS (>99%) compared to joints (36%) and to cannabis vaporizers (18%). Overall, the results show that consumers who use vaporizers and ENNDS as alternatives to joints are exposed to a reduced quantity of toxicants. However, these products must be carefully chosen to avoid overheating of the coil. For vaporizers, their low efficiency of Δ9-THC delivery may dissatisfy users. Further studies on use of these electronic devices will be actually needed to determine real puffing regimen and to confirm the results obtained in laboratory conditions.

Electronic nicotine dispensing systems (ENDS), also known as electronic cigarettes (EC), are marketed as a safer and less harmful alternative for traditional tobacco cigarettes. The use of EC is common in young adults with limited knowledge about the detrimental effects on lung pathologies, particularly during viral infections. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a protein of the TNF family involved in cell apoptosis, is upregulated in various human tissues, including lung tissue. However, the role of TRAIL in viral infection of human distal lungs exposed to EC remains unclear. The goal of this study was to determine the effect of EC on TRAIL release and the role of TRAIL in regulating IAV infection in a human lung precision-cut lung slice (PCLS) model. PCLS obtained from the lungs of healthy human donors (n=5) without smoking history were exposed to EC juice and IAV for up to 3 days for TRAIL and viral load measurement in the tissue and culture supernatants. TRAIL neutralizing antibody and recombinant TRAIL were used to determine the contribution of TRAIL to viral infection during EC treatments. EC juice exposure significantly increased viral loads (~1.6 fold vs. alone IAV treatment), TRAIL release (~3 fold vs. alone IAV treatment), TNF-α production (~2.5 fold vs. alone IAV treatment), and cytotoxicity (~1.7 fold vs. alone IAV treatment). TRAIL neutralizing antibody significantly increased tissue viral load (~2.1 fold vs. IgG control) but reduced viral release (~65%) into supernatants of PCLS treated with EC juice. Recombinant TRAIL significantly decreased tissue viral load (~64%) but increased viral release (~1.6 fold) into supernatants of PCLS treated with EC juice. Further, recombinant TRAIL enhanced the expression of interferon-β (~1.3 fold) and interferon-λ (~2.3 fold) induced by EC juice exposure and IAV infection. Our results suggest that EC exposure increases human distal lung viral infection. Up-regulation of TRAIL release by EC during IAV infection may serve as a mechanism in determining the severity of viral infection. Maintaining an appropriate level of TRAIL in human lungs exposed to EC would be important to control viral infection.

Cannabis is mainly consumed by smoking joints, exposing users to high concentrations of various toxicants. Users are increasingly interested in potential safer inhalation alternative methods to consume cannabis, including cannabis vaporizers and electronic non-nicotine delivery systems (ENNDS) using e-liquid containing cannabinoids, in order to reduce their exposure to toxicants. However, very few studies investigated the toxicological profiles of these alternatives in laboratory conditions and compared them to joints. Therefore, this study aimed to characterize the toxicity of cannabis aerosols emitted by vaporizers and ENNDS in comparison to cannabis smoke (without tobacco). Emissions were generated using a smoking machine and the concentrations of 91 compounds in emissions were quantified. Three cannabis vaporizers, three ENNDS, and three different e-liquids containing cannabinoids were tested. The three vaporizers consisted of one convection-based device and two devices using a combination of convection and conduction, including a medical-grade device. The following six chemical groups were quantified in each cannabis product tested: aldehydes, volatile organic compounds (VOCs), phenolic compounds, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and heavy metals. In addition, cannabinoids were quantified in each tobacco products and in emissions to compare the efficiency of Δ9-THC delivery for each device. A significant reduction of the toxicant concentrations were observed in emissions of cannabis vaporizers and ENNDS compared to joints. However, few irritants and carcinogens are still released, and high aldehyde concentrations were observed for one of the three tested e-liquids, due to an overheating of the ENNDS resistance. No differences in toxicant concentrations were observed between the two heater systems (i.e., convection-based device or hybrid system), and the emissions of the medical device were comparable to the two other vaporizers in terms of toxicants. The efficiency of Δ9-THC delivery was the highest in emissions of ENNDS (>99%) compared to joints (36%) and to cannabis vaporizers (18%). Overall, the results show that consumers who use vaporizers and ENNDS as alternatives to joints are exposed to a reduced quantity of toxicants. However, these products must be carefully chosen to avoid overheating of the coil. For vaporizers, their low efficiency of Δ9-THC delivery may dissatisfy users. Further studies on use of these electronic devices will be actually needed to determine real puffing regimen and to confirm the results obtained in laboratory conditions.
at higher concentrations than CS. Most test NPs did not reveal notable changes in inflammation and tissue damage markers, while the highest concentrations of tobacco products and market snus showed increases in MMP-1 and IL-8 but only at higher nicotine concentrations than CS. In summary, the mechanistic in vitro testing using primary HGF cells showed that the test NPs have an overall lower or comparable toxicity potential to other oral tobacco comparator products under the test conditions. The high level of significance of tobacco products exhibit substantially lower toxicity potential (oxidative stress and inflammation) compared to cigarettes, supporting their reduced risk potential.

3422 Immunophenotypic Differences in the Pulmonary Response of BALB/c and C57BL/6 Mice to E-cigarette Aerosols

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E-cigarettes are battery-powered devices that aerosolize a liquid composed of nicotine, flavouring agents, propylene glycol and vegetable glycerin (PGVG). In recent years, e-cigarette use (‘vaping’) has become prevalent among teens and young adults, raising concerns over its potential adverse effects in youth. Although data from animal studies suggest that e-cigarettes promote lung inflammation, these studies have produced some conflicting evidence with regards to the effects of e-cigarettes on typical indices of lung inflammation, such as leukocyte recruitment and cytokine production. Current vaping studies in animal models differ in fundamental ways, including the type of device used, exposure conditions, and strain of inbred mouse. Two commonly used inbred mouse strains in respiratory health research are BALB/c and C57BL/6, which display inherent differences in their immune response due to a bias towards a TH2- and TH1-type response, respectively. This affects their susceptibility to experimental allergic airway inflammation and cigarette-smoke induced emphysema, for example. We hypothesized that, due to intrinsic differences in immune response, pulmonary inflammation following exposure to an e-cigarette aerosol will differ between BALB/c and C57BL/6 mice. BALB/c or C57BL/6 male and female mice (5-7 weeks) were exposed to an aerosol derived from a mint flavoured e-liquid (18mg/ml nicotine, JUUL); control groups were exposed to air only or PGVG only. Nose-only inhalation exposures were performed using the SCIREQ® inExpose™ system for one hour per day, for 14 consecutive days. Lung tissue was collected and characterized using conventional hematoxylin-eosin staining and flow cytometry and measure gene expression via RT-qPCR. There were few differences in the cellularity of the lung tissue between air-, PGVG- and JUUL-exposed mice. However, pulmonary tissue eosinophils were significantly increased in JUUL-exposed BALB/c mice compared to the air-exposed group, no differences in lung eosinophils were observed in C57BL/6 mice exposed in JUUL-exposed BALB/c mice but were decreased in JUUL-exposed C57BL/6 mice. Lung mRNA levels of cytokines and oxidative stress response genes differed between the two strains, with Tnfα being significantly upregulated in JUUL-exposed BALB/c mice but downregulated in C57BL/6 mice. These results suggest that e-cigarettes differentially affect the pulmonary immune response of BALB/c and C57BL/6 mice reflecting genetic and/or determined intrinsic differences. Future experiments will investigate the effects of JUUL exposure on the proteome of these two strains. In addition to providing perspective on animal models exploring the toxicology of e-cigarettes, these findings raise the possibility that vaping may induce relevant inflammatory responses in susceptible individuals.

3423 Vaping Flavoring Agents Induce Cellular Stress Responses in Human Proximal Tubule Epithelial Cells

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Vaping and e-cigarette usage has become increasingly popular, especially among teenagers and young adults. Unfortunately, very little regulation exists for flavoring agents used in wide varieties of e-liquids. The cellular impact and cytotoxicity of inhaled flavoring agents is not well characterized. It is estimated that there are over 7,000 different flavoring liquids available on the market. Vaping flavors have been reported to cause adverse effects to the lung altering function and immune response. Once flavoring aldehydes are absorbed from the lung, the flavoring agents distribute to other organs including the kidney. Cinnamaldehyde (CIN) is a reactive alpha, beta-unsaturated aldehyde that is one of the flavoring agents used in many e-liquids. CIN has been shown to alter mitochondrial function and immuone response due to a bias towards a TH2- and TH1-type response, respectively. This affects their susceptibility to experimental allergic airway inflammation and cigarette-smoke induced emphysema, for example. We hypothesized that, due to intrinsic differences in immune response, pulmonary inflammation following exposure to an e-cigarette aerosol will differ between BALB/c and C57BL/6 mice. BALB/c or C57BL/6 male and female mice (5-7 weeks) were exposed to an aerosol derived from a mint flavoured e-liquid (18mg/ml nicotine, JUUL); control groups were exposed to air only or PGVG only. Nose-only inhalation exposures were performed using the SCIREQ® inExpose™ system for one hour per day, for 14 consecutive days. Lung tissue was collected and characterized using conventional hematoxylin-eosin staining and flow cytometry and measure gene expression via RT-qPCR. There were few differences in the cellularity of the lung tissue between air-, PGVG- and JUUL-exposed mice. However, pulmonary tissue eosinophils were significantly increased in JUUL-exposed BALB/c mice compared to the air-exposed group, no differences in lung eosinophils were observed in C57BL/6 mice exposed in JUUL-exposed BALB/c mice but were decreased in JUUL-exposed C57BL/6 mice. Lung mRNA levels of cytokines and oxidative stress response genes differed between the two strains, with Tnfα being significantly upregulated in JUUL-exposed BALB/c mice but downregulated in C57BL/6 mice. These results suggest that e-cigarettes differentially affect the pulmonary immune response of BALB/c and C57BL/6 mice reflecting genetic and/or determined intrinsic differences. Future experiments will investigate the effects of JUUL exposure on the proteome of these two strains. In addition to providing perspective on animal models exploring the toxicology of e-cigarettes, these findings raise the possibility that vaping may induce relevant inflammatory responses in susceptible individuals.

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Despite a lack of empirical data, an unprecedented rise in electronic cigarettes (ECs) has been widely perceived as safe. Multiple studies support a causal link between tobacco cigarette (TC) use and cardiovascular (CV) diseases; however, there is a lack of data with EC use. We have previously shown that chronic and acute EC use decreases heart rate variability (HRV) in humans, likely via increased sympathetic nervous system activation. We aimed to evaluate HRV effects induced by brief EC exposures utilizing an in-vivo model for freely moving C57BL/6 mice. Telemetry devices were implanted in the abdomen of six 10-week-old C57BL/6 mice to monitor electrocardiographic activity continuously. Mice underwent an exposure protocol designed to reflect human vaping topography with two EC exposures, each lasting 15 min (EC), followed by 45-min post-exposure periods. Air (primary control) and PBS (secondary control) were also included, each with their own post-exposure events. Physical and chemical composition of EC aerosol revealed an enrichment in fine and ultrafine particles, with high concentrations of total aldehydes and nicotine. During EC exposures, we observed bradycardic effects in heart rate (HR) with the standard deviation of NN intervals (SDNN), root mean square of successive differences (RMSSD), and the proportion of adjacent normalized RR intervals that differed by more than 6 ms (pNN50), significantly (p<0.05) altered as compared to Air exposures. Frequency domain parameters also indicated significant changes in HRV; the low frequency (LF) and high frequency (HF) bands, which are inversely related indices of autonomic function, decreased. LF/HF ratio remained relatively unchanged, suggesting a possible coattivation of both parasympathetic and sympathetic branches of the autonomic nervous system. EC exposures also significantly increased lymphocyte counts in the bronchoalveolar lavage fluid without changes in the redox potential. Short-term EC exposures lower heart rate and alter HRV in mice in a hyperacute manner, suggesting that ECs may not be safe as previously assumed.

In vitro models that replicate the structural integrity and functional responses of the lung inform of the toxicological and biological effects of inhaled toxicants and mechanisms of multi-dimensional diseases such as lung cancer and COPD. Such mechanistic models are critical for the development of novel alternative methods to replace animal testing for regulatory purposes. Here, we describe an application of a novel organotypic air-liquid interface (ALI) cellular model of lung airway cells for the evaluation of perturbed lung physiology and the potential risk of lung diseases from the usage of tobacco products. Normal bronchial epithelial cells were isolated from donors (with no identifiers, exempt status from the Institutional Review Board, and informed consent), cultured, and ALI culture models display pseudospheroidal epithelium of basal, ciliated, and goblet cell and phenotypic endpoints of ion channel function (CFTR protein and ENAC), which are key for the fluid homeostasis of ion and fluid balance, and mucociliary clearance. Thus, the ALI cultures replicate select structural and functional features of human lung epithelium. Whole smoke-conditioned medium (WS-CM) and particulate matter (TPM) from 3R4F reference cigarettes, and aerosol-conditioned medium (ACM) from a commercial electronic nicotine delivery system (ENDS) were used as test products. Non-cytotoxic, single exposures for 2h of WS-CM, TPM, and ACM did not impact the structural integrity of ALI cultures, however, Airway Surface Liquid (ASL) or ciliary beat frequency. However, significant declines in ClCH channel (GSR and ENAC) expression were detected treatment with the two control cigarette liquid preparations (WS-CM and TPM), but not with the ENDS-derived ACM. This suggests that disruption of ion channel function is one of the earlier perturbations from exposure to cigarette smoke toxicants. Repeated exposures of WS-CM, and ACM over 10 days, were not cytotoxic, and differentially impacted several endpoints. Treatment with WS-CM, and ACM, significantly inhibited ENAC and CFTR functions and reduced ASL, pointing to our disruptions leading to aberrant fluid homeostasis. Furthermore, at the molecular level, several markers of oxidative stress were also higher in WS-CM treated cultures. For example, the expression of ferritin heavy chain (FTH1), hemeoxygenase (HMox1), glutathione peroxidase-2 (GPX2), glutamate-cysteine ligase catalytic subunit (GCLC), and peroxiredoxin-1 (PRDX1) were elevated in WS-CM treated cultures, indicating a tilt towards an overall pro-oxidative state on exposure to combinatorial tobacco preparations. Global transcriptomic profiles revealed that single and repeat-dose treatments of WS-CM markedly enhance the expression of genes involved in xenobiotic metabolism, oxidative stress, cell proliferation, tumorigenesis, and mucociliary clearance, among others. In addition to acute effects, fundamental changes in the tissue architecture were induced by WS-CM as evident from the increased thickness of epithelia, goblet cell hyperplasia, and markers of cellular transformation (ki67 and carcinoembryonic antigen) within 10 days of treatment with cigarette smoke preparations. Collectively, we demonstrate that ALI cultures exposed to cigarette smoke preparations manifest early phenotypic and functional changes involved in the progression to COPD and lung cancer. Thus, the ALI cultures are a tractable model for assessing the re-glutaminate harm from tobacco products.

Host-derived danger-associated molecular patterns (DAMPs) contribute to immune/inflammatory responses during exposure to various environmental pollutants or pathogens. High mobility group box 1 (HMGB1) and heat shock protein 70 (HSP70) are two such DAMPs that serve as extracellular pro-inflammatory mediators which promote inflammation. Interestingly, HMGB1 has recently been proposed as a promiscuous receptor that can amplify immune/inflammatory responses. We have completed 10-day repeat-dose experiments and for our experiments we hypothesized that HMGB1 and HSP70 play important role in regulating TLR-4/RAGE-dependent inflammatory responses during exposure of cells to tobacco flavors (TF)-electronic cigarette vapor condensates nicotine (TF-EVC/CN). Since the U.S. FDA has banned all other flavors from the market, we used tobacco flavors for the study. TF-EVC was prepared by bubbling e-cig vapor through an FBS-free medium using the csm-estee-liquid puffing machine (CH Technologies, NJ, USA) at a flow rate pressure of 10 psi for 30 minutes. An optical density of 0.5 was considered as 100% TF-EVC/CN concentration, which was diluted with complete culture media to obtain the desired concentrations. Human type II lung alveolar epithelial cell s commonly called A549 cells were exposed to filtered air or e-cigarette condensates consisting of (1%) TF-EVC/CN (6 mg/ml) for 24hrs. Our preliminary findings revealed: a) an increase in the transcription of membrane-bound TLR-4 and RAGE (≥2.0 fold); b) increased levels and/or activation of transcription factors NF-kB (>1.75 fold) and STAT3 (>1.5 fold) which function downstream of TLR-4 and RAGE; c) increased expression of pro-inflammatory cytokine IL-6 (>1.3 fold) and chemokine IL-8 (>1.4 fold); d) translation induction of DAMPs like HMGB1 protein (>1.5 fold) and HSP70 (>1.6 fold); e) increased content of extracellular HMGB1 (>1.75 fold) and HSP70 (>2.0 fold); f) extracellular HSP70 mediated regulation of CCL2 and NF-kB transcription; and g) increase in the exosomal HSP70 levels (1.9 fold) in TF-EVC/CN challenged A549 cells compared to controls. Interestingly, our in-silico molecular docking results provide evidence of a strong interaction between nicotine-bound TLR-4 with HSP70 (>1.7286.1 KJ/mol) and HMGB1 (>1195.46 KJ/mol), compared to the interaction of the unbound receptor with HSP70 (>542.35 KJ/mol) and HMGB1 (>642.22 KJ/mol). Our results provide critical information about the possible molecular mechanisms associated with the release of DAMPs and downstream signaling events during ECVC-induced toxicity and inflammation.
For ICC, the number of cItH3 positive cells in BAL also increased significantly in PG/ VG and PG/VG+2.5%N samples compared to air controls. Global proteomics of BAL support exposure to e-cigarette aerosols containing PG/VG alone has the most significant effect on lung biologic responses independent of nicotine or vanillin flavoring with new findings of extracellular trap formation, acute phase responses, and coagulation significantly upregulated after exposure.

**3430**

The Cytotoxic Effects of Unvaped VUSE-Alto and JUUL E-liquids on Human Osteoblast-Like Cells MG-63 Cells


Electronic cigarettes (e-cigarettes) were introduced in the US as smoking cessation devices. In recent years however, manufacturers such as JUUL and VUSE have employed marketing strategies that are more appealing to adolescents than long-time smokers attempting to quit. These strategies are highly addictive. According to the 2022 National Youth Tobacco Survey, 30.1% of high school students use e-cigarettes daily. There has been limited research on the impacts of vaping on bone health. More than 90% of an individual’s lifetime bone mass is acquired by age 18. Our previous in vitro work demonstrates flavor-dependent osteotoxicity due to e-cigarette liquids (e-liquids). Therefore, this study focuses on three e-liquid flavors from the pod-based brand, VUSE Alto—Golden Tobacco, Menthol, and Rich Tobacco—containing 1.8% and 5% nicotine. We also investigate two e-liquid flavors from the historically popular JUUL—Virginia Tobacco and Menthol—containing 5% nicotine. The osteoblast-like cell line MG-63 was exposed to unvaped VUSE e-liquids (concentrations between 0.0001% - 2%) Cytotoxicity was determined using the MTT assay after 24-hour exposure. As we have previously reported that cinnamon flavored e-liquids can induce oxidative stress in MG-63 cells, we examined reactive oxygen species formation in this study. Oxidative stress was assessed using the reactive oxygen species (ROS) assay after 18-hour exposure. MTT experiments in both pod systems indicated that the Menthol flavors were more cytotoxic than the tobacco flavors. Preliminary studies suggest increased ROS formation only in response to Menthol exposure at the highest concentration (2%) in both brands. In addition, there were no significant differences between e-liquids with or without nicotine in any flavor category. Thus, our study demonstrates that in vitro exposure to VUSE and JUUL e-liquids results in flavor and not nicotine dependent osteotoxicity. Further research is needed to determine the effects of vaping on bone health. This research is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408.

**3431**

How Vitamin E Could Have Been Responsible for the 2019 THC Vaping Outbreak

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Vitamin E acetate was found in the tetrahydrocannabinol (THC) e-cigarettes or vaping products and detected in broncho-alveolar lavage (BAL) samples from most patients with the e-cigarette or vaping product use associated lung injury (EVALI). Hence, vitamin E acetate is considered to be a prime suspect by the CDC for the 2019 EVALI outbreak. However, the clinical findings from the EVALI cases provided few data explaining how vitamin E acetate could have contributed to the outbreak. The aim of this study was to conduct in vitro, molecular modeling and in vivo studies to investigate the possible role of vitamin E acetate in the 2019 EVALI outbreak. Our data showed evidence that vitamin E acetate when inhaled with THC caused a reduction in THC-CB2R binding which could cause reduced THC-CB2R mediated anti-inflammatory response in the lungs. Vitamin E acetate also decreased THC-CB1R binding which may lead to decrease in THC psycho effects in the brains, this proposes a possibility for increased vaping tendency when THC is smoked with vitamin E acetate. This likely increase in vaping frequency in addition to reduced THC-CB1R response may result in increased related acute responses of brain injury and subsequent healing by fibrosis in the lungs. Taken together, vitamin E acetate could have been the underlying causative agent in the 2019 vaping (EVALI) outbreak cases and fatalities by creating a sequela of pulmonary-cardiovascular effects. Lung inflammation, pulmonary hypertension and possibly congestive heart failure may have occurred that in some cases were severe enough to cause death.

**3432**

Fatal Hypoglycemia Associated with Dipping Tobacco Use: A Case Report

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There are limited and conflicting data as to whether nicotine induces severe hypoglycemia. In fact, there are very few studies available investigating nicotine on endpoints other than cancer, cardiovascular effects, reproductive effects, and local effects. We discuss the hypothesis of the impact of nicotine in the occurrence of hypoglycemia by reporting a case of fatal hypoglycemia in a consumer of dipping tobacco known, in Morocco, under the name «KALA». Case report: A 32-year-old male, was found unconscious at home. His medical history included active polysubstance abuse of benzodiazepines, alcohol and dipping tobacco. At the emergency department, his blood pressure was 110 mmHg /60 mmHg, pulse rate 110 beats per min, respiratory rate 22 breaths per min, SpO2 100 % on room air and temperature 37.5 °C. He was unconscious with Glasgow coma score of 7/15 and bilateral arm reflexes was absent. A severe hypoglycemia with 500 mL of 30 % glucose. Computed tomography scans showed diffuse brain edema. Bilateral aspiration pneumonia was found on the chest X-ray. Drug screening was positive for benzodiazepine and phenobarbital and was negative for other drugs. A routine blood investigation showed normal results. The magnetic resonance imaging of the brain showed multiple bilateral signal changes along caudate, lenticular nuclei and parahippocampal gyrus suggested hypoglycemia brain injuries Electroencephalography showed slowing of brain waves without physiological activity. Despite intensive medical treatment, the neurologic condition failed to improve. The patient developed nosocomial infections, but no more hypoglycemic episodes were observed. The patient died of central respiratory failure on day 90. Within the limits of laboratory tests and radiological exams performed in our patient, we have eliminated other causes of hypoglycemia. We draw attention, through this case, the eventual unknown danger of dipping tobacco which is largely consumed by Moroccan young people.

**3433**

Electronic Cigarette Wattage Used during Maternal Vaping Impacts Cerebrovascular Function in Offspring

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Electronic cigarettes (E-cigs) entered the market over 15 years ago since then, they have been marketed as a safer alternative to smoking even during pregnancy. Emerging studies find cerebrovascular dysfunction in offspring with in-vitro E-cig exposure. Our study was set on the hypothesis that the e-cig dysfunction is unknown. We hypothesize that maternal vaping at 30-watts will have a greater effect (vs. 5-watts) on cerebrovascular dysfunction in offspring and that the addition of nicotine would not further impact cerebrovascular function. Female Sprague Dawley rats (N=25) were time-mated, and once pregnant, the dams were randomly assigned to one of 5 groups (n=5 dams/group). E-cig groups comprised of a 5-watt setting with either 0 mg or 50 mg/ml nicotine (i.e. E-cig0-5w and E-cig50-5w) and a 30-watt condition with either 0 mg or 5 mg/ml nicotine (i.e. E-cig0-30w and E-cig50-30w). Ambient air exposed dams served as controls. E-cig exposure consisted of 60 puffs (1. Sh/rday; 5 days/week) and began on gestational day 2-4 using whole-body chambers. E-liquid used was 50% vegetable glycerin (VG), 50% propylene glycol (PG) and did not contain flavoring. One male & female offspring per dam are reported at 1- and 3-months of age, where the middle cerebral artery (MCA) was isolated, and reactivity assessed using dose-response (10-4M to 10-2M) to acetylcholine (ACH), serotonin (5-HT), and sodium nitroprusside (SNP) via pressure myography. In 1-month offspring, maximal MCA dilation to ACH was impaired in both, E cig0- 5- and 30-watts compared to air (33±3%, 50±3%, respectively, p<0.05) with 30-watt having a greater impairment than 5-watt (p<0.05). The E cig0 offspring had similar impairment with ACh challenge (E cig50-30w 34±3%, E cig50-30w 57±4%, p<0.05) compared to air controls, and wattage again affected ACh response. However, the SNP dilatation dysfunction associated with 5w was observed. These data show, regardless of the presence of nicotine, both 5w and 30w also showed blunted responses (20±6%, 39±4%, respectively, p<0.05) with 30w further impaired compared to 5w (p<0.05). SNP response in E cig group were also impaired (5w±18±6%, 30w±27±3%, p<0.05) compared to controls, with 30w greater than 9w (p<0.05). Vasocostruction response to 5-HT was blunted in both the Ecig0-5w and -30w (37±3%, 47±3%, respectively, p<0.05) and E cig50-5w and -30w (25±6%, 27±7%, respectively, p<0.05) with only E cig30w showing greater effect than E cig50-5w (p<0.05). In 3-month-old offspring, ACh responses were also impaired with E cig0 (5w±32±3%, 30w±44±2%, p<0.05) and E cig50 (5w±31±3%, 30w±50±2%, p<0.05) groups compared to controls. But here only E cig30 (and not E cig0) showed an effect of wattage (p<0.05). SNP response also showed impairment for both E cig50 (5w±19±4% vs 30w±25±2%, p<0.05) and E cig50 (5w±18±2% vs 26±4%, p<0.05), where only E cig50 had greater 30w effect than 5w. Vasocostruction was also blunted in E cig0 (5w±51±5% vs 30w±40±3%, p<0.05) and E cig50 (5w±13±3% vs 30w±20±4%, p<0.05), but no difference based on wattage was observed. These data show, regardless of the presence of nicotine, both 5w and 30w vaping conditions significantly impaired MCA reactivity via endotheli- al-dependent and -independent pathways in adolescent and young adult offspring. Although the magnitude of impaired vasodilation was greater with maternal vaping at higher compared to lower wattage, the relatively low maternal exposure (only 60-puffs/day - even from just 5w) indicates the threshold in triggering harm to the offspring is very low.
Electronic nicotine delivery systems (ENDS) are typically composed of propylene glycol (PG), vegetable glycerin (VG), nicotine, and flavoring chemicals. New ENDS products have begun to be brought to the market that utilizes synthetic nicotine, a racemic mixture of both R and S isomers of nicotine compared to the previous tobacco-derived nicotine, which is only composed of the S isomer. There is limited data about the health effects of the inhalation of synthetic nicotine, highlighting the need for research on the effects. We hypothesize that exposure to synthetic nicotine ENDS products will result in greater inflammation in the lungs of mice compared to tobacco-derived nicotine-containing ENDS-exposed mice. An equal number of male and female C57BL/6J mice, 8 - 12 weeks old, were exposed for 1 hour utilizing a nose-only exposure system to air, PG/VG (50/50), PG/VG (50/50) with 50 mg/ml tobacco-derived nicotine salt, Menthol-Mint with 50 mg/ml tobacco-derived nicotine salt and Menthol-Mint with 50 mg/ml synthetic nicotine salt ENDS product for 5 days and were sacrificed 2 hours after the final exposure. Total cell counts were collected using acridine orange and propidium iodide stain and inflammatory cytokines were measured using Luminox (M60009RDPS, Bio-Rad) in bronchoalveolar lavage fluid. Mice exposed to PG/VG with synthetic nicotine significantly increased total cell counts compared to both air-exposed and PG/VG with tobacco-derived nicotine. Mice exposed to PG/VG or PG/VG with tobacco-derived nicotine did not alter total cell counts compared to air controls. Mice exposed to all exposures significantly decreased TNF-α, IL-6, IL-1β, IL-12, and KC levels compared to air-exposed mice. Mice exposed to PG/VG, PG/VG with synthetic nicotine, and Menthol-Mint with tobacco-derived nicotine significantly decreased IL-6 levels compared to air-exposed mice. Although contrary to the initial hypothesis, exposure to both flavored and unflavored synthetic and tobacco-derived nicotine ENDS products resulted in differential effects on pro-inflammatory cytokines in the BALF indicating the potential for inflammatory/immunosuppressive effects of aerosol exposure.

Electronic cigarettes (e-cigarettes) deliver nicotine to the user by heating and aerosolizing a complex mixture of chemicals. The wide variety of e-cigarette products in the market adds complexity to this chemical mixture, with different chemical constituents, heating temperatures, and product features, which represents a significant research challenge. While many studies have examined the toxicity and effects of e-cigarette chemical constituents such as nicotine, propylene glycol, glycerin, and flavorings, fewer have assessed the aerosolized mixtures and which components land and interact in the airway. To address this gap, we utilized nasal sampling and an exposomics approach to help us identify what aerosolized e-cigarette chemicals reach and interact with the respiratory mucosa and potentially alter cellular metabolism. In this project, samples from the nasal epithelial lining fluid (NELF) of healthy e-cigarette users (n=32) and nonsmokers (n=27) were obtained. These samples were analyzed via untargeted high-resolution liquid chromatography-mass spectrometry (HRLC-MS). Using this methodology, we extracted 89,125 molecular features (MFs) and successfully annotated 101 significantly different features between our two study groups, nicotine and its metabolites being among the most abundantly detected compounds in e-cigarette user samples. Using a computational prediction model, we also identified the top 15 MFs associated with e-cigarette use. Further analysis will include annotating the missing MFs using public metabolomics/exposomics databases and pathway enrichment and cluster analyses to identify metabolomic differences between e-cigarette users and nonsmokers. So far, these results indicate that the NELF is a valuable sample for respiratory exposomics analyses and that there are several significantly different MFs between e-cigarette users and nonsmokers that require further analysis using exposomics/metabolomics tools to characterize the exposure associated with e-cigarette use.
Electronic cigarettes (e-cigarettes) have recently emerged as popular alternatives to combustible cigarettes for both current and former smokers but continue to attract novice users alike. The long-term effects of e-cig use are unknown and will take decades to observe in humans. Animal models are therefore necessary to identify the health hazard of this practice. We exposed adult, female Apoe−/− mice to either filter tip only or e-atomizer to smoke aerosols collected from a variety of commercially available e-cigarettes.

### Methods

#### Study Design

Four groups of mice were exposed to filtered air (controls) or e-atomizer smoke aerosols for 5 days. Mice were sacrificed, and BALF was collected for analysis.

#### Biomarkers

- **Nitric Oxide (NO)**
- **Oxidative Stress Markers**
- **Genetic Expression**

#### Findings

- NO levels were increased in e-atomizer-exposed mice compared to controls.
- Oxidative stress markers were also increased in e-atomizer-exposed mice.
- Genes involved in NO and oxidative stress pathways were upregulated.

### Conclusion

The results suggest that e-cigarette smoke aerosols can induce oxidative stress and inflammation in vivo. Further studies are needed to determine the long-term health effects of e-cigarette use.

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**References**

2. *New York University School of Medicine, New York, NY.*

Electronic cigarettes (e-cigarettes) came on the market over the last decade and have been progressively advertised as an alternative smoking product to conventional cigarettes, with fewer carcinogenic effects. Previous studies have found that the liquid in pulmonary diseases and environmental exposure, as well as other non-nicotin e-cig vapors containing caffeine, vitamin B12, vitamin C, and essential oils, among others.
The prevalence of electronic cigarette (e-cigarette) use among adolescents is of concern due to the potential for long-term deleterious impact on adolescent pulmonary development in this age group. It is hypothesized that adolescent alveolar epithelium may be more vulnerable to the inflammatory and cytotoxic effects of e-cigarette use. To test this hypothesis, primary cultures of rhesus macaque monkey organotypic alveolar epithelia were created to determine the mechanisms of cellular injury in alveolar epithelial cell following exposure to e-cigarette liquid. Primary juvenile rhesus monkey (~3-4 years old, N=4) airway epithelial cells were isolated by FACS sorting of lung homogenates immunostained with an antibody against epithelial cell adhesion marker (EpCAM). FACS sorted cells were cultured in 3D Matrigel domes with serum-free media supplemented with growth factors to direct differentiation toward alveolar duct like cultures. A panel of pro-inflammatory (NFκB, IL-1β, IL-6 and IL-8) and pro-fibrotic (TGFβ1) markers (Surfactant protein-C, Solute carrier family 34 member 2) and lack of alveolar epithelial type II (AT2) markers (Surfactant protein-C, Solute carrier family 34 member 2) were observed following exposure to GVP in two independent experiments. These results indicate that e-cigarette liquid exposure of the lungs of young rhesus monkeys may be responsible for the inflammatory and cytotoxic response. In addition, the study provides the first evidence of reduced expression of alveolar type II epithelial markers in response to e-cigarette liquid exposure.

Cigarette smoking during pregnancy is a major risk factor for complications in fetal development. Within the last 15 years, electronic cigarettes (Ecigs) have been marketed as safer alternatives to traditional cigarettes. However, there is a wide range of devices and settings that can be used. It has been reported that maternal vaping is associated with greater cardiovascular dysfunction in offspring, but the long-term health effects from maternal vaping at different wattages on offspring health remain poorly understood. This study tested the hypothesis that there would be an increase in aortic dysfunction with increasing wattage of the Ecig device that persists in early and adolescent life, and that the addition of nicotine will further impair aortic function. Female Sprague-Dawley rats (N=25) were time-mated and randomly placed into the following groups (N=5 dams per group): Ecig exposure 5W without nicotine (i.e. 111.08 mg/m³), Ecig50-30W (55% propylene glycol and 45% vegetable glycerin e-liquid vehicle) or media control (N=4 for each group) for 6 hours, and cytotoxicity was assessed via lactate dehydrogenase assay and gene expression analysis for inflammatory mediators IL-6 and IL-8. 3% JUUL e-cigarette liquid exposure resulted in significantly increased IL-6 mRNA (8-fold greater than control), and IL-8 mRNA (3-fold greater than control) IL-6 and IL-8. 3% JUUL e-cigarette liquid exposure resulted in significantly increased LDH release (20% increase over control) compared to controls (3439+94%, p<0.001). There was no difference in MCh dilation between air and Ecig50-30W (85±3%), or between 1% and 3% JUUL exposure without nicotine (Ecig50-30W=86±1%). SNP dilation showed no differences at any concentration range and were reproducible in three independent experiments. Conversely, no reproducible dose related increases in micronuclei were observed following exposure to GVP in two independent experiments. These results indicate that e-cigarette liquid exposure of the lungs of young rhesus monkeys may be responsible for the inflammatory and cytotoxic response. In addition, the study provides the first evidence of reduced expression of alveolar type II epithelial markers in response to e-cigarette liquid exposure.
usually contain vegetable glycerin and propylene glycol (PG/VG) as humectants, as well as nicotine and added flavors. While epidemiological outcomes related to cigarette smoking-mediated inflammatory responses have increased, the same is not true for E-cigs. During inflammation, contaminant-induced epigenetic changes influence the specificity and duration of gene transcription. Our previous mouse studies demonstrated altered PPARγ gene expression in the hypothalamus of young adult offspring exposed perinatally to E-cig aerosols, with and without nicotine. PPARγ plays a fundamental role in the immune response through its ability to inhibit the expression of inflammatory cytokines. Thus, characterizing the effects of E-cig aerosols on DNA methylation associated with lung inflammation contributes to our understanding of the pathophysiology of E-cig-related pathologies. We hypothesized that whole-body inhalation of E-cig aerosols (3h/d, 5d/wk) for 1- and 3-mo alter generation in genomic DNA methylation that modulate inflammatory responses in the lung. In this study, C57BL/6 and FVB/NJ male mice were exposed to either filtered air or PG/VG (50:50) with (18mg nicotine/ml) and without nicotine. Animals were euthanized immediately after exposure and the right caudal and middle lobe after were harvested. After a 1-mo exposure, alterations in DNA methylation in the right lung of both strains were analyzed using the Illumina Infinium MethylationEPIC kit. Sequences for 866,836 CpG probes were obtained from illumina and aligned to the NCBI NIH mouse genome. Results of this study demonstrated significant hypermethylation in the lungs of the PG/VG plus nicotine exposure group in both strains of mice, compared to their filtered air control counterparts. The methylation pattern has been reported in enhanced systemic inflammation as seen in previous animal models. This study is of significant clinical and biological importance, as E-cig use continues to increase, particularly among adolescents. To our knowledge, this is the first study demonstrating that exposure to E-cig aerosols induces altered DNA methylation in relation to adverse inflammatory response. Such findings, while more research is needed, could provide for greater and more relevant therapeutic treatments/intervention for E-cig related diseases.

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1. L. McFadden. In this study, a market map of MONPs was conducted for the determination of export solvent, artificial saliva without enzymes was selected for MONPs extractions due to physiological relevance. The types of oral nicotine products tested included nicotine pouches, tobacco pouches, and snuff, including flavors such as Wintergreen, Blood Orange, Peach, and others. Many of the MONPs tested failed to meet the requirements for in vitro toxicity monitoring unit (CFU/g) product. Three nicotine pouches tested had between 1-4 bacterial CFU, resulting in a maximum of <2.5x10^1 CFU/g product. The total bacterial results for tobacco pouches ranged from 3.77x10^3 CFU/g product to 2.16x10^4 CFU/g product. The total bacterial results for snuff products ranged from <1x10^3 CFU/g product to 1.94 x10^4 CFU/g product. The yeast and mold results for all products had a maximum of <10^3 CFU/g product. While the microbial content of tobacco leaf products is higher than that of nicotine products. While this study was a single point in time, it is important to determine microbial population viability over time for shelf-life justification. As products sit after manufacturing and prior to use, it is important to determine the microorganism stability of these reduced-harm products.

**Impact of Maternal Electronic Cigarette Exposure on Inflammation, Oxidative Stress, and Mitochondrial Function in Postnatal Brain**

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Electronic nicotine delivery system (ENDS), also commonly known as cigarette (e-cig) is considered as a safer alternative to tobacco smoke and therefore has become extremely popular among all age groups and sex. Around 15% of pregnant women are now using e-Cig in the US which keeps increasing at an alarming rate. Harmful effects of tobacco smoking during pregnancy are well documented for both pregnant and postnatal health however there is lack of preclinical and clinical studies to evaluate the long-term effects of prenatal e-Cig exposure on postnatal health. In this study, we have evaluated the consequences of maternal e-cig use on postnatal neuro-inflammation, oxidative stress, hypoxia and mitochondrial function at different age including both male and female offspring. In our study, pregnant CD1 mice were exposed to e-Cig vapor (2.4% nicotine) till postnatal day (PD) 7. Immunobead assay was performed to measure the level of pro-inflammatory cytokines at PD7, PD23, PD45 and PD90. The expression level of oxidative phosphorylation or OXPHOS Complex (I, II, III, IV and V) hypoxia inducible factor (HIF-1α), antioxidant glutathione (GSH), mitochondrial protein TOM20, Manganese superoxide dismutase (MnSOD), EGR1 and NRF2 were analyzed in offspring brain using western blot at PD7 and PD23. Significantly increased expression of OXPHOS complexes and HIF-1α and reduced expression of MnSOD, GSH and NRF2 were observed in prenatally e-Cig exposed offspring compared to control (P <0.05).

Additionally, prenatally e-Cig exposed offspring had higher level of pro-inflammatory cytokines (IL-6, TNF-α) at PD7, PD45 and PD90 (P<0.05). Our findings suggest that prenatal e-Cig exposure induces neuroinflammation and oxidative stress on neonatal brain by increasing cytokine level, oxidative stress and disrupting mitochondrial function which may alter fetal brain immune function and mitochondrial function. Further studies are warranted to make vulnerable in order to identify the affected structures. Currently, we are evaluating mitochondrial activity and making reactive oxygen species (ROS) in primary neuron from prenatal e-Cig exposed offspring. **Support:** NIH R01DA49737 and R10DA02912.

**Characterizing Sex-Dependent Effects in an HDM-Induced Asthma Mouse Model Exposed to Menthol-Flavored E-Cig Aerosol**

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Epidemiological studies have associated e-cigarette (e-cig) use with asthma diagnosis and exacerbations, with more severe asthma symptoms in asthmatics who vape. While some studies have demonstrated that e-cig exposure increases mucin production, inflammation, and induces airways hyperresponsiveness, few have examined this effect in animal models of allergic asthma, especially by using outbred mice and including both sexes. The aim of this study was to test the hypothesis that an outbred mouse model of allergic asthma would demonstrate sex-dependent effects in response to inhaled menthol-flavored aerosol (Juel Labs, Inc.). 7-8-week-old female and male ICR CD1 mice (Envigo, Inc.) were sensitized to either mouse house dust mite (HDM) extract (1 mg/ml), Citric Biologics (B.V.) or sham-sensitized to saline by subcutaneous injections a week apart prior to exposure. They were then exposed to menthol-flavored aerosol (714 ± 251 mg/m3) or filtered air for 1 hour daily for 15 consecutive days in a nose-only inhalation system (Inexposure chamber, SCIREQ). The menthol-flavored aerosol was generated with 20/80% menthol/propylene glycol and delivered at 3 puffs/min, with a 4-sec puff duration and 3.64 ml puff volume, for a total of 180 puffs/day. Intranasal challenges of either HDM (0.5 mg/ml) or saline occurred throughout days 6-15 of exposure within 2 h after exposure. Mice were necropsied 24 h after last exposure/challenge and underwent subsequent pulmonary mechanics measurements with methacholine...
challenge (Flexivent, SCIREQ) or bronchoalveolar lavage (BAL). Flexivent data showed that baseline dynamic respiratory resistance differed between sexes of HDM-sensitized mice that were exposed to menthol-flavored aerosol, with females being significantly higher than males (0.72 ± 0.05 vs. 0.54 ± 0.04 cmH2O.s/ml). In addition, total cell counts of macrophages, neutrophils, lymphocytes, and eosinophils in BAL fluid from HDM-sensitized/menthol-exposed mice were higher than HDM-sensitized/vehicle-exposed mice and were significantly modified the association of VPBs with HR and with SDNN among all relative to Air and Vehicle, although 2.5% WS-3 increased VPBs vs. Air. Coolants already diminished lung function. Future directions include looking at mucin- and proteins that are associated with inflammation and immune-related genomics. This work was funded by NIH P30 ES05065.

3450 Cooling Agents Exacerbate E-cigarette–Induced Cardiac Arrhythmias and Sympathetic Dominance in Mice

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Cooling agents can add minty or ‘icy’ attributes to e-cigarettes (e-cigs) and have become increasingly popular. Although these coolants can be toxic in vitro, their toxicity in vivo remains unknown, especially for synthetic coolants WS-3 and WS-23. To determine the potential for coolants to promote cardiac dysfunction, we tested the electrophysiologic and autonomic effects of e-cig aerosols with varying concentrations of menthol, WS-3, and WS-23. Mice (n=8 male C57BL/6J) were exposed to filtered air (Air) or e-cig aerosols from propylene glycol and vegetable glycerine (PG/VG, 30/70) + 2.5% nicotine benzoate alone (Vehicle) or with menthol, WS-3, or WS-23. On each exposure day, mice underwent a period of three puff sessions (9-min puffing then 9-min washout) per condition, increasing from 0.25%, to 1%, to 2.5% in e-liquids, with time-matched periods for Air and Vehicle controls. Telemetry-derived electrocardiograms were analyzed for ventricular premature beats (VPBs), heart rate (HR), and HR variability (HRV, i., standard deviation of RR [SDNN] and root means squared of successive differences [RMSSD]). Baseline-normalized minute averages of HR and HRV were analyzed by mixed models, and VPBs were analyzed by generalized estimating equations, with p = 0.05 for all reported differences. VPBs increased HR relative to Air during all puffing phases. During all washout phases, Vehicle modestly decreased HR (RMSSD and/or SDNN) vs. Air, suggesting sympathetic dominance. Menthol at 1% uniquely decreased HR during puffing and washouts relative to both Air and Vehicle. Similarly, during puffing WS-3 and WS-23 both at 1% and 2.5% increased RRMSD from Air and Vehicle, suggesting parasympathetic dominance. Conversely, during washouts 0.25% WS-3 increased HR, and 2.5% Menthol decreased SDNN and RRMSD, vs. Vehicle and Air. Further, during washouts WS-3 and WS-23 at both 1% and 2.5%, and WS-3 at 2.5%, not only increased HR but also decreased SDNN and RRMSD relative to Vehicle and Air, firmly indicating sympathetic dominance. Notably, only 2.5% WS-3 significantly increased VPBs relative to Air (p < 0.05). Importantly, these results suggest that menthol and WS-3 significantly modified the association of VPBs with HR and with SDNN among all e-cigs such that VPBs correlated with HR positively and with SDNN inversely for coolant exposures, but not for Vehicle. Cooling agents may enhance the cardiac risks of vaping by promoting sympathoexcitation and ventricular arrhythmia in a condition-dependent manner. If further validated, these findings may justify the regulation of specific cooling agents in e-cigarettes to lessen the adverse impacts of vaping on public health.

3451 Does Primary and Secondhand Exposure to E-cigarette Aerosols Impact Lung Function and Respiratory Symptoms?

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The rapid increase in electronic cigarette use (vaping) is a major public health concern. E-cigarette aerosols (vape) may contain toxic compounds that can cause adverse respiratory outcomes. Previous studies that have compared lung function of primary vapers to non-vapers have shown mixed/conclusive results on the effects of vaping on lung function. Importantly, there are limited data on the impacts on secondary exposed individuals. Therefore, the objective of this proposed study is to investigate the impact of vape exposure on lung function and symptoms, with a focus on comparative effects between primary and secondhand exposed individuals. This ongoing study aims to recruit a total of 30 individuals, consisting of 10 vapers, 10 non-vaping/non-smoking individuals (living in the same residence as vapers), and 10 non-vapers. Only participants ≥19 years old without any respiratory conditions were included. Repeated lung function measurements and questionnaire-based symptoms of the three groups will be obtained at three different time-points (within a period of 6 months at month 1, 3 and 6) and compared. Lung function measurements were performed using a Sibel 9.14.9 ventilated for flow analysis, with the following analyzed: Pulmonary function test (PFT) measurements including forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) were taken for three consecutive days. Forced oscillation technique (FOT) resistance measurements were also obtained. Vaping/smoking status was confirmed using a urinary cotinine test and an exhaled carbon monoxide test. The participant mean age was 27.2 and the mean (SD) FVC, FEV1, and FVC/FEV1 ratio were 3.70 (0.7), 4.30 (0.9), and 0.86 (0.05) respectively. The percent predicted PFT values for FVC ranged from 75-128% amongst participants. A general linear model analysis of the FEV1/FVC ratio demonstrated that vaping status was statistically significant. A post Tukey comparison showed that the mean FEV1/FVC ratio of primary vapers was significantly lower than the mean ratio of non-vapal participants (p<0.05). Due to the limited sample size, a Kruskal–Wallace non-parametric test was conducted which further confirmed that there was a significant difference between the FEV1/FVC ratios of primary vapers and controls. Our preliminary results indicate that vapers have a significantly lower FEV1/FVC ratio compared to controls. The FEV1/FVC ratio of the secondhand exposed group were also lower compared to controls, however due to the small sample size (n=4) further analyses were not conducted. FEV1/FVC <0.7 is used to diagnose obstructive respiratory diseases such as chronic obstructive pulmonary disease (COPD). Ratios below 0.7 were not observed for any study participants, but a statistically lower mean ratio in vapers compared to controls may suggest potential onset of airway obstruction amongst long-time vapers. More studies are needed to explore this phenomenon. Other PFT measurements such as percent predicted for FEV1 and FVC were considered; however, these measurements are affected by varying heights and BMIs. Further analyses are being conducted including symptoms and particularly FOT measurements to understand the impact on small airways.

3452 Extracellular Vesicle Signature Changes in the Urine of E-cigarette Users

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While the literature on the adverse effects of vaping continues to expand, at the present time, the long-term effects of using e-cigs are unknown and thorough research and longitudinal findings related to vaping are limited. Furthermore, many existing studies fail to adequately differentiate e-cigarettes/vaping with or without a history of smoking in their analyses. There is a strong link between cigarette smoking and systemic inflammation/oxidative stress, thus evaluating the association between vaping and systemic inflammation is needed. From November 2021 to November 2022, we recruited a cohort of tobacco product users in NYC (N=135) in order to assess whether or not primary users of combustible and electronic cigarettes present elevated systemic inflammation and demonstrate increased inflammation with sustained product use. Urine, nasal epithelial cells, and dried blood spot (DBS) samples were collected from this cohort of exclusive cigarette smokers, exclusive e-cigarette users, and dual users of both products. High throughput
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When the 2018 Farm Bill legalized non-psychoactive cannabinoid (CBD), it also opened the door to intentional chemical conversion of CBD to Δ8-tetrahydrocannabinol (Δ8-THC) by manufacturers. Δ8-THC is an unregulated psychoactive isomer of Δ9-THC, the main component of marijuana, and is now sold legally across the U.S. Since they were legalized, CBD and Δ8-THC vaping products have rapidly grown in popularity; however, little is known about their respiratory health effects. Based on their oxidation potentials and the high heat associated with vaping, CBD and Δ8-THC are likely to oxidize to reactive electrophilic quinones when vaped, a process that may be catalyzed by vaping product impurities. To determine the link between impurity-induced oxidation and potential respiratory health effects, we first explored whether pro-oxidant constituents of vaping liquids drive CBD and Δ8-THC oxidation towards CBDq and Δ8-THCq and then compared transcriptomic alterations induced by CBD, Δ8-THC, and their oxidation products in human lung cells. Cannabinoids in vaped condensates from CBD and Δ8-THC commercial oils (750-900 mg/mL) and strawberry flavored juices (16-7 mg/mL) were compared to lab-made juices (16.7 mg/mL) using liquid chromatography mass spectrometry (LC-MS). The pro-oxidant potential of vaping product constituents including heavy metals and flavoring chemicals were tested by measuring fatty acid oxidation products in a liposome system. Metal-induced oxidation was also determined by quantifying quinone formation after 108 and 24 hours of incubation with FeCl3 and CuCl2, to lab-made CBD and Δ8-THC juices. To identify biological pathways altered by CBD and Δ8-THC oxidation products, bulk RNA-sequencing was performed on human bronchial epithelial cells (16HBEs) exposed to CBD, CBDq, Δ8-THC, or Δ8-THCq (8.5 µM) for 12 or 24 hours (n=3 passage numbers). We observed that vapo-generated oxidation products, but not combustible tobacco smoke, generated CBDq and Δ8-THCq, indicating that impurities may be driving oxidation. In the liposome system, potential vaping product constituents iron (FeCl3) and the strawberry flavoring chemical furanone generated 30-50% more oxidation products than the control. After one hour of incubation at room-temperature, lab-made CBD juice containing FeCl3 had significantly more CBDq (0.044 nmol/mg) than juice with only CBD (0.007 nmol/mg). Addition of both FeCl3 and CuCl2 to Δ8-THC juice increased Δ8-THCq formation (0.029 and 0.020 nmol/mg respectively) versus the control (0.001 nmol/mg). While CBD and Δ8-THC did not significantly alter gene expression (≤1 gene altered per condition), CBDq and Δ8-THCq respectively altered 209 and 101 genes in the 12 hour and 48 hours at 108 hours (30.05 log2FC>1). Pathway analysis revealed significant activation of the Keap1-Nrf2 stress response pathway by CBDq and Δ8-THCq, indicating reactivity of CBDq and Δ8-THCq towards cysteine residues. In addition, predicted upstream activation of multiple pro-inflammatory cytokines was seen in all conditions, and pathways of ciliary dysfunction were induced by both CBDq and Δ8-THCq. Together, these data indicated that vaping-induced oxidation of cannabinoids generates reactive electrophilic quinones, that flavoring chemicals and potential metal contaminants contained in commercial vaping products enhance this process, and that these products have significant effects on human lung cells.

Cigarette smoking is one of the risk factors for the development of chronic obstructive pulmonary disease (COPD). Cigarette smoke contains a number of constituents such as heavy metals that can cause heart disease. Atherosclerosis develops through a pathway of endothelial dysfunction, lipid infiltration, macrophage recruitment and vascular remodelling. In the present study we sought to assess the cardiovascular impact of combustible cigarettes and next generation products on Human Coronary Artery Endothelial Cells (HCAECs) cultured on an OrganoPlate®2-lane chip (Mimetas BV). THP-1 monocytes were pre-incubated with smoke or aerosol bubbled PBS (bPBS) from combustible cigarettes (1R6F), Heated Tobacco Product (HTP) “Pulze” or E-Vapour Products (EVP) “myblu”. bPBS was generated by passing cigarette smoke or HTP/EVP aerosols through a series of impingers containing PBS, generating stock solution containing 108 and 4.8 puffs/ml for the 1R6F and EVP/HTPs, respectively. Following exposure of monocytes with bPBS, the resulting conditioned medium was then added to the HCAEC vessels. After exposure, glutathione depletion (measured 4 hours post exposure), ICAM-1 expression (measured 24 hours post exposure), and monocyte adhesion (measured 24 hours post exposure) was determined for each of the test articles. Glutathione levels were depleted by 5% 1R6F bPBS samples. Whilst the HTP and EVP samples did not cause any significant glutathione depletion up to 20% concentrations. The 1R6F test article caused a significant induction of ICAM-1 expression in the HCAECs at 5% concentration, whilst HTP or EVP samples caused no induction to a maximum concentration of 20%. The 5% 1R6F conditioned medium significantly increased the levels of monocyte adhesion to the HCAECs. HTP conditioned media also caused significant monocyte adhesion but at a higher (4-fold) concentration. EVP conditioned media did not cause any significant adhesion up to a maximum test concentration of 20%. The results from this study suggest that next generation products exhibit a marked reduction in biological activity in the early key events of atherosclerosis when compared to combustible cigarette.
3461 Nrf2 Responses in a 3D Human Airway Model Exposed to Whole Aerosol from Combustible Cigarettes or Heated Tobacco Products
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The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, activated in human lung cells by cigarette smoke, regulates genes involved in the antioxidative stress response. Here, we evaluated whole smoke/aerosol from two marketed combustible (full flavor and menthol) cigarettes (CC), 1RF6 reference cigarette, four HTP (glo®) styles, and a marketed HTP comparator on cell viability and Nrf2 response in a 3D human airway model (EpiAirway®) transplanted with a luciferase Nrf2 promoter. EpiAirway® tissues were exposed at the air liquid interface to whole smoke or aerosol generated on a Vitrocell® VC10®. Whole smoke/aerosol aerosols were comprised of dilution airflows of 0.5 to 8 L/min for CC, and undiluted to 5 L/min for glo® and the marketed comparator HTP aerosols. Maximum Nrf2 fold increase occurred at the undiluted dose for the HTPs versus 3 L/min for the CC. Moreover, the minimum exposure-correlated nicotine concentration required to induce a >2-fold increase (threshold response) in Nrf2 activation was >30x lower for CC than the HTPs. These data show that the 3D Nrf2 EpiAirway® in vitro model can be used to assess and discriminate responses for a biomarker (oxidative stress) from disease pathways associated with smoking (e.g., respiratory and cardiovascular disease).

3462 Cytotoxicity Assessment of Heated Tobacco Product and Combustible Cigarette Aerosols Utilizing Whole Aerosol Exposure in the Neutral Red Uptake Assay
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In vitro toxicological methods are used to assess the biological activities of combustible and next generation tobacco products (NGP), including Heated Tobacco Products (HTP). To determine the genotoxic potential of aerosols generated from four HTP (glo®) styles, a marketed HTP comparator, and three combustible cigarettes (CC), the bacterial reverse mutation (Ames) and in vitro micronucleus (IVMN) assays were conducted using test sample preparations of total particulate matter (TPM) combined with gas vapor phase (GVP). Equal volumes of the TPM and GVP fractions were combined (HC T-502) to represent “whole aerosol/ smoke” from each product and used for Ames and IVMN assays. Ames preincubation assays utilized tester strains TA98, TA100, TA102, TA1537 and TA102 (s9); HC T-501/OECD 471). For the IVMN assay, CHO cells were exposed under four conditions (3 HrS9 and 24 HrS9 with and without 24h recovery; HC T-503/ OECD 487, Thome et al, 2019). In the Ames assay, all CC were mutagenic based on positive responses in 3 of 5 test strains, while the HTP were negative across all strains and test conditions (s99) when tested at nicotine equivalent doses up to 10-fold greater than CC. In the IVMN assay, all CC produced positive genotoxic responses under all exposure schedules as indicated by dose-related increases in micronuclei. In contrast, genotoxic responses of only some HTP demonstrated positive genotoxicity under certain treatment. Additionally, nicotine-equivalent doses required for positive HTP genotoxicity ranged from 7.5-110x that of the CC. These results add to the weight of evidence from multiple studies showing that HTPs are less genotoxic/potentially harmful compared to CCs and align with the established tobacco harm reduction paradigm of NGPs.

3463 Genotoxicity Assessment of Heated Tobacco Product (HTP), and Electronic Nicotine Delivery Systems (ENDS) Aerosols and Modern Oral (MO) Nicotine Product Extracts in the Ames and In Vitro Micronucleus Assays
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In vitro genotoxicity assessment of tobacco products is an essential piece of the premarket tobacco product application (PMTA) process. Next generation tobacco products (NGPs) include Electronic Nicotine Delivery Systems (ENDS), Heated Tobacco Products (HTP), and Modern Oral (MO) nicotine products. The genotoxicity of combustible cigarette (CC), HTP and ENDS aerosols and MO extracts was determined using the Ames and in vitro micronucleus (IVMN) assays. Test samples from CC, HTP and ENDS included pad-collected total particulate matter (TPM: CC & HTP) or aerosol collected material (ACM: ENDS) and gas vapor phase (GVP) preparations, all generated under iso conditions. MO products were tested using complete artificial saliva (CAS) extracts. TPM/ACM and GVP were tested either separately (ENDS) or combined (CC, HTP) 1:1 (v/v). The Ames assay utilized Salmonella tester strains TA98, TA100, TA102, TA1535 & TA1537 (s99). The three standard exposure schedules were performed in the IVMN and an additional long-term exposure with a recovery period (24-hr, S9, with 24-hr recovery) for HTP and MO samples was incorporated into the assessment. CC (TPM+GVP) was genotoxic in both the Ames and IVMN assays. ENDS (ACM and GVP) and MO (CAS) tested negative in both the Ames and IVMN, while HTP (TPM + GVP) was non-mutagenic in the Ames but genotoxic in the IVMN, albeit less toxic (slope comparison, p<0.0001) at ~10X higher delivered nicotine concentrations compared to CC (TPM + GVP). Overall, the results from this series of studies provide data supporting the tobacco harm reduction paradigm, with a decrease in genotoxicity over a correlated panel of different representative tobacco product types along a decreasing risk continuum compared to combustible cigarettes.

3464 Genotoxicity Assessment of Heated Tobacco Product and Combustible Cigarette Aerosols in the Ames and In Vitro Micronucleus Assays
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In vitro toxicological methods were used to assess the biological activities of combustible and next generation tobacco products (NGP), including Heated Tobacco Products (HTP). To determine the genotoxic potential of aerosols generated from four HTP (glo®) styles, a marketed HTP comparator and three combustible cigarettes (CC), the bacterial reverse mutation (Ames) and in vitro micronucleus (IVMN) assays were conducted using test sample preparations of total particulate matter (TPM) combined with gas vapor phase (GVP). Equal volumes of the TPM and GVP fractions were combined (HC T-502) to represent “whole aerosol/ smoke” from each product and used for Ames and IVMN assays. Ames preincubation assays utilized tester strains TA98, TA100, TA1535, TA1537 and TA102 (s9); HC T-501/OECD 471). For the IVMN assay, CHO cells were exposed under four conditions (3 Hrs9 and 24 Hrs9 with and without 24h recovery; HC T-503/ OECD 487, Thome et al, 2019). In the Ames assay, all CC were mutagenic based on positive responses in 3 of 5 test strains, while the HTP were negative across all strains and test conditions (s99) when tested at nicotine equivalent doses up to 10-fold greater than CC. In the IVMN assay, all CC produced positive genotoxic responses under all exposure schedules as indicated by dose-related increases in micronuclei. In contrast, genotoxic responses of only some HTP demonstrated positive genotoxicity under certain treatment. Additionally, nicotine-equivalent doses required for positive HTP genotoxicity ranged from 7.5-110x that of the CC. These results add to the weight of evidence from multiple studies showing that HTPs are less genotoxic/potentially harmful compared to CCs and align with the established tobacco harm reduction paradigm of NGPs.

3465 In Vitro Cytotoxicity Assessment of Whole Aerosol/Smoke Generated from Heated Tobacco Products and Combustible Cigarettes in the EpiAirway® Tissue Model
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This study exposed EpiAirway® tissues to whole aerosol/smoke generated from four styles of a glo® Heated Tobacco Product (HTP), a market comparator HTP, two marketed combustible cigarettes (CC) and the 1RF6 Kentucky Reference cigarette. A Vitrocell® VC10® robot was used to generate whole aerosol/smoke with the Health Canada Intense (HCI) or modified HCI (market comparator HTP only) smoking regimen. Whole aerosol/smoke was diluted with clean air at increasing air flows (L/min) to achieve the delivered dose range. The exposure conditions were 5.0-10.0 L/min (undiluted) for the HTP and 10.0 L/min (undiluted) for the combustible cigarettes. Liquid traps containing PBS within the exposure module allowed quantification of delivered WA nicotine and carbonyl constituents. The CC’s delivered 24 - 54 µg nicotine per 24-minute exposure, the HTPs 620 - 2751 µg nicotine per 180-minute exposure. WA from the CC’s was cytotoxic, with IC50 values of 2.03 ± 0.51, 1.81 ± 0.17 and 1.68 ± 0.56 µg nicotine for the nonmenthol, menthol, and reference CC, respectively. HTP aerosols were cytotoxic, however the IC50 values ranged from 26.88 ± 13 cell viability and 97.12 µg nicotine, which were up to 80 times less cytotoxic, on a per nicotine basis, when compared to the CC. These results add to the weight of evidence from multiple studies on the harm reduction potential of HTPs when compared to CC, further supporting the tobacco harm reduction paradigm of NGPs.
Leachable investigations are routinely undertaken across a range of sectors (e.g., pharmaceuticals, medical devices, etc.) to determine if any chemicals from a container closure system can migrate or leach into a product. For Electronic Nicotine Delivery Systems (ENDS) that includes all materials in contact with the e-liquid that is aerosolized and subsequently inhaled by the user. Although there is no specific guidance for conducting leachable studies for ENDS products, there is general guidance for pharmaceuticals, more specifically orally inhaled drug products (United States Pharmacopeia, Chapters 1663 & 1664; ISO-10993-18, Product Quality Research Institute - Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, etc.). Based on existing guidance, leachable study conditions and analytical protocols are fairly straightforward, however, risk assessment of the analytical results can be challenging and may require additional data. Herein, we present a case study of the analytical investigation of two leachable compounds with little, if any, toxicological information (data deficient) that were found in simulated leachable studies using JUULpods filled with flavorless base formulation (PG/VO/nicotine/benzoic acid). Advanced LC-MS/MS methods and spectral interpretation proved critical in providing structural information for further elucidation of the data deficient compounds that allowed for tentative identification in the e-liquid. For one compound, additional structural information provided valuable insight into the source of the compound which allowed toxicologists to appropriately classify the data deficient compound as a nicotine-related impurity, i.e., reaction product data deficient compounds, no commercial authentic standards were available, and no reasonable synthetic route was found due to the molecular size and structural complexity of the compounds which prevents classical analytical approaches for the quantitation of these compounds. Analysis of the aerosol from the closed pod-based ENDS showed that the transfer efficiency of both data deficient compounds from e-liquid was minimal (<2% based on experimentally determined LOQ), which provided necessary analytical data pertinent to the route of exposure. Prior to experimental measurement of transfer efficiency, 100% transfer from e-liquid to aerosol was the conservative approach for exposure assessment. Overall, the advances in analytical instrumentation allow toxicologists to utilize creative approaches that ensure the most accurate health risk assessments can be conducted to meet the public health standards needed for ENDS consumer products.

**3467 External Factors Modulating Vaping-Induced Thermal Degradation of Vitamin E Acetate**

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Since their introduction, exposure to vaping emissions has become a major public health concern in the United States. Previous studies have shown that e-liquids readily decompose during the vaping process into alkenes, long-chain alcohols, carboxyl-containing compounds, and other electroplastic species that may induce oxidative stress in lung cells. Vitamin E acetate (VEA) in nicotine-containing e-liquids has been found to decompose into potentially toxic compounds such as duroquinone (DQ) at vaping temperatures below 200 °C. While most models simulate the thermal degradation of e-liquids under pyrolysis conditions, numerous factors - including device construction and the surrounding environment - may impact the thermal degradation process. In this study, we used VEA as a model e-liquid to investigate the role of the presence of molecular oxygen (O2) and transition metals in promoting thermal oxidation of e-liquids, resulting in greater degradation than predicted by pure pyrolysis at low temperatures. Thermal degradation of VEA was performed in inert (N2) and oxidizing atmospheres (clean air) in the absence and presence of Ni-Cr and Cu-Ni alloy powders – metals commonly found in the heating coil and body of e-cigarettes. VEA degradation was analyzed using thermogravimetric (TGA) and gas chromatography/mass spectrometry (GC/MS) analyses. While the presence of O2 was found to significantly enhance degradation of VEA at both high (356 °C) and low (176 °C) temperatures, the addition of Cu-Ni in oxidizing atmospheres was found to greatly enhance VEA degradation, resulting in the formation of numerous degradation products previously identified in VEA vaping emissions. O2, and Cu-Ni nanopowder together were also found to significantly increase the production of OH radicals, which has implications for e-liquid degradation pathways as well as the potential risk of oxidative damage to biological systems in real-world vaping scenarios. Ultimately, the results presented in this study highlight the importance of oxidation pathways in VEA thermal degradation and may aid in the prediction of thermal degradation products from e-liquids.

**3468 Assessment of Feasibility for an Alternate Transport Mode of ENDS Pods**


The JUUL® System is a pre-filled (closed pod) electronic nicotine delivery system (ENDS) that delivers nicotine via an inhalable aerosol generated by heating the nicotine containing e-liquid. The JUULpods contain the liquid storage tank,wick, a heating coil wrapped around the wick, gasket, absorbent pads, airpath tube and a mouthpiece; these are manufactured overseas and shipped empty to the U.S. where they are filled with e-liquid prior to distribution. Because environmental and handling conditions during shipping of the empty pods may influence product quality and product integrity, studies aimed to evaluate conditions related to seasonal differences in temperatures (winter and summer) and shipping routes (air and ocean) were conducted. To this end, simulated leachable studies were conducted on pods shipped empty by air and ocean, during winter or summer, and the leachable results were compared (percent difference for organic compounds and two-sample t-test analysis for inorganic compounds) between both shipping modes. Simulated leachable studies were conducted on a neutral flavor-free e-liquid, utilizing semi-quantitative methods (GC-MS & LC-MS with positive and negative ion mode) to identify volatile and non-volatile organic compounds; and quantitative methods (ICP-MS) to identify inorganic compounds. Leachables were reported based on method-specific analytical evaluation threshold (AET) calculated with a dose-based threshold (DBT) of 1.5 µg/day. Shipping mode differences were determined based on percent change (>100%) for non-targeted organic compounds and based on p-value (<0.05 as significant) calculated by two-sample t-test for metabolites and targeted compounds. In case of leachables exclusive to ocean-shipped pods, levels were compared to the method-specific AET and default TTC threshold of 0.75g/day, assuming two-pods per day. In both seasons one volatile and two non-volatile organic compounds (positive ion mode) had a percent change >100% and were subjected to risk analysis. First (Q)SAR was used to detect alerts for toxicological endpoints including mutagenicity and sensitization, then chemical-specific permissible daily exposures (PDE) or TTC-derived thresholds for inhalation exposures, based on Cramer Class were determined. Finally, the margin of exposure (MOE) was estimated. None of the identified leachables exceeding the acceptable change between both shipping modes were classified as mutagens or sensitizers. The risk assessment result was expressed in MOEs greater than unity indicating no toxicological concerns. Four (4) elemental impurities with statistically significant difference values (p-value<0.05) were observed and reviewed according to USP <232> proposed inhalation PDE limits and were found not to exceed these limits. The assessment of uniquely identified leachables were found to be acceptable overall, comparing results of both modes of transportation results show the two seasons indicate that only a few compounds are changed, show statistical difference, or are uniquely identified in ocean shipped pods. Overall, the risk assessment indicate minimal impact from ocean shipping on pod quality and integrity when compared to air shipping.

**3468a Vaping-Induced Transformation of Terpene Additives in Cannabis Vape Products and Its Impacts on Cell Membrane Integrity**

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The outbreak of vaping-induced lung injuries and deaths in late 2019 throughout the United States demonstrated the potential health risks of vaping. Multiple studies have shown thermal degradation of vaping additives are a potential cause of lung injury. Terpenes, often used as flavoring additives in commercial e-liquids, may undergo chemical transformation resulting in the formation of potentially harmful products. This study investigates the chemical transformation of terpene additives during vaping and the resulting impact on the cell membranes of lung epithelial cells (BEAS-2B). We hypothesized that terpenes exposed to nicotine containing e-liquid. The JUULpods contain the liquid storage tank, wick, a heating coil wrapped around the wick, gasket, absorbent pads, airpath tube and a mouthpiece; these are manufactured overseas and shipped empty to the U.S. where they are filled with e-liquid prior to distribution. Because environmental and handling conditions during shipping of the empty pods may influence product quality and product integrity, studies aimed to evaluate conditions related to seasonal differences in temperatures (winter and summer) and shipping routes (air and ocean) were conducted. To this end, simulated leachable studies were conducted on pods shipped empty by air and ocean, during winter or summer, and the leachable results were compared (percent difference for organic compounds and two-sample t-test analysis for inorganic compounds) between both shipping modes. Simulated leachable studies were conducted on a neutral flavor-free e-liquid, utilizing semi-quantitative methods (GC-MS & LC-MS with positive and negative ion mode) to identify volatile and non-volatile organic compounds; and quantitative methods (ICP-MS) to identify inorganic compounds. Leachables were reported based on method-specific analytical evaluation threshold (AET) calculated with a dose-based threshold (DBT) of 1.5 µg/day. Shipping mode differences were determined based on percent change (>100%) for non-targeted organic compounds and based on p-value (<0.05 as significant) calculated by two-sample t-test for metabolites and targeted compounds. In case of leachables exclusive to ocean-shipped pods, levels were compared to the method-specific AET and default TTC threshold of 0.75g/day, assuming two-pods per day. In both seasons one volatile and two non-volatile organic compounds (positive ion mode) had a percent change >100% and were subjected to risk analysis. First (Q)SAR was used to detect alerts for toxicological endpoints including mutagenicity and sensitization, then chemical-specific permissible daily exposures (PDE) or TTC-derived thresholds for inhalation exposures, based on Cramer Class were determined. Finally, the margin of exposure (MOE) was estimated. None of the identified leachables exceeding the acceptable change between both shipping modes were classified as mutagens or sensitizers. The risk assessment result was expressed in MOEs greater than unity indicating no toxicological concerns. Four (4) elemental impurities with statistically significant difference values (p-value<0.05) were observed and reviewed according to USP <232> proposed inhalation PDE limits and were found not to exceed these limits. The assessment of uniquely identified leachables were found to be acceptable overall, comparing results of both modes of transportation results show the two seasons indicate that only a few compounds are changed, show statistical difference, or are uniquely identified in ocean shipped pods. Overall, the risk assessment indicate minimal impact from ocean shipping on pod quality and integrity when compared to air shipping.
show that the oxygenated terpenes behave similarly to biosurfactants and other detergent-like products resulting in decreased membrane permeability. The results of this study show the changes that terpenes undergo during vaporing and how they induce the formation of more degradation products of the e-liquid solvents, which leads to increased membrane permeability. Terpene additives increase membrane permeability through oxidation of terpenes and increased degradation products of MCT oil. These findings are a steppingstone to better understanding the cytotoxic effects of terpenes in vaporing products.

3469 Toward Developing Novel Prostate Cancer Recurrence Suppressors: Acute and Subchronic Toxicity of Pseudomonas A, an Active Oral PCSK9 Axis—Targeting Small Molecule, in Swiss Albino Mice

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Proprotase convertase subtilisin kexin type 9 (PCSK9) emerged as a therapeutic target for cancer, cardiovascular diseases due to its ability to induce LDL-cholesterol by preventing LDL receptor (LDLR) recycling. Preliminary studies revealed that pseudomonin A (PSA), a spiro-heterocyclic γ-lactam alkaloid from several Aspergillus and Penicillium species, can modulate the oncogenic role of PCSK9 in breast and prostate cancers progression and recurrence due to its dual ability to suppress PCSK9 expression and protein-protein interaction (PPI) with LDLR. Thus, a preliminary assessment of PSA acute toxicity represents an important step to developing PSA as a novel orally active PCSK9 axis-modulating cancer recurrence inhibitor. Male and female Swiss albino mice administered by oral gavage PSA single doses of 10, 250, and 500 mg/kg for the acute toxicity study and daily doses at 10, 40, and 80 mg/kg over 3 months for the sub-chronic study versus vehicle controls. Mice continuously observed over the experiments course to monitor any abnormalities in their behavioral, neuromuscular, and autonomic responses. Mice sacrificed at each study end, their body and organ weights recorded and collected. Mice plasma samples subjected to comprehensive hematomical and biochemical analyses. Collected mouse organs histopathologically analyzed. No morbidity detected following any PSA oral dosing. The Up-and-Down methodology determined PSA LD50 value of >550 mg/kg. No observed histopathological abnormalities in liver, kidney, brain, and heart, suggesting a high safety profile as a prospective valid lead for future use as a first-in-class small molecule prostate cancer recurrence suppressor via PCSK9 axis modulation.

3470 Evaluation of the Effect of Orally Administered Histacel Extract on Respiratory Functions on Anaphylactic Shock in Male Wistar Rats

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Anaphylaxis is a life-threatening form of hypersensitivity characterised by the activation of mast cells and basophils, resulting in release of bioactive mediators that influence vascular permeability. The present study was undertaken to explore the antiasthmatic activity of histacel extract (poly herbal extract). This study was conducted to evaluate the effect of histacel extract on the respiratory function in conscious male rats following repeated gavage administration for 14 days using whole body plethysmography. The test item and Montelukast were administered once daily through gavage for 14 days to rats belonging to three treatment groups (G3 - G5) and a positive control (G6) group, respectively. Rats (G2 to G6) were immunised by 0.5 ml SC injection of DTPa vaccine and horse serum. Rats belonging to the vehicle and the sensitised control groups received RO water. Rats were observed daily for mortality and clinical signs. Respiratory parameters like respiratory rate, tidal volume, minute volume, etc., were recorded after the treatment on days 0, 7, and 14 for 3 hours, 1 hour, and 30 minutes respectively. Rats were challenged on treatment day 14 by a 1 ml IV injection of horse serum. Rats were observed for respiratory functions immediately after the challenge injection for 20 minutes. The test item at mid (250 mg/kg b. wt./day) and high (500 mg/kg b. wt./day) doses caused an increase in the tidal volume, minute ventilation, and a decrease in the frequency of respiration, post-challenge with horse serum. Thus improving the respiratory function in the anaphylactic shock-induced male Wistar rats. Similar effects were observed in the respiratory function for the Montelukast (positive control) administered group, post-challenge with horse serum. The findings of the study suggest that histacel extract has an anti-anaphylactic activity as it improves the respiratory functions in the experimental models, similar to Montelukast, a known anti-allergic compound.

3471 Toxicity of the Insensitive Explosive FOX-7 in Sprague Dawley Rats via Oral Gavage

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The insensitive explosive 1,1-diamino-2,2-dinitroethene (DADNE, referred to as FOX-7) is a bright yellow, crystalline powder developed by the Swedish military in the late 1990s. Compared to the legacy explosive RDX, FOX-7 shows significant improvements in response to impact and friction stimuli while maintaining nearly equal performance. A complete oral toxicological test series in rats is currently being performed to assess the toxicity of FOX-7 prior to its use by the U.S. Army. Acute oral studies yielded estimated median lethal doses of 875 (708-1082) mg/kg in male rats and 805 (668-971) mg/kg in female rats. Gross pathological evaluation indicated delayed clearance and moderate irritation throughout the gastrointestinal tract at higher doses. To determine subacute oral toxicity, rats were repeatedly exposed to FOX-7 at 0, 6.25, 12.5, 25, 50, 100, 200, and 400 mg/kg for 14 days via oral gavage. No rats in the 200- and 400-mg/kg-d groups survived. Weight loss, irritability, lethargy, and porphyrin staining were frequently observed prior to death or moribund euthanasia. Distended stomachs and evidence of moderate to severe irritation throughout the gastrointestinal tract were found in the majority of the pre-death terms. Body weight gain in the male 100 mg/kg-d group was lower than controls throughout the treatment period although food consumption was only decreased from days 0 through 7. Kidney and spleen weights were elevated in 100 mg/kg-d males and females. Differences in clinical pathology parameters were mainly confined to the 100 mg/kg-d group. Blood urea nitrogen/creatinine ratios in male rats and alkaline phosphatase concentrations in female rats both exhibited dose-dependent increases. The micronucleus assay was performed on 25, 50, and 100 mg/kg-d males, and the results indicated that FOX-7 is not genotoxic in rat peripheral blood at the doses tested. The FOX-7 subchronic oral study has not been initiated to date, but limited results should be available for the conference. These studies are critical to determining safety thresholds for use of FOX-7 by US Army soldiers.

3472 Evaluation of Antioxidant Activities of Brown Macroalgae Bifurcara bifurcata Extract

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Research in natural products of marine algae has made significant advances in recent years and marine algae have been proven to produce a variety of compounds and some of them have been shown to possess biological activity of potential nutritional and medicinal value. Epidemiological evidence demonstrated that the consumption of marine algae has lowered the occurrence of chronic diseases, such as hypertension, type 2 diabetes, hyperlipidemia, and coronary heart disease which are in part caused by oxidative stress. The aim of this research was to evaluate the response of brown seaweed Bifurcara bifurcata extract to an oxidative stressor, tert-butyl hydroperoxide (t-BUOOH), in Caco-2 cells. Selected seaweed represents a common specie in northern Atlantic coasts of Spain. This study demonstrates that seaweed extract has the ability to protect human colon carcinoma Caco-2 cells against oxidative insult by modulating reactive oxygen species (ROS) generation, malondialdehyde (MDA) production, antioxidant enzymes [NADPH quinone dehydrogenase 1 (NQO1), glutathione S-transferase (GST)] activities, and caspase 3/7 essential enzyme during apoptosis. These results show that treatment of Caco-2 cells in culture with B. bifurcata extract confers a significant protection against an oxidative insult and suggest that the seaweed B. bifurcata extract can be used as a natural antioxidant in the food industry as well as dietary supplement against oxidative damage. This work was supported by the Project Ref. PID 2020-15979RR-C33 from the Ministerio de Ciencia e Innovación, Spain.

3473 In Silico Occupational Exposure Banding Framework for Data-Poor Compounds in Biotechnology

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Occupational exposure limits (OELs) and occupational exposure bands (OEBs) are exposure benchmarks intended for the protection of worker health. OEBs are typically assigned when there is not sufficient data to derive a quantitative OEL. OEB assignments are typically based on defined methodology and industry best practices often relying on empirical data. However, there is no harmonized methodology available to assign OEBs for compounds with little to no data. Thus, an in silico framework approach for OEB assignment was developed for compounds lacking in vivo toxicology data. Existing tools, including quantitative structure-activity relationship (QSAR) tools and the NIOSH occupational exposure banding tool, were adapted and used in sequence to assign OEBs to data poor compounds. Briefly, two QSAR tools were used to evaluate standard toxicological endpoints and the results were assigned a reliability rating based on agreement between the QSAR tools. Subsequently, a hazard category per Global Harmonization System (GHS)
The output of this framework is adequately protective of worker health. Together, this analysis demonstrates that this framework provides a practical methodology for the evaluation of biotechnology application, this framework can ensure that worker health is protected. As additional data becomes available, the compound may be de-risked using harmonized approaches for deriving OELs or other limits. This approach provides a tool to bridge the existing banding tools available for industrial chemicals (NIOSH OELs) and pharmaceuticals (that have their own banding to reflect high potency). Together, this analysis demonstrates that this framework provides a practical methodology for the evaluation of data poor biotechnology compounds and shows that the output of this framework is adequately protective of worker health.

**3474 Evaluation of Complex Compounds in the UV-Vis Absorption Spectra Method**

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An ultraviolet-visible (UV-Vis) spectral analysis is one of the first steps in a photosafety assessment to determine absorption of light by test compounds. Adopted in 1981, OECD Test Guideline (TG) 101 provides guidance on evaluation of compounds without defined molecular weights and limited solubility need photosafety evaluation. We evaluated compounds using an adaptation of the procedures described in TG 101 to incorporate a 96-well high throughput format, and we investigated additional solvents for test compound preparation and potential filter effects. Test compounds were prepared at multiple concentrations in three different solvent buffer systems (acidic, basic, and neutral), added to a quartz 96-well plate and then absorbance (OD) determined at wavelengths of 230 to 800 nm in 2 nm increments using a Tecan® Infinite M Nano+. Spectral scans and OD values of selected peaks were analyzed using Magellan™ Tracker Software, and Molar Extinction Coefficient (MEC) values were determined using peak absorption and molarity. Compounds with MEC values >1000 L mol⁻¹ cm⁻¹ are considered to have significant light absorption and may require further photosafety testing. For compounds without defined molecular weights, a theoretical evaluation using an absorption threshold of OD ≥ 1.0, as described in Nishida, et al. 2015, was incorporated. To investigate this alternate absorption threshold, fragrance compounds, p-methoxy-cinnamaldehyde and acetovanillone, with MEC values of >1000 L mol⁻¹ cm⁻¹ were also prepared at a high volume in water or methanol, an alternative method with an expected shift in absorbance as concentrations decreased, we prepared a sunscreen formulation at 8 concentrations in methanol. At concentrations > 9.54 mg/mL, the absorbance exceeded the limit of the plate reader (e.g. OD ≥ 4.0) to ~400 nm and then remained flat through 700 nm. A peak at 306 nm was visible at 2.98, 0.93, 0.29, and 0.09 mg/mL concentrations, resulting in OD values of 3.30, 1.60, 0.84, and 0.49. UV-Vis analysis is an important first step for screening of test compounds prior to evaluation in more complex and costly test systems. As industry test needs become more complex, the importance of UV-Vis analysis will increase.

**3475 DOD Fit-for-Purpose Integration of Photosafety: Application of the OECD 432 Phototoxicity Assay**

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Photosafety assessments are conducted to assess the photoactivity of a candidate chemical. Once a chemical is exposed to ultraviolet (UV) light, its level of photoactivity may induce an acute tissue response of variable phototirritation (phototoxicity) and/or an immune-mediated response (photallergy). Integration of photosafety assessments for military-relevant chemicals identifies potentially photosensitive candidate chemicals that may lead to a non-photosensitive user exposed to simulated UV. The BALB/c 3T3 mouse fibroblast cell-line is used to simulate an acute tissue response to UV, which is relevant for military-candidate chemicals that become a part of occupational or outdoor products. Available international standards, OECD 432 and ICH S10, provide guidance to establish protocols for fit-for-purpose testing and to ensure that compounds are fully evaluated and exposed to simulated UV. The CPS+ Suntest was selected to simulate UV-exposures with our first experiment testing guidance as recommended with an irradiation setting of 251 W/m². A second experiment assessed the effects of mid to high irradiation settings (269, 314, 443 W/m²) on cell viability and optical densities (OD). Results from these two experiments showed a range of cell viabilities with the fibroblasts clustering in the center and edges of the well. These results suggested cellular loss and/or detachment likely occurred during one or all of the following steps: 1) plating, 2) aspiration/washes, and 3) UV-exposure. To assess plating techniques, two experiments with two different ethnic (white and black) cells were left in the biosafety cabinet 60 minutes post-plating versus a plate immediately placed in the incubator. Results showed waiting 60 minutes post-plating improved cellular adherence. A fourth experiment assessed the effect of a vacuum aspirator versus a manual multi-channel pipette aspiration, where the latter technique improved OD values and cell viability. A fifth experiment assessed the effect DPBS with glucose, calcium, magnesium, and sodium pyruvate on OD and cell viability as our first two experiments using EBSS or DPBS without calcium and magnesium as a solvent vehicle. These experiments yielded low OD values and viability. Even though DPBS with supplements improved cell-attachment and viability, our sixth experiment confirmed the OD values in the solvent vehicle. The final experiment combined previously described findings and a range of irradiation settings (251, 269, 403 W/m²). Except for the highest irradiated plate, all the dark plates consistently maintained an average OD of 0.37 indicating our adapted protocols combined previously described findings and a range of irradiation settings (251, 269, 403 W/m²). Results from these two experiments showed a range of cell viabilities with the fibroblasts clustering in the center and edges of the well. These results suggested cellular loss and/or detachment likely occurred during one or all of the following steps: 1) plating, 2) aspiration/washes, and 3) UV-exposure. To assess plating techniques, two experiments with two different ethnic (white and black) cells were left in the biosafety cabinet 60 minutes post-plating versus a plate immediately placed in the incubator. Results showed waiting 60 minutes post-plating improved cellular adherence. A fourth experiment assessed the effect of a vacuum aspirator versus a manual multi-channel pipette aspiration, where the latter technique improved OD values and cell viability. A fifth experiment assessed the effect DPBS with glucose, calcium, magnesium, and sodium pyruvate on OD and cell viability as our first two experiments using EBSS or DPBS without calcium and magnesium as a solvent vehicle. These experiments yielded low OD values and viability. Even though DPBS with supplements improved cell-attachment and viability, our sixth experiment confirmed the OD values in the solvent vehicle. The final experiment combined previously described findings and a range of irradiation settings (251, 269, 403 W/m²). Except for the highest irradiated plate, all the dark plates consistently maintained an average OD of 0.37 indicating our adapted protocols combined previously described findings and a range of irradiation settings (251, 269, 403 W/m²). The extracts of the different parts of these plants are commonly used as nutritional supplements or in traditional medicines, mainly for their laxative properties. The above-mentioned botanical species have among their components hydroxyanthracenes as aloe-emodin and emodin, which were considered as genotoxic by the 2018 EFSA-ANS Panel due to the related potential for genotoxic carcinogenicity. The aim of the study was to evaluate the genotoxic potential with the micronucleus assay (OECD 487) in vitro of the extract of Rheum palmatum L., Rhusnus purshiana DC, Rhamnus frangula L., Cassia senna L. Extracts: Absence of Genotoxicity in OECD 487 Micronucleus Assay In Vitro

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Anthraquinones are natural substances contained in several botanical species. Among these species there are Rheum palmatum L., Rhamnus purshiana DC, Rhamnus frangula L., and Cassia senna L. The extracts of different parts of these plants have been used as dietary supplements for anti-inflammatory and free radical scavenging effects, mainly for their laxative properties. The above-mentioned botanical species have among their components hydroxyanthracenes as aloe-emodin and emodin, which were considered as genotoxic by the 2018 EFSA-ANS Panel due to the related potential for genotoxic carcinogenicity. In the Department of Pharmacy, our research has been focused on evaluating genotoxicity in OECD 487 micronucleus assay.
Read-across is a data gap filing approach commonly used when suitable analogues with robust legacy data are available. When uncertainties are identified, new approaches – e.g. NAMs and read-across – are needed to fill in the gaps.

### Integration of New Approach Methodologies and Next-Generation Risk Assessment in Safety Assessment of Naturally Sourced Cosmetic Ingredients: A Case Study of Skin Compatibility Assessment of Botanically Sourced Cosmetic Ingredients

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Due to the rising consumer interest and demand for naturally sourced products, botanical and other naturally sourced ingredients are increasingly used in cosmetic formulations. Many naturally sourced ingredients being isolated and prepared as cosmetic ingredients are new and novel and require evaluation for their safety by consumers. For a new ingredient where a safety assessment did not previously exist, traditionally, animal studies had to be conducted to prove that it is not toxic to human health. With the implementation of animal testing ban on cosmetic products and their ingredients and on the sale of cosmetics that rely on new animal test data in many countries, however, it became apparent that the animal studies can be no longer used in the cosmetics or their ingredients safety assessments. For this reason, we have used New Approach Methodologies (NAMs) and Next Generation Risk Assessment (NGRA) approaches in the safety assessment of naturally sourced cosmetic ingredients. For our purpose, NAM is defined as non-animal-based approaches, such as in silico, in chemico and in vitro assays, used to support the chemical hazard and risk assessment. These new approaches also include integrated testing and assessment of chemical exposure and read-across. NGRA is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates NAM. An example of NAMs and NGRA approaches in the safety assessment of naturally sourced cosmetic ingredients, we are presenting a case study of skin compatibility assessment of botanically sourced cosmetic ingredients. The new approaches involve: (1) review of documented historic use of botanicals in comparison to existing and/or intended products (considering the effect of processing/extraction), (2) characterization of the intended finished product categories and consequent exposure, (3) in vitro assessment of irritation and sensitization potential, and (4) confirmation of skin compatibility with human volunteers. From the skin sensitization studies, the skin sensitization potential of certain botanicals was confirmed. In particular, the first step focused on: (1) review of history of use, (2) identification of potential sensitizer(s) of concern, and (3) if practical, selected elimination of identified potential sensitizers(s) of concern from botanical extracts. The utility of in vitro irritation battery testing, together with historic use data review, as a useful screening tool in the skin compatibility evaluation of botanical ingredients was demonstrated from confirmatory human patch testing (total number of volunteer panelists > 3,000; number of botanically sourced cosmetic ingredients = 20).

### Biological Action Mechanism-Based Safety Assessment of Raspberry Ketone in Cosmetics

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Advances in cheminformatics, bioinformatics, quantitative structure-activity relationship (QSAR), and in vitro assays in the context of biological systems are now at a point that these tools can be applied to mechanism-based cosmetic ingredient safety assessment and prediction. These integrated efforts will also require active collaboration of non-animal-based approaches, such as in silico, in chemico and in vitro assays, as well as chemical grouping and read-across approaches. Here, we describe the approaches for the mechanism-based cosmetic ingredient safety assessment of raspberry ketone in cosmetics. In cosmetics, raspberry ketone has been used as a skin conditioning or fragrance ingredient. The use of raspberry ketone as a multifunctional antimicrobial ingredient in cosmetics is relatively new. Raspberry ketone is structurally similar to rhododendrol that is known to cause leukodermia. The chemical leukodermia caused by rhododendrol is an acquired hypopigmentation caused by repeated exposure to rhododendrol damaging to epidermal melanocytes. Rhododendrol is a good substrate for tyrosinase and causes a tyrosinase-dependent cytotoxicity to melanocytes through the tyrosinase-catalyzed oxidation to cytotoxic o-quinones. An analysis is conducted to confirm that no significant or detectable level of rhododendrol is present in raspberry ketone (rhododendrol is not detectable at detection limit of 50 ppm). In addition, comparative cytotoxicity and melanin quantification assays have been conducted to assess the relative potency of chemical leukodermia caused by rhododendrol and to cause the depigmentary disorder (or leukodermia). The results of these studies demonstrate that: (1) raspberry ketone is less cytotoxic compared to rhododendrol and (2) the relative potency of raspberry ketone to cause chemical leukodermia is low compared to that of rhododendrol. In the case of chemical leukodermia associated with phenolic and catecholic derivatives, symptoms of contact dermatitis, such as itching, erythema, or edema, are present before the occurrence of depigmentation. No symptoms of contact dermatitis at 1%, 5%, 10% and 12% raspberry ketone in multiple and repeated-exposure human patch tests conducted in agreement with Good Clinical Practice (GCP) Human Patch Testing (N = 30) and Human Repeat Insult Patch Testing (N = 204) provide favorable clinical evidence that the raspberry ketone can be safely used as a multifunctional antimicrobial ingredient in cosmetics at ≤ 1 wt %.

### A Tiered Approach for Assessing the Safety of Polymeric Ingredients in Cosmetics and Personal Care Products

L. Brown1, D. McMillan2, J. Urbani2, and A. Mihalchik2


Polymers are commonly used in cosmetic and personal care products as film-formers, emulsifiers, thickeners, conditioners and more, given they can be high performing and cost-effective substances. From a human safety perspective, polymers can sometimes be classified as PLC (polymers of low concern) due to their unique physicochemical properties, such as the potential to be minimally absorbed by the skin. However, conducting safety assessments on polymers can pose unique challenges due to their large size, the monomers from which they are synthesized, impurities in the ingredient and the potential for residuals in the final product. Consequently, there is a need for a holistic, evidence-based method that
addresses these various aspects of a safety assessment for polymeric ingredients. The objective of this work is to propose a tiered approach that can be used for the safety assessment of polymers used in cosmetics and personal care products. Through practical application by toxicologists who are expert in cosmetic safety assessment, read-across, QSAR and risk assessment, the approach was developed to be fit-for-purpose for cosmetic and personal care product scenarios. The result of this work is a method which begins with understanding the physical chemical properties of the polymer (e.g., average molecular weight and molecular weight distribution, water solubility, logP, presence of residual monomers) and progresses from a more simplistic (confirming PLC) to increasingly complex (calculating multiple margins of safety for individual constituents within a polymer) evaluation.

A study sponsored by the Equity-League Pension and Health Trust Funds concluded there was no evidence of serious health effects associated with exposure to mineral oil haze at a mean total mineral oil concentration of 0.74 mg/m³ and wide daily subject averages. A series of Canadian studies found no significant association between acute symptoms and exposure to mineral oil fogs and non-physiologically significant declines in lung function after 4 hours exposure. The average breathing zone aerosol concentration of mineral oil of all productions were between 0.41-1.21 mg/m³ as inhalation with 0.5% of the NOAEL. A NICOP study of theater production found oil mist concentrations were less than 0.13 mg/m³ with no evidence theatrical smoke caused occupational asthma. A study conducted to assess stage fog exposure and worker populations are less likely to die of these respiratory diseases than U.S. communicators, and photographers) than is expected based on the proportion of deaths from respiratory disease among workers in general. Thus, studies to assess stage fog exposure and worker populations are less likely to die of these respiratory diseases than U.S. communicators, and photographers) than is expected based on the proportion of deaths from respiratory disease among workers in general.
Endocrine-disrupting chemicals (EDCs) have been intensively studied regarding their harmful effects on human brain development. Despite increasing evidence that early developmental EDC exposure causes developmental neurotoxicity (DNT), regulatory EDC risk assessment does not feature DNT endpoints. Therefore, the incorporation of DNT testing strategies into the risk assessment of EDCs is urgently required. Currently, the identification of chemicals causing adverse neurodevelopmental effects is based on animal studies. However, insufficient test throughput, species differences, and ethical concerns demand alternative in vitro models with high predictivity for humans. Therefore, a DNT in vitro testing battery has been assembled including a multiplexed high-content assay based on human neural progenitor cells (hNPCs), the Neurosphere Assay. To identify hormone-sensitve neurodevelopmental key events for ED-DNT in vitro assays development, we investigated the effects of hormone receptor agonists and antagonists targeting T4 nuclear hormone receptors on key neurodevelopmental processes modeled within the human Neurosphere Assay including NPC proliferation, migration, and terminal differentiation into neurons and oligodendrocytes. In addition, RNASeq analyses were performed to confirm hormone receptor target gene activation and cell-type specific gene modulation. Strikingly, oligodendrogenesis was especially sensitive to endocrine modulation, being influenced by activation of the aryl hydrocarbon receptor (AhR), liver X receptor (LXR), retinoic acid receptor (RAR), peroxisome proliferator-activated receptors (PPARs), progesterone receptor (PR), prostaglandin E2 receptor (PG2ER) and vitamin D3 receptor (VDR). By incorporation of both male and female NPCs of human and rat origin in our testing strategy, we identified several neurodevelopmental key events impacted by hormones in a species- or sex-specific manner. Since hormone-regulated neurodevelopmental processes provide putative targets for EDCs, the established ED-DNT in vitro assays will be used to screen libraries of known and putative EDCs and identify ED-induced DNT. Therefore, one of the ED-DNT assays, the NPC1_RAR_GR assay is currently under validation at the French ED-validation platform PEPPER for future ED-induced DNT. Thus, development of test battery has been considered as a simple screening test for developmental neurotoxicity requires significant resources, a simple screening test for identifying lower doses than those at which significant changes in serum T4/TSH levels were observed, suggesting that histopathological analysis can be a more sensitive parameter for detecting antithyroid effects, regardless of the mechanism. Similarly, significant increases in thyroid weight and pituitary TSH+ area were detected at doses comparable to those at which significant changes in serum T4 and TSH levels were observed, indicating that these parameters are also useful for evaluation of antithyroid effects. Immunohistochemistry for T4 was more sensitive than serum hormone levels for detection of thyroid peroxidase inhibitors and was useful for distinguishing them from thyroid hormone metabolism promoters. Hepatocellular hypertrophy and significant increases in UGT1A6+ area in the liver were found in lower dose groups, and histone acetylation examination and UGT1A6 immunostaining could be used to detect thyroid hormone metabolism promoters.

Some xenobiotic substances disrupt thyroid homeostasis including effects on thyroid hormone (TH) synthesis, signaling, metabolism and excretion. Thus, a concern has been raised that TH disrupting chemicals may have potential to interfere with the developing brain since THs are essential for normal brain development in humans and animals. Since standardized studies to identify developmental neurotoxicity (DNT) issues requires significant resources, for chemically induced TH perturbations, a simple screening test investigating whether maternal chemical exposure reduces brain TH levels in offspring (a necessary precursor event for brain developmental disorders) would be valuable. The comparative thyroid assay (CTA) is a screening test for TH disruptors in peripheral blood of offspring (US.EPA, 2005), but it requires several animals and is criticized as reliant on peripheral THs alone as predictive markers of brain malfunc-1436
tion being inadequate. Recently, we began verifying the sensitivity, feasibility and/or reliability of a modified CTA with reduced numbers of rats and additional parameters such as examination of brain THs levels and brain histology. We showed that the modified CTA detected 10 ppm 6-propylthiouracil (6-PTU)-induced severe (>70%) suppression of serum THs in dams, with ~50% suppressed serum/brain TH levels in offspring and brain heterotopia in PND 21 pups. It also detected 1000 ppm sodium phenobarbital (NaPB)-induced mild (<35%) suppression of serum THs in dams, with mild (<35%) reduction of serum/brain TH levels in fetuses but not in pups, and no increased heterotopia formation. Within lab repeatability of the results was shown, and the test appears highly repeatable in the range of 0-3500 ppm NaPB. We showed that NaPB, 1000 ppm NaPB were again detected. The NaPB data indicate that mild reduction of THs in maternal rats by enzyme inducers may have little impact on brain development of offspring in contrast to the 6-PTU-like phenotype in brain THs and morphology, although further studies may be needed to evaluate the assay’s reproducibility by using other chemicals having NaPB-like effects. Since our findings suggest that the modified CTA may have potential as a screening test for offspring brain TH disruptors, we propose using the modified CTA as a screening test for developmental neurotoxicity. This research is partly supported through a grant of LRI (The Long-range Research Initiative, #20-3-02) by Japan Chemical Industry Association.

In response to increasing regulatory attention to the potential “endocrine disruption (ED)” issues, exhaustive research efforts have been initiated to better understand the way in which these chemicals interact with their targets. There is strong support for the hypothosis that chemically induced thyroid hormone (TH) perturbations, specifically those for which the mode of action (MoA) is mediated via hepatic enzyme induction and increased biliary excretion, are only relevant in rodents, especially in rats, with little or no relevance for human exposures. The objective of the present study was to further investigate peculiarities of T4 metabolism in 2D-sandwich-cultured cryopreserved primary (Wistar) rat (PRH) and human hepatocytes (PHH) following daily exposure to reference hepatic enzyme inducers up to 7 days. We confirm that in the previously described long-term culture configuration (Parmentier et al., 2013, 2017), both PRH and PHH efficiently respond to specific reference inducers beta naphthoflavone (BNF, in PRH/PHH), pregnenolone 16α-carbonitrile (PCN, in PRH) or rifampicin (RIF, in PHH) and phenobarbital (PB, in PRH/PHH), activators of AhR, PXR and CAR nuclear receptors, respectively. Specific CYP mRNA induction and increases in related enzyme activities are proofed in the above-mentioned induced cells. In addition, we confirm the prominent induction of UGT2B mRNA in PRH and of UGT1A1 in PHH by these inducers as we previously reported (Wiemann et al., in revision). Altogether, induction responses of PRH were much stronger than PHH. With respect to thyroxine (T4) metabolism, glucuro-no-conjugates (T4-G), sulfo-conjugates (T4-S, 3,3’,5’-triiodothyronine (T3) and reverse T3 (T3) were identified. The obtained results indicate that T4 transformation rate was much higher in PRH (363 pmol/106 cells/24h) compared to PHH.

The Organisation for Economic Co-operation and Development test guideline for testing battery has been considered as a simple screening test for investigating lower doses than those at which significant changes in serum T4/TSH levels were observed, suggesting that histopathological analysis can be a more sensitive parameter for detecting antithyroid effects, regardless of the mechanism. Similarly, significant increases in thyroid weight and pituitary TSH+ area were detected at doses comparable to those at which significant changes in serum T4 and TSH levels were observed, indicating that these parameters are also useful for evaluation of antithyroid effects. Immunohistochemistry for T4 was more sensitive than serum hormone levels for detection of thyroid peroxidase inhibitors and was useful for distinguishing them from thyroid hormone metabolism promoters. Hepatocellular hypertrophy and significant increases in UGT1A6+ area in the liver were found in lower dose groups, and histone acetylation examination and UGT1A6 immunostaining could be used to detect thyroid hormone metabolism promoters.

This research is partly supported through a grant of LRI (The Long-range Research Initiative, #20-3-02) by Japan Chemical Industry Association.
Effects of Sodium Phenobarbital in a Downsized Comparative Thyroid Assay with Additional Examination of Brain Thyroid Hormone Levels and Brain Histology

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Since thyroid hormones (THs) are essential for normal brain development in humans and animals, concern has been raised that TH disrupting chemicals may have potential to interfere with the developing brain. In contrast to strong anti-thyroid agents such as propylthiouracil (PTU), suppression by liver enzyme inducers (e.g., phenobarbital, PB) is generally mild. Effects of mild suppression of maternal TH levels on brain development in offspring are not well documented. While standardized studies to identify developmental neurotoxicity requires significant resources, a simple screening test for investigating whether maternal chemical exposure reduces brain TH levels in offspring would be valuable. Recently, we began verifying the feasibility of the Comparative Thyroid Assay (CTA) by downsizing the number of rats but with additional examination of brain TH levels and brain histology. We had shown that the modified CTA could detect 10 ppm 6-PTU-induced severe suppression of serum THs in dams, with suppressed serum/brain TH levels and brain heterotopia in offspring. The modified CTA also detected 1000 ppm NaPB-induced mild suppression of serum THs in dams, with mild reduction of serum/brain TH levels in offspring but not in pups, and no a full-blown brain heterotopia. 8-Anilino-1-naphthalenesulfonic acid (ANSA) is a probe which fluoresces when bound to TTR or TBG. In our modified CTA, ANSA was used to screen possible suppression of TH binding to TTR and TBG in both PRH and PHH. Daily treatment of PRH with reference compounds BNF, PCN or PB for 7 days induced an additional increase in T4 metabolism over control (572, 586 and 188 ppm/10^10 cells/24h relative increases, respectively), which is caused by only additional T4-G formation. The metabolism of T4 was also, however to a much lesser extent, increased in PHH by BNF, Rif and PB (45.6, 47.6 and 48.5 ppm/10^10 cells/24h relative increases, respectively) and consisted mainly in T4-G formation, between 88 and 96 %. Overall, this in vitro system is a useful supporting tool to evaluate the human relevance of the rat liver mediated TH disruption induced by environmental chemicals, as required in the new European regulations.

In Vitro Screening of 149 PFAS Chemicals for Potential Inhibition of the Sodium Iodide Symporter (NIS)

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There is increased concern for environmental chemicals that can target various sites within the hypothalamic-pituitary-thyroid axis to disrupt thyroid synthesis, transport, metabolism and/or function. One well known thyroid target that has been described in both humans and wildlife is the sodium iodide symporter (NIS) that regulates iodine uptake into the thyroid gland, the first step of thyroid hormone synthesis. Our laboratory pre-validated a radioactive iodide uptake (RAIU) high-throughput (HTP) assay using a stably transduced human NIS cell line (NNSI-HEK293T-EPA) to identify chemicals that have the potential to inhibit NIS activity. We previously used this RAIU to test more than 2000 chemicals (US EPA’s ToxCast chemical libraries Pl,v2, Pl and e1K) and identified a subset of those chemicals that significantly inhibited iodide uptake. Here, we used this HTP screening assay to evaluate a new test set of 149 unique per- and polyfluoroalkyl substances (PFAS) chemicals (ToxCast PFAs library) for potential NIS inhibition. In the current evaluation, the 149 blinded chemicals were screened using a tiered-approach, first in a single-concentration (≤100µM) RAIU assay and subsequent evaluation of the active chemicals (≥20% iodide uptake inhibition) using multi-concentration (MC) response (0.001µM-100µM) testing in parallel RAIU and cell viability assays. Of the chemicals tested in the MC assay, 38 of the PFAS chemicals inhibited iodide uptake by at least 20% and 25 of the chemicals showed over 50% inhibition as compared to the control wells. To further prioritize the most potent PFAS NIS inhibitors in this set, chemicals were ranked based on iodide uptake and concurrent cytotoxicity and normalized to percolate, the known positive. We repeated our previous findings that both PFOS and PFHxS were potent NIS inhibitors, but also identified novel PFAS chemicals in this test set that inhibited NIS activity. Although further studies are needed to confirm these effects in vivo, this initial screening effort identifies NIS as a potential thyroid target for PFAS chemicals and suggests several persistent and structurally diverse PFAS chemicals. This abstract does not necessarily represent the views or policies of the US Environmental Protection Agency.

Identifying Thyroid-Active Chemicals Using High-Throughput Screening

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High-throughput screening (HTS) assays allow for rapidly testing many chemicals for bioactivity at specific molecular targets. One goal of HTS is to reduce the time and cost of generating data to support evaluation of chemicals for potential endocrine disruption. Data from these assays can be used to develop a framework to predict in vivo effects with the goal of reducing animal testing. The US EPA’s Toxicity Forecaster (ToxCast) has a library of HTS data which can be used to prioritize chemicals of concern. Further, HTS can be used to identify potential molecular initiating events (MIEs) in adverse outcome pathways (AOPs). One gap in thyroid-related HTS is the sodium iodide carrier proteins transthyretin (TTR) and thyroxine-binding globulin (TBG) as potential MIEs of thyroid system disruption. TTR and TBG maintain the levels of free versus bound thyroid hormone and serve as circulating hormone transport proteins to deliver thyroid hormone to target tissue. To address this gap, a fluorescent high-throughput assay has been developed to assess inhibition of TTR or TBG. 8-Anilino-1-naphthalenesulfonic acid ammonium salt (ANSa) is a probe which fluoresces when bound to TTR or TBG. In the assay, displacement of ANSa from the protein by inhibitory chemicals results in a loss of fluorescence. A two-tiered approach was utilized to rank and prioritize chemicals for further testing. The first tier consisted of screening approximately 1800 chemicals from the ToxCast phase 1, phase 2, and e1k libraries for activity at a single high concentration. The total number of active chemicals in each assay are also enriched in the positive chemical space, particularly those used as surfac- tants. We considered surfactants to be false positives that likely interfered with the protein-based assay. Considering these two factors increased our confidence in a final list of 101 chemicals with bioactivity for at least one enzyme. Finally, the in vitro bioactivity data were used to predict administered equivalent doses (AEDs) using high-throughput toxicokinetic models for in vivo to in vitro extrapolations. Future work will explore how internal doses in a maternal-fetal physiological-ty-based toxicokinetic model compare to bioactive concentrations from thyroid-re- lated assay endpoints. This work suggests an extensible approach to other MIE groups of thyroid-related bioactivity data from ToxCast. A broad analysis of all ToxCast assays for MIEs in the thyroid AOP network may provide the weight of evidence necessary to connect MIEs to potential adverse outcomes in susceptible human populations. This abstract does not necessarily reflect US EPA policy.
assays will support ranking and prioritization of chemicals to be tested in vivo and will aid in the development of a framework to predict in vivo effects from in vitro data. The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.

3492 Characterization of Maternal Liver Enzyme Induction Effects on Thyroid-Related Endpoints in Offspring

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Adequate supply of maternal thyroid hormone (TH) is essential for normal brain development. Since maternal TH comprises a significant portion of the fetal TH supply, a maternal TH deficiency can impair the mother's thyroid resources. There has been a public health concern to protect the fetus from potential neurodevelopmental health risks posed by the maternal exposure to chemicals. Thus, TH endpoints have been incorporated into many regulatory guideline studies used for safety testing of chemicals. However, there are many uncertainties regarding the relationship between TH changes and neurological development. These uncertainties prevent us from fully understanding the impact of TH endpoints collected in regulatory guideline studies. To better understand the effects of changes in maternal TH level on offspring, this study focuses on characterizing the dose-response effects of a classical liver enzyme-inducer compound, phenobarbital, on maternal TH homeostatic endpoints and on the offspring in utero. In this study, pregnant Sprague-Dawley rats were dosed with different concentrations of phenobarbital (15, 25 and 80 mg/kg/day) from gestation day (GD) 6 to 20, followed by thyroid related endpoints assessment in both dams and fetuses. Dams displayed dose-response increase in relative liver-to-body weight ratio (by 30%), upregulations in hepatic Phase I (Cyp2b1 by 52-fold and Cyp3a2/3 by 8-fold) and Phase II (Ugt2b1 by 3-fold) enzyme gene expressions, and the induction in hepatic T4-glucuronidation activity (80% increase), and reductions in serum T3 and T4 levels (30% and 29%, respectively), when compared to the control sodium perchlorate (NaClO₄) group. The effect was observed in fetal liver, suggesting that reduced T4 levels observed in fetuses in serum T3 and T4 levels (30% and 29%, respectively), when compared to the

3493 Validated In Vitro Assays for Identification of Chemicals with Direct Effects on Thyroid Function


Thyroid hormone (TH) homeostasis depends upon the coordination of multiple key events including iodide uptake, hormone synthesis, metabolism, and elimination to maintain proper TH signaling. Perturbation of TH signaling has been linked to abnormalities in brain development, cognitive impairments, and other adverse outcomes in humans. These perturbations can occur via several mechanisms, which include inhibition of the sodium/iodide symporter (NIS) that enables uptake of iodide to the thyroid gland, or thyroid hormone-thyroid peroxidase (TPO) that incorporates iodide into tyrosine residues from thyroglobulin to produce TH, and inhibition of iodothyronine deiodinase types I, II, or III (DIO1, DIO2 or DIO3, respectively), which regulate the levels of TH via removal of specific iodine substituents. To increase chemical safety screening efficiency and better understand the direct effects of chemicals on thyroid function, we developed and validated three functional in vitro assays in rats: (1) the NIS (2) the TPO, and (3) the DIO inhibition assays. These assays are simple, sensitive, inexpensive, robust, and highly reproducible. Furthermore, they give the possibility to screen many compounds in a short time. The NIS inhibition assay allows to identify NIS-inhibiting chemicals by assessing iodide accumulation in cells, using a spectrophotometric assay based on the Sandell-Kolthoff reaction. We first compared the iodide uptake capacities of three cell lines: the rat thyroid-derived cells (FRTL-5) and HEK293 stably transfected cells overexpressing human (HEK-NIS) or rat NIS (HEK-NIS). We found that HEK-NIS and HEK-NIS incorporated iodide in greater amounts and in a more reproducible way than FRTL-5 cells. Thus, we developed the HEK-NIS cell-based assays using known positive control sodium perchlorate (NaClO₄) and negative controls 6-N-Propyl-2-Thiouracil (PTU) and sodium fluoride (NaF). NaClO₄ inhibited NIS function as previously shown in the literature, while PTU and NaF did not have any effect, as expected. The TPO inhibition assay allows to identify TPO-inhibiting chemicals by assessing TPO activity in rat and human thyroid microsomes, using the TPO-mediated conversion of the Amplex UltraRed (AUR) substrate to a fluorescent product. We validated both rat and human thyroid systems using known positive control PTU and negative control NaClO₄. As previously described in the literature, PTU inhibited rat and human TPO activity, while NaClO₄ did not have any effect. Finally, the DIO inhibition assay allows to identify DIO-inhibiting chemicals by assessing DIO activity in HEK-stable transfected cells, overexpressing human, or rat DIO1, DIO2, and DIO3 using the DIO-mediated conversion of thyroxine (T4) substrate into triodo-L-thyronine (T3) by DIO1 and DIO2, and into 3,3',5'-triiodo-L-thyronine (reverse T3, rT3) by DIO3. We validated both rat and human test systems using known positive controls Aurothioglucose (GTG) and PTU for DIO1 and GTG for DIO2 and DIO3, and negative controls NaClO₄, for the three DIOs and PTU for DIO2 and DIO3. Both GTG and PTU inhibited rat and human DIO1 activity, while NaClO₄ did not have any effect. GTG inhibited both rat and human DIO2 and DIO3 activities, while PTU and NaClO₄ did not affect them. All these results were consistent with those reported in the literature. The above-mentioned assays, successfully validated in both rat and human test systems using specific positive and negative controls, represent valuable support tools that allow to quickly identify potential chemical-mediated changes in thyroid function as required in the new European regulations.

3494 Transcriptomic Signatures of Thyroid Hormone Disruption in the Rat Liver

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Thyroid hormone (TH) signaling is critical for metabolism and mammalian development, including neurodevelopment. Disrupting TH regulation and action can thus have severe consequences. A number of environmental chemicals, for example pentabromodiphenyl ether (PentaBDE, DE-71) and octyl-methoxycinnamate (OMC), can disturb the TH system by lowering circulating TH levels without a compensatory increase in thyroid stimulating hormone (TSH). This effect pattern in response to certain chemicals is poorly understood, but emerging evidence suggests that the liver plays a role. For instance, in the liver DE-71 exposure affects expression of genes related to phase I and phase II enzymes, transporters and ion channels. In this study, we sought to investigate how chemicals disrupting the TH system by different mechanisms of action affect the liver transcriptome in rat pups after developmental exposure. Pregnant rats were exposed from gestational day 7 to postnatal day 16. The treatment groups were either control (corn oil, n = 14); propylthiouracil (PTU, 1 and 2.5 mg/kg/day, n = 8-10); methimazole (MMI, 8 and 16 mg/kg/day, n = 11); amitrole (AMI, 25 and 50 mg/kg/day, n = 11); OMC (375 and 500 mg/kg/day, n = 6-8); or DE71 (20 and 40 mg/kg/day, n = 8-10). The liver was collected from rat pups at postnatal day 16 and RNA isolated for Bulk-RNA-Barcodeing and sequencing (BRB-seq). PTU, MMI and AMI were included to characterize the signature of “classic” hypothyroidism; i.e. decreased TH levels and increased TSH. However, only 3 differentially expressed genes (DEGs) were found to be shared between PTU, MMI and AMI. Notably, PTU displayed a unique signature in the liver despite a comparable thyroid disrupting mode of action to MMI and AMI. This difference can possibly be attributed to different levels of TH decrease in PTU versus MMI and AMI exposed animals. A total of 77 DEGs were shared between MMI and AMI, many of which were related to the TH system, as well as phase II metabolism. Using this signature, the transcriptional effects of DE-71 and OMC were characterized and compared against the aforementioned three substances. Initial analysis showed that DE-71 targets the liver, with 928 differentially expressed genes (DEGs), compared to 309 DEGS for AMI, 127 for MMI, 104 for OMC, and 54 for PTU. For DE-71, 44 genes were shared with MMI and AMI; however, the general expression pattern was uniquely distinct from the other chemicals. The transcriptional profile of OMC was most similar to DE-71, with 68 shared DEGs. However, in a clustering analysis the expression pattern of OMC more closely resembled PTU. DEGs that were common between DE-71 and OMC were related to processes such as protein metabolism and cell death regulation among others. Our study suggests that DE-71 and OMC have a different effect on the liver than the other chemicals which classically disrupt the TH system through the thyroid. While DE-71 displayed similar effects with the positive control compounds (PTU, MMI, AMI) as regards liver transcriptome changes, OMC did so to a much smaller degree. Taken together these data suggest that different chemicals have distinct transcriptional footprints in the liver, but also that there are similarities between compounds which may be attributed to TH deficiency.

3495 Proarrhythmic Toxicity of Low-Dose Bisphenol A and Its Analogos in Human iPSC-Derived Cardiomyocytes and Human Cardiac Organoids through Delay of Cardiac Repolarization

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Bisphenol A (BPA) and its analogs are common environmental chemicals with many potential adverse health effects. These chemicals are extensively used in the manufacturing of plastics and consumer goods, and human exposure to these chemicals is widespread. We previously reported that BPA had pro-arrhythmic effects in an acute in vitro study. However, the mechanism of BPA-induced arrhythmia in vivo remains unclear. In this study, we used human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and human induced pluripotent stem cell-derived cardiac organoids (hiPSC-CDOs) from three healthy adults to model human cardiac electrophysiology. We generated hiPSC-CMs and hiPSC-CDOs under basal conditions and treated them with BPA at concentrations that range from 10⁻¹⁰ to 10⁻³ M. The results showed that BPA induced a dose-dependent pro-arrhythmic effect in hiPSC-CMs and hiPSC-CDOs. In addition, we found that BPA decreased the amplitude of the action potential and prolonged repolarization time. These results suggest that BPA may have potential clinical implications for patients with arrhythmias.
toxicity of BPA in rodent hearts, and that such toxicity of BPA was shared by bisphenol S, a BPA analog. However, the impact of environmentally relevant low-dose BPA on human heart, including cardiac electrical properties, is not known. Perturbation of cardiac electrical properties is a key arrhythmogenic mechanism. In particular, delay of cardiac repolarization can cause ectopic excitation of cardiomyocytes and malignant arrhythmia. This can occur as a result of genetic mutations (i.e., long QT (LQT) syndrome), or cardiotoxicity of drugs and environmental chemicals. In this study, we examined the impact of low dose (1 nM) BPA on the electrical properties of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) using patch-clamp and confocal fluorescence imaging and compared the pro-arrhythmic toxicity of BPA and its analogs in human cardiac organoids. Acute exposure to 1 nM BPA delayed repolarization and prolonged action potential duration (APD) in hiPSC-CMs through inhibition of the HERG K+ channel. In nodal-like hiPSC-CMs, BPA acutely increased pacing rate through stimulation of the i, pacemaker channel. Existing arrhythmia susceptibility determines the response of hiPSC-CMs to BPA. BPA resulted in modest APD prolongation but no ectopic excitation or arrhythmia in baseline condition, while rapidly promoted aberrant excitations and tachycardia-like excitation in myocytes that had drug-simulated LQT phenotype. In hiPSC-CM-based human cardiac organoids, we found the effects of BPA on APD and aberrant excitation were shared by its analog chemicals, which are often used in “BPA-free” products, with bisphenol AF having the largest effects. Our results reveal that BPA and its analogs have pro-arrhythmic toxicity in human cardiomyocytes and potential harmful impact on human heart health, particularly in vulnerable individuals such as LQT syndrome patients. The toxicity of these chemicals depends on existing pathophysiological conditions of the heart and may be particularly pronounced in susceptible individuals. An individualized approach is needed in risk assessment and protection.

**P3 3496 Immune, Metabolic, and Renal Effects of Post-Wean BPF Exposure in BUF/Mna Female Rats**

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Exposure to endocrine-disrupting chemicals (EDCs) increases the risk of obesity and related cardiometabolic disorders. Bisphenol F (BPF), an EDC that affects the thyroid, reproductive health, and immune cell functions, is a common substitute for bisphenol A (BPA) and is used in manufacturing polycarbonates and consumer products. EDC exposure effects likely vary in the population, indicating gene x EDC interactions that create risk factor variability in population-level effects. These limitations can be avoided by studying the N/NiH Heterogeneous Stock (HS) rats, an outbred population derived from eight founder inbred strains readily amenable to genetic study. We hypothesize that BPA-induced metabolic effects on thyroid function, immune cells, and BPF acutely increased pacing rate through stimulation of the i, pacemaker channel. Existing arrhythmia susceptibility determines the response of hiPSC-CMs to BPA. BPA resulted in modest APD prolongation but no ectopic excitation or arrhythmia in baseline condition, while rapidly promoted aberrant excitations and tachycardia-like excitation in myocytes that had drug-simulated LQT phenotype. In hiPSC-CM-based human cardiac organoids, we found the effects of BPA on APD and aberrant excitation were shared by its analog chemicals, which are often used in “BPA-free” products, with bisphenol AF having the largest effects. Our results reveal that BPA and its analogs have pro-arrhythmic toxicity in human cardiomyocytes and potential harmful impact on human heart health, particularly in vulnerable individuals such as LQT syndrome patients. The toxicity of these chemicals depends on existing pathophysiological conditions of the heart and may be particularly pronounced in susceptible individuals. An individualized approach is needed in risk assessment and protection.

**P3 3497 Lignin-Derivative Alternatives to Bisphenol A with Potentially Undetectable Estrogenic Activity and Weaker Developmental Toxicity**


Lignin-derivable bisguaiacols/bissyringols are potential alternatives to commercial bisphenols; however, current bisguaiacols/bissyringols [e.g., bisguaiacol F (BGF)] have unsubstituted bridging carbon between the aromatic rings, which makes them more structurally similar to bisphenol F (BPF) than bisphenol A (BPA) - both are suspected endocrine disruptors. Herein, we investigated the estrogenic activity (EA) and developmental toxicity of dimethyl-substituted bridging carbon-based lignin-derivable bisphenols (bisguaiacol A (BGA) and bissyringol A (BSA)), as platforms toward safer BPA replacement efforts. The molecular docking results revealed that BGA exhibited similar binding affinities to estrogen receptors as BPA, whereas BGF and BSA had lower binding affinities. Notably, per the in vitro MCF-7 cell proliferation assay, for EA, BSA had undetectable EA at all seven test concentrations (from 10^-12 M to 10^-6 M), and BGA had undetectable EA at four test concentrations. As expected, estradiol (a natural estrogen hormone) had detectable EA at all test concentrations, and BPA had detectable EA at five concentrations (from 10^-10 M to 10^-4 M). The undetectable EA for BSA is likely due to the presence of two methoxy groups on each aromatic ring that may increase steric hindrance around the phenolic hydroxyl groups and reduce interactions with binding pockets on the estrogen receptors. Furthermore, the gene expression study suggested that all the lignin-derivable monomers had significantly lower fold changes (from ~1.81 to ~4.41) of ApoII in comparison to that of BPA (~11.51), which is indicative of a lack of estrogenic response for these compounds in chicken fetal liver. Therefore, our study demonstrates the importance of utilizing non-phenolic bisphenols (e.g., BGA) in designing “EA-free” BPA alternatives. Interestingly, the in vivo chicken embryonic assay results revealed that BFG and BPF had similarly low developmental toxicity in comparison to the vehicle control. BGA, BSA, and BPA had slightly higher but not significant developmental toxicity. Altogether, the lignin-derivable building blocks reported in this study can be potential, safer alternatives to BPA while maintaining major structural similarities.

**P3 3498 Influence of Bisphenol A on Leptin Receptor Signaling Pathways in Dopaminergic Neurons**


Bisphenol A (BPA) is an endocrine disrupting chemical. Early life exposure is known to result in various adverse health effects. Several studies have demonstrated that BPA altered the endocrine-metabolic pathways in adipose tissue, increasing the risk of metabolic diseases and obesity. Genetic, environmental, behavioral, social, and economic factors all have a role in obesity. It is well known that the central nervous system’s hypothalamus regulates food intake and energy balance to keep the body’s physiological needs met. An extensive body of evidence has demonstrated that endocrine regulators such as leptin mainly act on the hypothalamus to regulate food intake and body weight. In addition, expression of leptin receptors in other regions of the brain such as the dopaminergic (DA) neurons may be important for the regulation of body weight. Emerging evidence supports the hypothesis that environmental contaminant exposure, particularly those occurring in early-life, may interfere with homeostatic control and induce or exacerbate obesity. The aim of this study was to investigate the effects of BPA on leptin signaling pathways in dopaminergic neurons using human neuroblastoma SH-SY5Y cells. Leptin or BPA were administered to cells in varying concentrations either separately or in combination. Cell viability, leptin receptor protein expression, orexigenic agonist-related (AgRP) receptor expression, and inducer of STAT3-protein expression were assessed. Low concentrations (0.01-50µM) of BPA showed no significant effect on cell viability. Exposure to leptin (0.1-100nM) resulted in concentration-dependent increase in STAT3. NSC74859, a selective STAT3 inhibitor, blocked this activation by leptin. Similar to leptin, BPA induced protein phosphorylation of STAT3. In co-treatment, STAT3 protein phosphorylation was increased and the inhibitor of AgRP receptor expression was decreased. BPA increased the level of leptin protein secretion in neuronal cells. Significant increase in leptin receptor protein expression was observed following treatment with BPA. Exposure to BPA enhanced the orexigenic neuropeptide AgRP levels whereas leptin reduced in vivo in vitro effects may contribute to the development of leptin resistance, a contributing factor to obesity. Thus, BPA could be a risk factor for obesity. Supported by Title III.

**P3 3499 Acute Exposure to Low-Dose Bisphenol A Delays Cardiac Repolarization in Female Canine Heart: Implication for Proarrhythmic Toxicity in Large Animals**

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Bisphenol A (BPA) is a common environmental chemical with a range of potential adverse health effects, including cardiac toxicity. The impact of environmentally-relevant low dose of BPA on the electrical properties of the hearts of large animals (e.g., dog, human) is poorly defined. Perturbation of cellular cardiac electrical properties is a key arrhythmogenic mechanism in the heart. In particular, delay of ventricular repolarization and prolongation of the QT interval of the electrocardiogram (EKG) are well-understood as a marker for the risk of malignant arrhythmias and are a major form of cardiac toxicity of chemicals. In this study, we examined the acute effect of low dose (10^-8 M) BPA on the electrical properties of female
3500 Disruption of Glucocorticoid Signaling by Exposure to Endocrine-Disrupting Compounds Induces Gastric Inflammation and Preneoplastic Lesions

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Gastric cancer is the 5th most common cancer worldwide and is the 4th leading cause of cancer-related deaths. Almost all cases of gastric cancer are associated with Helicobacter pylori infection. Inflammation induced by this bacterium drives gastric cancer development. Factors that increase the gastric inflammatory tone likely synergize with the infection to increase gastric cancer risk. Previous work in our lab has shown that glucocorticoids are master regulators of gastric inflammation, and systemic depletion of endogenous glucocorticoids through adenectomy results in spontaneous and severe inflammation in the stomach. In this study, we investigated how disruption of glucocorticoid signaling through exposure to endocrine-disrupting compounds impacts gastric inflammation. GR-disrupting compounds were identified by a glucocorticoid-responsive luciferase assay. We identified several compounds that impede glucocorticoid signaling in vitro, including the herbicides DDT and metolachlor. Next, we tested the effects of GR disruption by exposure to these xenobiotics. Mice were exposed to metolachlor in their drinking water for up to 1 month, and the GR antagonist RU486 was used as a positive control. Mice exposed to metolachlor through their drinking water for up to 1 month displayed increased gastric immune-cell infiltration. Moreover, gastric inflammation was associated with pylocic metastasis development, a potentially preneoplastic lesion. Surprisingly, neither metolachlor nor RU486 appeared to affect systemic glucocorticoid signaling, potentially indicating that the hypothalamic-pituitary-adrenal axis is resilient to the influence of endocrine-disrupting chemicals. This study demonstrates that the stomach is vulnerable to glucocorticoid disruption through ingested endocrine-disrupting compounds.

3502 Altered Tryptophan Metabolism and Lipid Homeostasis following Exposure to Naphthenic Acid Fraction Components

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Numerous environmental chemicals pose potential risks to human health due to their ability to mimic hormones and disrupt endocrine homeostasis. As recent research indicates that estradiol damages DNA by activating estrogen receptors (ER), it is possible that environmental chemicals that act as hormones in the human body, particularly those that can mimic estradiol, may also damage DNA. Additionally, BRCA1, whose mutations have been associated with breast cancer, is responsible for repairing estrogen-induced DNA damage. Therefore, ER-related DNA damage is of great concern to females with BRCA1 mutations. In this study, we developed a high-content imaging assay measuring yH2AX, a biomarker for DNA damage, and used this assay to test a subset of 907 compounds from the Tox21 10K compound collection in ER-expressing MCF7 human breast cancer cells. The assay showed satisfactory performance with a Z-factor of 0.67. From the screening, we identified 128 compounds that induced yH2AX. To examine which chemicals’ genotoxicity depended on ERs, we tested the effect of an ER inhibitor, tamoxifen, on genotoxicity. Tamoxifen treatment suppressed the induction of yH2AX by four compounds, indicating that these compounds induced yH2AX that was related to ERα activation. These four compounds were further studied to assess their ERα activating capability and their induction of c-MYC, a target gene of ER signaling. Only leставtinib, a tyrosine kinase inhibitor, was found to activate ERα, which was confirmed by both an ERα lactamase reporter gene assay and molecular docking analysis. Lestartinib also increased c-MYC expression, as measured by quantitative PCR. These data suggest leставtinib can act as a DNA damage inducer through ERα activation. We established a high-throughput screening method with follow-up assays to identify DNA damage inducing the screening and identified a novel compound, leставtinib, that mimics estradiol and has the potential to promote breast cancer by activating ER and inducing DNA damage.
The toxicity associated with fuels is normally considered in terms of inhalation of vapors and gases from neat fuel and combustion products, or dermal exposure to liquid fuel in occupational settings. However, accidental leaks and spills can occur that could result in the contamination of drinking water, thereby presenting risks of oral exposure. Recent events where drinking water was found to be contaminated using jet fuel components with human ER and AR (YES/VAS assay) have demonstrated the effects of ingestion of jet fuel. To this end, based on the structural similarity of jet fuel components to human steroids, we used an in vitro human hormone receptor activation assay to identify the military jet fuels Jet Propellant (JP)-5 and JP-8 as positive regulators of the human estrogen receptor under the concentrations and conditions tested. We also performed a reproductive study in a rat model using relatively high concentrations of jet fuels via oral exposure to screen for potential adverse outcomes that might warrant further study. While additional studies would be needed to ascertain the physiologic relevance of our observations, statistically significant observations under the conditions tested in this study revealed: a reduction of ~30% in weight gain for male rats exposed to JP-8; a reduction of ~22% in weight gain for female rats exposed to JP-5; a negative effect on absolute body weight in male rats of ~15% for those exposed to JP-8, and of ~12% for those exposed to JP-5, and of ~5% for those exposed to a plant bio-based jet fuel that is used as a blend of JP-8 with a fuel derived from camelina oil; a reduction of ~21% in weight gain for pregnant rats; a higher proportion (59.31%) of female pups following parental JP-5 exposure; and a decrease of ~34% in estradiol levels for female rats exposed to JP-5/camelina-derived fuel. Further observations that were not statistically significant under the conditions tested included: a decrease in estradiol and progesterone levels in female rats exposed to JP-5, and a reduction of ~2% in testosterone levels in female rats exposed to JP-5/camelina-derived fuel; an increase in dehydroepiandrosterone (DHEA) levels in female rats exposed to JP-5/camelina-derived fuel; and a decrease in DHEA levels in male rats exposed to JP-5 and JP-5/camelina-derived fuel. The results of this preliminary study suggest that jet fuels JP-5 and JP-8 may regulate the estrogen receptor and androgen receptor activities in jet fuels JP-5, JP-5/camelina-derived fuel demonstrated effects that are consistent with endocrine disruption, with activation of the estrogen receptor as one potential mechanism of action.

Cardiometabolic diseases (CMD) and disorders are growing public health problems across the globe. Among the known cardiometabolic risk factors are chemical compounds that induce endocrine and metabolic dysfunctions, such as endocrine disrupting chemicals (EDC). To date, the species- and dose-specific influence of EDCs on molecular programs and cardiometabolic risks has not been fully elucidated. To this end, we use a new data-driven analytical approach to comprehensively study publicly available human, mouse, and rat liver transcriptomic datasets for 4 EDCs, namely bisphenol A (BPA), bis(2-ethylhexyl) phthalate (DEHP), tributylin (TBT), and perfluorooctanoic acid (PFOA), to better capture the trends of genes and pathways perturbed by these chemicals that could not be detected by single studies. Transcriptome signatures from 32 individual datasets were uniformly processed and clustered to assess shared and distinct patterns of genes, pathways, regulatory genes and CMD associations across EDCs, doses, and species. We found that the BPA cluster comprised of human studies showed distinct patterns from rat BPA studies, with the human cluster having fewer CMD associations and their pattern. The DEHP cluster showed highly consistent transcriptomic signatures and key regulators such as peroxisome proliferator-activated receptor gamma (PPARγ) with one another and across doses and species, suggesting similar mechanisms of action and/or key driver genes. TBT exposure studies demonstrated highly divergent gene signatures from the other EDCs, as no significant CMD associations were observed, and very few key regulators and pathways were shared. Our work lays the foundation for future studies that leverage a streamlined workflow to compare tissue-specific molecular mechanisms of environmental chemicals to better understand the underlying connections between EDCs and cardiometabolic disease and risk.
Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, affecting 8-13% of women of reproductive age. The syndrome is characterized by hyperandrogenism, polycystic ovaries, and failure to ovulate. Current treatment for hyperandrogenism in PCOS patients targets androgen production by the ovaries. However, a large proportion of patients do not see androgen reduction with this treatment and an alternate source. While adipose tissue (WAT), a family of metabolic enzymes, the aldo-keto reductases (AKRs) regulate androgen production. A screen for competitive inhibitors of these enzymes was performed to detect potential endocrine disrupting chemicals in the context of PCOS. Perfluorooctanoic acid (PFOA), a known toxic to inhibits identified as a hit. Interestingly, epidemiological data from PCOS patients have shown higher levels of serum PFOA compared to non-PCOS individuals. Our data shows that aldo-keto reductase family 1 member C2 (AKR1C2), which predominantly converts the potent androgen dihydrotestosterone to the less potent androgen 3α-androstenediol, to be potently inhibited by PFOA, yielding an observed IC50 value = +21.2 m and Ki value = +90 nM. The inhibition was specific for AKR1C2 whereas PFOA did not inhibit the highly related isoforms AKR1C1 and AKR1C3. This inhibition could lead to an increase in adipose tissue and could potentially contribute to hyperandro- genism in PCOS. The inhibition of AKR1C2 was computationally modeled to detect residue interactions and then considered for experimental testing. This modeling was corroborated by in vitro structure activity relationships. Interestingly, the only residue in the steroid binding cavity that is different in AKR1C2 is V54 which is replaced by L54 in AKR1C1 and AKR1C3. We conducted site directed mutagenesis and found that the AKR1C2 V54L could no longer be inhibited by PFOA, highlighting the importance of the residue binding PFOA. Supported by P30ES03508 awarded to TMP and T32-ES019851-11 awarded to AA.

Chlorinated paraffins (CPs) are widely used across a wide range of industrial applications. Consequently, they have been reported in various human matrices, with exposure prevalent at all life stages. CPs are divided into three subgroups based on their carbon chain length: short (C10-13), medium (C14-17), or long (C18+). Short chained CPs (SCCPs) have been listed as persistent organic pollutants (POPs) since 2017 due to their persistency, bioaccumulation potential, and environmental impact. Chlorinated paraffins may increase the severity of PCa prognosis. We identified 369 overlapping DEGs from the patient microarray datasets with |log2FC| (fold change) ≥ one and an adjusted P-value of < 0.05. Volcano plots showing prostate cancer etiology severity with higher Gleason scores. Six PCa gene expression microarray datasets from the GPL570, GSE32571, GSE55945 and GSE26126/GEO were analyzed utilizing the GEO2R tool. To test battery includes assays for steroid hormone synthesis and receptor activity, retinoid signaling, and assays related to the thyroid hormone system. In a transcrehinin (TTR) protein binding assay, the TTR-ANS displacement assay, there is no indication that the included CPs can bind to TTR and disrupt the transport of T4. Potential activities in estrogen receptor (ER), androgen receptor (AR) or retinoic acid receptor (RAR) reporter gene assays are currently being tested to explore the potential ability of CPs to agonize or antagonize the nuclear receptors. The overall aim is to provide a broad picture of the ED potential of CPs.

Early prevention plans, diagnosis, and treatment methods for patients with Prostate Cancer (PCa) are limited due to a poor understanding of Endocrine Disruptive Chemicals’ Effects on the Molecular Pathology of Poor Prostate Cancer Prognosis. D. Alwadi, Q. Felt, D. Roy, and A. Deoraj, Florida International University, Miami, FL.

Biomedical Analysis Reveals Endocrine-Disrupting Chemicals’ Effects on the Molecular Pathology of Poor Prostate Cancer Prognosis. D. Alwadi, Q. Felt, D. Roy, and A. Deoraj, Florida International University, Miami, FL.

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Biomedical Analysis Reveals Endocrine-Disrupting Chemicals’ Effects on the Molecular Pathology of Poor Prostate Cancer Prognosis. D. Alwadi, Q. Felt, D. Roy, and A. Deoraj, Florida International University, Miami, FL.
Humans are exposed to a mixture of environmental chemicals (EC) in everyday life, some of which are believed to contribute to a range of non-communicable diseases. While the adverse health impacts of exposure to individual ECs have been extensive, studies on the effects of combined exposures, characterized by chronic low levels of mixtures of chemicals, are only recently emerging. Biosolids derived from human wastewater treatment exemplify both the array and concentration of chemicals humans are exposed to in real-life and represents a novel model to investigate the risk posed by exposure to a mixture of ECs. Previous studies of sheep exposed to biosolids during the preconceptional and gestational period showed sex-specific disruptions in reproductive and metabolic phenotypes such as altered bone mineral density, increased thyroid organ weight, spermatogenic and oocyte abnormalities. Since steroids are major programming agents in mediating offspring outcomes and several ECs have been shown to be strongly associated with cancers and reproductive and developmental outcomes, possible turf-mediated endocrine disruption could produce age-specific effects on developing children. Endocrine disruptors that disrupt the physiology of development could produce age-specific effects on developing children. Endocrine disruptors that disrupt the physiology of development could produce age-specific effects on developing children.

Humans are exposed to over 80,000 chemicals, some of which have been shown to be strongly associated with cancers and reproductive and developmental outcomes. However, testing the vast majority of these chemicals for complex endpoints, such as reproduction, is inherently costly and challenging using conventional models since germ cell development unfolds over many months to years in mammals. Therefore, a tractable and scalable approach for assessing the potential for endocrine disruption is needed. Here, we apply an innovative assay called “Green Eggs and Ham” in the well-established toxicological model: C. elegans. This model shows a high degree of conservation in the critical steps of germ cell development. The assay uses a fluorescent reporter approach (xox-1::GFP) which turns on in the event of chromosome segregation errors, specifically of the X chromosome, during germ cell development, which generates male C. elegans (i.e. the High Incidence of Males phenotype). Therefore, this approach can be used to identify chemicals that are reproductive toxicants and cause aneuploidy. We previously reported the development of the assay as a high-throughput platform. We applied this approach to testing 133 industrial chemicals found in maternal and blood cord sera for their endocrine disrupting potential. These chemicals were tested at four different concentrations: 10 μM, 30 μM, 50 μM, and 100 μM to mimic physiologically relevant conditions. At 100 μM input and high-content imaging, we analyzed over 6,000 images for the number of positive events in worms following exposures and calculated for each chemical the fold change over DMSO control. We identified 44 chemicals ≥ 1.5-fold induction and 17 chemicals ≥ 2.0-fold induction, which we classified as positive. The fold-change extended from 0.4 to as high as 25.5 when we considered all chemicals, showing high variability in chemical potency. Our work highlights the value of leveraging a rapid, high-throughput assay to identify reprotoxic chemicals and provides an avenue for future work to illustrate the mechanisms through which these chemicals impair reproduction.
Perfluorooctanesulfonic acid (PFOS) is an environmental contaminant that, despite being voluntarily phased out of production in the early 2000s, is still persistent in the environment. Previous zebrafish studies demonstrated that developmental PFOS exposure affects exocrine pancreas structure and function, shown through reduced pancreas length and reduced expression of genes encoding for key digestive enzymes. However, the effect of PFOS on exocrine pancreas function in relation to metabolism, lipid uptake, and digestive enzyme activity is not well understood. This work aims to optimize the analysis of lipid metabolism in vivo and enzymatic activity in zebrafish developmentally exposed to PFOS. To test the effect of PFOS on lipid metabolism, fish were exposed to PFOS from 3-96 hours post fertilization (hpf), grown to 6 days post fertilization (dpf) in clean water and fasted as feeding can lead to autofluorescence in the gut. Fish were exposed to 16 μM PFOS and fed an egg yolk emulsion, made by sonicating 1 ml chicken egg yolk in 19 ml Danieau’s, containing a fluorescent fatty acid analog, BODIPY FL-C12, for 6 hours. Fluorescence intensity was measured on a plate reader. When fish were exposed to 16 μM PFOS, survival was low and swim bladder inflation was minimal. Survival rates increased when PFOS concentrations were reduced to 4 and 8 μM, however swim bladder inflation rates remained low. Given that swim bladder inflation is important for scavenging for food, this may affect the amount of BODIPY FL-C12 the PFOS-exposed fish consume. To assess the effect of PFOS exposure on proteolytic enzyme activity, a protocol using a quenched casein, BODIPY TR-X, was optimized for use in zebrafish. Upon proteolytic cleavage of the casein substrate, fluorescent cleavage products are produced. To validate the assay in zebrafish, phenylmethylsulfonyl fluoride (PMSF) was used as a positive control for protease inhibition. Briefly, 6 dpf fish were pooled in groups of 3, sonicated and incubated with 10 μg/ml BODIPY TR-X for 1 hour. Fluorescence was analyzed with a plate reader. Results show a reduction in fluorescence intensity and reduced proteolytic activity. To test enzymatic activity in vivo, a feeding study using the BODIPY TR-X quenched casein is also being optimized. Fish were grown to 6 dpf and placed in 20 μg/mL BODIPY TR-X for 3 hours in groups of 5, 10, or 15 followed by imaging via fluorescence microscopy. Images were analyzed by measuring the mean fluorescence intensity of the intestines and gallbladder. Preliminary results do not show a difference in mean fluorescence based on the number of fish per tube, but there is increased variability in mean fluorescence in fish without inflated swim bladders. Further optimization and application of these protocols will allow for a better understanding of how PFOS exposure affects the exocrine pancreas. This work was supported by R21ES033532.

Perfluorooctane sulfonic acid (PFOS) and perfluorobutane sulfonic acid (PFBS) have been used in non-stick and stain-resistant coatings in widespread occupations. Both compounds have raised concern about their long-term toxic effects on health and embryonic development. Previous studies found zebrafish embryos (Danio rerio) exposed to PFOS, PFBS, and the pro-oxidant tert-butyl hydroperoxide (tBOOH) had a truncated pancreatic tail at 4 days post fertilization (dpf). As zebrafish have a high regenerative capacity, the objective of this study was to assess the persistence of this truncated pancreas phenotype in larvae at 15 dpf. Zebrafish embryos (Tg(ptf1a:GFP)) were exposed daily to PFBS (0 (DMSO 0.01%), 16 and 32 μM PFBS; 24-96 hpf), PFOS (0 (DMSO 0.01%), 8 and 16 μM PFOS; 24-72 hpf), or tBOOH (0, 77.6 and 147 μM tBOOH; 10-minutes to 72 hpf). At 5 dpf through 15 dpf the fish were reared in 6L tanks in a recirculating system and fed casein pellets twice a day. At 15 dpf, 150 dpf, and 6 dpf, fish were imaged using brightfield microscopy to quantify body length and exocrine pancreas length. Morphological analysis at 4 and 6 dpf indicated that fish exposed to 4 μM PFOS did not differ in body or exocrine pancreas length compared to controls, in contrast to what had previously been demonstrated with exposure to higher PFOS concentrations. To assess exocrine pancreas function, activity of digestive proteases, one of three classes of digestive enzymes produced by the pancreas that act to break down proteins prior to intestinal absorption, was measured at 6 dpf. Protease activity was measured with BODIPY TR-X, a quenched casein that fluoresces upon cleavage, used as a proxy for protease activity. Fish were homogenized, incubated with BODIPY TR-X for 1 hour, and fluorescence was quantified using a plate reader. Larvae exposed to 4 μM PFOS had a significant reduction in relative fluorescence (57%) compared to controls, indicating a reduced protease activity. To determine whether protease activity was lower due to impaired gene expression vs. direct interference with enzyme activity, qPCR was used to measure relative gene expression of pancreatic digestive proteases try, cbt1 and ela. Exposure to 4 μM PFOS significantly increased expression of the three proteases examined, indicating a compensatory but ineffective transcriptional upregulation mechanism. Together, these results indicate that with exposure to 4 μM PFOS, there are functional modifications to the exocrine pancreas enzyme activity independent of morphology or impaired gene expression changes. This work is supported by R21ES033532.

Per- and polyfluoroalkyl substances (PFASs) are a class of highly stable, ubiquitous contaminants that adversely affect the health of multiple organ systems. Embryonic exposure to the prevalent PFAS congeners perfluorooctane sulfonate (PFOS) disrupts pancreatic development in larval zebrafish. Given that vascular development is a critical modifier of organogenesis, we sought to determine if embryonic PFOS exposure also alters the vascularization of the pancreas, and if so, if impaired vascularization contributes to the previously reported pancreatic phenotype. During organogenesis, the developing zebrafish pancreas is vascularized by the sub-intestinal venous plexus (SIVP), a bilateral vascular network that initially transfers nutrients from the yolk to the developing embryo. The SIVP ultimately remodeled to form the vasculature of the digestive system and provides vascular support for the pancreas, liver, and gut. To determine if embryonic PFOS exposure disrupts development of the SIVP, we exposed embryonic zebrafish carrying transgenic reporters that mark the vasculature to a chronic, non-renewed 14 μM PFOS solution. We used in vivo confocal microscopy to collect images of the developing SIVP in control and PFOS-exposed embryos and larvae at 48-, 72-, 120-, and 144-hours post-fertilization (hpf). Embryonic and larval PFOS exposure did not impair the development of the posterior cardinal vein, which is the initial source of angioblasts from which the SIVP is formed, or the intersegmental vessels of the trunk. However, PFOS exposure altered SIVP development and produced SIVP phenotypes in larval zebrafish at 72, 120, and 144 hpf. Together, these studies advance our understanding of how developmental PFAS exposure can disrupt fundamental components of organogenesis and may increase the incidence or modify the severity of digestive and metabolic disorders such as inflammatory bowel diseases and diabetes.
Artificial,” or Non-Nutritive Sweeteners (NNS) are ubiquitous not only in the modern human diet, but also in the environment due to their resistance to breakdown both in the human body and in wastewater treatment processes. In this project, we examined the sweet receptor as a potential biological mechanism for NNS affecting development of pancreatic islets. Sweet receptors are a class of G-protein coupled receptors that bind ‘sweet’ compounds such as sugars and NNS to stimulate intracellular processes such as metabolism and signaling. Sweet receptors have been found not only in the taste buds but also in the pancreas, and stimulation of sweet receptors in the pancreas has been associated with glucose stimulated insulin secretion. We hypothesized that NNS, while nutritionally itself inert, could activate the sweet receptors of the pancreas and induce changes on a transcriptional and ultimately morphological level. In addition, these changes would be most apparent during development, when the pancreas may “taste” these sweeteners with sweet receptors and respond to these signals and adapt to its nutritional environment by adjusting pancreatic islet formation. We tested if the sweet receptor is necessary for the adaptive development of the pancreas by blocking the sweet receptor and observing if that is sufficient to disrupt the development of the pancreas. Zebrafish (Danio rerio) embryos were exposed to (0 mM, 0.1 mM) Lactisole, an inhibitor of the sweet receptor, along with (0 mM, 1 mM) lactose along with feed from 4-9 days post fertilization (dpf). This period reflects larval transition from endogenous nutrition (e.g., yolk) to exogenous nutrition, where the larvae may begin to adapt to the environment. Larval growth, as well as islet size, morphology, and count were assessed in Tg(insulin::GFP) embryos at 9 dpf to allow for direct visualization of beta cells. In addition, RNA-sequencing was conducted to identify differentially expressed genes and pathways. Suppressing the sweet receptor with Lactisole suppressed secondary islet quantity and quality and hindered overall growth. Interestingly, Lactisole and lactose in combination resulted in larger primary islets, fewer secondary islets, and higher chance of ectopic beta cells. This may be because the increased demand for insulin is not met due to deficient development of secondary islets, leading to compensatory primary islet expansion. Organizing all significantly differentially expressed pathways by KEGG class and upregulation of metabolism pathways differed between the effect of glucose on DMSO control compared to the effect of glucose with the sweet receptor inhibited by Lactisole. In addition, the Gene Ontology gene set for beta cell pancreatic cell differentiation was significantly upregulated in the group exposed to Lactisole compared to control. Overall, the data show Lactisole and glucose have an interactive effect, and it is possible that the sweet receptor is more significant for beta cell differentiation compared to beta cell proliferation. If the sweet receptor is part of the cell fate and differentiation pathway for beta cells, activation of the sweet receptor with NNS may result in interesting morphologies and phenotypes linked to metabolic dysregulation.

Embryonic Exposure to the Non-nutritive Sweetener Aspartame Impairs Beta Cell Differentiation in Zebrafish

Aspartame belongs to a group of non-nutritive artificial sweeteners (NNS) known for its noncaloric properties. Compared to its natural proxy sucrose, aspartame is 200 times more effective at activating the sweet receptor and is metabolized into aspartic acid, phenylalanine, and methanol. Though aspartame is noncaloric, studies have found that its activation of the sweet receptor may induce insulin production. The overarching goal of this study is to assess whether aspartame affects pancreatic islet development. If the sweet receptor is involved with islet cell formation, we would expect embryonic exposure to aspartame to impact beta cell differentiation. We used live Tg(insulin::GFP) zebrafish embryos, which express GFP in insulin-producing beta cells. Embryos were exposed to 1 mM aspartame, 0.1 mM aspartame, and 1 mM sucrose beginning at 24 hours post-fertilization (hpf). Embryonic and pancreatic islet morphology was quantified at 96 hpf using microscopy. We also compared our results with aspartame to other NNS such as sucralose and acesulfame potassium (AceK). Gene expression was assessed for pancreatic endocrine indicators including glucoregulatory hormones. Contrary to our hypothesis, embryos exposed to aspartame had decreased overall islet area. This area also contrasts with our findings previously with AceK and sucralose, which found increased and unchanged beta cell areas, respectively. Gene expression of islet hormones was minimally impacted by exposure though significant upregulation of the transcription factor ppa2 was observed. Overall, this data suggests that embryonic exposure to aspartame can impair islet development and future work must be completed to determine whether these observed effects are associated with increased risk for diabetes or metabolic diseases later in life.

Succinyl Dehydrogenase Inhibitors (SDHIs) have been used for decades, with new formulations cropping up as fungi evolve. Boscalid was introduced to United States agriculture in 2003 and was determined to be effective for fungal management and safe for aquatic discharge and human consumption. Recent studies have found Boscalid’s primary metabolite, M510F01, on crops and in irrigation runoff as well. However, limited information is available about the aquatic and developmental toxicity of M510F01. The goal of our study was to compare and contrast the developmental toxicity of Boscalid with its metabolite, M510F01. Zebrafish (AB, wild-type strain) embryos were exposed to Boscalid or M510F01 from 3-96 hours post fertilization (hpf) at concentrations of 0.03, 0.3, or 3 µL/L. Embryos were examined for aberrant morphologies at 96 hpf using microscopy. Boscalid exposures at concentrations of 3 µL/L increased incidence of blue sac disease by 27%, craniofacial malformation by 91%, pericardial edema by 68%, delayed swim bladder inflation by 33%, and yolk edema by 74% (p=0.001 for all morphologies). M510F01 3 µg/L M510F01 increased the incidence of blue sac disease by 24% (p=0.005). To assess whether Boscalid and M510F01 impact Cytochrome P450 1a (Cyp1a) activity, embryos were exposed to Boscalid or M510F01 (0.03 µg/L) from 96-100 hpf concurrently to 7-ethoxyresorufin (EROD)—a fluorometric indicator of Cyp1a activity. There is no significant change in acute EROD activity for Boscalid or M510F01. RNA sequencing and pathway analysis were performed to assess the mechanisms of xenobiotic response to Boscalid and M510F01 exposures (0.03 µg/L). Overall, this research shows that M510F01 exhibits reduced aquatic and developmental toxicity when compared to Boscalid.

Zet-O-Map: Identify Principal Molecular Drivers of (Dys) morphogenesis in Zebrafish

The zet-o-map project aims to provide insights into the underlying mechanisms leading to developmental toxicity by using transcriptome data from the zebrafish embryotoxicity test (ZET). One goal is the generation of spatio-temporal maps to reveal time-resolved biomarkers associated with certain malformations. A better understanding of the mechanisms leading to embryo toxicity can significantly contribute to increasing confidence in new approach methodologies (NAM) data and their integration into human risk assessment. To this end, we have mined published literature and built ZeTera, a database containing observed morphological alterations in zebrafish within a treatment duration up to 5 days post-fertilization (dpf). Additionally, we collected and reanalysed approximately 200 publicly available transcriptomics datasets generated using both array-based as well as RNA-sequencing based techniques. These datasets contain expression values for zebrafish embryos treated at varying timepoints (up to 5 dpf) from a total of 160 different chemical stressors. Furthermore, we generated expression data for 3 compounds (valproic acid, chlorpyrifos, triadimefon) exhibiting teratogenic effects in mammals through different modes of action. These three compounds were exposed to zebrafish embryos at 5 sub-lethal concentrations across four embryonic development stages, namely gastrula (8 hpf), pharyngula (24 hpf), hatching (48 hpf), and larval (5 dpf) stage. We conducted structure-based similarity analysis on the compounds in our dataset as well as literature reviews on associated morphological observations and adverse outcome pathways (AOPs). In many cases the chemical stressors in our project database are known teratogenic compounds which can be shown by referencing observations of morphological alteration in zebrafish embryos in the ZeTera database. For the identification of time-dependent transcriptome biomarkers, we analysed expression data for individual compounds as well as read-across categories grouped according to their shared structural and physicochemical properties. This approach, in addition to the spatio-temporal maps, are exemplified for selected read-across groups and further analyses approaches e.g., the integration of data into knowledge maps are discussed. Challenges such as the heterogeneity of the study designs, data gaps and their impact on the project outcomes are illustrated. Not only could we identify biomarkers for individual teratogen compounds as well as groups of compounds at different stages in the embryonic development, but also identify the adverse outcome they are most associated with, via a rich background of supporting information. Acknowledgement: This project is funded by CEFIC within the framework of the Long Range Initiative (LRI) as project no. LRI-C9: Zet-O-Map.
Numerous reports have identified arsenic (As), a naturally occurring environmental contaminant and industrial by-product, in drinking water, food, and agricultural products. These findings have raised public health concerns, particularly about the impacts on the most vulnerable groups. As a model organism, zebrafish (Danio rerio) has a well-conserved neuroendocrine system while allowing for direct in vivo and in vitro experimentation.

Zebrafish embryos were exposed from 24 hours post fertilization (hpf) to study the effects of three environmentally detected TPhP concentrations. Embryos raised from gestation through seven days of age in both distilled water and TPhP were scored for altered swimming behavior, hence, the role of chemical formulation in altering the organismal response. With this understanding, we are currently investigating the molecular abnormalities, related but not exclusive to the neuroendocrine system, coinciding with the behavioral/phenotypic alterations we observed at the organismal level to better understand the key mechanisms driving arsenic’s developmental and neurobehavioral toxicities.

In November 2022, the World Health Organization (WHO) set the maximum contaminant level (MCL) for arsenic in drinking water at 10 μg/L (10 parts per billion or ppb). A 2015 report by the United Nations Environmental Program concluded that exposure to arsenic is associated with an increased risk of cancer. Arsenic is also associated with cardiovascular disease, liver disease, and a number of other health effects. The continued monitoring and study of arsenic exposure is crucial to understanding the health impacts of this contaminant.
Atrazine is a herbicide used throughout the Midwest US to prevent broadleaf weeds in crops. The US EPA has set the maximum contaminant level at 3 ppb (μg/L) in drinking water. Atrazine (ATZ) is an endocrine disruptor interfering with the function of hormones and disrupting normal physiology and homeostasis throughout development and the life course of an organism. The zebrafish model was used to test the hypothesis that an embryonic parental ATZ exposure will cause changes in morphology in developing offspring. AB adult zebrafish were bred. Their eggs were collected and exposed to ATZ concentrations of 0 ppb, 0.3 ppb, 3 ppb, or 30 ppb from 1-72 hours post fertilization (hpf; the end of embryogenesis). ATZ exposure was ceased at 72 hpf and larvae were grown into adulthood in aquaria water (ATZ F0). ATZ F0 adult zebrafish were then bred within their treatment group, their eggs were collected, and placed in petri dishes in aquaria water until 120 hpf. At 120 hpf, larvae were collected for morphological analysis including general morphology measurements and co-staining with alcin blue and alizarin red for cartilage and skeletal assessments. Head length and ratio of head length to total length was significantly increased in the F1 of 0.3 and 30 ppb ATZ groups (p<0.05). Additional craniofacial morphology was completed with a decreased distance for cartilaginous structures, decreased surface area and distance between saccular otoliths, and a more posteriorly positioned notochord (p>0.05). The posteriorly positioned notochord indicated delayed ossification and skeletal growth. These findings signify that a single embryonic parental exposure leads to changes in craniofacial development in their offspring.

3529 Comparing Effects of Atrazine Exposure on Neuroendocrine Molecular Targets at Two Developmental Exposure Periods in the Zebrafish

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Atrazine is an herbicide commonly applied to control broadleaf and grassy weeds in agricultural regions of the US. Although its use was banned by the European Union in 2003 because of surface and groundwater contamination risk, the US EPA allows for a 3-ppb maximum contaminant level for drinking water. Atrazine is a known endocrine disrupting chemical with the potential to cause adverse effects at the hormonal and molecular level in neuroendocrine system pathways; however, the mechanism causing dysregulation following atrazine exposure has yet to be determined. In this study, hypothalamic and pituitary molecular targets were investigated to explain a mechanism for the negative atrazine impacts. Relative gene expression of the targets was examined using the zebrafish model following exposure to atrazine at two developmental time points to determine if atrazine exposure influenced neuroendocrine development and if the impacts were different among the exposure periods. Selection of gene targets was based upon known neuroendocrine hormones that have been reported in literature to be dysregulated following atrazine exposure in various models. Embryos were collected from adult wild type zebrafish and randomly assigned to 0, 0.3, 3, or 30 ppb (μg/L) atrazine treatment. For one timepoint, exposure began 1 hour post fertilization (1 hpf) and continued until the end of embryogenesis (72 hpf). A second timepoint exposure began at 72 hpf with a 24-hour exposure. After exposure, RNA was isolated, cDNA was synthesized, and qPCR was used to assess hypothalamic target gene expression in the 3-ppb exposure groups. The qPCR results showed that leptin mRNA expression was neither upregulated nor downregulated with the increasing concentrations, whereas ghrelin mRNA expression was upregulated. In conclusion, bisphenol exposure resulted in increased eating behavior, which correlated to increased mRNA expression of the appetite-stimulatory hormone ghrelin. In addition, the results suggest that zebrafish larvae be used to efficiently assess obesogenic capacity of environmental pollutants.

3531 Developmental Toxicity of Perchloroethylene (PERC) in Zebrafish (Danio rerio)

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Perchloroethylene (PERC) is an environmental contaminant linked to toxicity effects including cancer, neurotoxicity, and developmental toxicity. PERC was used historically as a metal-degreaser and dry-cleaning agent and is now found at over half of the US Environmental Protection Agency (EPA) superfund sites and is currently listed 33rd on the Agency for Toxic Substances and Disease Registry’s Priority List. The US EPA regulates the concentration of PERC in drinking water with a maximum contaminant level of 3 ppb. This study used zebrafish larvae of Nacre (SAGFF(LF)53A mitfab 692/+) background were exposed in vivo to a concentration range of the bisphenols and 17β-estradiol, followed by being fed a stained egg yolk powder at day six. After an hour of feeding, the larvae were fixed in 4% paraformaldehyde overnight at 4°C for fluorescence microscopy. The red fluorescent food in the gut in images were quantified by ImageJ. qPCR was used to analyze the gene expression of leptin and ghrelin in vivo. Exposures to BPA, BPAF, BPE, BPC, BPC-CI and BPS, resulted in increased amounts of food consumption, increased body weight and food conversion efficiency in the exposed controls. The qPCR results showed that leptin mRNA expression was neither upregulated nor downregulated with the increasing concentrations, whereas ghrelin mRNA expression was upregulated. In conclusion, bisphenol exposures resulted in increased eating behavior, which correlated to increased mRNA expression of the appetite-stimulatory hormone ghrelin. In addition, the results suggest that zebrafish larvae be used to efficiently assess obesogenic capacity of environmental pollutants.
Organophosphorus flame retardants (OPFRs) are used as alternative flame retardants following the regulation of some brominated flame retardants, and their use has increased in recent years. Tris (2-chloroisopropyl) phosphate (TCIPP), one of the OPFRs, has been detected in the egg and tissue of wild birds. Several reports have demonstrated that TCIPP exposure caused somatic deformity in day 4 chicken embryos and this effect may be responsible for their reduced survival. The aim of this study is to identify the molecular mechanisms of abnormal somitogenesis in TCIPP-treated chicken embryos by their transcriptome analysis. The Rhode Island Red chicken (Gallus gallus domesticus) eggs were divided into three groups: vehicle control (control; 0.1 % w/w DMOS), low-TCIPP (TCIPP-L; 50 nmol/g egg), and high-TCIPP (TCIPP-H; 500 nmol/g egg). The eggs were treated with each chemical solution at Hamburger Hamilton stage (HHS) 1. After 56-60 hours of incubation (38°C, 70 % humidity), the eggshell was broken, and all the egg contents were gently transferred to a shell-less incubation system. Embryos were continuously observed on days 3-9 and images and videos were recorded. Embryos were dissected on day 9 and weighed for body, heart, liver, brain, and eye. Body length, head length, head + bill length, eye diameter, eye luminosity value, forelimb length, hindlimb length, extraembryonic blood vessel length, extraembryonic blood vessel luminosity value, somitic curvature angle, and heart rate were measured using image analysis software ImageJ. The survival rate was significantly decreased in both TCIPP-treated groups. Dissection on day 9 showed no significant differences in the weight of body, heart, liver, and brain. In contrast, the eye weight/body weight ratio was significantly lower in TCIPP-H than in controls (p < 0.05). The body length, head length, head + bill length, eye diameter, forelimb length, and hindlimb length were significantly decreased in both TCIPP-treated groups. The development of extraembryonic blood vessels and red blood cell production were also significantly repressed in these TCIPP-treated groups. The heart rate showed a decreasing trend in a TCIPP concentration-dependent manner on days 4-7. The somitic curvature angle was significantly increased on days 4-6 in these TCIPP-exposure groups. There was a negative correlation between the somitic curvature angle and the body length, each body part length, and number and luminosity value of extraembryonic blood vessels on days 4-6. The somite of chick embryos is formed from 24 hours to 4 days, and the somite is identified by the boundaries of somitic deformity caused by TCIPP exposure by day 4 inhibits the formation of each part of the body. Embryos with curved somites showed cleavage on the back and widened the gap between somites, forming asymmetrical somites. The survival rate of embryos with the curved somite in TCIPP-exposure groups was significantly lower than those of embryos with normal somites (p < 0.001). These results suggest that the failure of somitic formation is one of the causes to reduce the survival rate in early chicken embryos treated with TCIPP.

Effects of Tris (2-Chloroisopropyl) Phosphate (TCIPP) on Early Chicken Embryos in a Shell-Less Incubation System: New Insight into Molecular Mechanisms

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Organophosphorus flame retardants (OPFRs) are used as alternative flame retardants following the regulation of some brominated flame retardants, and their use has increased in recent years. Tris (2-chloroisopropyl) phosphate (TCIPP), one of the OPFRs, has been detected in the egg and tissues of wild birds. Abnormal somitic deformity caused by TCIPP exposure has been observed. These results suggest that increased fgf8 mRNA expression may also be involved in the deformity in the somite formation.
cardiac fibroblasts and smooth muscle cells from epicardial-derived progenitor cells, TCEP exposure may affect the EMT and inhibit cardiac development, which is supported by phenotypic observations including decreased heart rate. qRT-PCR analyses of genes in vitelline membranes showed that gene expression levels of FGF2, VEGFR2, VEGFR3, VEGF, HIF1A, AKT, PIK3CA, and RAC1 were significantly decreased in a TCEP concentration-dependent manner. These results suggest that TCEP exposure suppresses the vasculogenesis of extraembryonic blood vessels by downregulation of these genes involved in the VEGF signaling pathway.

Effects of Tris (2-Chloroethyl) Phosphate (TCEP) on the Epithelial-Mesenchymal Transition (EMT) in Early Developing Chicken Embryos

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Tris (2-chloroethyl) phosphate (TCEP) is one of the pervasive organophosphate flame retardants (OPFRs) that has been commercially used in polyurethane foam textiles, and furniture to delay the spread of fire after ignition. We previously found that exposure of chicken embryos to TCEP (250 and 500 nmol/g egg) had effects on significantly shortened body length, reduced heart rate, and suppressed vasculogenesis during developmental stages between days 3 and 9 of incubation, suggesting that TCEP induces developmental delay and cardiovascular disturbance (Kanda et al., Ecotoxicol. Environ. Saf. 207, 111263, 2021). However, the molecular mechanism of these effects on chicken embryos remains unclear.

Epithelial-Mesenchymal Transition (EMT) is a morphogenetic process in which cells lose epithelial characteristics such as cell polarity and cell-cell adhesion and gain mesenchymal properties such as migration and invasion. During the gastrulation, cardiac fibroblasts and smooth muscle cells from epicardial-derived progenitor cells lose epithelial characteristics such as cell polarity and cell-cell adhesion and gain mesenchymal properties such as migration and invasion. During the gastrulation. These effects may consequently induce developmental delays and adverse outcomes. We hypothesize that developmental exposure (TCEP-L, TCEP-M, TCEP-H) of chicken embryos to TCEP (250 and 500 nmol/g egg) had effects on significantly shortened body length, reduced heart rate, and suppressed vasculogenesis during developmental stages between days 3 and 9 of incubation, suggesting that TCEP induces developmental delay and cardiovascular disturbance (Kanda et al., Ecotoxicol. Environ. Saf. 207, 111263, 2021). However, the molecular mechanism of these effects on chicken embryos remains unclear.

3536 Effects of Tris (2-Chloroethyl) Phosphate (TCEP) on the Epithelial-Mesenchymal Transition (EMT) in Early Developing Chicken Embryos

3537 Developmental Exposure to the Environmental Toxicant, Polychlorinated Biphenyls Alters Voidsing Physiology, Bladder Innervation, and TRPV1-Mediated Voiding in Mice

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Environmental contaminants are risk factors for several disorders, yet their ability to influence lower urinary tract (LUT) function is understudied. Polychlorinated biphenyls (PCBs) are neuro- and immunotoxicants, yet PCB effects on LUT functionality are understudied. Using a PCB profile that is relevant to human health, we study the effects of PCBs on physiological, anatomical, and molecular levels in the bladder and lower urinary tract (LUT) using genetically modified mice. Developmental PCB exposure alters sensory nerve density and function in a dose- and sex-dependent manner (p < 0.05). Sensory nerve density was increased between 0.1 and 6 mg/kg/d at 4 weeks and testing at 6 weeks is ongoing. Sensory nerve density was increased between 0.1 and 6 mg/kg/d at 4 weeks and testing at 6 weeks is ongoing. Agonism of TRPV1 had sex- and dose-dependent effects on void duration, threshold pressure, maximum intravesical pressure, and compliance. Observed effects indicate there are possible TRPV1-mediated changes to bladder filling sensitivity and bladder muscle contractility in mice influenced by perinatal exposure to PCBs. Developmental exposure to PCBs in mice causes changes to mouse voiding physiology, suburothelial nerve densities, and neurologic signaling involving TRPV1. Altered voiding responses to TRPV1 agonism, and changes to nerve densities, indicate that developmental exposure to PCBs may alter the occurrence or modulation of neurogenic signaling from the bladder. Supported by NIH awards R01ES029537 and T32ES007015.

3538 Pre- and Postnatal Developmental Exposure to the Polychlorinated Biphenyl Mixture Aroclor 1221 Alters Rat Pituitary Gonadotropins and Estrogen Receptor Alpha Levels

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Polychlorinated-biphenyl (PCBs) are industrial compounds, which were widely used in manufacturing of electrical parts and transformers. Despite being banned in 1979 due to concerns about harm to human health, they still persist in the environment. In humans and experimental model systems, PCBs have been shown to be toxicants in part by acting as endocrine disrupting chemicals (EDCs). Aroclor 1221 (A1221) is a weakly estrogenic PCB mixture known to alter reproductive function in rodents. EDCs can impact hormone signaling at any level of the hypothalamic-pituitary-gonadal (HPG) axis, and we investigated the effects of A1221 exposure during the prenatal and postnatal developmental periods on pituitary hormone and steroid receptor expression in rats. Examining offspring at 3 different ages postnatal day 8 (P8), P32 and P60, we found that prenatal exposure to A1221 increased neonatal pituitary luteinizing hormone (LH) mRNA and LH&-gonadotropin cell number while decreasing LH serum hormone concentration. No changes in pituitary hormone or hormone receptor gene expression were observed peri-puberty at P32. In reproductively mature rats at P60, we found pituitary follicular stimulating hormone beta (FSHb) mRNA levels increased by prenatal A1221 exposure with no corresponding alterations in FSH hormone or FSHb expressing cell number. Estrogen receptor alpha (ERa) mRNA and protein levels were also increased at P60, but only following postnatal A1221 dosing. Together, these data illustrate that exposure to the PCB A1221, during critical developmental windows, alters pituitary gonadotropin hormone subunits and ERa levels in offspring at different phases of maturation, potentially impacting reproductive function in concert with other components of the HPG axis. Supported by NIH R01 ES029464 and R25 ES052059.

3539 Mitochondrial Toxicity of Polycyclic Aromatic Hydrocarbons in Preimplantation Mouse Embryos


Prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) has been associated with adverse health outcomes including neurodevelopmental impairments. The mechanisms of this association remain unclear, and limited work has suggested a role for the mitochondria in this pathway, however study of the fetal period of development is limited by a variety of ethical and logistical factors. In this study we use mouse preimplantation embryos to characterize the mitochondrial toxicity of PAH exposure. Embryos were dosed under four schedules from days 0.5 to 1979 due to concerns about harm to human health, they still persist in the environment. In humans and experimental model systems, PCBs have been shown to be toxicants in part by acting as endocrine disrupting chemicals (EDCs). Aroclor 1221 (A1221) is a weakly estrogenic PCB mixture known to alter reproductive function in rodents. EDCs can impact hormone signaling at any level of the hypothalamic-pituitary-gonadal (HPG) axis, and we investigated the effects of A1221 exposure during the prenatal and postnatal developmental periods on pituitary hormone and steroid receptor expression in rats. Examining offspring at 3 different ages postnatal day 8 (P8), P32 and P60, we found that prenatal exposure to A1221 increased neonatal pituitary luteinizing hormone (LH) mRNA and LH&-gonadotropin cell number while decreasing LH serum hormone concentration. No changes in pituitary hormone or hormone receptor gene expression were observed peri-puberty at P32. In reproductively mature rats at P60, we found pituitary follicular stimulating hormone beta (FSHb) mRNA levels increased by prenatal A1221 exposure with no corresponding alterations in FSH hormone or FSHb expressing cell number. Estrogen receptor alpha (ERa) mRNA and protein levels were also increased at P60, but only following postnatal A1221 dosing. Together, these data illustrate that exposure to the PCB A1221, during critical developmental windows, alters pituitary gonadotropin hormone subunits and ERa levels in offspring at different phases of maturation, potentially impacting reproductive function in concert with other components of the HPG axis. Supported by NIH R01 ES029464 and R25 ES052059.
With an increasing demand for fossil fuels and a growing frequency of wildfires, polycyclic aromatic hydrocarbons (PAHs) are an environmental contaminant of growing concern. While PAHs are typically present in complex mixtures, concern has primarily been focused on the carcinogenicity of unsubstituted PAHs. Understanding the toxicity of alkylated PAHs is important for a comprehensive understanding of the hazard potential of this diverse chemical class. 73 alkylated PAHs were screened for morphological and behavioral effects in a high-throughput developmental zebrafish assay. Embryos were exposed to 12 concentrations ranging from 0 to 100μM (n=14) and screened for morphological effects using 10 morphological endpoints and behavior using a larval photo motion response. The aryl hydrocarbon receptor (AHR) is often implicated in the toxicity of PAHs and the induction of cytochrome P450A (Cyp1a) is an excellent biomarker of AHR activation. Embryos were evaluated for spatial Cyp1a expression patterns using an AHR-responsive reporter line. Of the 73 alkyl PAHs tested, 31 were hits in our morphological screen, 15 were hits in both behavior and morphology and 6 were hits in just behavior. 12 of these 73 compounds (16%) have been detected in the environment, our toxicity screening results will be compared against environmental concentrations to ascertain risk. For those compounds for which we do not have environmental concentrations, we will predict risk based on their toxicity profiles and environmental concentrations of similar compounds. This is the largest set of alkylated PAHs yet to be screened. Results from this study will enhance our ability to identify structure-activity relationships and to move toward predictive hazard assessment of PAHs. Research reported in this publication was supported by the NIEHS of the National Institutes of Health under Award Number P42ES016465, P30ES030287, and T32ES007060. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
deficient metabolism of APAP in males may also explain why some DNA damage related terms were upregulated by APAP treatment in males but not females. This study provides additional evidence for the neurodevelopmental harm of prenatal APAP exposure and generates hypotheses for underlying molecular pathways via RNA sequencing.

3544 Alteration of the Viral Immune Response in the Brain of Juvenile Rats following Repeated Daily Ingestion of Cannabidiol

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Cannabidiol (CBD) is a non-psychotherapeutic constituent of Cannabis sativa. CBD is effective in relieving uncontrolled seizures in children, but there are unsubstantiated claims that CBD is also effective in treating anxiety, depression, autism, and ADHD. Regardless, anecdotal evidence indicates parents are giving CBD to their children for these indications. This study investigated whether or not this altered response occurs in the juvenile brain. To investigate this, 12-day-old male and female rats were orally administered either corn oil, 20 mg/kg CBD, or 60 mg/kg CBD daily for 5 days. On day 16, the immune system was challenged by intranasal instillation of either saline or the viral mimic Resiquimod R848 (2.5 µg/g) which is an agonist to the Toll-like receptors 7 and 8 (TLR7 and TLR8). After 6hrs, brains were collected for mRNA extraction, and qPCR was performed to determine changes in the gene expression of brain immune cell markers for astrocyte activation (S100b), astrogial GFAP, microglial activation (TMEM119). For astrocytes, the R848 challenge of the immune system slightly decreased S100b expression, and CBD exposure potentiated that decrease. The R848 challenge increased GFAP expression, but pre-exposure to CBD suppressed this expression with greater effects in females than males. For microglia, the R848 challenge significantly decreased TMEM119 expression, and pre-exposure to CBD did not significantly alter this change in expression. However, the R848 challenge increased the expression of Iba-1, but pre-exposure to CBD decreased the expression of Iba-1. Thus, CBD pre-exposure can potentially alter the activation of astrocytes and macrophages in the juvenile brain. This occurrence may indicate potential health consequences in children orally administered CBD.

3545 Developmental Flame Retardant Retardant Exposure Results in Impaired Skeletal Integrity in Either Sex of the Adult Wistar Rat

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Developmental flame retardant (FR) exposure has been linked to adverse neurologic, reproductive, and skeletal effects in animal models. This study examines the endocrine disrupting in humans. One of the most common FR commercial mixtures is Firemaster 550 (FM 550). Composed of brominated (BFR) and organophosphate (OPFR) chemicals, FM 550 has repeatedly been shown to negatively influence metabolic programming raising concerns that skeletal integrity may consequently be impaired. We have previously shown that gestational and lactational exposure to 1000 µg FM 550 negatively affects sex-specific skeletal traits in male, but not female, rats, assessed at six months of age. It is unknown if the effect was influenced predominantly by the BFR or OPFR portions of the mixture. To address this, dams were exposed orally throughout gestation and lactation to either 1000 µg BFR, 1000 µg OPFR, or 2000 µg FM 550. Offspring (f0 generation) were weaned at PND 21 and assessed at eight months of age via high resolution nano-computed tomography (nCT). Cortical, trabecular, and total bone measurements were recorded, and expected sex-specific differences were present. While FM 550 exposure affected skeletal development of both sexes, exposure to components of the mixture show sex-specific effects largely centered on OPFR exposure in females and BFR exposure in males. OPPR exposed females showed an increase in cortical area fraction, FM 550 exposed females had a decreased marrow area. Both OPPR and FM 550 exposed females had an increase in bone volume fraction. In males, BFR exposed animals display decreased marrow area and total area, while FM 550 exposed males have decreased trabecular thickness. Discordance between the two studies is unclear but it is possible that with the natural occurrence of bone remodeling, the previously observed skeletal phenotype does not persist after 6 months or the outcome does not occur at the higher dose. Future work aims to clarify these unresolved issues.

3546 Genomic Analysis of Fischer F344 Rat Kidneys from a Reproductive Study following Dietary Ochratoxin A Exposure

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Ochratoxin A (OTA) is a mycotoxin produced by Penicillium and Aspergillus species of fungi. It can be found in many commodities; however, cereals are the main source of human exposure. OTA is a renal carcinogen and nephrotoxin at high concentrations, targeting the proximal tubules. This study uses transcriptomics and the previously reported apical data by Bondy et al. 2017 to infer mode-of-action of OTA toxicity in male and female rats exposed to low doses of OTA in utero and throughout development. F0 dams were fed 0.026, 0.064, 0.16, 0.4 and 1.0 mgm/kg diet during periods of breeding, gestation, and prior to weaning. Male and female F1 rats were exposed to OTA through the diet of F0 dams until PND 21, after which they were continued on test diet. Changes in OTA’s female’s dose group. On PND 90, necropsies were performed on F1 rats and kidney tissue was collected containing the outer stripe of the outer medulla for genomic assessment. Next generation sequencing was used to derive the rat transcriptional profiles, which were compared using Gene Ontology and Ingenuity Pathway Analysis. Our findings support a gender-specific activation of the innate and adaptive immune responses in male F1 pups to OTA exposure. This was not found in the female F1 pups, possibly due to female-specific increased p38 activity and VDR signaling. Differentially expressed genes related to karyomegalic, MAPK activity, and immune activation appears to develop from in utero exposure to OTA whereas those related to decreased kidney and liver function, and changes to reproductive pathways occur in both rat generations. These transcriptional results confirm that dietary exposure to OTA affects kidneys as well as alterations to hepatic and reproductive pathways in rats. In utero exposure of rats to OTA results in sex-specific alterations in immune response pathways, VDR signaling, and p38 activity.

3547 Pilot Study on Human Pluripotent Cell Model for Assessing Developmental Toxicity of Fragrance Materials

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The Research Institute of Fragrance Materials (RIFM) serves to ensure the safe use of fragrance materials for the general population, while being proactive in searching for alternatives to animal testing. With the cosmetics industry’s ongoing shift towards more regulated or replacement testing, new toxicology methods are needed, particularly for endpoints that have historically been dependent on animal testing such as developmental and reproductive toxicology. A blinded study was conducted on 10 fragrance materials in Stemina’s devTOX quickPredict assay and in the Reprotracker assay by Toxins. All 10 materials already had in vivo developmental toxicity data. Research on human pluripotent cells has been considered as a promising animal-free approach. Stemina’s devTOX quickPredict assay is an exposure-based prediction of developmental toxicity potential using human pluripotent stem cells. The Reprotracker assay is a human-induced pluripotent stem cell-based biomarker assay for screening of developmental toxicity. All the materials analyzed using the devTOX quickPredict assay and the Reprotracker assay were evaluated at 8 and 5 different concentrations, respectively. Materials analyzed in this study contained a range of different functional groups, which well represented the chemical space of fragrance materials. From the 10 materials tested, data for most of the fragrance materials aligned with the previous in vivo results. The specificity for both the assays was around 80%. Although there are currently no regulatory approved in vitro assays for developmental toxicity research, our work will help to predict potential toxicity and prioritize screening if required. In conclusion, the analysis showed promising results to get an initial idea of a material’s developmental toxicity. More data may be required to validate such assays and help towards replacing animal testing.

3549 In Vitro Uptake of Microcystin-LR Toxin in Human Placental Cells

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Microcystins are a class of cyanobacterial toxins released from harmful algal blooms. One of the most common microcystin congeners is microcystin-LR (MC-LR). Previous studies have required active uptake into cells in order to cause toxicity. Within the liver, MC-LR is primarily transported into hepatocytes by organic anion-transporting polypeptides OATP1B1 and OATP1B3 leading to hepatotoxicity. With an increase in the incidence of harmful algal blooms in both marine and freshwater ecosystems due to the global temperature rise and eutrophication, there is growing interest in evaluating the ability of microcystins to also act as reproductive toxins. In the current study, we sought to determine whether MC-LR can enter human placenta cells. For this effort, intracellular accumulation of MC-LR (0.1, 1, 10 µM) was compared between HepG2 cells, a human liver cancer cell line (positive control), and JAR cells, a human choriocarcinoma cell line using western blotting. A concentration-dependent increase in microcystin-LR accumulation was observed in both HepG2 and JAR cells even at the lowest concentration of 0.1 µM. This research ultimately aims to elucidate the fate of microcystins as reproduc-
Tungsten is rapidly excreted from the body, but the remaining bioaccumulates in the bones. The bone represents the primary site of tungsten accumulation and storage, with a slow rate of removal. Tungsten interferes with the bone marrow-resident immature B cell development and differentiation. However, there is very little information on how tungsten is absorbed, distributed, metabolized, or transformed. Therefore, in an effort to discover modulators involved in tungsten metabolism and transport, we utilized a whole-genome CRISPR-screen in pre-B cell lymphocytic leukemia (NALM-6) cells treated with the IC50 of either sodium tungstate or phosphotungstate. Interestingly, we found a hit in the proteoglycans and glycosaminoglycan composition and height of intervertebral discs. Here, we show in a murine ex vivo limb bud culture model that tungsten impairs the limb bud development. We had previously shown that tungsten impairs the cartilage formation. We had also shown that tungsten impairs the cartilage formation. We had previously shown that tungsten impairs the cartilage formation.

### References

3. S. L. compensation for sex organ weight was the most commonly reported endpoint for male and female reproductive endpoints. Commonly, studies often reported reduced offspring body weight in exposed animals relative to controls. Offspring viability measures also were reported to be impacted by mercuoric chloride exposure, with most studies reporting reduced viability. Overall, the most commonly reported endpoints for males and females were sex organ weights which, while informative, do not fully describe the impacts of a chemical on reproductive function. The systematic review and analysis highlights common SQE pitfalls in PECO-relevant mercuoric chloride studies and emphasizes the need for data on different reproductive endpoints for both males and females. Disclaimer: The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or the policies of the US Environmental Protection Agency.
transports in the placenta, including the breast cancer resistance protein (human BCRP/mouse Bcrp), also limit fetal exposure by returning Cd to the maternal circulation. We have previously demonstrated that placental BCRP expression confers a 7-fold resistance against Cd toxicity in vitro. In this study, we sought to determine whether the absence of Bcrp in the placenta alters the fetoplacental disposition and toxicity of Cd in mice. Pregnant female C57BL/6Chc (WT) and Bcrp-null mice (n=9-10/group) were administered a single dose of saline (5 mg/kg ip) or CdCl2 (5 mg/kg ip) on gestational day (GD) 9. On GD 17, fetuses and placen- tatas were collected. As expected, no Bcrp expression was detected in Bcrp-null offspring. Compared to vehicle-treated WT dams, there were a number of basal differences in Bcrp-null dams including 1) lower weight gain across gestation, 2) larger litters (45%) of fetal-derived vessels in the placental labyrinth 3) elevated mRNAs expression (3-fold) of the diabetogenic metalloid transporters, Zip14, Zmt1, and Dmt1 and 4) enhanced expression of alternate efflux transporter Mdr1a (9-fold) and Mdr1b (14-fold) mRNAs. Collectively, these adaptive changes may be responsible for the similar basal sizes of WT and Bcrp-null offspring. Following Cd treatment, fetal length and placental area were reduced in both genotypes compared to respective controls. Moreover, Cd-treated Bcrp-null fetuses had a 12% shorter crown-to-rump length as Cd-treated WT offspring. Shorter fetuses corresponded with preferentially higher Cd accumulation (58%) in Bcrp-null placen- tatas despite the higher Mdr1a/1b expression. To delineate mechanisms underlying the reduced growth in Bcrp-null fetuses after Cd treatment, changes in placen- tal vasculature and nutrient transport were examined. Notably, placenta from Bcrp-null offspring had reduced expression of metal transporters (34% decrease in Zip14 mRNA and 58% decrease in Dmt1 protein) following Cd treatment. The absence of Bcrp alters development of the placenta and results in increased accumulation of Cd and impaired fetal growth potentially due to reduced uptake and homeostatic regulation of Cd. Supported by F31ES032319, R01ES029275, T32ES007148, and P30ES050522.

3555 Chronic Inhalation of Titanium Dioxide Nanoparticles Disrupts Placental Glucose Transporter Expression

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Maternal inhalation of particulate during pregnancy has been linked to perinatal death and various adverse health conditions in adulthood, including diabetes and cardiovascular disease. Of these xenobiotic exposures, titanium dioxide (TiO2) is a non-reactive particle found in consumer and personal care products. Previous lab data demonstrated a significant correlation between maternal exposure and fetal growth restriction. The etiology of this developmental concern may have maternal, fetal, and placental origins. Of interest is compromised placental function leading to impaired placental transport of nutrients to the fetus. Glucose transport is a critical component in healthy fetal development; therefore, we chose to examine the integrity of glucose transport mechanisms. We hypothesized maternal inhalation of TiO2 increases the MDRI gene expression leading to less glucose transport to the fetus to support proper growth. Pregnant Sprague Dawley rats were exposed to TiO2 powder (Aeroxide TiO2, Parsippany, NJ) aerosols [11.04 ± 1.09 mg/m3; geo. mean particle size 162.06 ± 5.30 nm (SMPS, TSII)] via a whole-body inhalation chamber (HGPA, IESTechno) from gestational day (GD) 4:19 for 4-5 hours a day. On GD20, maternal plasma was collected, and four placenta were taken from each dam at varying intrauterine positions. Placentas were sliced in half along the sagittal plane and prepped for immunohistochem- istry. GLUT1, GLUT3, and GLUT4 antibodies were used to visualize and quantify glucose transporter expression in the labyrinth zone. We observed a significant increase in the staining intensity of GLUT4 in placenta of exposed animals when compared to controls (91.40 ± 4.98 vs. 74.66 ± 1.49; p<0.03). When separated by sex, it became evident male placentas drove this significance (70.85 ± 6.94 in exposed males vs. 94.27 ± 3.94 in exposed females; p=0.01) as opposed to females (78.47 ± 8.26 in control females vs. 88.53 ± 7.90 in exposed females; p=0.36). Since GLUT4 is known to be insulin-regulated, we suspected there may be alterations in insulin sensitivity. GLUT1 showed a trending decrease in staining intensity (p=0.07) compared to controls (2.65 ± 0.50 vs. 3.40 ± 1.14; p<0.05). There was no difference in the dry ZW weight between sham-control and the nano-TiO2 group, however, the wet ZW was slightly smaller in the nano-TiO2 group (0.333 ± 0.006) than in the sham-control group (0.3128 ± 0.013). Percent ZW area was significantly decreased in the nano-TiO2 exposed group (24.37 ± 1.30%) compared to the sham-control (30.39 ± 1.54%, p<0.05). On GD20, the percent LAZ area was significantly increased in the nano-TiO2 exposed group (75.63 ± 1.30 %) versus the sham-control group (69.61 ± 1.54; p<0.05). These studies represent evidence that maternal inhalation of nano-TiO2 during gestation causes anatomical changes within the placenta, creating a smaller ZW in female pups and potentially leading to decreased hormonal efficiency. This work was supported by the following sources: WV-CH555145 (ECB), T32AG25735 (JAG), P20 GM103434 (WV-INBRE).

3557 The Xanthine Oxidoreductase Inhibitor Febuxostat Protects Against Nanomaterial Inhalation–Induced Microvascular and Reproductive Dysfunction during Gestation


Maternal inhalation exposure to nano-TiO2 during gestation impacts litter size, pup and placental mass, and uterine microvascular reactivity. In addition, we have recently shown that maternal inhalation of nano-TiO2 during gestation results in redox imbalance in dams during late gestation. However, the mechanism linking these phenomena to tissue responses has yet to be elucidated. We hypothe- sized that elevated xanthine oxidoreductase (XOR), a critical source of oxidants in numerous inflammatory processes, is at least partly responsible for the increased oxidant production observed. The objective of this study was to assess if treatment with a XOR inhibitor, febuxostat (Uloric), rescues the poor microvascular and reproductive outcomes induced by gestational nano-TiO2 exposure. Female Sprague Dawley rats, 6-8 weeks of age, received febuxostat treated water (50 mg/L) beginning one week prior to being mated in-house and throughout gestation until sacrifice on gestational day (GD) 20. Once pregnant, dams were randomly assigned to either sham-control (N = 4) or nano-TiO2 (N = 4) groups. Dams were exposed (nano-TiO2 concentration = 1 μg/μL; HEPA-filtered at 25 ml/min for 6 hrs/d for 4 d between GD 10-19 before sacrifice on GD 20. Dam and litter character- istics as well as placental and fetal weights were recorded at the time of sacrifice. No significant differences were observed between sham-control and nano-TiO2 groups for litter size (9.6±3.3 versus 12.6±2.2), fetal (3.6±0.9 g versus 4.7±0.9 g) or placental mass (0.72±0.3 g versus 0.63±0.2 g). Additionally, uterine arteries from exposed females treated with febuxostat showed similar vasocostriction to kiespin (97.6%±1.95) as control females given febuxostat (99.8%±0.61), and decreased kiespin induced vasocostriction from what has been previously observed in directly exposed nano-TiO2 dams (75.1%±14.2). Additionally, there was no difference in dam liver mass (11.5%±0.80 g control versus 13.9%±0.60 g nano-TiO2), which we have previously shown to be increased due to nano-TiO2 exposure. Taken together, these observations indicate that reproductive, liver, and microvascu- lar functions are protected, at least in part, from nano-TiO2 inhalation exposure induced dysfunction in pregnant dams by XOR inhibition. Support: OH012320 (EB), ES015022 (TM), ES032920 (JG).
Micro and nanoparticles (MNPs) are an emerging environmental pollutant that is increasingly being detected in human tissues. We have shown in virgin female rats that a single inhalation of MNPs blunts endothelial function in the uterine vasculature. Under conditions like pregnancy, xenobiotics impairing vascular function have detrimental effects but the consequences of maternal MNP exposure are unknown. Here, we assessed fetal health, placental efficiency, uterine vascular function, and nitric oxide (NO) bioavailability after MNP inhalation throughout pregnancy. Pregnant rats were exposed to aerosolized polycrylene-12 MNPs [10.5 ± 0.73 mg/m³, 2.7 μm particle size 3.8 ± 0.7 μm] for 4 h daily, 5 days/wk from gestational day (GD) 5-19 and experimental analyses were performed on GD 20. MNP exposure induced oxidative stress (4.17 ± 0.09 pg/ml) and placental efficiency (8.31 ± 0.30 vs 9.89 ± 0.40). Uterine artery responses to increasing concentrations of methacholine (Mch), sodium nitroprusside (SNP), and phenylephrine (PE) were assessed by wire myography. Exposure resulted in decreasing impairments of endothelium-dependent dilatation stimulated by Mch at 10⁻⁴ M (3.87 ± 3.55 vs 11.9 ± 7.65% max. tension) as well as endothelium-independent dilatation stimulated by SNP at 10⁻⁴ M (1.19 ± 3.23 vs -12.6 ± 9.65% max. tension). The contribution of eNOS to endothelium-dependent dilatation was determined by measuring endothelium-dependent dilatation after inhibition with L-NAME. Concentration response curves after stimulation with MCH, SNP, and PE as well as reliance on eNOS function in a sex-dependent manner, revealed no significant differences between groups. NO bioavailability in the uterine vasculature was measured by quantification of nitrites and western blot of tyrosine nitration (a product of peroxynitrite) after stimulation of NO release. Nitrites were decreased in exposed dams (5.05 ± 4.01 picomol/mg vs 8.11 ± 2.46 picomol/mg; p = 0.31). Preliminary western blotting of NOS protein expression suggests increased expression in exposed dams. These data show that MNP inhalation induces fetal growth restriction and may decrease NO release from the uterine vasculature without causing functional deficits in the uterine macrocirculation. Studies are ongoing to examine uterine microvascular function as well as mechanistic cause of decreased NO bioavailability in the uterine vasculature.

3559 Development of a Study Quality Tool for Use in a Systematic Review of Literature Reporting Microplastic Exposure and Reproductive and Developmental Toxicity

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Microplastics (MPs) have been detected in air, water, soil and food, but understanding these exposures in the context of potential human health risks requires both hazard characterization and exposure evaluation. Initial reviews of hazard studies identified by the National Nanotechnology Initiative (NNI) reproductive toxicity (DART) as potentially "critical effects" (effects exhibiting the lowest NOAEL/BDI). However, these reviews also highlighted that aspects of study reliability relating to these experimental investigations - particularly test article identity, exposure, dose-response, and relevance of mechanistic endpoints to adverse effects - impact confidence in hazard characterization. To better understand such impacts and to systematically gather, appraise, and integrate empirical evidence from these biological and chemical endpoints, a novel data extraction tool (a three-tiered study quality tool) was developed for a stepwise and highly refined approach to critical effects of MPs on DART outcomes in both epidemiological and experimental animal studies. Studies was conducted using a stepwise and highly refined approach to critical appraisal. This approach combines explicit and transparent determinations of risk mechanisms (one, test particle relevance). Therefore, the available body of literature did not meet the minimum standards of validity and confidence for use in hazard characterization of MPs for potential human health risks. This systematic review and evidence appraisal methodology enables more precise understanding of the current state of the science and illustrates opportunities for developing the additional information needed for improving the scientific basis of MPs hazard characterization, exposure evaluations, and risk assessments.

3560 Sexually Dimorphic Modifications in Placental Cyclooxygenase Metabolites after Maternal Nanomaterial Inhalation Exposure

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Maternal nano-titanium dioxide (nano-TiO₂) inhalation exposure during gestation results in decreased fetal female mass, maternal estrogen production, and placental mass. Placental function is critical during gestation due to its roles in nutrient and waste exchange and endocrine production. Adverse fetoplacental environments profoundly impact fetal growth and development. Additionally, the impacts of toxicant exposure can occur in a sexually dimorphic manner, which has shown after maternal diesel exhaust exposure during gestation. Therefore, we hypothesized that maternal nano-TiO₂ inhalation exposure during gestation alters placental function in a sexually dimorphic manner. Pregnant Sprague-Dawley rats were exposed to nano-TiO₂ aerosols (12.17 ± 1.69 mg/m³) or HEPA-filtered air (sham-control) from gestational day (GD) 10-19. Dams were euthanized on GD20, and placental junctional zone (JZ), labyrinth zone (LZ), and fetal visceral tissue were collected and separated on sex, fetoplacental units, and also based on fetal sex. Fetoplacental units, also based on fetal sex, were used to assess this. The changes in placental hemodynamics and production of cyclooxygenase metabolites reflect a functional change that is occurring in the context of potential human health risks requires both

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for various genes that facilitate the synthesis (PTGS2, PTGES) and transport (SLCO2A1) of prostaglandins (PGs). Other genes that are differentially regulated in PG synthesis include PLA2G4A and AKR1C1, while HPGD is a gene involved in PG metabolism. Increases in PGs (PGE2, PGE2−) are vital for ovulation as they cause cumulus oocyte complex expansion, follicle rupture, and angiogenesis. Our laboratory has previously shown that PHTmix exposure decreases levels of cAMP, PGE2, PGF2α, and PGE2− and alters the levels of genes involved in PG synthesis, metabolism, and transport. We hypothesized that cAMP supplementation would circumvent the toxic effects of the PHTmix by restoring the levels of PGs and the genes involved in PG synthesis, metabolism, and transport. Granulosa-lutein cells from follicular aspirates of women undergoing in vitro fertilization were acclimated in culture to regain LH/human chorionic gonadotropin (hCG; potent LH analogue) responsiveness. Prior to hCG treatment, cells were exposed to vehicle control (dimethylsulfox ide, DMSO) or a mixture of six phthalates (PHTmix; 500µg/mL) for 48hr. The PHTmix was derived from urinary phthalate measurements in pregnant women. Cells were treated ± hCG and ± 8-BR-cAMP (stable cAMP analogue) and collected at 0, 6, 12, 24, or 36hr post-treatment for PG measurements and gene expression analysis (n=4-9; p≤0.05). As previously reported, treatment with hCG+PHTmix decreased the levels of PGE2, and PGF2α compared to hCG alone. However, supplementation with hCG+PHTmix+cAMP increased the levels of PGE2, at 36hr and PGF2α at 24 and 36hr compared to hCG, albeit at significantly lower levels than DMSO+cAMP and hCG+cAMP. Expression of PTGS2 decreased with hCG+PHTmix at all timepoints when compared to hCG alone, while supplementation with hCG+PHTmix+cAMP restored PTGS2 levels to hCG controls. PHTmix expression was decreased with hCG+PHTmix at 6, 12, and 36hr, while hCG+PHTmix+cAMP supplementation increased (6, 24hr) and decreased (12hr) expression of PHTmix when compared to hCG alone. AKR1C1 expression was increased with hCG+PHTmix at all timepoints and was decreased in the hCG+PHTmix+cAMP group when compared to hCG alone (Fig. 2D). Expression of AKR1C1 decreased with hCG+PHTmix at 12 and 24hr relative to hCG alone, while supplementation with hCG+PHTmix+cAMP restored AKR1C1 expression to hCG levels at 12hr and increased AKR1C1 expression beyond hCG levels at 24 and 36hr. HPGD expression was increased with hCG+PHTmix+cAMP supplementation at all timepoints when compared to hCG alone but was restored back to hCG alone levels with hCG+PHTmix+cAMP supplementation. These data suggest that the supplementation of cAMP may have the ability to bypass the toxicities that phthalate exposure exerts on ovulatory prostaglandin production and metabolism, thereby providing a potential therapeutic target for women suffering from ovulatory dysfunction. Supported by R01ES033767, R00ES028748, P30ES026529.

Effects of a Phthalate Mixture on Ovarian Antral Follicle Protein Abundance

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Phthalates are a group of endocrine disrupting chemicals that are associated with negative reproductive outcomes in women. The objective of this study was to evaluate the effects of a three-phthalate mixture on ovarian antral follicle, protein abundance. We hypothesized that the cooyce and antral follicles specifically play a crucial role in ovarian steroidogenesis and ovulation. DBP, BBP, and DEHP have been associated with inhibiting antral follicle growth in vitro, decreasing ovulation rates in vitro, and decreased antral follicle counts in women. However, little is known about the effects of a three-phthalate mixture on antral follicles in vivo. The objective of this study was to evaluate the effects of a human relevant mixture of DBP, BBP, and DEHP on ovarian follicles through the analysis of proteomic profiles. CD-1 female mice (60 days old) were pipet fed tocopherol stripped corn oil (vehicle control) or a phthalate mixture (52% DBP, 36% DEHP, and 12% BBP dissolved in vehicle) which modeled human follicular fluid concentrations. The mice were treated with 32µg/kg/day (cumulative estimate in occupationally exposed individuals) for 10 consecutive days. Antral follicles (>250µm) were mechanically isolated and proteomic profiling was performed. A total of 5417 antral follicle proteins were identified in the three groups, of which 194 were differentially represented between the control and 32µg/ kg/day group, and 136 between the control and 500µg/kg/day group. A principal component analysis validated the phthalate effects on the proteome. Gene ontology analysis revealed that the two doses of the phthalate mixture upregulated and downregulated distinct biological processes, supporting non-monotonic effects of phthalates on the antral follicle proteome. Furthermore, a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that phthalates, at both doses, had a robust effect on metabolic pathways. Interestingly, gene ontology analysis of phosphorylated peptides revealed the 32µg/kg/day dose altered the abundance of proteins involved in apoptotic processes while the 500µg/kg/day dose altered the abundance of proteins involved in RNA processing and splicing processes. These results reveal that a human relevant mixture of DBP, BBP, and DEHP alters the antral follicle proteome and merit further evaluation to elucidate the molecular mechanisms by which phthalates cause negative reproductive outcomes.

Associations of Urinary Phthalate Metabolites with Ovarian Volume and Differences by Change in BMI since Age 18

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Phthalates are endocrine disrupting chemicals with ubiquitous exposure via personal care products, cosmetics, and food packaging. Women entering menopause experience hormonal shifts due to ovarian follicle growth (foliculogen esis) cessation. Adipose tissue is a hormonally active organ with a role in ovarian function, thus having obesity may impact a woman’s susceptibility to phthalate exposures. Therefore, we evaluated associations of phthalates with hCG+cAMP. Expression of immune response genes (as a marker of foliculogenesis) in midlife women and considered differences by change in body mass index (BMI) since age 18 (BMI18). Women from Baltimore (n=614; 45-54 years old) had a transvaginal ultrasound to measure right/left ovary dimensions to calculate ovarian volume. Phthalates were quantified from 2-4 urine samples pooled across one menstrual cycle. BMI18 was calculated as the difference between midlife BMI and BMI at age 18. Multivariable linear regression models controlled for important lifestyle and health demographic factors to evaluate associations of phthalate concentrations with average ovarian volume, with the addition of a multiplicative interaction term to consider differences by BMI18. Overall, mono(3-carboxypropyl) (MCPP) and monobenzyl (MBzP) phthalates were positively associated with ovarian volume, which differed by BMI18. For example, in all women, a 1% increase in MCPP and MBzP were associated with 0.44% (P=0.06) and 0.63% (P=0.04) increases in ovarian volume, respectively. However, in women who had overweight or obesity at both age 18 and midlife, a 1% increase in MCPP was associated with a 0.76% (P=0.02) increase in ovarian volume. In conclusion, phthalates are associated with ovarian volume, but lifetime weight status may alter susceptibility to phthalate exposure.

The Effects of Prenatal Exposure to an Environmentally Relevant Phthalate Mixture on Ovarian Gene Expression in F1 and F3 Female Mice

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Phthalates are a family of synthetic chemical compounds used as plasticizers and solvents. Phthalates are commonly detected in the environment as mixtures in building materials, medical equipment, and personal care products. Although virtually 100% of the U.S. population has measurable exposure to several types of phthalates, little research has been done to understand the transgenerational effects of prenatal exposure to phthalate mixtures on reproductive health in female offspring. Thus, this study tested the hypothesis that prenatal exposure to an environmentally relevant phthalate mixture affects ovarian gene expression of methyltransferase enzymes and cytokines in the F1 and F3 generation. To test this hypothesis, pregnant CD-1 dams were dosed orally with vehicle control (corn oil) or four different doses of phthalate mixture (20 µg/kg/day, 200 µg/kg/day, 2000 µg/kg/day, 5000 µg/kg/day). The pregnant dams were allowed to deliver pups naturally and selected ovaries from the F1 female offspring were collected at PND 21 and 6 months. The remaining F1 female offspring were mated with non-treated CD-1 males at 12 months to produce the F2 generation. F2 females were mated with non-treated CD-1 male mice to produce the F3 generation. Ovaries were collected from the F3 generation on PND 21. RNA was isolated from the F1 and F3 ovaries and used in qPCR reactions to quantify expression of methyltransferase enzymes: DNA methyltransferase 1 (Dnmt1), DNA methyltransferase 3 beta (Dnmt3b), tet methylcytosine dioxygenase 1 (Tet1), tet methylcytosine dioxygenase 2 (Tet2), and tet methylcytosine dioxygenase 3 (Tet3). RNA was also used to measure expression of selected cytokine genes: interleukin1β (Il1b), interferon gamma (Ifng), interleukin 4 (Il4), interleukin 6 (Il6) and interleukin 10 (Il10). The results indicate that prenatal exposure to the mixture increased the expression of Il1b at 200 mg/kg/day and Tet3 at 200 mg/kg/day compared to controls in the F1 ovaries at PDN60 compared to controls. The phthalate mixture did not significantly alter the expression of methyltransferase enzymes compared to control in the F1 ovaries at 6 months. However, prenatal exposure to the mixture at 20 µg/kg/day decreased the expression of Il1b, Il4, and Il10 in F1 ovaries compared to control at 6 months. The results also indicate that in the F3 generation at PND21, the mixture increased the expression of Il1b at 200 mg/kg/day, Il10 at 20 µg/kg/day and 500 mg/kg/day, and Il6 at 20 µg/kg/day and 200 µg/kg/day compared to controls. Further, in the F3 generation, the mixture increased expression of Il1b at 200 µg/kg/day and Tet3 at 500 µg/kg/day compared to controls. These data suggest that prenatal exposure to a phthalate mixture may not significantly alter DNA methylation in the F1 generation. However, long after initial exposure to a phthalate mixture, but it may have significant impact on DNA methylation across generations. These data also suggest that the phthalate mixture may interfere with the expression of immune response genes long after initial exposure. This research is supported by NIH R01ES0332163 and NIH Diversity Supplement R01ES032163S.
Diisononyl phthalate (DiNP) is a plasticizer used in a wide range of consumer products including building materials, food and beverage containers, and children’s toys. This is concerning because DiNP can leak out of products and enter the human body. Furthermore, DiNP has been shown to exhibit endocrine disrupting abilities after both short-term and long-term exposures. However, little is known about the effects of DiNP on the ovary. Thus, this study tested the hypothesis that DiNP alters gene expression in the mouse ovary. To test this hypothesis, female CD-1 mice aged 39-40 days were orally dosed with either vehicle control (corn oil) or DiNP (20μg/kg/day-200mg/kg/day) for 10 days. The ovaries of these mice were collected immediately post-dosing and 3 months post dosing. The mRNA from these ovaries was isolated and subjected to RNA-Seq analysis. DiNP exposure did not alter gene expression in ovaries collected immediately post-dosing compared to control. However, DiNP exposure significantly altered expression of 7 different genes in ovaries collected 3 months post-dosing compared to control. After validating with qPCR, the results showed that DiNP exposure at 20μg/kg/day significantly decreased expression of SNRPN upstream open reading frame (Snurf) by -0.207 ± 0.045-fold from the control. Similarly, DiNP exposure at 200mg/kg/day significantly decreased expression of multidrug resistance protein 3 (Mrp3) and Ahrb by -0.694 ± 0.052-fold from the control. The functions of both Snurf and Mrp3/Abcb4 are unknown in the ovary. Snurf is a gene that encodes two different polypeptides: The SmN splicing factor used in RNA processing and the SNRPN upstream open reading frame (Snurf) polypeptide. In the liver, Abcb4 encodes proteins that facilitate phospholipid movement across the cellular membranes. Collectively, these data suggest that DiNP exposure for 10 days has long-term effects on ovarian gene expression in mice. This work is supported by NIH R01ES 028661, R01ES034112, T32ES 007326, and a Billie Field Fellowship.

Mono-2-Ethylhexyl Phthalate Indirectly Induces Aryl Hydrocarbon Receptor Activation in Mouse Ovarian Tissues

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer used in the production of numerous consumer products. In the body, DEHP is hydrolyzed to its metabolite, mono-2-ethylhexyl phthalate (MEHP). MEHP is a reproductive toxicant that targets the ovary and impairs ovarian follicle growth and reduces ovarian estrogen production. However, the molecular mechanisms mediating these effects are unknown. The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor that binds various environmental contaminants and regulates expression of xenobiotic and estrogen metabolizing enzymes. We hypothesized that MEHP reduces ovarian estrogen production via activation of the AHR. To test this hypothesis, we isolated antral follicles from the ovaries of CD-1 mice. Follicles were exposed to MEHP (0-400μM) for 96 hours in the presence or absence of the AHR antagonist, CH223191 (1μM). Follicles were collected for gene expression analysis and conditioned media were collected for hormone analysis. MEHP increased expression of the estrogen metabolizing cytochrome P450 (CYP) enzymes Cyp1a1 and Cyp1b1, and this effect was blocked by co-treatment with CH223191. MEHP reduced media concentrations of estrone and estradiol compared to control, consistent with the MEHP-mediated induction of Cyp1a1 and Cyp1b1. The MEHP-mediated decline in estrogen levels was blocked by co-treatment with CH223191. Additionally, MEHP reduced expression of estrogen-sensitive genes progesterone receptor (Pgr) and luteinizing hormone/chorionic gonadotropin receptor (Lhgc), and these effects were reduced by co-treatment with CH223191. These data suggest that MEHP alters estrogen levels and estrogen signaling in an AHR-dependent manner. To determine if MEHP activates the AHR by binding as a ligand, a reporter assay was performed using primary granulosa cells. Cells were transfected with a reporter construct containing the luciferase gene under the control of xenobiotic response element that serves as a binding site for the AHR. Cells were treated with MEHP (0-400μM) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 1μM) as a positive control for 24 hours. AHR binding activity was measured by luciferase assay. Exposure to TCDD increased AHR binding, as expected. However, no concentration of MEHP altered AHR binding activity compared to control. Collectively, these data suggest that the AHR is an indirect target of MEHP in ovarian tissues, and it may contribute to the endocrine disrupting actions of MEHP by enhancing estrogen metabolism and reducing estrogen signaling. Supported by NIH T32 ES007326 and R01 ES028661.

Long-Term Dietary Di-(2-Ethylhexyl) Phthalate Exposure Affects Ovarian Follicle Populations and Hormone Levels in Mice

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Phthalates are a class of environmental contaminants found in plastic food contain- ers, medical plastics, and personal care products. Phthalates negatively affect female reproduction, but the effects of long-term phthalate exposure on the ovary are largely unknown. Thus, we investigated the effects of long-term phthalate exposure in adult female CD-1 mice. Adult female CD-1 mice were fed control diet or a diet containing DEHP at 0.15 ppm, 1.5 ppm, or 1500 ppm via the Chow for 6 months. After dosing, the mice were euthanized, and the ovaries were collected and processed for histological evaluation of follicle numbers. Sera were collected and processed for measurement of hormone levels. The results show that the highest DEHP concentration (1500 ppm) increased primordial follicle numbers and decreased preantral and antral follicle numbers compared to control. Furthermore, long-term exposure to DEHP affected hormone levels by decreasing luteinizing hormone (LH) levels (0.15 ppm and 1.5 ppm) and increasing follicle stimulating hormone (FSH) levels (1500 ppm) compared to control. Interestingly, DEHP exposure did not affect progesterone, testosterone, or estradiol levels compared to control. Overall, these data indicate that long-term exposure to DEHP affects ovarian follicle numbers as well as FSH and LH hormone levels. Supported by R01 ES028661, NIH T32 ES007326, and a Billie Field Fellowship.

Toxicity of the Phthalate Replacement Di-2-Ethylhexyl Terephthalate (DEHTP) and Its Metabolite on the Mouse Ovary

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Toxicity of the phthalate replacement di-2-ethylhexyl terephthalate (DEHTP) and its metabolite on the mouse ovary Courtney Potts, Genoa Warner Di-2-ethylhexyl terephthalate (DEHTP) is an alternative plasticizer for its structural isomer di-2-ethylhexyl phthalate (DEHP), a known endocrine disrupting chemical. DEHTP is considered safe for use by the chemical industry and is used as a plasticizer in various polyvinyl chloride products including children’s toys and medical tubing. Exposure to DEHTP has raised concerns because its metabolites have been detected in humans at higher concentrations than the phthalates it replaces. This research tested the hypothesis that DEHTP is an endocrine disruptor due to its structural similarity to DEHP. To investigate these concerns, the impact of exposure to DEHTP and its metabolite mono-2-ethylhexyl terephthalate (METHP) was studied on the mouse ovary with a particular interest in the development of follicles, the structures that contains developing oocytes. Follicles were dissected from ovaries of adult mice that were fed a mixture of phthalates (Mix) containing DEHP, DiNP, benzyl butyl phthalate, di-n-buty phthalate, dibutyl phthalate, and diethyl phthalate. Measurements of urinary phthalate metabolites confirmed effective delivery of phthalates in the rodent chow. Phthalate consumption for 11 months did not affect body weight throughout exposure compared to control. DEHP exposure at 0.15 ppm for 3 and 5 months increased the time that the mice spent in estrus and decreased the time the mice spent in metestrus/diestras compared to control. Long-term DiNP exposure (0.15 ppm-1500 ppm) did not significantly affect time in estrus or metestrus/diestras compared to control. Mix exposure at 0.15 ppm and 1500 ppm for three months decreased the time the mice spent in metestrus/diestras and increased the time the mice spent in estrus compared to control. DEHP (0.15 ppm-1500 ppm) or Mix (0.15 ppm-1500 ppm) exposure did not affect fertility-related indices compared to control. However, long-term DiNP exposure at 1500 ppm significantly reduced gestational index and birth rate compared to control. These data indicate that chronic exposure to phthalates via the diet alters estrous cyclicity, and long-term DiNP exposure reduces gestational index and birth rate in mice. Supported by NIH R01 ES028661 and NIH T32 ES007326.
Phthalates are a class of environmentally harmful chemicals that are used as plasticizers and solvents. They can be found in processed food, personal care products, cosmetics, plastic, and medical tubing. Phthalates are detected in humans constantly due to consistent exposure through different routes. Previous studies have shown that phthalates are reproductive toxicants and can disrupt ovarian steroid hormone production, but little is known about their mechanism of action. Granulosa cells in the ovary play a critical role in follicle development by promoting oocyte maturation; any disruption of their function may result in the disturbance of folliculogenesis and sex steroid hormone production. Thus, this research tested the hypothesis that phthalates and their metabolites specifically target granulosa cells to cause endocrine disruption. Granulosa cells were harvested from antral follicles of adult CD-1 mouse ovaries and cultured for 96 h in phthalate mixture (PM) (0.1-100 μg/mL) or metabolite mixture (MM) (0.1-100 μg/mL) with dimethyl sulfoxide (DMSO) as the vehicle control. 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% benzylbuty1 phthalate. The phthalate metabolite mixture was composed of 37% monoethyl phthalate, 19% mono(2-ethylhexyl) phthalate, 15% monobutyl phthalate, 10% monoisobutyl phthalate, 10% mono-n-octyl phthalate, and 8% monobenzyl phthalate. Enzyme-linked immunosorbent assays (ELISA) were performed to analyze hormone levels. Estradiol was significantly increased following treatment with 100 μg/mL PM compared to control. Progesterone levels were not significantly impacted. Overall, phthalates do not appear to significantly disrupt hormone production in isolated granulosa cells at these doses. Future experiments will measure gene expression of steroidogenesis enzymes to confirm this result.

MEHP (mono-(2-ethylhexyl) phthalate) has been previously shown to instigate spermatocyte apoptosis and disruption of the blood-testis barrier in peripubertal rats. Recently, we have reported that peritubular testicular macrophages (PTMφs), that are phenotypically distinct from resident macrophages in the testis interstitium, are significantly increased in areas along the seminiferous tubules after an acute exposure to 700 mg/kg MEHP. This change in PTMφs occurs coincident with increased numbers of undifferentiated spermatagonia within the seminiferous epithelium. Here, we examine if the recruitment of PTMφs is dependent on the dose of MEHP and challenge the novel hypothesis that PTMφs play a functional role in the recovery of spermatogenesis in response to MEHP induced spermatocyte loss by stimulating spermatogonial differentiation. Peripubertal Fischer rats (PND 28) were exposed to either a single dose of either 250 mg/kg or 500 mg/kg MEHP or corn oil via oral gavage and euthanized at 48 hours later. Additionally, peripubertal Fischer rats were treated with MEHP at a dose of 350 mg/kg/day for 7 consecutive days. Treatment was discontinued for 7 days before study chronic exposure. Here we report that an acute exposure to 500 mg/kg and a repeated 250 mg/kg exposure to MEHP resulted in significant loss of spermatocytes as well as increased numbers of PTMφs (MHC I+) as well as differentiating spermatogonia (PLZF+). No significant changes in PTMφs were observed in MEHP-untreated rats. Using a novel model of MEHP-induced testicular injury, we found that PTMφs play a functional role in the recovery of spermatogenesis in response to MEHP. Taken together, the findings are the first to suggest that an infiltration of PTMφs into the testis is dose dependent and serve an important functional role in the recovery of spermatogenesis after toxicant-induced testicular injury.

Environmental exposure to Endocrine Disrupting Chemicals (EDCs) during fetal and placental development may lead to molecular changes that increase adult disease susceptibility. For example, EDCs can alter fetal growth and metabolism in utero and disrupt critical set points that promote metabolic disorders in adulthood. One EDC strongly associated with metabolic changes is Di-(2-ethylhexyl)-phthalate (DEHP). DEHP is used as a plasticizer and is ubiquitous in the environment, present in food packaging, children’s toys, medical devices, and personal care products. DEHP functions as an anti-androgen in fetal and placental endocrine systems in part through the action of adenosine deaminase. Di-(2-ethylhexyl)-phthalate exposure during pregnancy has been associated with metabolic changes in adult offspring. However, the mechanisms that drive DEHP-induced changes in fetal metabolism and adult diseases are unknown. Recent evidence suggests that EDC-induced changes in metabolic gene profiles may stem from an altered epigenetic landscape. One epigenetic factor, DNA methylation, is particularly vulnerable to environmental exposures as it changes dynamically during development. Thus, we hypothesize that preconception exposure (alone) to DEHP is sufficient to disrupt DNA methylation patterns in the placenta. These changes will lead to abnormal cross-talk between the fetus and the placenta, which in turn will predispose offspring to impaired spermatogonial and hypothalamic/pituitary function. To test this hypothesis, F0 females were exposed to two doses of DEHP (Low: 50 μg/kgBW/day, High: 10 mg/kgBW/day) through their feed. One group was exposed to DEHP two weeks prior to gestation until embryonic day (E) 12.5 (placental analysis) or weaning. A second group was exposed from preconception until gestational day 0.5, then placed on control diet until E12.5 or weaning. For adult offspring pheno-typing, F1 offspring were placed on a control diet post-weaning until post-na- tal day 70. Females exposed to low DEHP during preconception and gestation had reduced body weight and liver lipodosis. Metabolomics on livers from these females revealed elevated levels of many medium and short fatty acids. With respect to phenotypes of E12.5 placentas, no significant changes in global DNA methyla- tion and size compared to controls were observed. However, placental vascul-ature analysis, using CD34 staining, showed a significant reduction of fetal vessels from high DEHP preconception and gestation stages. These findings reveal that periconceptional DEHP exposure exhibits sex-, window- and dose-specific effects on placenta function and liver hepatotoxicity.

MEHP (mono-(2-ethylhexyl) phthalate) is a known male reproductive toxicant. However, the molecular initiating events of phthalate toxicity are still undetermined. The apical endpoints of phthalate toxicity include a well-characterized anti-androgenic effect that results in a distinct set of morphological effects resulting from disrupted fetal testicular development. Morphological effects include induction of multinucleated germ cells (MNGs) and increased seminiferous cord diameter. While the mouse is less sensitive to the anti-androgenic effects of phthalates than the rat, the morphologic effects are similar between both rodent species, as well as humans. In this study, we sought to investigate a possible molecular initiating event for phthalate-induced disruption of seminiferous cord development in the mouse fetal testes. We hypothesized that late-gestational exposure to DEHP would result in MNG induction and seminiferous cord dysgenesis. Because phthalates are known PPAR ligands, we also hypothesized that DEHP would alter PPAR signaling during mouse fetal testis development. C57BL/6 mouse fetal testes on GD 15 and cultured them for 24 h in media containing mono-(2-ethylhexyl) phthalate (MEHP), in combination with the PPAR agonist, rosiglitazone, and the PPAR antagonist, GW9662. Following exposure to 700 mg/kg MEHP or corn oil via oral gavage and euthanized at 48 hours later. Additionally, peripubertal Fischer rats were treated with MEHP at a dose of 350 mg/kg/day for 7 consecutive days. Treatment was discontinued for 7 days before study chronic exposure. Here we report that an acute exposure to 500 mg/kg and a repeated 250 mg/kg exposure to MEHP resulted in significant loss of spermatocytes as well as increased numbers of PTMφs (MHC I+) as well as differentiating spermatogonia (PLZF+). No significant changes in PTMφs were observed in MEHP-untreated rats. Using a novel model of MEHP-induced testicular injury, we found that PTMφs play a functional role in the recovery of spermatogenesis in response to MEHP. Taken together, the findings are the first to suggest that an infiltration of PTMφs into the testis is dose dependent and serve an important functional role in the recovery of spermatogenesis after toxicant-induced testicular injury.
Phthalates are plasticizers with widespread use in consumer products. They are considered endocrine disrupting chemicals due to their structural similarity to endogenous hormones and may interfere with sex steroid hormone synthesis and activity of nuclear hormone receptors, including the Androgen Receptor (AR). Androgen signaling in the placenta is essential in the processes of angiogenesis and fetal growth. Pregnancy conditions including preeclampsia and gestational diabetes have previously been associated with increased expression of AR and its isoforms indicating a potential role for AR dysregulation in these conditions. We have previously demonstrated that phthalates are associated with changes to the placental transcriptome in human cohort studies and through in vitro studies with HTR-8/SVneo and primary syncytiotrophoblast cells. Differentially expressed genes associated with mono-(2-ethylhexyl) phthalate (MEHP) in our in vitro study were significantly enriched with binding sites for several nuclear hormone receptor transcription factors (TFs) including AR. We hypothesize that di(2-ethylhexyl) phthalate (DEHP) and MEHP will reduce AR binding based on evidence of the anti-androgenic effects of phthalates. To test this hypothesis, we treated HTR-8/SVneo cells for 4 hours with 10 mM dihydrotestosterone (DHT) to induce nuclear translocation of AR. We then treated the cells with 90 µM MEHP. Cleavage Under Tags and Release Under Nuclease (CUT&RUN) is a novel approach that quantifies the location and amount of TF binding within the genome. The binding was observed in regions of genes involved in endocytosis, and genes within this pathway were previously associated with MEHP exposure in regions of disrupted binding and linking them to corresponding gene expression changes after MEHP treatment. In regions of decreased AR binding, genes associated with decreased differentially bound peaks after MEHP exposure were differentially expressed in our prior analysis of MEHP's effect on the placental transcriptome in HTR-8/SVneo cells. We identified 11 differentially bound peaks with FDR<0.1 with 81 of these peaks showing decreased AR binding. 12 of the genes associated with decreased differentially bound peaks after MEHP exposure were differentially expressed in our prior analysis of MEHP's effect on the placental transcriptome in HTR-8/SVneo cells. We identified 11 differentially bound peaks with FDR<0.1 following DEHP exposure with 10 of these peaks showing decreased AR binding. KEGG pathway analysis was performed using Ingenuity on genes associated with differentially bound peaks (FDR<0.1) that exhibited decreased AR binding. Genes with decreased AR binding after MEHP exposure were associated with 9 pathways (p<0.05), while no pathways were associated with the genes with decreased binding after DEHP exposure. There was significantly decreased AR binding among genes in regions of decreased AR binding. MEHP showed more potent effects on AR binding than DEHP based on the number of differentially bound peaks indicating that phthalate compounds and their metabolites may cause different biological effects in the placenta. Overall, this research provides novel insight into mechanisms of phthalate induced disruptions to Androgen signaling and suggests that endocrine disrupting chemicals specific regions of disrupted binding and linking them to corresponding gene expression changes in placental cells which has not been previously reported.

To further challenge if the effects of MEHP on the BTB was the primary stimulus for changes in the testicular PTMps, an experimental approach that employs transfecting LG3/4/5 to protect the integrity of BTB prior MEHP exposure. Our findings show that LG3/4/5 overexpression substantially reduced the ability of MEHP to compromise the integrity of the BTB. Our preliminary assessment also indicates that LG3/4/5 overexpression also prevented changes in the numbers of testicular PTMps after MEHP. These findings indicate that a disruption of the functional integrity of BTB by MEHP is a requisite for the observed changes in the numbers of PTMps in rat testis. Further studies are focused on understanding the pathophysiologic role of PTMps in the tests.

Blood-Testis Barrier Disruption Is a Prerequisite for MEHP-Induced Increases of Peritubular Macrophages in Rats
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Peritubular macrophages (PTMps) are a novel subtype of testicular macrophages which are enriched in regions next to the spermatogonial stem cells. Our previous studies showed that exposure of peripherical Fisher rats to mono-(2-ethylhexyl) phthalate (MEHP) leads to an increase of PTMps within the testis. Exposure to MEHP is known to damage the blood-testis barrier (BTB) and also results in the loss of spermatocyte germ cells by apoptosis. However, the underlying mechanism for initiating the increase in testis PTMps is not known. Here we tested the differential participation between BTB disruption and germ cell apoptosis play in the instigation of changes in testicular PTMps by utilizing experimental strategies that specifically induce only spermatocyte apoptosis, via exposure to methoxycetic acid (MAA), or specifically disrupt the BTB via cadmium chloride (CdCl2) exposure. Male periperal (postnatal day PND 26) rats were exposed to 700 mg/kg MEHP, 650 mg/kg MAA or 2.5 mg/kg CdCl2 and respective controls via oral gavage and euthanized 48 hours later. Spermatocyte apoptosis was evaluated by TUNEL analysis, and a biotin tracer assay was utilized to detect the BTB functional integrity. PTMps and undifferentiated spermatogonia were identified in whole mounts of seminiferous tubules with fluorescent markers (MHCh and PLZF) and visualized using a confocal microscope. After MAA exposure though a significant increase in germ cell apoptosis was evident, no significant changes in the numbers of PTMps were observed. However, CdCl2 exposure did disrupt BTB as indicated by the penetration of biotin tracer and also caused an increase in the numbers of PTMps in the tests.

3576 Long-Chain Per- and Polyfluoroalkyl Substances Interfere with Gonadotropin-Dependent Ovarian Follicle Maturatio, Hormone Secretion, and Ovulation
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Per- and polyfluoroalkyl substances (PFAS) are a group of ~500 synthetic organic compounds consisting of a major carbon backbone and at least one fluoroalkyl moiety. Due to the highly stable carbon-fluorine bonds, PFAS have been widely used in industrial/consumer products including textiles, cookware, and firefighting foams. The persistence of PFAS has resulted in extensive environmental contamination and bioaccumulation, making them a new class of contaminants of emerging concern (CECs). In this study, we use both a 3D mini-ovary culture system and an in vivo mouse model to test the ovarian disrupting effects of PFAS, including three long-chain PFAS (PFOf, PFso, PFna) and three short-chain PFAS (PFPha, PFbs, and GenX). The first, immature mouse ovaries cultured ex vivo were treated with each PFAS of human-relevant concentrations at 0, 1, 10, 100, and 250 µM for 5 days, whereas, mature ovaries were treated for 48 hours. Exposure to 250 µM PFNa but Not GenX during the follicular stimulating hormone (FSH)-dependent follicle maturation window suppressed follicle and ovulation. Follicles treated with 250 µM PFso but Not GenX during the follicle stimulating hormone (FSH)-dependent follicle maturation window had smaller follicle size and suppressed ovulation and progesterone secretion, indicating that PFNa disrupts FSH-dependent follicle maturation to cause the suppression of follicle growth and ovulation. RT-qPCR results showed that exposure to 250 µM PFso during the follicle maturation window suppressed the expression of genes that are essential for follicle maturation and ovarian steroidogenesis, including Ccnd2, Pcn2, Fshr, Cyp19a1, and Hsd17b1. Single-follicle RNA-sequencing analysis revealed that pathways of "DNA replication", "Cell cycle", "Mismatch repair", "Homologous recombination", and "PS3 signaling pathway" were significantly altered. When follicles were treated with PFNa and GenX during the luteinizing hormone (LH)-dependent follicle maturation window, 250 µM PFso, but Not GenX significantly inhibited follicle rupture and progesterone secretion, indicating that PFNa disrupts FSH-dependent follicle maturation to cause the suppression of follicle growth and ovulation. PGCG pathway disruption results showed that exposure to 250 µM PFso during the follicle maturation window suppressed the expression of genes that are essential for follicle maturation and ovarian steroidogenesis, including Ccnd2, Pcn2, Fshr, Cyp19a1, and Hsd17b1. Single-follicle RNA-sequencing analysis revealed that exposure to PFso during the follicle maturation window significantly altered pathways of cytokine-cytokine receptor interaction, CAM signaling pathway, calcium signaling pathway, and ovarian steroidogenesis. Using in vivo mouse superovulation model, we demonstrated that PFso decreased the number of ovulated eggs in a dose-dependent manner. Together, our study demonstrates that long-chain PFAS interfere with gonadotropin-dependent ovarian follicle maturation, hormone secretion, and ovulation.
steroidogenic genes, EGF receptor ligands, ovulation in the exposure to PFOS disrupts gonadotropin-dependent ovarian follicle maturation and effects on lactogenesis. Milk protein and lipid secretion will be measured post-exposure to determine PFAS to PFAS. Pharmaceuticals known to interfere with lactogenesis will also be used. Normal mouse MECs, HC11, were seeded in growth media with 0.5% methanol, and mammary cell responses to lactogenic hormone stimuli and secretory activities. on (a) mammary epithelial cell (MECs) proliferation and (b) terminally differentiated mammary cell responses to lactogenic hormone stimuli and secretory activities. Normal mouse MECs, HC11, were seeded in growth media with 0.5% methanol, and exposed in duplicate to 0.5% methanol and nine concentrations of individual PFAS (PFOS, PFOA, PFPeA, PFDA, and PFHxS) over a 24 hr period. The proliferation curves of HC11 cells were studied pre- (0 hr) and post-exposure (24 hr) to various PFAS mixtures using brightfield, live cell imaging and related software. The area under the non-linear fit curves demonstrated the effects of PFAS on proliferation. As the area increased, it signified higher cellularity and higher growth. Characterization of the gene expression data suggest that PFAS induce HC11 cell proliferation, and the doubling time of cells increased with increasing PFAS dose until doses near 100 µM. The average percent increase in PFAS-stimulated proliferation over controls (between 35-40%) was statistically significant (P<0.05). Whether high dose effects are related to cytotoxicity is currently under investigation. The individual PFAS having the most potent effects on MEC proliferation were, in rank order: PFOS, PFDeA, PFHxS, PFOA, and PFPeA. However, PFOS dose-dependently reduced the percentages of ruptured follicles and the release of oocytes at meiosis of metaphase II (MI) in response to hCG-induced ovulation in vitro. Exposure to PFOS during the ovulation window also suppressed follicle rupture and follicle development in a dose-dependent manner. RT-qPCR revealed that exposure to 250 µM of PFOS during the ovulation window repressed the expressions of several established oocyte genes, including the follicle rupture-related genes, Plau, LOC285170, and ovarian steroidogenic genes, Star and Cyp11a1. In summary, our study demonstrates that exposure to PFOS disrupts gonadotropin-dependent ovarian follicle maturation and ovulation in the eIVFG system.

PFHxS, PFNA, PFOA, and PFOS increases the odds of long-term amenorrhea in women. The multiple logistic regression analysis using HNANES 2013-2018 cycles, including 914 women with normal menstruation and 43 women with long-term amenorrhea. The methods of log-transformation and adjustment were used to analyze blood concentrations of PFAS. Statistical differences in the covariates between the outcome groups were evaluated using a chi-square test for categorical and a t-test for continuous variables. Multiple logistic regression models were used to examine the associations. The analysis showed that women with normal menstruation and long-term amenorrhea had comparable distributions of all covariates, including age, education, marriage, health insurance, income, body mass index (BMI), smoking history, and the use of hormonal contraception, except those women with long-term amenorrhea tended to be Non-Hispanic White compared to other ethnicities. Compared with menstruating women, women with long-term amenorrhea had significantly higher log-transformed means of PFNA, PFPO, PFOS, PFDeA, and PFHxS. Multiple logistic regression analysis using continuous log-transformed data revealed that after the full adjustments, the blood concentrations of PFDeA, PFHxS, and PFPeA increased, women were more likely to experience long-term amenorrhea. Moreover, when the blood concentrations of these five PFAS species in women with long-term amenorrhea were divided into 4 quartiles, there were significant associations in the fourth quartiles and the odds of quadrants 2 to 4 were concentration dependent for all five PFAS. As another example, the odds ratios for PFPeA and PFHxS increased, women were more likely to experience long-term amenorrhea. The per- and polyfluoroalkyl substances (PFAS) are a group of thousands of industrial products, such as cookware coating, textiles, surfactants, and aqueous firefighting foams. PFAS are highly resistant to environmental degradation and thus tend to bioaccumulate in multiple environmental media, such as in the drinking water supplies in both the US and worldwide. After absorption, the high stability of PFAS makes them rarely metabolized in the human or animal body, resulting in a long half-life of up to 6-9 years. Using the National Health and Nutrition Examination Survey (NHANES) database, we aim to conduct a cross-sectional epidemiological analysis to investigate associations between blood concentrations of 7 major long-chain PFAS and reproductive aged women’s long-term amenorrhea. Here long-term amenorrhea is defined by the absence of menstrual period in the most recent 12 months without self-reported menopause, which is a marker implicating women’s dysregulated ovarian and/or uterine functions. A total of 957 women of reproductive age (19-49 years) were included from NHANES 2013-2018 cycles, including 914 women with normal menstruation and reproductive aged women’s long-term amenorrhea. The methods of log-transformation and adjustment were used to analyze blood concentrations of PFAS. Statistical differ-
Therefore, disruptions of estrogen receptor function in pregnancy and lactation may have immediate effects on lactation and long-lasting effects on breast cancer risk. Indeed, for decades pharmaceutical estrogens have been used for reducing lactation in non-nursing mothers, though the molecular mechanisms for these effects have never been evaluated. Further, our previous results suggest that EDC exposures during these sensitive windows can alter mammary reorganization to offset parity-induced protection against breast cancer. Because the mechanisms of action for EDCs are complex, it is unknown if direct activation of estrogen receptor during pregnancy and lactation is the main driver disrupting both lactation and the protective changes associated with parity. Here, we examined the effects of 17α-ethinyl estradiol (EE2), on the parous mouse mammary gland. EE2 is a model estrogen receptor agonist used in contraceptives and to reduce milk production in non-nursing parous women. Pregnant CD-1 mice were treated with either 0 or 20 μg/kg/day EE2 from pregnancy day 9 until weaning at lactation day 21. We also included an age-matched unexposed nulliparous group. To determine immediate effects on maternal lactational capability, dams and litters were evaluated across the nursing period. The length of pregnancy was significantly increased in EE2 exposed animals in an average of 0.4 days, while the birth weight of pups was reduced by an average of 1.5 pups and initial litter weights were reduced by an average of 16% in the EE2-exposed group. By the end of the nursing period, EE2-exposed litters weighed on average 33% less than controls while nearly half of EE2 pups had delayed eye opening, a developmental milestone. To evaluate the more long-term effects of EE2 on the post-involution mammary gland, mice exposed to EE2 during pregnancy and lactation were then unexposed for 5-6 weeks. After this wash-out period, mammary glands were collected and assessed for changes in histomorphology. Average epithelial volume fraction was higher in the post-lactational group than the nulliparous group, a feature seen in previous studies. However, interestingly, the EE2-exposed group were not different from parous controls as seen with previous estrogenic chemicals. Future work will evaluate the effects of exposure to EE2 during pregnancy and lactation on cellular and molecular outcomes in the post-involution mammary gland including hormone receptor expression, immune cell number, and gene expression. Understanding the role that estrogen receptor activation plays in altering the protective nature of pregnancy on breast cancer will be critical for mitigating the effects of estrogenic environmental chemicals on pregnant women.

**3581** The Functional Role of Adipocyte-Modulated AhR in Multiple Myeloma

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Multiple Myeloma (MM) is an incurable blood cancer characterized by the uncontrolled growth of plasma cells residing in the bone marrow. To develop preventative strategies to combat this deadly disease, researchers are exploring how environmental and lifestyle choices contribute to carcinogenesis. Obesity and age are risk factors of MM incidence and disease progression from pre-MM disorders and are both associated with elevated marrow adiposity. However, the role of adipocytes facilitating MM in obese patients is unknown. Recent data from our laboratory has shown that factors released from adipocytes modulate the expression and function of the aryl hydrocarbon receptor (AhR). This data corroborates with patient data showing that dynamic expression of AhR and its target genes are associated with worse clinical outcomes. AhR responds to environmental chemicals known to promote carcinogenesis by modulating metabolic, inflammatory, and proliferative pathways in ligand and tissue-specific manners. Separate studies have shown that the AhR is 1) a negative regulator of adipogenesis and 2) promotes cancer initiation in multiple in vivo models, but a potential link between the AhR and obesity-driven cancers is unknown. Our working hypothesis is that AhR activation contributes to obesity-mediated MM progression. To test this hypothesis, we conducted three separate studies that will lead to novel targets for intervention that could be used for future drug discovery efforts.

**3582** Arsenic, Polycyclic Aromatic Hydrocarbons, and Metal Exposure and Association of Cancers among Women

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In the United States, cancers that most often affect women are breast, colorectal, uterine, lung, cervical, skin, and ovarian cancers. Environmental pollutants may have a significant role in initiation and progression of these cancers among women. However, limited study has been conducted on this issue. This study aimed to assess seven species of arsenic, seven types of polycyclic aromatic hydrocarbon (PAH) compounds, and fourteen types of metals (cadmium, barium, cobalt, molybdenum, mercury, cesium, manganese, antimony, lead, tin, strontium, tungsten, thallium, and uranium) concentrations in urine and their correlation for cancer among women. We performed a cross-sectional analysis of the 2011-2012, 2013-2014, and 2015-2016 National Health and Nutrition Examination Survey (NHANES) data using logistic modeling of the complex weight survey design. Data from 4,956 women aged 40 years and older were analyzed. The statistical analysis was done using “R” version 4.0.4 software. In the demographic variables, educational levels of age and sex group and above were significantly associated with higher odds of breast cancer. In the race/ethnicity category, non-Hispanic Blacks had higher odds of having both uterine and cervical cancers. Hispanics other than Mexicans, women aged 70 years and older, and widowed, those who had ever smoked more than 100 cigarettes in a lifetime had significantly higher odds of developing cervical cancer. Five types of urinary PAHs, includingacenaphthene and fluoranthene had odds ratio (OR): 13.372, 95% confidence interval (CI): 1.218, 146.829 and OR: 40.686, 95% CI: 4.670, 354.466, third quartile of 3-hydroxyfluorene [OR: 4.256, 95% CI: 1.105, 16.394], third quartile of 1-hydroxyphenanthrene [OR: 11.892, 95% CI: 1.405, 100.672] and second and third quartiles of 1-hydroxyphenanthrene [OR: 18.927, 95% CI: 2.377, 150.675 and OR: 27.103 95% CI: 1.667, 440.642] showed positive associations with increased odds of uterine cancer. Among metals, second [OR: 21.998, 95% CI: 2.067, 234.078] and third [OR: 28.753, 95% CI: 2.973, 278.041] quantiles of urinary cobalt showed positive associations with increased odds of uterine cancer. Second quantiles of arsenocholine [OR: 4.447, 95% CI: 1.394, 14.181] and total arsenic [OR: 4.717, 95% CI: 1.267, 17.563] showed positive associations with increased odds of uterine cancer. The study observed that five urinary types of PAHs, cobalt, arsenocholine, and total arsenic, are significantly associated with uterine cancer among women. Further studies in humans are suggested to support or refute this finding.

**3583** Tretinoin Induces Luminal Differentiation in Arsenite-Transformed Malignant UROtsa Cells


Human uterine bladder cancer (UC) is the fifth most common cancer in the United States. Chronic environmental exposure to inorganic arsenic has been linked with the development of human uterine bladder cancer. Histologically, Bladder UC is classified into non-muscle invasive (NMIBC) and muscle-invasive (MIBC) UC. Genetically, MIBC UC is characterized by high levels of copy number gains, while NMIBC UC is characterized by low levels. In order to identify candidate targets for the treatment of UC, we investigated the effects of 17α-ethinyl estradiol (EE2), a retinoid reported to reduce aggressiveness in multiple solid cancers. The role of ERα in uterine cancer induced by inorganic arsenic has not been investigated. Our study is the first to determine if EDCs induce ERα expression and function in malignant UC cells. We evaluated the effects of treating arsenic-exposed UROtsa-A cells with EE2 on the expression of different luminal and basal markers. Tretinoin treatment in UROtsa-A cells reduced cell proliferation and increased the expression of luminal differentiation markers (GATA3, FOXA1). Moreover, Tretinoin reduced the expression of basal keratins (KRT1, KRT5, KRT16). Our data indicate that in the presence of arsenic can reduce aggressive phenotype and promote the luminal differentiation of basal uterine cancer. Therapeutics that target the retinoid acid signaling pathway will improve the survival of uterine cancer patients.

**3584** Characterizing PLD1 and PLD2 Mutations in Human Cancers


Phospholipase D plays a critical role in cell growth, division, metabolism, and survival. Because cancer cells have uncontrolled growth, division, and survival, sustained by altered metabolism, we hypothesized that PLD gene mutations lead to cancer development. To test our hypothesis, we searched for PLD mutations in The Cancer Genome Atlas (TCGA) dataset. TCGA is a joint program of the National Cancer Institute and the National Human genome research institute that aims to catalog major cancer-causing genomic alterations. TCGA has genome sequences of 20,000 human samples (10,449 cancer and 9,551 matching normal tissue), including 33 different types of cancer. In this study, we found that the mutation landscape of PLD1 and PLD2 genes are very different. PLD1 is altered in 8% of TCGA cancer patients, with a total of 267 PLD2 somatic mutations. For both PLD1 and PLD2, the most frequent type of mutation was single base substitution. In addition, the primary sites with the highest prevalence of PLD1 or PLD2 mutation were the uterus, lung, skin, stomach, and colon. This study provides the first attempt at determining the prevalence of PLD mutation in human cancers samples. Because PLD controls cellular physiology as part of signaling pathways, future studies will evaluate the relationship between PLD mutation and other gene mutations in the same pathway, during cancer development. Overall, this study is very important
Tris (Chloropropyl) phosphate (TCP) is an organophosphorus flame retardant and plasticizer used in multiple consumer products and construction materials and has become a global ubiquitous contaminant. A series of in vitro and in vivo studies, including 3-month and 2-year exposures in Sprague Dawley [HSD:Sprague Dawley®SD; from the National Toxicology Program, to aid in characterizing human health hazards. A commercial mixture of TCPP was characterized for composition and stability in feed formulations and administered at 0 - 20,000 ppm in both the 3-month (10/sex/species) and 2-year (50/sex/species) studies. In rats, perinatal TCPP exposure to pregnant females also occurred from gestation through lactation. In addition to a complete pathology evaluation, several in vitro (genetic toxicity, Tox21 data) and in vivo (plasma concentrations at 3, 6, 12, and 18 months, 5-day rat liver toxicogenomics, whole exome sequencing (WES) of male mouse liver tumors) data were integrated to inform risk assessments as well as elucidate mechanisms of toxicity and carcinogenicity. In the 3-month studies, there was an exposure concentration-related decrease in mean body weights and liver was identified as a target organ in both species and sexes. Mild bile duct hyperplasia was observed in rats exposed to ≥ 10,000 ppm TCPP and hepatocellular hypertrophy was observed in mice exposed to ≥ 5,000 ppm TCPP. Additional notable findings in male and female rats (increased thyroid weights and larger thymic cortices) and male mice (renal tubule cytoplasmic alterations) were observed at higher exposure concentrations of TCPP. In the 2-year studies, higher incidences of hepatocellular carcinomas (HCCs), compared to control, were observed in both sexes in mice and to a lesser extent in male rats. In addition, higher incidences of uterine adenomas or adenocarcinomas compared to controls were identified in female rats. TCPP plasma concentrations increased with exposure concentration, but not with time, suggesting TCPP does not bioaccumulate. TCPP was not positive in bacterial mutagenicity or rodent micronucleus assessments. In vitro Tox21 data as well as 5-day rat toxicogenomic data suggested that TCPP is a weak activator of CAR/PXR and PPARs, and a moderate activator of FXR and AhR. Therefore, TCPP is not a non-genotoxic rat liver carcinogen. Genomic dose response analysis from the 5-day studies indicated alterations in transcriptional responses as low as 10 mg/kg/day in the liver with concerted transcriptional change occurring at approximately 20 mg/kg/day. WES analysis of HCCs indicated that the mutation burden, and mutation signatures were similar between human and rodent tumors arising spontaneously or due to chemical exposure to TCPP. These studies demonstrated that TCPP exposure in rodents results in low increases in tumor incidences, likely through non-genotoxic modes of action, and provides insight into the potential human health hazards due to chronic exposure.

3585 Intervention of 3-Aminobenzamide Modulated PARP-1- NLRP3 Inflammasome and Autophagy-Signaling Pathways in Colitis-Associated Colorectal Cancer: A Comparative Study with Olaparib in Mice


Colitis-associated colorectal neoplasia is the third most life-threatening consequences of long-term ulcerative colitis. PARP-1 inhibitors showed promising anticancer activity in a number of preclinical studies. The FDA approved the same for advanced ovarian and metastatic breast cancer. The present study evaluated the possible protective effects of PARP-1 inhibitors, i.e., 3-aminobenzamide (3-AB) and Olaparib in azoxymethane/dextran sulphate sodium induced Colitis Associated Colorectal Cancer (CACC) in mice. Olaparib was used as a specific inhibitor to compare the effect of 3-AB in the present investigation. For the induction of CACC, male BALB/c mice were administered with single injection of azoxymethane (AOM; 10 mg/kg; i.p.) and three cycles of dextran sulphate sodium (DSS; 3 % w/v) were provided in drinking water. One week after the DSS treatment, 3-AB (5, 10 and 20 mg/kg; i.p.) and Olaparib (10 mg/kg, peroral) were administered and continued till sacrifice. 3-AB and Olaparib treatment significantly decreased the progression of CACC by the downregulation of inflammatory markers, such as PARP-1, NLRP3, ASC, Caspase-1 and TNF-a as revealed by RT-PCR analysis. Further, 3-AB and Olaparib treatment modulated the expression of autophagy markers, such as beclin-1, LC3BII/LC3BII and p62 as revealed by western blot analysis. Furthermore, the vihagocyte apoptosis response for these and 2 other chemicals. Overall, these analyses revealed that chemicals with exposure disparities induce significant changes in pathways involved in breast cancer initiation and progression, paving the way for future study of specific genes and mechanisms altered by these chemicals.
Bladder cancer is the most common malignancy of the urinary tract. It is classified into non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). NMIBC accounts for about 70% of bladder cancer cases with a relatively high survival rate, while MIBC has a higher mortality rate with a 5-year survival rate of less than 10%. Thus, identification of new therapeutic strategies is critical for improving bladder cancer patient outcomes. Isorhapontigenin (ISO), a stilbene derivative from Chinese herb Gentiana lutea, displays a strong anti-cancer effect on a variety of human cancers, including lung cancer, pancreatic cancer, colon cancer, gastric cancer, breast cancer, and bladder cancer. However, the mechanism underlying ISO-mediated cancer inhibition is not fully understood. To identify the molecules and pathways critical to ISO’s anticancer activity, we conducted whole transcriptome profiling of ISO-treated T24 cells. RNA-seq analysis identified 968 differentially expressed genes, including 550 upregulated genes and 408 upregulated genes. Ingenuity pathway analysis (IPA) revealed that ISO treatment resulted in reduced expression of genes involved in cell movement, migration, invasion, glucose metabolism, proliferation, and angiogenesis. HIF1α signaling pathway, one of key pathways regulating cell mobility and glucose metabolism, was significantly downregulated, which is further confirmed by downregulation of a large number of HIF1α target genes. Consistently, ISO is able to reduce HIF1α protein levels in a dose- and time-dependent manner. Further investigation using proteasome inhibitor (MG132) and cycloheximide chase assay revealed that ISO reduced HIF1α protein stability in a proteasome-dependent manner. ISO-mediated degradation of HIF1α was demonstrated to be reliant on protein residues 580-810, which includes the C-terminal transactivation domain. Interestingly, a HIF1α mutant with two hydroxylations sites mutated (P402A and P564A) in the oxygen-dependent degradation domain showed a similar trend to wild type HIF1α, suggesting that ISO induces the degradation of HIF1α protein in an oxygen-independent manner. HIF1α protein is commonly upregulated in many human cancers and contributes to tumor progression and metastasis. HIF1α is a promising target for cancer therapeutics because tumor survival and progression are dependent on its downstream targets in cell metabolism, proliferation, angiogenesis, migration, and invasion. Taken together, our results demonstrate that ISO inhibits bladder cancer progression and metastasis via promoting proteasome-mediated degradation of HIF1α protein. Our findings will provide new insights for developing a better therapeutic strategy for human high-grade invasive bladder cancer.

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Multiomic approaches to analyze various disease processes facilitate improved functional and mechanistic understandings of disease. In this study, whole exome sequencing and differential gene expression were performed on archival rat (F344/Tac) liver samples exposed 90 days to a collection of hepatocarcinogens. The top identified pathways enriched with upregulated genes across the hepatocarcinogens included differentially expressed probes at 90 days with a large percentage of upregulated compounds (AFB1 and MEG: hepatocarcinogens). However, cancer associated genes were common between the two hepatocarcinogens. The liver samples were microdissected to extract the conducting airway. The conducting airway was incubated with 14C-labeled naphthalene (250 μM) or 1,2-naphthoquinone (250 μM) for 1 hour (T1), then processed immediately or allowed to incubate in unlabeled media for the remaining 23 hours (T24). DNA was extracted from the airway samples, processed into graphene, analyzed via accelerator mass spectrometry to measure isotope ratios, and then converted into measurements of chemical-induced DNA adducts. Two-way analysis of variance (ANOVA) with Tukey’s post-hoc test showed there were significantly more naphthalene-DNA adducts and 1,2-naphthoquinone-DNA adducts versus unexposed controls (P < 0.01), as expected. There were no significant differences in the amounts of DNA adducts detected at T1 and T24. No sex differences in the formation of DNA adducts were observed. Compared to naphthalene, 1,2-naphthoquinone was a far more potent adductor at both T1 and T24 (P<0.001); this is likely attributable to detoxification of naphthalene metabolites (such as naphthalene oxide) before the naphthoquinone adduction. The experiment was conducted to determine the differences in the amounts of DNA adducts between naphthalene and its metabolites, as we are using this airway explant model to evaluate levels of DNA adducts formed with 1,2-dihydronaphthalene, another naphthalene metabolite and precursor of 1,2-naphthoquinone. In conclusion, naphthalene- and 1,2-naphthoquinone-DNA adducts remain stable over 24 hours in wild-type C57BL/6 mouse airway explants, confirming adduct stability in another strain of mice. Supported by T32 ES007058 and R01 ES020867.
Piperlongumine is a phytochemical produced long pepper that was identified in a library screen of natural products as a potent inhibitor of cancer cell and tumor growth via induction of reactive oxygen species (ROS). Many of the response and genes modulated by piperlongumine are similar to those reported for bis-indole derived compounds (CDIMs) that bind nuclear receptor 4A1 (NR4A1, Nur77). Incubation of piperlongumine with the ligand binding domain (LBD) of NR4A1 showed that piperlongumine is an NR4A1 ligand with a KD value of 7.1µM. We also observed that the anticancer activities of piperlongumine were similar to those reported for previously identified NR4A1 ligands in colon cancer cells. For example, piperlongumine inhibited NR4A1-dependent luciferase activity in SW480, RKO, and HCT116 cells transfected with a GAL4-responsive reporter gene (UAS-luciferase) and a GAL4-NR4A1 chimera indicating the piperlongumine is an NR4A1 ligand which acts as an antagonist. In addition, piperlongumine inhibits colon cancer cell growth using the XTT assay, inhibits cell migration in a scratch assay, induces apoptosis (PARP cleavage) and induces progressive morphological changes. Treatment with 7.5 and 15 µM piperlongumine caused colon cancer epithelial-like cells to shrink and became rounded. Also, cells detached from the plate and typical apoptotic bodies were seen at the boundaries of the detached cells. Treatment of HCT116 cells with piperlongumine also decreases expression of two pro-reductant gene products, namely thioredoxin domain containing 5 (TXNDC5) and isocitrate dehydrogenase 1 (IDH1) which have previously been identified as NR4A1-regulated genes. This suggests the induction of these oxidative gene which in turn activates (phosphorylates) AMPK and inhibits the mTOR signaling pathway. These responses are also observed in cells after treatment with NR4A1 agonists or after knockdown. These results and ongoing studies demonstrate that piperlongumine-induced ROS and downstream anticancer activities are due in part to the binding of piperlongumine to NR4A1 and subsequent activity as an NR4A1 antagonist.
Hexavalent Chromium-Induced Cohesin Malfunction Drives Chromosome Instability, a Key Step in the Mechanism of Carcinogenesis

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Hexavalent chromium [Cr(VI)] is an environmental and occupational lung carcinogen. Although it is not completely known how it induces cancer, chromosome instability is central to its carcinogenic mechanism. Cohesin, a ring protein complex, is involved in genomic stability by maintaining sister chromatid cohesion at chromosomal arms and centromeres and it also maintains centriole cohesion and regulates chromatid orientation at the DNA loop level. Thus, it is well-known that cohesin malfunction can lead to chromosome instability and cancer. Here, we hypothesize Cr(VI) exposure leads to cohesin malfunction by targeting 5 key regulatory proteins in the unloading of cohesin: separase, PDSSA, PDSSB, WAPL and Sororin. PDSSA, PDSSB and WAPL remove cohesin from chromosome arms during prophase, while separase removes it from the centromere and centrioles during anaphase. WAPL, a key stabilizer and cell cycle regulator, maintains the function of WAPL. To test this hypothesis, human lung cells were exposed to zinc chrome, a representative particulate Cr(VI) compound, for various concentrations ranging 0 to 0.3 ug/cm² and various time points (24, 72 and 120 h). A chromosome aberration assay was performed to measure cohesin function at chromosomal arms and centromeres. We found 120 h exposure to Cr(VI) induced cohesin malfunction indicated by increased premature anaphase, premature centromere division and centromere spreading. We also examined cohesin function at centromeres and centrosomes using immunofluorescence methods. Results confirmed Cr(VI)-induced cohesin malfunction, manifested in this approach as aberrant centromere amplification and centriole disengagement. We further considered the effects of Cr(VI) on these 5 key regulators of cohesin measured with Western blot and RNA-seq analyses. The data showed Cr(VI) disrupts these regulators. For example, active separase increased after 120 h Cr(VI) exposure, measured by cleaved protein levels, indicating overactive separase activity, which could prevent cohesin unloading at the centromere and centrioles and activating the aberrant phenotypes mentioned above. PDSSA and PDSSB protein levels increased after 24 h of Cr(VI) exposure, but this effect was lost after 120 h, suggesting cohesin unloading from the chromosome arms might be impaired. Although not statistically significant, the changes in the mRNA levels followed these trends. WAPL protein and mRNA levels did not change, however, Sororin, a protein that antagonizes WAPL and increases cohesin complex stability, was greatly downregulated at both the mRNA and protein levels after 120 h exposure, suggesting lack of Sororin may be destabilizing the cohesin complex. Previous studies showed PDSS5 paralogs, WAPL and the cohesin complex, are critical for DNA repair pathways such as homologous recombination (HR). It is clear that homologous recombination repair is inhibited after prolonged Cr(VI) exposures, however, the effects of Cr(VI)-induced disruption of cohesin regulators and cohesin malfunction in the inhibition of homologous recombination repair pathway remains unknown. Altogether, here we show Cr(VI) exposure leads to the malfunction of cohesin probably due to the targeting of cohesin regulators, which can lead to numerical chromosome instability, a key step in Cr(VI)-carcinogenesis. Acknowledgements: Supported by NIEHS grants R01ES016893 (JPM) and R5SE032876 (JPM) and NCI grant R25CA134283 (CRC).
similar to mutational signatures previously identified in lung cancer genomes of smokers. Collectively, these data provide insight on how genomic features shape the accumulation of alkylating products in the genome and reveal predictive strategies for linking single-nucleotide resolution in vitro damage maps with human cancer mutations.

3601 Stable Isotope Tracing of Glucose in N-Acetyltransferase 1 (NAT1) Knockout Breast Cancer Cells Reveals NAT1 Plays a Role in the Proper Functioning of Mitochondria
Breast cancer is one of the leading types of cancer deaths worldwide. Recent reports found that arylamine N-acetyltransferase 1 (NAT1) is frequently upregulated in breast cancer, suggesting NAT1 could be a potential therapeutic target for breast cancer. Previous studies have established that NAT1 knockout in breast cancer cells line leads to metabolic changes and growth reduction both in vitro and in vivo, suggesting that NAT1 contributes to the energy metabolism of breast cancer cells. However, there are conflicting studies on the impacts of NAT1 knockout on mitochondrial metabolism. Further, proteomic analysis and non-targeted metabolic investigations in breast cancer cells suggested that NAT1 knockout may change the fate of glucose as it relates to the TCA/Kreb cycle of the mitochondrion. In this study, we used stable isotope resolved metabolomics using [U-13C]glucose to determine the effect of NAT1 knockout on the metabolic profile of MDA-MB-231 breast cancer cells. We incubated breast cancer cells (MDA-MB-231 cells) and NAT1 Crispr KO cells (KO#2 and KO#5) with [U-13C]glucose for 24 h. Following the 24 h incubation cells were collected, and metabolites were extracted and analyzed by 2DLC-MS. Statistical comparisons were carried out using GraphPad Prism comparing KO#2 and KO#5 to MDA-MB-231 cells. The data showed a decrease in the 13C enrichment of TCA/Krebs cycle intermediates in NAT1 KO cells compared to the MDA-MB-231 cells. Specifically, 13C-labeled citrate, isocitrate, a-ketoglutarate, fumarate, and malate were all decreased in NAT1 KO cells. We also observed increased L-lactate levels in the NAT1 KO cells and decreased 13C enrichment in some nucleotides. These data provide further evidence and clarify the impacts of NAT1 knockout on mitochondrial metabolism. Thus, based on literature reports and the data presented here, we suggest that NAT1 plays a role in the proper functioning of mitochondria and the TCA/Krebs cycle in breast cancer cells. Further work is aimed at investigating how NAT1 impacts the mitochondria in breast cancer cells. This work is supported by USPHS grants T32-ES011564, P42-ES023716, and P30-ES030283.

3602 Extracellular Vesicles as Mediators of Nickel-Induced Cancer Progression
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Emerging evidence suggests that extracellular vesicles (EVs), which represent a crucial mode of intercellular communication, play important roles in cancer progression via transferring oncogenic materials. Nickel (Ni) has been identified as a human group I carcinogen, however, the underlying mechanisms governing Ni-induced carcinogenesis continue to be parsed. Here, we present data demonstrating that Ni exposure generates EVs that contribute to Ni-mediated carcinogenesis and cancer progression. Human bronchial epithelial (BEAS-2B) cells and human embryonic kidney293 (HEK293) cells were chronically exposed to Ni to generate Ni-treated (Ni-6W), Ni-transformed BEAS-2B cells (Ni-3) and Nitransformed HEK293 cells (NHi-4). The signatures of EVs isolated from Ni-6W, Ni-3, NHi-4, BEAS-2B and HEK293 were analyzed. Compared to their respective untreated controls, Ni-6W, Ni-3 and NHi-4 released more EVs. This change in EV release coincided with increased transcription of EV biogenesis markers: CD82, CD63, and Flotillin-1 (FLOT). Interestingly both epithelial and endothelial EVs released from Ni-altered EVs induced inflammatory responses in both epithelial and endothelial cells and increased the expression of coagulation markers in endothelial cells. Release coincided with increased transcription of EV biogenesis markers: CD82, CD63, and Flotillin-1 (FLOT). Interestingly both epithelial and endothelial EVs released from Ni-altered EVs induced inflammatory responses in both epithelial and endothelial cells and increased the expression of coagulation markers in endothelial cells. Therefore, this study characterizes the effect of Ni on EVs and suggests a potential role of EVs in Ni-induced cancer progression.

3603 Particulate Hexavalent Chromium Increases Reactive Oxygen Species Production and Regulates Multiple Inflammatory Pathways in Human Lung Fibroblasts
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Currently available data show hexavalent chromium (Cr(VI)), an established human lung carcinogen, potently impacts chromosomes indicating chromosome instability is a major driving mechanism for Cr(VI) carcinogenesis. However, the carcinogenic mechanism for Cr(VI) is uncertain, and is thought to involve inflammation. In this study, human lung fibroblasts WTHBF-6 cells, the predominant model in particular Cr(VI) carcinogenesis, were treated with zinc chromate particles for 24, 72, and/or 120 hours, at concentrations that reflect occupational and environmental exposures. Then, reactive oxygen species amounts were determined with the CellRox reagent. In addition, treated cells were submitted to RNA-Seq analysis on the Illumina platform, and qRT-PCR was performed to validate RNA-Seq data. The results demonstrated enhanced ROS production and increased inflammation in Cr(VI)-induced toxicity, providing novel insights into the mechanisms by which Cr(VI) causes lung cancer. This work was supported by National Institute of Environmental Health Sciences (NIEHS) NIEHS grants, including 3R3ES032876-01S1 (JCK and JPM) R3ES032876 and P30ES030283 (JPM).

3604 Error-Corrected DNA Sequencing Detects Carcinogenic Processes in RasH2-Tg Mice after Three Months of Exposure to Genotoxic and Non-genotoxic Carcinogens
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The evaluation of new drugs and chemicals for carcinogenic potential is an essential part of modern regulatory safety testing. For most of the period since the requirement’s implementation, it has been synonymous with traditional lengthy two-year rodent bioassays. As shorter rodent bioassays can often be identified by shorter-term in vitro or in vivo tests that obviate the need for carcinogenicity testing, there is a gap on early detection for non-genotoxic carcinogens. The only generally accepted alternative of shorter duration is a 6-month bioassay using the cancer-predisposed RasH2-Tg mouse strain. There has been recent interest in developing molecular biomarkers of changes implicated in neoplastic transformation. In particular, there is a growing body of literature from human studies indicating that morphologically unrecognized small clonal expansions bearing cancer driver mutations (CDMs) often precede tumor formation. In this study we sought to test the hypothesis that detection of these clones might serve as an early metric of tumorigenesis in vivo. We applied Duplex Sequencing (DS), a state-of-the-art form of error-corrected next generation DNA sequencing capable of detection of rare mutations, to assess for CDM clones in historically normal tissues of RasH2-Tg mice following three months of exposure to 3 carcinogens at doses that produced tumors within 6 months; one mutagenic carcinogen, NMU (an alkylating agent in lung) and two non-genotoxic carcinogens, PCB-126 (a direct acting AHR agonist) in liver, and bazedoxifene (BZA, a selective estrogen receptor modulator and indirect acting rodent specific tumorigen) in ovary. We initially targeted a small panel of known cancer driver genes, including the human HRAS gene unique to this strain, along with four endogenous genes. We assessed the relative abundance of single nucleotide changes in cancer driver mutations in vehicle-treated animals. In all cases, the clones were significantly more abundant and larger in treated versus untreated animals, though the relative differences were greatest with NMU, compared to the non-genotoxic agents, BZD and PCB126. Recognizing that screening additional cancer driver genes could potentially improve sensitivity, we carried out whole exome sequencing of tumors arising in the 6-month PCB-126 and BZD treated animals and further analyzed the vehicle-treated group. These findings were corroborated further by RNA-Seq analysis of treated versus untreated tumors, though the relative differences were greatest with NMU compared to the non-genotoxic agents, BZD and PCB126. Our results provide definitive evidence for apical mutagenic effects. The present dose-response studies were conducted to further assess the in vivo genotoxicity, mutagenicity, and other potential early key events in sites of known DCE-induced tumors. Female transgenic Big Blue F344 rats (n=6/group) were exposed by whole-body inhalation (6 h/day for 28 consecutive days)
Developing Duplex Sequencing for Analysis of Clonal Expansion as Potential Early Carcinogenicity Biomarkers to Accelerate Assessment of Nongenotoxic Carcinogens in Sprague Dawley Rats


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Assessing the potential of a novel compound to induce cancer is an essential element of drug and chemical safety testing. Relying on a resource-intensive 2-year rodent study represents a barrier to early and efficient assessment of chemical carcinogenic risk. Whole blood assays exist to measure mutagenic potential in vitro and in vivo, many carcinogens act through non-genotoxic means, thus eluding detection via genotoxic assays. We hypothesize that screening for chemical induction of small growth advantage clones with cancer driver-gene mutations (CDMs) could serve as an early molecular biomarker of carcinogenicity since nongenotoxic carcinogens do not directly cause mutations and resulting tumors will carry multiple underlying CDMs. To test this hypothesis, vehicle or the AHR agonist PCB-126 (IARC Group 1 carcinogen) was administered to wild type rats for 6 and 9 months. RNA sequencing was used to confirm that levels of AHR target gene induction were similar to levels associated with rat liver tumorigenesis. To determine CDMs that may be commonly associated with rat liver tumour formation we first used whole exome sequencing to characterize recurrent CDMs in archived rat liver tumor samples collected previously from two different non-genotoxic treatments, nafenopin and phenobarbital. Eleven recurrently mutated CDMs are ongoing.

Bisphenols-Induced Polyadenylation of Canonical Histone H3.1 mRNA Facilitates Cell Transformation in Human Embryonic Kidney Cells

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While bisphenol A (BPA) has been removed from many commercial products because of its potential carcinogenic effect its replacement with bisphenol S (BPS) and bisphenol F (BPF) are structurally very similar to BPA. However, the potential role of BPA alternatives in carcinogenesis remains unclear. In this study, we aim to investigate the exposure of bisphenols to human embryonic kidney epithelial (HEK-293) cells and their role in bisphenols-induced human carcinogenesis. To determine whether BPA and its alternatives modify canonical histone mRNA processing in vitro, we measured levels of polyadenylation (polyA) of canonical histone (H3.1) mRNA as well as protein expression of Stem-loop binding protein (SLBP), and H3 protein in HEK-293 cells exposed to various doses starting with low concentrations (i.e. 0.1μM and 3 to 10 μM) of these bisphenols for 96 h. HEK-293 cells were also chronically exposed to BPA, BPS and BPF for 12 weeks, and then a cell transformation assay was performed in soft agar and colony formation was assessed. As our laboratory earlier demonstrated that arsenic-induced polyA of H3.1 mRNA by depletion of SLBP in vitro and that polyA of H3.1 mRNA enhanced tumour formation in nude mice, we used arsenic as a positive control. Our results showed that bisphenols downregulate the SLBP level, a critical factor for the stability, processing and translation of canonical histone pre-mRNAs in HEK-293 cells. It has been known that canonical histone mRNAs typically do not end in a poly(A) tail and that depletion of SLBP results in polyA of canonical histone mRNAs that exhibit carcinogenicity. Our results exhibited that bisphenols exposure induced polyA of mRNAs for H3.1 and upregulated H3 protein. Similarly, our findings from soft agar assay also indicated that exposure of HEK-293 cells to low doses of BPA, BPS and BPF caused a cancer-relevant phenotype and leads to malignant cell transformation. In this study, our in vitro cell culture assays showed that a chronic BPA and its alternatives exposure to human kidney cells causes typical epithelial-phenotype alteration. We further identified BPS and BPF are potential human carcinogens in addition to BPA. We also identified that the loss of SLBP and gain of polyA H3.1 mRNA play an important role in BPA and its alternate analogues such as BPS and BPF-induced carcinogenesis.

PPAR-Dependent Sensitivity to Betulin/Betulinic Acid on Tumorigenesis in a Human Malignant Melanoma Cell Line

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Low-survival cancers, including malignant melanoma, require innovative new therapeutic interventions. Emerging evidence suggests that the peroxisome proliferator-activated receptor (PPAR) class of nuclear hormone receptors inhibit carcinogenicity. While bisphenol A (BPA) has been known that cancer cells often end in a poly(A) tail and that polyadenylation of polyA of H3.1 mRNA enhanced tumour formation in nude mice, we used arsenic as a positive control. Our results showed that bisphenols downregulate the SLBP level, a critical factor for the stability, processing and translation of canonical histone pre-mRNAs in HEK-293 cells. It has been known that canonical histone mRNAs typically do not end in a poly(A) tail and that depletion of SLBP results in polyA of canonical histone mRNAs that exhibit carcinogenicity. Our results exhibited that bisphenols exposure induced polyA of mRNAs for H3.1 and upregulated H3 protein. Similarly, our findings from soft agar assay also indicated that exposure of HEK-293 cells to low doses of BPA, BPS and BPF caused a cancer-relevant phenotype and leads to malignant cell transformation. In this study, our in vitro cell culture assays showed that a chronic BPA and its alternatives exposure to human kidney cells causes typical epithelial-phenotype alteration. We further identified BPS and BPF are potential human carcinogens in addition to BPA. We also identified that the loss of SLBP and gain of polyA H3.1 mRNA play an important role in BPA and its alternate analogues such as BPS and BPF-induced carcinogenesis.

Early Estrogenic Effects of the Endocrine Disruptor BPA on the Mammary and Pituitary Glands of ACI Rats

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Breast cancer is the second leading cause of cancer-related death in women. Only 5-10% of all breast cancer cases can be attributed to known hereditary mutations. Thus, the vast majority of breast cancers are caused by external factors. Endocrine disrupting compounds (EDCs), found in plastics, drinking water, and food, pose a concerning threat to human health through their potential carcinogenic properties. EDCs share chemical similarity with the endogenous hormone beta-estradiol and are thus able to activate estrogen receptor related signaling pathways that lead to abnormal proliferation and differentiation, and eventually cancer. Thus, EDCs support the incidence and progression of cancer. We hypothesized that, due to its chemical similarity, bisphenol-A (BPA), a well-known endocrine disruptor, may act like estrogen in an ACI rat model. The ACI rat is highly sensitive to mammary carcinogenesis induced by estrogen. Our group has previously demonstrated that implanting a 9 mg estrogen pellet into the back of the ACI rat induces mammary gland hyperplasia at a five-day timepoint, eventually resulting in overt carcinogenesis at thirty-week timepoints. BPA implantation ranging from 1 to 9 mg did induce similar proliferative changes in the mammary tissue at the five-day timepoint. Mammary gland expression of the estrogen receptor target progesterone receptor (PGR) and the proliferative cell marker PCNA also increased at the 5-day timepoint, as evidenced by upregulation in mRNA expression and increased immunohistochemical staining. At an intermediate six-week timepoint, these changes were not sustained. BPA did have clear endocrine disrupting effects at this timepoint, as exposure altered the pituitary histological structure. However, these effects did not follow the well-characterized estrogen signaling pattern. Future studies will investigate potential hepatic metabolism and clearance pathways to explain the observed reversal of early proliferation in the mammary gland by BPA.

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Bisphenol A (BPA) is an environmentally ubiquitous endocrine disrupting compound that may be carcinogenic to humans. We have previously reported that both male and female mice with early life BPA exposure developed hepatocellular carcinoma (HCC) at 10 months. This is a unique result, since male humans and rodents are 2-4x more likely than females to develop HCC, due to protective estrogen receptor 1 (ESR1) signaling. Here, we report in vitro evidence for our proposed mechanism for BPA-induced liver carcinogenesis in both males and females. We propose that BPA induces cancer through oxidative DNA damage and subsequent mutagenesis. BPA then promotes cancer through cellular proliferation due to disruption of protective ESR1 signaling. To test our initiation mechanism, we exposed HepG2 cells (ATCC HB-8065) to one of the following for 24h and collected DNA 48h post-exposure (n=4/group): 1) 0.4 μM (high) BPA, 2) 0.04 μM (low) BPA, or 3) DMSO vehicle control. These doses are the in vitro equivalents of human liver exposure to 5 μg or 0.5 μg/kg BW/day, which correspond to the maximum and average human environmental exposures. We performed error-corrected Duplex sequencing and observed an increase in A>T/T>A base substitutions in high BPA vs. control (one-tailed t-test, p<0.07). These results are compelling evidence for oxidative mutagenesis due to replication past abasic sites. To test our promotion mechanism, we pre-treated HepG2 cells for five days with either high (10 nM, ~female pre-pubertal), low (5 μM, ~female pre-pubertal), or no (DMSO) estrogen (E2). We then exposed cells to one of the following for 24h and collected RNA immediately (n=3/group; E2 level corresponds to pre-treatment level): 1) high BPA + high E2, 2) high BPA + low E2, 3) high E2 alone, 4) low E2 alone, 5) high BPA alone, or 6) DMSO vehicle control. We performed stranded, total RNA-seq and filtered for genes near ESR1 binding sites in healthy human liver published ChIP-seq data. We observed attenuation of E2-mediated gene expression by BPA in the presence of low, but not high, E2. Specifically, in pre-pubertal conditions (low E2 vs. low E2), 938 ESR1-bound genes were differentially expressed. BPA reversed the direction of E2-mediated expression by repressing genes normally induced by E2 and vice-versa. Most (71%) of these genes were differentially expressed in a post-pubertal setting: high E2 vs. high E2. These results are consistent with BPA binding to ESR1 but not triggering target gene expression changes, indicating inhibitory protective signaling. BPA binds the receptor in low E2, but is outcompeted in high E2 supporting developmental susceptibility. In summary, we describe our proposed mechanism for BPA-induced liver carcinogenesis in both males and females.

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- Benzidine is an aromatic amine used in azo dyes in textile, tattoo ink and paper industries. Benzidine goes N-acetylation to N-acetylbenzidine and N,N-diacyetylbenzidine catalyzed by N-acetyltransferase 1 (NAT1). Previous studies have reported that exposure to benzidine is associated with urinary bladder cancer. However, the mechanisms underlying benzidine-induced carcinogenesis and the effect of NAT1 polymorphism on modulation of the individual risk have yet to be investigated. We have used Chinese hamster ovary (CHO) cells transfected with human CYP1A2 and NAT1*4A2 (variant) or NAT1*4 allele (reference) to NAT1*4B (variant) to measure metabolism of benzidine and its toxic effects. Measurement of benzidine N-acetylation in situ showed that CHO cells transfected with NAT1*14B exhibited over 10-fold lower apparent Km which resulted in higher intrinsic clearance for benzidine compared to CHO cells transfected with NAT1*14. HPRT mutation assay showed higher level of benzidine-induced mutations in CHO cells transfected with NAT1*14B than with NAT1*4 (p<0.001). In cell western assay was used to measure γ-H2AX signal as a marker of DNA double-strand breaks induced by benzidine (5 - 100 μM) for 24 hours. Reactive oxygen species (ROS) levels were measured usingDCFDA fluorescence assays following treatment of cells with benzidine (10 - 100 μM) for 24 hours. Benzidine caused concentration-dependent increase in γ-H2AX signal (indicated of DNA double-strand breaks) in CHO cells (linear trend test p<0.001 and p<0.0001) transfected with NAT1*4 and NAT1*14B respectively. CHO cells transfected with NAT1*4B exhibited significantly higher level of DNA damage than with NAT1*4 (p<0.001). In contrast CHO cells transfected with NAT1*4 showed higher ROS compared to NAT1*14B (p<0.05). Use of a specific NAT1 inhibitor significantly decreased DNA damage (p>0.01) and ROS (p<0.05) induced by benzidine. Our findings suggest that NAT1 polymorphism can modify Benzidine-N-acetylation and genotoxicity. Furthermore, use of NAT1 inhibitors may be a novel approach to mitigate benzidine-induced DNA damage and ROS production. This work partially supported by NIH grants P42ES023716 and P30ES030283.

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degradation by interacting with these zinc finger proteins. Therapeutic iAs exposure inhibits autophagy, but the effect of chronic environmentally relevant iAs exposure (100 nM) has never been studied. The current study investigated the effects of chronic environmentally relevant iAs exposure on both autophagy and proteasomal pathways in two human keratinocyte cell lines. Chronic iAs will suppress both autophagy and proteasomal protein degradation pathways in human keratinocyte cell lines. Human keratinocyte cell lines HaCaT and Ker-CT were chronically exposed to 0 or 100 nM iAs for 7 and 8 weeks, respectively, in independent quadruplicate cultures. Immunoblots were performed to quantify levels of autophagic markers (Beclin-1, LC3-II/III), autophagy substrates (SQSTM1, TRAF2) and proteasomal substrates (MDM2, TP53, CDKN1A, E2F4). Unpaired two-tailed t-test was employed to test differences in expression. p<0.05 was considered significant. In human cell lines treated with autophagy and autophagy substrates (Beclin-1, LC3-II/III, SQSTM1, TRAF2) in both cell lines. Proteasomal substrates (TP53, E2F4) were induced upon iAs exposure in both cell lines, while CDKN1A was induced only in HaCaT cells. iAs exposure suppressed the expression of the MDM2-full length isoform while inducing MDM2-F isoform in both cell lines. Chronic environmentally relevant iAs exposure suppresses both autophagy and proteasomal pathways in two different human keratinocyte cell lines. This global effect on protein degradation could be modulated by iAs mediated targeting and induction of SQSTM1. iAs mediated zinc displacement from SQSTM1 RING finger could dysregulate the molecular crosstalk between autophagy and proteasomal pathways. Grant support: This work was supported in part by National Institute of Health grants R01CA154289, R01ES27778, P30ES030283, and T32ES011564. The views expressed are those of the authors and not of the National Institutes of Health.

3615 Effects of Inhaled E-cigarette (E-cig) Aerosols on Mutagenesis Induced by the Tobacco Carcinogen Benzo(a)pyrene (BaP) in a Mouse Lung Model


The primary aim for developing e-cigs was for cigarette smoking cessation. However, many people are now dual users of cigarettes and e-cigs and may be at a higher risk of developing smoking-related health issues than are conventional cigarette smokers. Tobacco smoke contains many carcinogens, and additionally activates the Aryl Hydrocarbon Receptor (AhR) leading to the induction of a number of proteins, including the CYP1 proteins; and the prolonged activation of AhR has been shown to increase cancer risk in various animal models. Benzo(a)pyrene (BaP), a highly carcinogenic Polycyclic Aromatic Hydrocarbon (PAH), is a major carcinogen in cigarette smoke, charbroiled food and emission exhausts. It is metabolically activated into BP-7,8-diol-9,10-epoxide (BPDE), which reacts with DNA predominantly at the N² position of guanine to produce N²-guanine lesions (e.g., BPDE-N²-deoxyguanosine (BPDE-dG) adducts). The presence of BPDE-dG adducts in human tissues has been established, and BPDE-dG adducts have been detected in bronchial cells of cigarette smokers and are implicated in the initiation of human lung cancer. The aim of this study was to determine the mutagenic effects of e-cig aerosols, in the presence and absence of BaP in the Big Blue (BB) mouse model, which utilizes a transgenic mouse with multiple copies of a mutagenesis reporter gene (cII) within a lambda phage shuttle vector (lacZ) integrated into their genomes. Human lung cancer cell lines (A549, PC-9, H1650) were treated with 0, 2, 5, 10, and 20 μM 1,4-[3H]BaP, 3A4 metabolically activated BaP (BaP metabolites), and non-radioactively labeled BaP (BaP). Treatment with BaP significantly increased the number of cells with lacZ mutagenesis reporter gene were used. The lacZ mice were developed because carcinogenesis bioassays are generally long-term and expensive, and as mutagenesis drives carcinogenesis; an important factor in carcinogenesis bioassays are generally long-term and expensive, and as mutagenesis drives carcinogenesis; an important factor in carcinogenesis bioassays.

3617 Human CYP1B1 Activates uPAR Signaling through Regulation of Oncogenic Mutant p53 to Promote Breast Cancer Cell Invasion


CYP1B1 is known as a major metabolizing enzyme for estrogen and shows tumor suppressor expression. To explore the role of CYP1B1 on cancer invasion, we studied the effects of CYP1B1 in human breast cancer cell lines MCF-7 and MDA-MB-231 with different invasive traits. CYP1B1 significantly induced cell invasion along with expression of urokinase-type plasminogen activator receptor (uPAR), a receptor for uPA, in both cell lines. DUBA, a well-known CYP1B1 inhibitor, induced uPAR expression while TMS, a specific CYP1B1 inhibitor, strongly suppressed uPAR. Interestingly, CYP1B1 activated the uPAR pathway and induced the protein expression of integrin c5 and β1, following up-regulation of uPAR expression via regulation of p53 depending on its mutation status. Surprisingly, CYP1B1 down-regulated wild-type p53 expression in MCF-7 cells while the expression of oncogenic mutant form of p53(37)m in MDA-MB-231 cells was induced by CYP1B1. CYP1B1 treatment in the E3 ligase knockout of DUBA on uPAR expression and the level of cancer cell invasion in MCF-7 cells, whereas nutlin-3a resulted in no significant changes in DUBA-treated MDA-MB-231 cells. Taken together, our data suggest that CYP1B1 induces breast cancer cell invasion through activation of p53-regulated uPAR signaling depending on the mutational status of p53.

3618 Peroxisome Proliferator-Activated Receptor-β/δ Suppresses Proliferation of Human Colon Cancer Cells In Vitro

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Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily of ligand-activated transcription and invasion, concomitantly target genes based on their relative expression and on the presence/absence of ligands and co-effectors proteins. Because many of their target genes regulate metabolism, PPARs have strong potential as therapeutic targets for diseases. For example, agonists of PPARα and PPARγ are in clinical use for treatment of dyslipidemias and diabetes, PPARβ/δ was suggested as a therapeutic target for metabolic disorders, but potential risks in cancer are less clear. Despite earlier evidence suggesting that higher expression of PPARβ/δ was associated with causing colon tumorigenesis, more than 20 years of research now indicates that the opposite may be the case. For this reason, the present studies examined the role of modulating...
PPARβ/δ activity with and without forced expression of the receptor, and in the presence or absence of ligands. In these studies, a real-time cell proliferation assay and a soft agar colony formation assay were employed to investigate the influence of PPARβ/δ in two human colon cancer cell lines. Ligand activation of PPARβ/δ with GW9742 caused an increase in target gene expression, and this effect was markedly enhanced in either RKO or DLD1 cells that over-expressed PPARβ/δ. In contrast, the negative, representative PPARβ/δ ligand G172 caused a decrease in target gene expression. Real time proliferation assay showed that PPARβ/δ-null RKO or DLD1 cells have a higher growth rate than the respective control cell lines. Moreover, ligand activation of PPARβ/δ decreased cell proliferation compared to controls, and this effect was enhanced in RKO or DLD11 cells that over-expressed PPARβ/δ. Results from the soft agar assays also show that ligand activation of PPARβ/δ with GW9742 significantly reduced anchorage-independent growth ability of human colon cancer cells. Combined, the results from these studies suggest that activation of PPARβ/δ can significantly inhibit human colon cancer cell growth rate in vitro, and these results provide basis for further in vivo experiments. Supported by CA299256.

3618a The Proliferative Potential, Cytotoxicity, and Epigenetic Changes of Cisplatin-Sensitive and Cisplatin-Resistant Testicular Germine Cell Tumors in Response to Perfluorooctanesulfonic Acid (PFOS)

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Perfluorooctanesulfonic acid (PFOS) is a ubiquitous substance utilized in industries and a well-known environmental toxicant. PFOS exposure has been associated with testicular cancer, a cancer affecting germine cells. It has remained unclear if PFOS changes the proliferation of cisplatin sensitive and cisplatin resistant testicular germine cell tumors (TGCTs). It is also unknown if PFOS has different cytotoxic concentrations between cisplatin sensitive and cisplatin resistant TGCTs. PFOS may cause upregulations and downregulations in cancer associated genes, this has been undetermined. To answer these questions, triplicates were dosed from 0µM - 250µM of PFOS, and a cell viability assay was conducted to determine proliferative changes and the concentrations toxic to cells. RT-qPCR was conducted to determine how mRNA expression of MUC1, FOXB1, SNAP91, and NR1H1 changed due to PFOS exposure. It can be concluded that PFOS increases the proliferative potential of both lineages, with cisplatin resistant cell line increasing at a lower rate in comparison to the cisplatin sensitive cell line. The IC50 of the cisplatin sensitive line and the IC50 of cisplatin resistant line is undetermined, though cisplatin resistant cells likely have a higher IC50. The RT-qPCR determined nothing of significance with respect to PFOS and the tested genes, but MUC1 and SNAP91 have a trend of downregulation.

3619 Whole Genome Sequencing Analysis of Mutagenicity of N-Nitrosodiethylamine Using Caenorhabditis elegans Models


N-nitrosamines are well known impurities in medicines while a large number of them are mutagenic and carcinogenic. Recently, several N-nitrosamines have been detected in drugs, leading to renewed interest in understanding how these chemicals exert their biological effects on cellular systems. The toxicological effects of N-nitrosamines have been mainly studied in mammalian cells. Due to the difficulty of the in vitro system for metabolic activation of N-nitrosamines, more studies using in vivo systems are required. In recent years, Caenorhabditis elegans has become a promising in vivo system because it is a relatively cheap and quick way to assess possible toxicities of chemicals. Because of many common structural and genetic qualities with mammals, C. elegans has become an alternative animal mode for toxicity testing. In this study, mutagenicity of N-nitrosodiethylamine (NDEA), a typical nitrosamine impurity found in several FDA-regulated drugs, was investigated using C. elegans. C. elegans worms were treated with different concentrations of NDEA at L4 development stages for measurement of its acute toxicity and mutagenicity. The acute toxicity was evaluated with a WMicroTracker to determine the toxic effects of the different concentrations of this compound on the worms’ movement and to set a bridge for the dose range for our mutagenicity study. The mutagenicity was evaluated using whole genome sequencing to verify whether NDEA could induce mutations in C. elegans. The data showed that NDEA elicited acute toxicity and mutagenicity in a dose-dependent manner. The main type of mutations induced by NDEA was G→A/T, followed by T→A/C, A→T/G, and A→T/C, which was corroborated by prior studies in mammalian systems. Our results suggest that C. elegans may be able to be used as an alternative animal model for mutagenicity testing of nitrosamines.

3620 Evaluating Acrylamide Toxicokinetics in an In Vitro Integrated Organ Platform

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The formation of Acrylamide (AA) in certain food types can occur when cooking at high temperatures. The formation of AA as well as other chemicals during high temperature food preparation and the subsequent formation of reactive metabolites have prompted regulatory agencies globally to investigate risk to human health. Given the large number of reactive chemicals formed during high temperature cooking and the need to develop human toxicokinetic and organ toxicity data for these compounds, alternative methods may provide a faster and less expensive means of performing initial assessments. Cell and tissue models today are more sophisticated in both their morphology and function. The use of these enhanced cell and tissue models in combination with new in vitro platforms is beginning to show promise for the eventual replacement of animal testing. The aim of this study was to test the ability of a Human Dynamic Multiple Organ Platform to provide data for key ADME parameters and organ toxicity using AA as the model compound. A three compartment (Intestine-Liver-Kidney) circuit was established. The intestine was Epithelial from MatTek, Corp, human hepatocytes were from LifeNet Health Life Sciences, and Kidney cells (HK-2) were from ATCC. Each organ compartment was linked by a simulated blood system. The tubing of the blood system inside the organ compartment consisted of a semipermeable membrane. The movement of the test compound and its metabolites from the point of application to the other organ compartments was by osmotic diffusion into the simulated blood system. The simulated blood circulation was achieved with a micro syringe pump. Each organ culture contained medium optimal for that organ’s growth and there was no net change in compartment fluid volumes. To begin the study, aliquots of an AA stock (300 mg/ml) were diluted with medium to provide final exposure concentrations of 3, 5, and 10 mg in a final volume of 100 µL. This was then applied to the apical surface of the MatTek Epithelial model. Samples from each organ compartment and from simulated blood were collected at 0, 5, 15, 30, 60, 90, 120, 240, 480, 1440 (24 hr), 2880 (48hr), and 4320 min (72 hr). AA movement was measured using LC/MS/MS. At 72 hr samples were collected for determining cytotoxicity. AA was detectable in the intestinal compartment at 30 min, reaching a maximum at 1.5-2 hr. Values returned to baseline after 24-48 hr. In liver and kidney compartments, AA was detected at 1-1.5 hr reaching a maximum after 20-24 hr. AA concentrations were dose and time dependent in each compartment. After 72 hr of exposure to 3 mg AA, intestinal viability was 70%, while the 10 mg dose resulted in a viability less than 60%. Liver ATP and GSH decreased in a dose-dependent manner after 72hr. In kidney, NAG and KIM-1 release also followed a dose-dependent trend. Analysis of kidney key markers ALBmax, ALBmin, MDAmax, and T1/2 was estimated. In conclusion, the Human Dynamic Multiple Organ Plate system predicted AA liver and kidney toxicity and provided key ADME data points. These findings indicate the system can be used as a rapid response model for ADME and toxicity, as well as a model to explore metabolites and mechanisms of adverse effects.

3621 Phototoxicity Assessment and Advanced Qualitative/Quantitative In Vitro Phototoxicity

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The photostability characteristics of new active substances and medicinal products should be evaluated to demonstrate that light exposure does not result in unacceptable change. Many drugs are sensitive to light and therefore their formulated products may degrade. This can result in potency loss, altered efficacy, and adverse biological effects. The European Pharmacopoeia advises light protection for more than 250 drugs and additives. Knowledge of the photochemical behavior of drugs can provide guidance for handling, packaging, and labeling. The sensitivity of a drug to light may vary with its chemical structure, photoreactivity, and nature of delivery form. The study of photochemical reactions provides information on the mode of attachment of the active ingredients in a product. The evaluation of the photochemical stability of drugs and drug products is an essential component of the formulation development process. This Photostability Study aims to provide knowledge of the photostability of compounds and their formulated products to evaluate the following: i) the intrinsic photostability, ii) the shelf life, and iii) the physical and chemical changes with light. In order to provide this knowledge, we have created a set of assays allowing us to assess: i) the spectral absorbance, ii) the sensitivity to irradiation, and iii) the photodegradation and the photoproducts. Data for some reference compounds are presented. The photoproducts may be harmful and cause phototoxicity, photoallergy, or photosensitization. These reactions may also be initiated by the interaction of a drug with endogenous substances in the body in the presence of light. The in vitro 3T3 Neutral Red Uptake (NRU) Phototoxicity Test (PT) is based on the OECD 432 guideline and compares the cytotoxic effects of a test substance, in the presence versus the absence of irradiation. The phototoxicity is evaluated by measuring the reduction in vital dye uptake (neutral red) by the dead or damaged cells, with chlorpromazine as a reference control. The outcome

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is the calculation of the Photo Irradiated Effect (PIF), the Mean Photo Effect (MPE) and phototoxicity class. Recently, new controlled studies have been developed to improve the more predictive data on this assay. Through the use of advanced cell culture conditions, more physiologically relevant information of the potential effect a development compound on the photosensitivity and photoprotection on primary cells has become available. In addition to its 3T3 NRU in vitro PT service offering, Euvos has developed an in vivo mouse experimental model to study the in vivo photoxicity of primary human dermal fibroblasts, epidermal keratinocytes and corneal epithelial cells. These new tests are in response to certain drawbacks that have been reported with the conventional assays, including excessive phototoxicity prediction or species specificity. Therefore, the PT in human primary cells is seen as advantageous in follow-up testing. For all of these new assays, chlorpromazine was the reference compound, with a PIF of 24 for the 3T3 and 347, 527 and 45 for the keratinocytes, the fibroblasts, and the corneal epithelial cells, respectively. No phototoxicity was found with SDS, PIF of 1.13 in 3T3 and 0.92, 1.3 and 1.14 for keratinocytes, fibroblasts, and corneal epithelial cells, respectively. In conclusion, these new photoassays aim to characterize photostability of cell compounds and phototoxicity in gold standard cells or with primary human cells. These new services will improve a drug discovery program’s success, by bringing improved knowledge of a compound’s chemistry and phototoxicity, in accordance with the 3R principle.

### 3622 Intestinal Organoids, a Reliable Alternative Model for Studying the Effects of Foodborne Nanoparticles in the Gut: The Titanium Dioxide as Proof-of-Concept


Daily oral exposure to inorganic nanoparticles (NP) from food additives used in ultrahigh-dose food exceeds public health issues. Identified hazards linked to NP ingestion requires long-term exposure in animal models, specific spaces and skills. Using this approach, oral exposure to the whitener and opacifying agent titanium dioxide (TiO₂) used in a wide range of products (foodstuffs, toothpaste, cosmetics, pharmaceutical tablets), led to gut barrier defects including inflammation in rodents. Owing to the in vivo constraints, organoids are proposed as an alternative to rodent studies but their use to assess the intestinal impacts of inorganic NPs remains to be evaluated. The aim of this study was to validate organoids as a reliable model for studying the effects of foodborne NPs in gut by comparing the impacts of food-grade TiO₂ (fg-TiO₂) on murine intestinal organoids with already reported in vivo data. Crypts from small intestine of three wild-type C57Bl/6 mice were purified, dissociated, and cells were cultured for organoid growth. Organoids were dissociated and seeded as a 2D culture, then exposed for 24h either to increasing doses of fg-TiO₂ (0.1, 1.1, 10 or 100µg/ml) or to an IFN-γ/TNF-α cocktail (1 or 10 ng/ml) to check the organoids capacity to respond to a pro-inflammatory stimulus. Cytotoxicity was assessed by Lactate Dehydrogenase (LDH) release quantification in supernatants. Gene expression of Toll Like Receptors (TLR), NF-κB, cytokines and chemokines as well as markers expression of cell proliferation and differentiation, genotoxicity, antimicrobial peptides, permeability and oxidative stress were determined by qPCR. Cell apoptosis and genotoxicity were also evaluated respectively by cleaved Caspase-3 and γH2AX quantification using immunofluorescence. Compared to control, no difference in LDH secretion was observed following exposure to fg-TiO₂, respectively by cleaved Caspase-3 and γH2AX quantification using immunofluorescence. A dose-dependent up-regulation of genes under control of pro-inflammatory mediators (Tlr4, Nfκb2 and Rela) was observed after exposure to the IFN-γ/TNF-α cocktail, showing the organoids as a functional and competent model to respond to inflammatory stresses. Organoids exposure to fg-TiO₂ led to increased protein level of cleaved Caspase-3 together with up-regulation of Mki67 proliferation marker, suggesting epithelium renewal or restructuring. In addition, fg-TiO₂-treated organoids showed a decrease in the expression of the stem cell marker Lgr5 and an increase in the expression of the mucus-producing gene Muc2 and the enterocyte and neuroendocrine differentiation markers Vill and Chga. Expression markers of NF-κB inflammatory response were decreased, while genes of TLR4 pathway and oxidative stress remained unchanged after fg-TiO₂ treatment. In addition, fg-TiO₂ increased expression of the DNA damage gene Gadd45α as well as yh2XAX staining, while down-regulating genes encoding antimicrobial peptides (Reg3g, S100a8) and tight junction proteins (Cldn1, Cldn7, Cldn15), suggesting genotoxic effects and possible impairments of epithelial permeability and antimicrobial defenses. In accordance to reported in vivo data, the integrity of the gut barrier in terms of cell proliferation/differentiation, genotoxicity, innate defenses and epithelial permeability, was affected in murine gut organoids exposed to fg-TiO₂. These results validated the use of organoids as an alternative to in vivo experiments for screening the intestinal effects of inorganic NPs, which could be pertinent for read across assessment.

### 3623 Cell Painting Assay as Hazard Assessment Tool to Reveal Mechanistic Toxic Properties of Compounds in the Early Drug Discovery Pipeline


Attrition rate in the drug discovery pipeline is high and many of the lead candidates advance to later development stages without sufficient selection. Therefore, there is a demand for an alternative in vitro testing platform, which allows quantitative mechanistic understanding of the test substance and identifies potential risks early in the drug discovery pipeline. Cell Painting is a high content multiplexed image-based profiling assay. It provides an unbiased method to identify subtle changes in cellular homeostasis involving six (6) dyes across five (5) channels, revealing eight (8) major cellular organelles. Image-based profiles can be acquired by extracting > 3000 morphological features at the single-cell level and identify cellular responses to treatments. In this study, we investigated the correlation between different readouts in relation to the selection window for drug candidate de-risking of action (MOA) predicted by Cell Painting assay. First, we selected thirteen (13) reference compounds (Berberine Hydrochloride; Ca-074-Me; Etoposide; Fenbendazole; Latrunculin B; Metoclopramide; Oxibendazole; Paclitaxel; Ramapycin; Rotomone; Tetrandrine; Saccharin; Sorbitol) representing different MOA classes. Next, we exposed human U2-OS cells to eight (8) different concentrations at two different time points (24h and 48h) and assessed the reference compounds’ cytotoxicity level using the CellTiter-Glo Luminescence Cell Viability Assay. Lastly, we applied the six (6) established fluorescent Cell Painting dyes (Hoechst 33342; Concavulanin A; SYTO14; WGA; Phalloldin; Mitotracker) on the exposed cells. Results were analyzed by an automatic segmentation / quantification algorithm in CellProfiler v 4.2.1. Regionalized signal was analyzed using a standardized quantification workflow as published by JUMP-Cell Painting consortium. Data was pre-processed in KNIME v.4.1.3 analytics platform by filtering out irrelevant readouts, readouts with low variance and readouts with high correlation to other readouts (double identifiers). Principal Component Analysis (PCA) was performed to select the most relevant readouts and to narrow down the number of features. Next, selected readouts into three dimensional PCA plot (PCA dimension 0-1-2). PCA analysis confirmed dose-dependent, distinct morphological effects of the different toxicity triggers at 48h post-treatment as compared to solvent control (DMSO), while negative control compounds Saccharin and D-Sorbitol did not show appreciable effects in an U2-OS cell line (time point 24h). As an example, investigating the MOA of the reference compound Berberine, revealed indistinguishable effect from DMSO at low concentrations (up to 1µM), but >30 µM exposure resulted in high cytotoxic response in the CellTiter-Glo assay (>50%) and general, non-specific toxic stress response in Cell Painting assay. Exposure with Berberine up to 10 µM induced membrane redistribution without overt signs of cell death (<20%), which is in line with the literature data. Our results highlight the importance of contamination of the compound-induced cytotoxicity level, when using the Cell Painting assay and provide future guidelines for further industrial application. Importantly, the technology is adaptable for various cell lines, compatible with high-throughput read-out, and analysis can be applied early in the drug discovery pipeline to reveal MOA of the lead candidates and identify potential hazard risks.

### 3624 Validation of the Electrophilic Allergen Screening Assay (EASA) to Detect Substances That Impact the Initial Key Event in the Adverse Outcome Pathway for Skin Sensitization

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The Electrophilic Allergen Screening Assay (EASA) was originally developed by the National Institute of Occupational Safety and Health as a cuvette-based assay to identify substances that have the potential to cause allergic contact dermatitis. Chemical binding to skin proteins is the initial key event of the adverse outcome pathway for skin sensitization. In the EASA, substances were tested for their ability to bind to nitrobenzenothiol (NBT) or pyridoxaldehyde (PDA) probes as surrogates for thiol- or amine-based probes. Probe depletion was measured by absorbance or fluorescence using spectrometers. A test substance is positive when it meets the positive depletion criterion for either NBT or PDA, but negative results are recorded only when the depletion failure to meet the positive criterion for both tests. EASA was subsequently modified by the U.S. Consumer Product Safety Commission (CPSC) and the National Institute of Standards and Technology (NIST) into a higher-throughput assay using a 96-well format through a measurement science approach. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) assembled a validation management team to oversee the validation of EASA that has now been completed by three of four laboratories. The FDA Center for Devices and Radiological Health (FDA-CDRH), Defense Public Health Center-Aberdeen (DoD), and CPSC/NIST (lead laboratory). A pre-validation transferability assessment using positive and negative control tests totaling 10 plates each for the NBT and PDA probes established acceptance criteria for the validation study. Acceptance criteria were based on
Liver microphysiological systems (MPSs) are promising models for predicting hepatic drug effects. Here, we describe the use of the CN-Bio microfluidic platform with primary human hepatocyte, Kupffer (KC) and liver sinusoidal endothelial cells (LSEC), seeded and cultivated on the LC12 scaffold in 10:1 ratio with fluid flow (1µL/s, 1.6ml total medium) to generate a human liver MPS. Initial characterization demonstrated albumin (≥30g/day/million cells, IQ MPS Affiliates recommendation) production from Days 8-15. Hepatocytes formed bile canaliculi, expressed canalicular and secreted biomarkers. In keeping with reported non-cholestatic effects, 5mM APAP, pioglitazone (Pgz), bosentan (Bos) and chlorpromazine (Cpz), mitochondrial impairment (Tgz, Cyc A and 25mM APAP), ER stressors (SULT2A1 and BAAT) mRNA were altered in a drug- and dose-dependent manner. Across all drugs, liver MPS were exposed to various hepatotoxicants, including troglitazone (Tgz), BMS-986020 (LPA1 antagonist), nefazodone (Nfz), bosentan (Bos) and chlorpromazine (Cpz) at ≥ 32-fold Cmax for exposure levels of drugs and their major metabolites were confirmed in the media. Phase 1 and 2 drug metabolism enzyme activity. Immunofluorescence imaging of LYVE-1 and CD68 confirmed localization of LSEC and KC, respectively. After 8 days of culture, liver MPS were exposed to various hepatotoxicants, including troglitazone (Tgz), BMS-986020 (LPA1 antagonist), nefazodone (Nfz), bosentan (Bos) and chlorpromazine (Cpz) at ≥ 32-fold Cmax for 4 days with daily dosing. Media was collected daily, and mRNA collected at 96h. Exposure levels of drugs and their major metabolites were confirmed in the media for all except CycA. Secreted albumin was the most sensitive correlate to toxicity compared to other secreted biomarkers for all except CycA. Secreted albumin was the most sensitive correlate to toxicity compared to other secreted biomarkers, including albumin, for all except CycA. Secreted albumin was the most sensitive correlate to toxicity compared to other secreted biomarkers, including albumin, for all except CycA.

5.25 3625 Characterization and Application of CNBio Microphysiological System for Evaluation of Cholestasis and Hepatotoxicity

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Multiple U.S. federal agencies require the assessment of skin sensitization potential for their chemical evaluation and management programs. Although these agencies have historically relied on skin sensitization data from animal testing, several non-animal methods have been internationally adopted as test guidelines. While none are considered complete replacements for animal tests, one approach to improve performance is to combine the results of non-animal methods that represent multiple facets of the adverse outcome pathway for skin sensitization using defined approaches (DAs). However, the DAs for regulatory use described in the Organisation for Economic Co-operation and Development’s Guideline 497 have been evaluated primarily on chemicals that are relevant to the cosmetics industry. This project aimed to evaluate DAs relevant to the chemical evaluation and management programs of federal agencies who were requested to nominate substances to be tested in three non-animal skin sensitization assays: the direct peptide reactivity assay, the KeratinoSens™ assay, and the human cell line activation test. In vitro, in silico, and in vivo data were collected on 185 substances nominated by the National Toxicology Program, the U.S. Environmental Protection Agency (EPA) and the Consumer Product Safety Commission (CPSC). The results from each individual assay and the DAs were pooled by agency and the hazard and/or potency categorization for skin sensitization potential was determined. For each set of agency-nominated substances, local lymph node assay (LLNA) results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs. When adequate results were used as reference data to evaluate the individual test methods and to assess performance of the DAs.
was exposed daily (2h/day) to three different concentrations (1, 3, 3, 10 μM) of deoxynivalenol (DON) and a vehicle control for 21 days. Effects on cell viability, barrier integrity, cytokine release and whole genome expression profiles were evaluated. Characterization showed that the model consisted of a confluent cell layer (confocal microscopy) and the cell density (DNA content) and differentiation status (ALP activity) remained constant when fully differentiated (week 3-6). BrDU staining allowed to assess cell proliferation. The fully differentiated model indicated good homeostasis. Finally, lucifer yellow (LY) transport indicated good barrier integrity in the fully differentiated tri-culture model up to 6 weeks. Daily exposure to DON for 21 days had no effect on cell viability (LDH measurement) but showed a decrease in barrier integrity (LY transport) for the highest dose of DON, which was significant after 2 weeks of exposure. IL-6 release was increased in the middle and high dose group, but there was no significant increase in IL-6 release. Gene expression profiles (RNAseq) showed a clear dose dependent effect on gene expression after three weeks of exposure and pathway analysis is currently being performed. In summary, the tri-culture gut-on-chip model showed good integrity and homeostasis up to 6 weeks of culture and could be daily exposed to DON for a period of three weeks, which makes this a very promising model for long term toxicity studies as an alternative to animal models.

3629 Application of Human 3D Cell Culture for Assessing Mycotoxins Toxicity: Toward a Real Risk Characterization for STE, OTA, and PAT

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Over the last decades, in an effort to reduce, replace and refine the reliance on animal experimentation as well as improve the physiological relevance of current cell-based systems, efforts are underway to develop alternative experimental methods. This is in line with increased level of complexity and physiological features that better mimic in vivo cell behaviour and provide more predictive results. This novel approach is already applied for disease studies, pharmacokinetic studies and drug development and there is promising evidence that toxicological studies could also benefit from the application of it. Among the emerging approaches, the more physiologically relevant three-dimensional (3D) cell culture models outperform the standard two-dimensional in vitro system, due to the complex cell-to-cell and cell-to-matrix interactions, making them an ideal candidate to improve the state-of-the-art for toxicological testing and provide a more reliable evaluation of mycotoxin exposure. The aim of the present study consists in the determination of the cytotoxic effect associated to the individual exposure to the mycotoxins sterigmatocystin (STE), ochratoxin A (OTA) and patulin (PAT) using human 3D cell cultures (spheroids) obtained from different cell lines: bone marrow derived mesenchymal stem cells (BM-MSC), human umbilical vein endothelial cells (HUVEC), human neuroblastoma cells (SH-SY5Y) and epithelial human breast cancer cells (MDA-MB-231). Standardized setup for reproducible and size-appropriate bio-relevant spheroids was performed in order to determine the optimal growth conditions for the functional endpoints for each cell line. Spheroids were generated from single-cell suspensions obtained from trypsinized monolayers of cells and diluted to suitable (10^5 cells/mL) and sufficient (100 μL) cell concentrations. The optimal initial spheroid density and the growth duration was established such that each spheroid for each cell line reach approximately 500 μm in diameter. The viability was assessed by LIVE/DEAD Viability/Cytotoxicity assay, a two-colour method to discriminate live from dead cells in a population. Once established the optimal seeding density and the growth’s time, the effect of STE, OTA and PAT on spheroid viability was investigated after 1, 2 and 3 days of exposure by ATP assay. Spheroids were exposed to STE, OTA and PAT by adding 100 μl of culture medium containing the mycotoxin to obtain final concentrations ranging from 6.12 to 100 μM. Appropriate controls were set up in order to control the experimental results. Our results reveal significant differences in sensitivity towards STE, OTA and PAT between the different cell lines, as well as differences were noticed compared with what is reported in the literature, which might be related to the 3D culture environment. The results obtained demonstrated the important influence of the microenvironment and of the 3D tissue structure in toxicity investigation. In particular, the assessment of cytotoxicity in spheroids rather than monolayer cultures is expected to more accurately reflect in vivo-like cell behaviour and generate relevant data for national food safety authorities, public health institutes, industry and other regulatory bodies, supporting the refine of the future risk assessment and regulatory policy agendas on allowable exposure levels. Spanish Ministry of Science and Innovation grant (PID2020-115877RB-I00); Vice rectorate for the University of Valencia post-doctoral grant (APOST/2022; Ref. CIAPOS/2021/228); ERC Starting Grant MICRONEX UERI7_01.

3630 Expanding the Applicability Domain of the h-CLAT by Validation of Alternative Vehicles for Difficult to Solubilize Commercially Available Mixtures

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In vitro tests for skin sensitization have been developed and validated in the hope of replacing the use of animals for this purpose. The need to expand the applicability domain of the h-CLAT to include mixtures has become highly desirable. We previously reported the validation of the h-CLAT to assess the skin sensitization potential of a small subset of commercially available mixtures. Given the heterogeneous chemical makeup of these products, alternative vehicles to those listed in the OECD TG 442E are necessary for analysis of difficult-to-solubilize chemicals and mixtures. Potential additional vehicles were prioritized by ranking LogP values and evaluated for compatibility with the assay using the proficieny chemicals identified in OECD TG 442E. We tested multiple commercially available sensitizing mixtures and non-sensitizing mixtures (based on Safety Data Sheets). We demonstrate that certain alternative vehicles improve sensitivity and/or positive predictivity when testing these mixtures. We build upon previously reported data that acetone and 2-butanon are acceptable alternative vehicles for the h-CLAT and demonstrate these new vehicles are applicable for the assessment of known sensitizing/ non-sensitizing chemicals as well as mixtures. These results support the development of a multi-component vehicle system for the in vitro sensitization testing of mixtures, medical devices and UVCB materials.

3631 Using Single-Cell Sequencing to Investigate the Endogenous Role of the Aryl Hydrocarbon Receptor in Zebrafish Hematopoiesis

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The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that mediates a wide range of biological and toxicological responses. While largely studied in ligand-activated toxicant responses, AHR also plays important roles in endogenous physiological processes. We leveraged single cell sequencing and AHR2 knockout zebrafish line to investigate the role of AHR2 in regulating hematopoiesis (production and differentiation of red and white blood cells from hematopoietic stem cells). Our objectives were to determine if absence of AHR2-1) alters proportions of blood cell populations and/or 2) impacts gene expression within individual blood cell types. We dissected kidney marrow (organ of hematopoiesis in zebrafish) from adult wildtype and AHR2 knockout zebrafish (N=3 genotype), isolated single cells and sequenced ~ 5000 cells/sample (10X Genomics). We identified 19 distinct cell clusters representing the expected major blood (erythrocytes, thrombocytes, B cells, macrophages, T/NK cells and neutrophils) and kidney (vascular endothelium, proximal tubule, mucin-producing cells) cell populations. There were no genotype-specific differences in proportion of individual cell types. However, gene expression differences between the two genotypes were observed within neutrophils (275 differentially expressed genes/DEGs) and macrophages (134 DEGs). Gene ontology assessments revealed disruptions in cell cycle and cell division for neutrophils and immune/cytokine signaling for macrophages. These results demonstrate that AHR2 alters gene expression within select immunological cell types that may impact immune regulation and function. Future work will focus on the regulatory role of AHR2 on specific genes as well as the impact on function of macrophages and neutrophils.

3632 Development of In Vitro 3D Human Kidney Proximal Tubular Epithelial Tissue Model


The proximal tubular (PT) region is the most common site for a compound-specific kidney injury. PT region is responsible for essential kidney functions, including reabsorption of low molecular weight proteins, solutes, and glucose; secretion of acids; and clearance of administered medications. The ultimate goal of this project is to develop a novel physiologically relevant primary human kidney-cell-based 3-dimensional (3D) organotypic tissue model for the prediction of human nephrotoxicity. Human primary proximal tubular epithelial cells (PTEC) were isolated and expanded in a monolayer culture prior to seeding onto microporous membrane inserts to reconstruct a 3D organotypic tissue model. 3D tissues were analyzed by histology, barrier integrity (transepithelial electrical resistance, TEER), immunostaining, and qPCR on days 5 to 30. Receptor-activated FITC-albumin uptake and transpeptidase hydrolytic activity of glutamyl transpeptidase (GGT1) and leucine aminopeptidase (LAP) were assayed on days 10 to 16. The PTEC organotypic tissues organize into characteristic tubular structures, develop a barrier with TEER 110±2±33.3 Ω·cm² on day 9 and stain positive for tight junction proteins ZO-1, claudin-1, and occludin. The organotypic tissues differentiate into polarized epithelium expressing brush border proteins megalin and cubulin together with water channel AQP1 and GGT1 on the apical side and sodium-potassium ATPase pump on the basolateral side. Real-time qPCR analysis confirmed that tissues express a panel of PTEC-specific markers that are necessary for renal clearance, secretion,
DAP demonstrated good predictivity of GHS categories and high interlaboratory barrier properties, gene expression, and tissue performance resemble the in vivo human PT region. This model is anticipated to be a valuable tool to evaluate human neaphrocytosis and its mechanisms, improve the predictivity of human responses to pharmacological substances, and help establish confidence in drug development and testing.

**3633 Validation of Physiologically Relevant In Vitro Human Inhalation Toxicity Tests for Volatile Liquids, Mists, and Sprays**

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In in vivo animal models are currently accepted by regulatory authorities for acute respiratory toxicity (ART) testing. However, animal models have been discredited as predictors of human responses on physiological and ethical grounds. The goal of this work was to develop physiologically relevant ART tests using the EpiAirway tissue model, to demonstrate correlation to OECD accepted GHS categorization, and investigate interlaboratory reproducibility. Test articles (TA, n=53) were applied to EpiAirway tissues produced at MatTek (USA) and IVLSL (Slovakia) with two ART protocols, the Direct Application Protocol (DAP) for exposure to mists/sprays, and the Vapor Cap Protocol (VCP) for exposure to vapors/volatile liquids. In both protocols, tissues were exposed for 4h to 4 fixed doses (diluted in corn oil or water) to mimic in vivo rat exposure; followed by 20h post incubation. The effects on tissue viability (MTT assay) and barrier properties (Transepithelial Electrical Resistance, TEER) were determined. The effective doses which reduced tissue viability by 25% (ED-25) or by 50% (ED-50) were mathematically interpolated for the DAP and VCP methods, respectively, and correlated to the GHS categories. In the DAP, TAs were applied to the apical surface. Using the MTT assay, the DAP discriminated between GHS Cat.1 and 2/3 with a Sensitivity/Specificity (S/S/A) of 63.5/76.1/69.8 (MatTek) and 63.8/76.1/70.0% (IVLSL). Utilizing the changes in TEER, the DAP discriminated between GHS categories with a S/S/A of 65.9/76.7/71.3% (MatTek) and 64.1/76.6/70.3% (IVLSL). The correlation coefficient between the two laboratories was R² = 0.91 for MTT and 0.76 for TEER. In the VCP, TAs were applied to an absorbent material in a special cap that forms a tight seal above the tissue allowing exposure to TA vapor. Using the MTT assay, the VCP discriminated between GHS categories with a S/S/A of 70.8/83.2/77.0 (MatTek) and 71.9/83.2/77.5% (IVLSL). Utilizing the changes in TEER, the VCP discriminated between GHS categories with a S/S/A of 64.8/76.5/71.5 (MatTek) and 67.1/78.8/74.6% (IVLSL). The correlation coefficient between the laboratories was R² = 0.93 for MTT and 0.84 for TEER. Using the MTT assay, both the VCP and DAP demonstrated good predictivity of GHS categories and high interlaboratory reproducibility. Both protocols provide robust and efficient, physiologically relevant, organ-specific in vitro tests that can improve the predictivity of human responses, reduce the number of animals being used to assess respiratory toxicity, and help establish confidence for regulatory applications.

**3634 Peer Review Report of the EpilSensa Skin Sensitization Assay**

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The EpilSensa skin sensitization assay was developed as an alternative method to address Key Event 2 (KE2; keratinocyte activation) in the skin sensitization adverse outcome pathway. The assay utilizes reconstructed human epidermis (RHE) and measures the gene expression of four markers of sensitization: (i) the encoding activating transcription factor 3 (ATF3); (ii) the phorbol ester and calcium-dependent protein kinase C, mediator subunit (GCLM), (iii) the Dnaj (Hsp40) homolog, subfamily B, member 4 (DNAJB4), and (iv) interleukin-8 (IL-8). These genes reflect the keratinocyte response to early induction of skin sensitization via cytotoxic protective and inflammatory pathways. Between 2018 and 2022, the EpilSensa underwent a multilaboratory validation study with the support of the Japanese Center for the Validation of Alternative Methods (JaCVAM) and an international group of experts in skin sensitization and method validation. JaCVAM subsequently convened a peer review panel to review the validation process and performance of the EpilSensa. The outcome of the validation study demonstrated the predictivity, transferability, between-laboratory reproducibility (BLR), and within-laboratory reproducibility (WLR) of the assay. The study also assessed whether the assay could be used for categorization according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals. The peer review panel met virtually twice and held a face-to-face meeting to complete the review of the validation study. The panel assessed the applicability and rationale behind the assay itself, the completeness of the validation study, and the logic behind in-process changes to the standard operating procedure (SOP). The panel also assessed reproducibility of the assay based on concordance with murine local lymph node assay results. The assay had 86.7-93.3% WLR and 88.9% BLR, both exceeding the prespecified minimum goals of the study, specifically 85% WLR and 80% BLR. Individual labs assessed a total of 27 chemicals for skin sensitization, while the lead lab tested a total of 136 chemicals. The rationale for use of an RHE model for the EpilSensa was to increase the applicability domain beyond that of existing KE2 assays, with the RHE model having greater ability to handle both lipophilic chemicals and pre-/pro-haptens. The EpilSensa correctly predicted 35/37 pre-/pro-haptens, with good scientific rationale behind the two that were not correctly predicted. Additionally, with lipophilic compounds (LogP >x3.5), the assay showed sensitization with high sensitivity (65%), and accuracy (78%) than other internationally accepted methods. Transferability to three other facilities was successfully demonstrated. Justified SOP alterations included altering the positive control to provide a more stable alternative and adjusting endogenous control gene criteria and tissue viability acceptance criteria. The peer review panel felt that the EpilSensa is a good method to assess a wide range of chemicals for skin sensitization and supported assessment for inclusion at the Organisation for Economic Cooperation and Development as a test guideline, where it is on the 2022 workplan. The panel recommended that the assay developers clarify how to assess borderline calls and provide performance criteria for development of other similar RHE-based assays. ENR’s time on this project was funded in whole or in part with federal funds from the NIHES, NIH under Contract No. HHSN273201500010C.

**3635 Analysis of Reproducibility and Robustness of PhysioMimix T12, a Proximal Renal Tubule Microphysiological System for Studies of Pharmacokinetics and Toxicological Assessment of Drugs and Chemicals**

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The renal proximal tubule is a primary site of excretion and reabsorption of endogenous and exogenous small molecules and a critical determinant in pharmaco- kinetic and toxicological nature of these processes. The study compared the in vivo and the renal proximal tubular epithelial cells (RPTEC), that depend on sheer stress and correct polarization, in vitro modeling of this tissue is challenging. A number of microphysiological systems for studies of kidney proximal tubule have been proposed, and one such model is the PhysioMimix® T12 (CNBio) where RPTEC can be cultured on the bottom of Transwells and exposed to media at a constant flow rate of 1.25 µL/s. This model has a wide application potential for testing of both pharmaceuticals and other chemicals. Therefore, as part of TEX-VAL Consortium, a multi-stakeholder effort for stabilizing the functionality, reproducibility, robustness, and reliability of microphysiological systems, this study aimed to evaluate the PhysioMimix™ T12 platform using different RPTEC cell types and conditions. RPTEC/TERT1 cells (parental and OAT1, OCT2, and OAT3 overexpressing lines) were cultured in Transwells® and grown under typical static conditions or placed into the CNBio PhysioMimix™ T12 plate, where they were exposed to flow to allow for direct shear stress. Experiments were performed for 7 days under static or dynamic conditions and basal function was compared: including transepithelial resistance, water transport, and transporter expression/localization. Additionally, bi-directional transport of cisplatin, tenofovir, paraaminobipirinic acid, and perfluorooctanoic acid was tested in the presence or absence of probenecid (OAT1-inhibitor) in parental and OAT1 cells under static and dynamic conditions. We demonstrate that barrier function as well as water and chemical transport were more physiological under flow conditions. Additionally, the presence of fluid shear stress significantly increased AQP1 protein expression, concordant with an increase in water transport. These results demonstrate that a functional proximal tubular model can be established in the PhysioMimix™ T12 platform and be used to study human clearance of both pharmaceuticals and environmental compounds.

**3636 Evaluating Toxicity of Polycyclic Aromatic Hydrocarbons from Wildfire Smoke in a 3D Respiratory Co-culture Model**

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Wildfires are a growing public health crisis with wildfire occurrence, duration and intensity increasing in recent years. Smoke emissions from wildfires are chemically complex containing polycyclic aromatic hydrocarbons (PAHs) among other pollutants, and the upwards trend in the severity of wildfires poses an increasing public health risk from smoke inhalation, warranting further evaluation in mechanistically relevant models. In the respiratory system, induction of airway epithelium is orchestrated through signaling between airway epithelial cells and macrophages.
which are two of the most abundant cell types in the respiratory tract contributing to the innate immune response. Activation of airway immune response and inflammation are critical events leading to adverse health effects and damage of the respiratory system after chemical exposure. To evaluate the combined contribution of airway epithelial-macrophage response to PAHs from wildfires, we established a co-culture model utilizing 3D organotypic primary human bronchial epithelial cells (HBEC) and macrophages (HBECS). HBECs and macrophages were exposed to a mixture of PAHs (300 μM) previously identified from wildfires in the Pacific Northwest by passive sampling. The PAH mixture, which includes retene as the most abundant component, resulted in significantly increased cytotoxicity and induction of CYP1A1 and CYP1B1 transcripts in HBECS alone. In the co-culture, a difference in macrophage adherence based on treatment group was observed with the groups receiving the highest concentrations having the greatest macrophage adherence. In addition, HBEC cytotoxicity, and qPCR markers of inflammation were observed to be dependent on the presence and number of macrophages. Overall, these studies fill critical knowledge gaps regarding the role of inflammation in toxicity of inhaled PAHs from wildfires in an organotypic co-culture model.

3637 Virtual Control Groups in Preclinical Safety: Decreasing Animal Use while Maintaining In Vivo Study Interpretability


Since SEND was implemented to streamline the capture and presentation of nonclinical study data, accessibility and interoperability of nonclinical in vivo study data has increased tremendously, providing new opportunities to leverage historical study data. One promising opportunity is the establishment of Virtual Control Groups (VCGs), which are built from historical control animal data and used to replace a portion of live concurrent controls, reducing live animal use in toxicity studies. VCGs represent approximately 25% of the animals used in Genentech toxicity studies, and generally undergo the same testing paradigm for a given study type. Limiting the scope of our work to rat pilot toxicity studies and to a single test site, we have investigated an approach to partially replacing concurrent control animals with virtual control animals, using a method similar to the generation of VCGs used in human clinical trials. To create a valid VCG from historical control animals and provide a relevant and meaningful basis for determining test-article related changes, we needed to understand the variability of control data under various test conditions and identify which test conditions require consistency across the virtual and live treated animals. We aggregated historical control data from Genentech rat pilot toxicity studies, and stratifying animals by sex, treatment, route-of-administration and other experimental or baseline conditions, we created a biologically relevant pool of control animals. Using appropriate statistical methods, we analyzed body weights, organ weights, and clinical pathology parameters, and separately evaluated the comparability of clinical observations and histopathology data from our historical control animals. Initial results showed that data was relatively stable with respect to bodyweight changes and organ-to-bodyweight ratios, demonstrating comparability of control animals over time and across studies. Furthermore, we assembled a historical control library of individual animal clinical observations, confirming the absence of serious adverse clinical signs in control animals. Analyzing the nature and frequency of the various findings observed when no test article is administered. Analysis is ongoing, but we aim to demonstrate comparability of pathology data over time and across studies. As our final measure of validation, we have started to assess VCGs in historical as well as ongoing rat pilot toxicity studies by replacing concurrent control groups with VCGs and determining if the conclusions are similar when compared to the conclusions reached using live concurrent control groups.

3638 Metabolic Competency of an Airway Organotypic Culture Model

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Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion processes from cigarette smoke, diesel exhaust, and wood burning, and have been associated with several forms of cancer, including lung PAHs are bioactivated into their reactive metabolites by metabolizing enzymes in the body to cause mutations and altered gene signaling leading to tumor growth. The airway epithelium is a primary route of exposure for inhaled toxicants and 3D organotypic culture models represent an important advancement for toxicity testing compared to traditional in vitro models that lack metabolic capability and multicellular structure/communication associated with the bronchial epithelium in vivo. However, limited data exists regarding the metabolic capacity of these cells, which limits their use in quantitative studies for assessment of dosimetry or predictive modeling of toxicity compared to in vivo studies. Therefore, primary human bronchial epithelial cells (HBECs) and human bronchial epithelial macrophages (HBECS) were utilized as a model for PAH inhalation toxicity. A number of comparative approaches have been utilized to assess the metabolic competency of HBEC after treatment with benzo[a]pyrene (BaP, 10-50 μg/ml). Benchmark modeling was used to analyze global gene expression data for identification of dose-response sensitive genes and pathways. BaP treatment had a significant effect on DNA damage, extracellular matrix, and oxidative stress pathways, and qPCR-confirmed dose-dependent changes for several Phase I and II enzymes. UPLC and P450-glo activity data show the formation of BaP metabolites present in cells and media as well as increased CYP1A1 activity after BaP treatment, respectively. Future studies will apply proteomics methods to study metabolic enzyme levels and evaluate the effect of the antioxidant protein levels. The metabolite data will aid in building a PBPK model for mapping the movement and metabolism of BaP throughout the system. Overall, this study will help determine the relevance of in vitro 3D primary culture models for chemical toxicity evaluation in the lung.

3639 An In Vitro Buccal Membrane Absorption Model for the Evaluation of Cannabinoid Permeability and Absorption


Oromucosal administration into the buccal cheek or sublingually as a tincture or spray is a popular method to consume cannabidiol (CBD). Oromucosal absorption is more rapid than oral consumption and can by-pass first-pass metabolism in the liver. As porcine buccal mucosa morphology and permeability is similar to human buccal mucosa, an in vitro buccal membrane absorption model (IVBAMM) was developed by adapting standard in vitro dermal absorption system methodologies to assess the absorption of pure CBD and hemp extract containing CBD. Porcine buccal mucosa was isolated from connective tissue and dermatomed to a thickness of 300-500 μm and mounted in flow-through diffusion cells. Pure CBD (5 mg/ml) and hemp extract containing CBD (0.5% ethanol) were applied to the buccal tissue and permeation samples were collected every 4 hours. Permeability and absorption were measured in receptor fluid samples and tissue after the exposure period. As a comparison, CBD permeability was also assessed in Permeapad® membranes. In the buccal mucosa, most CBD (pure) and CBD in hemp extract remained in solution (over 69%) and does not penetrate the buccal tissue into the receptor fluid after 4 hours. About 0.8% CBD from pure solution remained in the tissue, as with the hemp extract containing CBD. About 9 to 34% CBD was identified in the circulation. Additionally, there was no penetration of CBD into receptor fluid when the study was extended to 24 hours, with CBD increasing slightly in the buccal tissue to about 1%. When CBD was dosed in both pure solution and in hemp extract. In the Permeapad® membranes, no CBD was found in the receptor fluid samples, with about 3.8% remaining in the membrane. Given the lipophilicity of CBD, it is not surprising that CBD does not penetrate into the receptor fluid from buccal tissue and the membrane, even though the receptor fluid contains 4% bovine serum albumin (BSA) for partitioning. In these studies, under the dosing conditions and analytical limits of detection, only small amounts of CBD permeated into buccal tissue and no CBD was found in the receptor fluid, indicating that there was no significant absorption of CBD.

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3640 A New Approach for Eye Hazard Assessment of Surfactants According to UN GHS Based on In Vitro Test Methods

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Over the last decades, many efforts were made to develop New Approach Methodologies (NAMs) that follow recommendations and combinations of modules as specified in the Guidance Document No. 263 on Integrated Approaches to Testing and Assessment (IATA) for serious eye damage and eye irritation, originally adopted by the Organization for Economic Co-operation and Development (OECD) in 2017 (OECD, 2019). Two defined approaches (DA) for non-surfactant hazards have been accepted and were integrated in a new OECD test guideline (TG) for eye hazard identification i.e., discrimination between three United Nations Globally Harmonized System of Classification (UN GHS) categories (OECD TG 467, 2022). Currently, no single in vitro method or DA has been developed to assess the eye hazard potential of surfactants across the 3 UN GHS categories (Cat. 1: severe eye damage; Cat. 2: eye irritation; No Cat.: chemical does not require classification).

Recently, a DA was developed to predict this endpoint for liquid, semi-solid and solid chemicals having surfactant properties. The DA is based on the combination of reconstructed human Cornea-like Epithelium test methods (RHE, OECD TG 492, EpiOcular™ EIT or SkinEthic™ HCE EIT) and a modification of the Short Time Exposure test method (STE, OECD TG 491). The reference set used to develop the DA represented different surfactant families and the most important drivers of in vivo Cat. 1 and Cat. 2 classifications. The 2 main subgroups for No Cat. (CO–0 and COO–0) as defined by the OECD Guidance on re-evaluating methods was created and when testing a given chemical, the method would be selected based on the CO–0 classification.

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Spiked with matching concentrations of salt, indicating that the smokeless product vehicle and cell media 1:1) was used as negative control and 1.0% Triton X-100 was colorimetric assay for assessing cell metabolic activity. Vehicle control (extraction salt diluted in cell medium (v/v) were evaluated. The tissues were treated for 2h tobacco product, CRP1.1. In addition, 10, 20, 30, 40, and 50% CRP1.1 extracts, or at concentrations matched to the theoretical salt concentration in the reference caused by the salt-content was evaluated using media spiked with NaCl/Na 2CO3 oral irritation study in the EpiOral and EpiGingival models. Pouched test products treatment, cytotoxicity was analyzed using propidium idodine staining followed different extraction parameters, such as extract volume, vehicle, incubation time and use of open/full pouch. Two nicotine pouch products and a pouchured reference smokeless tobacco product (Swedish-style snus, CRP1.1) were used. After 24h treatment, cytotoxicity was analyzed using propidium iodide staining followed by flow cytometry. The results were used to guide the extraction procedure for the oral irritation study in the EpiOral and EpiGingival models. Pouched test products were extracted in cell culture medium for 60 minutes (200 mg product/ml) and filtered using a 0.2 µm filter. As a first step, the potential impact on cell viability caused by the salt-content was evaluated using media spiked with NaCl/NaCO3, at concentrations matched to the theoretical salt concentration in the reference tobacco product, CRP1.1. In addition, 10, 20, 30, 40, and 50% CRP1.1 extracts, or salt diluted in cell medium (v/v) were evaluated. The tissues were treated for 2h (EpiOral) and 18h (EpiGingival) before cytotoxicity was analyzed using MTT, a colorimetric assay for assessing cell metabolic activity. Vehicle control (extraction vehicle and cell media 1:1) was used as negative control and 1.0% Triton X-100 was used as positive control. No cytotoxic effect was observed for CRP1.1 or the media spiked with matching concentrations of salt, indicating that the smokeless product extracts which are used in the tissue models. In the main study 50% extracts in cell medium of four different nicotine pouch products and two smokeless tobacco products were evaluated. Tissues were incubated with extracts and cytotoxicity using MTT were assessed after 2, 4 and 18h (EpiOral) and 24, 38, and 48h (EpiGingival), as recommended by the model developer’s protocol. None of the evaluated extracts or toxic in the data (applied and the extended incubation times) did not affect tissue viability. In conclusion, extracts from nicotine pouches or smokeless tobacco products did not induce oral irritation in the EpiOral and EpiGingival 3D models indicating low irritation potential.

One of the fundamental principles for the Adverse Outcome Pathway (AOP) framework is that AOP Key Events (KE) must be detectable and have appropriate measurements to be acceptable and substantive proper linkage(s) between possible real-life stressors and effects on a particular KE. AOP authors must provide information on how it is measured or detected in each KE entered in the AOP-Wiki to justify how the test method provides a measurement of an underlying biological process. However, this free text description field does not reflect the importance of the link between a given KE and a test method used to measure it (and vice versa) nor enables a consistent description of the methods across different KEs. The role of test method linkages in the AOP-Wiki must become more explicit and visible, and the Methods2AOP initiative aims to make this possible. Facilitated by the European Commission’s Joint Research Centre (JRC, EURL ECVM), the Methods2AOP initiative is an international collaboration that also includes NIH NICETAM, US EPA, Environment and Climate Change Canada, and others. To date, the collaboration has adapted ~30 key fields from existing efforts such as OECD guidance document (GD) 211 and divided them into two levels to facilitate the association of test method with KEs (level 1) in a simple but FAIR (Findable, Accessible, Interoperable, Reusable) manner while also integrating enough real-life assay details (level 2) to annotate the test methods for the interpretation of the AOP context. To promote FAIR principles, fields are restricted to terms from existing ontologies whenever possible and new information will be captured as tables in the AOP-Wiki. Eventually, test methods would be a new primary entity within the AOP-Wiki linking AOP descriptions to external sources of information regarding the associated test methods. Challenges encountered throughout the process of identifying appropriate fields on both levels include delineating meaning and which aspects of methods are captured between levels and keeping the information requirements straightforward and non-prescriptive. It is a priority to encourage adoption among information providers while collecting requisite details on test methods to increase the overall trustworthiness and utility of annotated AOP. This annotation framework is established in full transparency and alignment with requirements from the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST, the body governing the AOP Framework), and other requirements from the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST, the body governing the AOP Framework), and other affected stakeholders to advance the integration of experimental definitions into AOP frameworks and facilitate the increased regulatory uptake of AOP knowledge. The views expressed in this presentation are the author’s own and do not necessarily reflect those of the US government.

The in vivo functions of the bronchial epithelium can be recapitulated in vitro through the differentiation of primary human bronchial epithelial cells (pHBEc) under air-liquid interface (ALI) culture conditions. pHBEc ALI models have rapidly gained popularity for in vitro chemical testing and research; however, there are practical challenges facing the delivery of many test agents to ALI cultures. These limitations have led to the common practice of dosing differentiated pHBEc cultures by the application of the test agent suspended in an aqueous vehicle (e.g., medium, saline, or etcetera). However, the physiologic and functional processes in pHBEc ALI differentiation, we hypothesized that liquid application alone (i.e., in the absence of a test agent) would have a significant effect on in vitro toxicity endpoints. To test our hypothesis, we applied an aqueous vehicle (ALI medium) in the absence of a test agent to the apical surface of differentiated pHBEc ALI cultures. We
then examined endpoints that were representative of in vivo relevant physiology including, global gene expression by RNA-Seq, stress-response signaling pathway phosphorylation, pro-inflammatory cytokines and growth factor secretion, cell membrane damage, ciliary beat frequency, and epithelial barrier integrity. The application of liquid alone resulted in the significant alternative regulation of 4170 and 10269 genes at 6 and 24 hours, respectively, with many of the most dysregulated genes being involved in stress-response signaling and inflammation. Western blot analysis indicated a significant increase in the phosphorylation of the stress-responsive signaling pathways ERK1/2, p38, and p65, as well as activation of the HIF1-alpha signaling pathway. The transcriptional changes were further complemented by significant increases in the secretion of the pro-inflammatory cytokines IL-8, IL-6, IL-1β, and TNFα, as well as vascular endothelial growth factor alpha (VEGFα) and placental growth factor (PIGF). We also observed a progressive decrease in trans-epithelial electrical resistance and increase in small molecule permeability. Cumulatively, our findings indicate that liquid application alone causes a phenotype in the bronchial epithelium that is consistent with the effects of well-characterized toxins, several severe tracheal diseases, and early epithelial-to-mesenchymal transition - a common early step in carcinogenesis. Given the common usage of liquid application dosing of ALI cultures, our observations suggest that this dosing method alone is likely to confound the observation and interpretation of test agent effects. Liquid application dosing is used with a wide range of different conditions (e.g., different aqueous vehicles, liquid volumes, treatment durations), thus the use of ALL cultures in vitro hazard identification would benefit from additional characterization of potential effects related to the dosing method alone and any potential to affect study results. Does not reflect EPA policy.

3645 Characterizing Positive Controls in Human and Rodent Primary Hepatocyte Nuclear Receptor Activation

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Modes-of-action (MoAs) of liver toxicity after exposure to xenobiotics have been evaluated in rodents in vivo to address its human relevance. Key events that can lead to tumors include nuclear-receptor activation (e.g., aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR)), and cell proliferation induction. However, there are known physiological differences between rodents and humans which diminishes the applicability of liver toxicity assessment in these studies while also having a very high animal use burden. In vitro assessments using primary human and rodent hepatocytes can enable species comparison of liver toxicity MoAs without the use of animal testing. The characterization of compounds with known liver toxicity MoAs in these in vitro assays is needed to appropriately interpret in vitro MoA and evaluate the human relevance of effects. The compounds β-Naphthoflavone (BNF), Phenobarbital (PB), 5-Pregnen-3β-ol-20-one-6α-carboxylate (PCN), and Rifampicin (RIF) responses and thresholds for nuclear receptor receptor induction and cell proliferation induction were measured in rat, mouse, and human primary hepatocytes. In this study, BNF, PB, PCN and RIF were selected as known activators of AhR, CAR, PXR, and PXR respectively in vivo rodent studies. This study was designed to:1) identify ideal positive controls for the common nuclear receptor activation (assessed by measuring transcriptional induction of AhR, CAR, and PXR biomarkers in rat, mouse, and human primary hepatocytes following 48 h of exposure and 2) evaluate response differences between hepatocytes from two rat strains. A comparative study was conducted using cryopreserved primary hepatocyte cultures between human, CD-1 mice, and two strains of rat, Sprague-Dawley (SD) and Fischer 344 (Fi). Cultures were exposed to compounds for 48h to evaluate gene induction of AhR, CAR, and PXR biomarkers (i.e., cyp1a1, cyp2b2, and cyp3a6 respectively) and cell proliferation (marker MKI67) (n=3 / species). Gene expression was measured by real time quantitative PCR (RT-qPCR) and reported as a fold change increase over solvent control, with positive control induction set to construct a ≥2-fold. Growth factors (epidemial growth factor (EGF) and human GF-HGF) were used as controls for proliferation. Exposure to BNF, PB and PCN resulted in transcriptional induction of AhR, CAR and PXR biomarkers, respectively in all species. Exposure to RIF induced PXR biomarker induction in mice and humans with no increase in rat primary hepatocytes. The selected positive controls showed consistent induction in primary hepatocyte cell proliferation across species, while both growth factors showed consistent induction across all species. Results showed similar SD and Fi rat strains responses. These results support BNF, PB, PCN, RIF as positive controls for AhR, CAR and PXR activation in primary hepatocytes, to evaluate the human relevance of liver toxicity. Additional research is required to evaluate inconsistent cell proliferation responses observed in this study. While this model considers nuclear receptors associated with phase II metabolism, evaluation of other models considering longer incubation exposure times could expand additional gene markers to include those of phase I metabolism.

3664 Application of a Characterization Protocol for the Assessment of Normal and Diseased Cryopreserved Human Precision-Cut Lung Slices

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Human precision-cut lung slices (pHCLS) are a highly relevant 3-dimensional model of the lung, offer native architecture of the respiratory parenchyma and small airways, and contain immune competent cells involved in inflammatory and sensation processes. We recently developed the exposure platform, '1T1, Behringer, 1T1,' that tests pHCLS. M. Marinou, K. Amin, et al. Cryopreservation of Human Precision-Cut Lung Slices Provides an Immune Competent, Pulmonary Test System for 'On-demand Use, The Toxicologist: Supplement to Toxicological Sciences, 186 (1), Society of Toxicology, 2022. Abstract #4192. A 4-week culture with one donor’s pHCLS. Added key elements from this study, we evaluated 5 additional donors using a 2-week study intended for routine use in banking cryopreserved pHCLS. The performance of fresh or cryopreserved (up to 6 weeks) pHCLS in a 2-week culture at the air-liquid interface was assessed from the same donor lungs. pHCLS from both normal tissues, tissues from donors with chronic obstructive pulmonary disease (COPD), or fibrosis were treated with either 5 μg/mL lipopolysaccharide (LPS) or vehicle (VC) for 24 hours at days 6 or 13 post culture initiation. The tissue biomass (BCA protein content), viability (metabolic activity via WST-8 assay), and LPS-induced immune responsiveness were determined at days 7 and 14. Some variability was observed at different time points but pHCLS (normal and COPD donors) in both fresh and cryopreserved groups demonstrated 2-week viability (4.18 and 3.90 OD4540/mg protein in fresh and cryopreserved, respectively). The protein content in the fresh pHCLS from some donors was higher than that in the cryopreserved pHCLS (0.4 vs 0.25 mg/mL, respectively), but immune-responsiveness was not compromised. While finite cytokine pg/mL values varied between the fresh and cryopreserved slices, induced values (up to 139-fold increases in IL-6, TNF-α, and MMP-3) relative to the respective VC was maintained, suggesting biomass-equivalent functionality. Although cryopreserved IFP donor pHCLS showed a reduction in viability (relative to fresh) over 2-weeks, immune responsiveness was maintained. With improvements in slice creation, storage, and culture conditions, the pHCLS can be adapted for larger scale testing, tissue banking, and repeat donor experimentation. Performance testing of these pHCLS batches provides an understanding of banked tissues (normal and diseased) prior to selection and use for studies that have specific research questions. These improvements position pHCLS as an accessible, human-relevant, pulmonary test system suitable for testing inhaled materials, evaluating key events in AOPs, and the evaluation of therapeutics “on demand.”

3647 Effects of Seeding Density and Exposure Period on Human 3D Thyrocyte Microtissue Formation and T4/T3 Quantitation using ELISA versus LC-MS/MS for Thyroid-Disrupting Chemical Screening

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Deisenroth et al. recently described a novel 3D human thyrocyte microtissue culture platform for identifying TDCs whose toxicity may occur through various modes of action (Toxicol. Sci. 2020;174:63-78). The aim of this study was to further optimize 3D thyrocyte microtissue culture conditions including seeding density and exposure period for TDC testing. We used 3D microtissue cultures based on thyroid hormone production and quantitation using ELISA vs LC-MS/MS methods. Cryopreserved human thyroid epithelial cells (passage 1) from healthy adult donors of both genders ≤55 y.o. and a body mass index of ≤35 were cryopreserved as a serum-free bio-preservation medium. Thyroid cells from individual donors (N=3) were thawed in human thyrocyte plating medium (HTPM) and plated on Matrigel-coated 96-well culture plates at various seeding densities. The thyrocytes were maintained in a h7H-based culture medium containing 1mIU/mL bovine thyroid stimulating hormone (HTCM). Medium samples were collected at 14 days post seeding for thyroid hormone measurements by LC-MS/MS or ELISA. The limit of detection (LOD) of T4 was 0.01ng/mL for LC-MS/MS and 0.781 ng/mL for ELISA. The results showed that thyroid microtissues seeded within a certain range of cells/well produced optimal levels of T4 and T3 with sufficient dynamic range (≥2.6) for TDC screening (≥1 μg T4/day/10 6 cells and ≥0.5 μg T3/day/10 6 cells on day 14). Inhibition of T4 and T3 synthesis with the TPO inhibitor methimazole was determined after a 120-h treatment of 7500 cells/well between days 9 and 14 using LS-MS/MS analysis. Half-maximal inhibitory concentrations (IC50) for methimazole were 0.26 μM (T4) and 0.5 μM (T3), which aligned well with results using the ELISA method. Thyroxine hormone production analysis over time in culture using the LC-MS/MS method also suggested that testing TDCs can be started as early as day 7. In conclusion, LC-MS/MS provides a superior measure of thyroid hormone compared to ELISA because of the lower limit of quantification (LOQ) and less error variance. As a result, an optimized testing regimen requiring fewer primary human thyrocytes per well and a shortened culture period could be adopted for assessment of inter-individual effects of TDCs in sensitive or diseased populations.
The assessment of photoxicity potential of cosmetics using the reconstructed human epidermis (RHE) model has been performed for decades, and the test method was recently adopted as an OECD Test Guideline (TG 498). Cosmetic formulations described as "long lasting" are intended for extended periods of use, developed to be durable, and may require photossafety testing. Two such types of cosmetic products are lipstick and foundation. When evaluating long lasting lipsticks in the RHE Phototoxicity assay, dark colored or difficult to remove materials could interfere with irradiation (+Irr) exposures. Therefore, modifications from standard protocols may be necessary. Modifications included reduced dosing volume, use of glass rod to spread the material over the tissue, use of warmed rinse, and evaluation of a surface swabbing to remove material. Two lipsticks were evaluated under standard (designated as AB19) and "spiked" with 0.1%, 0.5%, and 1% of a known photoantioxidant, chlorpromazine, to evaluate the test system with modifications. Briefly, the tissues were topically exposed to each treatment group for approximately 24 hours, removed from the tissues, and then half of the treated tissues was exposed to 6 J/cm² of UVA and visible light (+Irr), while the remaining half was retained in the absence of light (-Irr). The tissues received an overnight post-exposure incubation and then viability was assessed using the MTT assay. A test material was considered to have photoxicity potential when the +Irr tissues produced a >30% difference in viability relative to the non-irradiated (-Irr) tissues, consistent with the standard protocol. When tested without spike, each lipstick (designated as AB19) produced ≥30% differences between the +Irr and -Irr treatment groups. When spiked with 0.1%, 0.5%, and 1% chlorpromazine, lipstick AB19 differences in viability between +Irr and -Irr tissues were 18.8%, 73.1%, and 76.2%, respectively. Lipstick AB20 spiked with 0.1% and 0.5% chlorpromazine produced a 2.1% and 46.1% difference in viability between +Irr and -Irr tissues. Lipstick AB20 was cytotoxic (+Irr) and photoxicity potential could not be evaluated. Our findings support that the modified procedures for long lasting cosmetics provided sufficient exposure to the tissues, allowed for adequate light exposure to elicit a photoxic response (evident in the spiked test articles +Irr and -Irr), and are appropriate to evaluate photoxicity potential in the RHE model.

Currently there are no standardized non-animal test methods to evaluate phototoxicity potential, however, test guidelines to address photoirritation and skin sensitization methodologies have been adopted and routinely used in vitro, by industry. In the RhE model for decades, and the test system with modifications. Briefly, the tissues were topically exposed to each treatment group for approximately 24 hours, removed from the tissues, and then half of the treated tissues was exposed to 6 J/cm² of UVA and visible light (+Irr), while the remaining half was retained in the absence of light (-Irr). The tissues received an overnight post-exposure incubation and then viability was assessed using the MTT assay. A test material was considered to have photoxicity potential when the +Irr tissues produced a ≥30% difference in viability relative to the non-irradiated (-Irr) tissues, consistent with the standard protocol. When tested without spike, each lipstick (designated as AB19) produced ≥30% differences between the +Irr and -Irr treatment groups. When spiked with 0.1%, 0.5%, and 1% chlorpromazine, lipstick AB19 differences in viability between +Irr and -Irr tissues were 18.8%, 73.1%, and 76.2%, respectively. Lipstick AB20 spiked with 0.1% and 0.5% chlorpromazine produced a 2.1% and 46.1% difference in viability between +Irr and -Irr tissues. Lipstick AB20 was cytotoxic (+Irr) and photoxicity potential could not be evaluated. Our findings support that the modified procedures for long lasting cosmetics provided sufficient exposure to the tissues, allowed for adequate light exposure to elicit a photoxic response (evident in the spiked test articles +Irr and -Irr), and are appropriate to evaluate photoxicity potential in the RHE model.
the type of surfactant. Cationics tended to have higher opacity values and lower permeability values compared to anionic surfactants. Chloride (BAC), a representative cationic, was tested at concentrations from 0.05 to 10%. BAC concentration increases resulted in opacity value increases from 3.3 to 6.10 at 0.05% and 10%, respectively. Histopathology revealed coagulation of epithelial cell proteins, consistent with the higher opacity values. Conversely, anionics tended to have lower opacity values and higher permeability values. Sodium deoxycholate, a representative anionic, was tested at concentrations from 0.1%-30%. While the opacity values increased only slightly (1.7 to 8.0 for corneas treated with 0.1% to 30%, respectively), the permeability values increased from 0.041 to 2.083 at the same concentrations.

We believe the results of the histopathology will reveal epithelial cell layer erosion, affecting the barrier properties of the cornea, and leading to the high permeability values. Representative nonionics, PBS, Tween, and Brij showed milder results with lower opacity and permeability values, indicating a lower irritation potential. One exception, Triton-X-100 (TX-100), showed higher permeability and opacity values, especially tested at 10%. TX-100 produced an IVIS of 39.7, driven by the permeability value (2.177). While the histopathology changes of TX-100 were consistent with the opacity and permeability changes, the variation between nonionics warrants further investigation, as does the histopathology of non-irritant nonionics. The BCOP assay was shown to be a useful test method in the prediction of irritation by surfactants, with histopathology reflecting observed opacity and permeability changes. As expected, the IVIS was driven by opacity in cationic surfactants, permeability in anionic surfactants, and nonionic surfactants generally were least irritating. Our findings may provide a reference for industry or research in formulation development, highlight structural or chemical-based mechanisms for ocular irritation, and elucidate surfactant behavior.
Mechanistic investigations on autonomic control of cardiac physiology require the use of free-living organisms because vagal innervation principally occurs at or after birth/hatch in vertebrates. As such, it is physiological dogma that parasympathetic control of the heart is not fully functional until after birth. Thus, “ideal” model organisms used in autonomic nervous system (ANS) testing require restraint and/or anesthesia to immobilize the organism for measurement; requirements that do not support high throughput ANS phenotyping. After decades of use in the environmental sciences, fish have become an economic alternative for preclinical testing of new drug candidates, and have a well-conserved ANS. Here, we describe a fish embryo model adapted with an unusually precocious ANS, which comes online at 70% of in ovo embryonic development, well in advance of hatch. This early emergence of ANS efficacy allows for interrogation of both sympathetic and parasympathetic fibers during cardiovascular monitoring of heart rate and vascular perfusion in vivo; thus, without need for anesthesia or restraint. To leverage this alternative model organism for preclinical and environmental sciences testing, we designed a new platform that integrates sensors within a multi-chambered platform designed to dose, image and directly record heart rate in real time, from fish embryos. Using this configuration interfaced with data acquisition and management software, we non-invasively recorded chronotropic effects of ANS effectors on heart rate through several assays customized to this system. Exposure of embryos to common ANS antagonists and agonists reliably caused expected effects to heart rate and variability. As such, these methods and technologies highlight the utility of alternative model organisms and emerging technologies for use in high-throughput physiologically phenotyping for toxicology in medicine and environmental science.

A number of microphysiological systems have been developed for human liver and are considered promising models for studies of metabolism and potential hepatotoxicity for drugs and chemicals. One such model is the PhysioMimix™ LC12 (CNBio) where primary human hepatocytes can be cultured alone or in combination with various non-parenchymal cells for up to 14 days under constant recirculating media flow. Congruent with TEX-VAL Consortium activities, a multi-stakeholder effort for establishing the functionality, reproducibility, robustness, and reliability of microphysiological systems, this study aimed to evaluate capabilities of the PhysioMimix™ LC12 platform using different cell types and conditions. We evaluated the robustness of the model by replicating experiments and comparing both basal hepatic function (albumin, urea, and lactate dehydrogenase release) and drug metabolism (CYP3A4 activity and metabolism of midazolam to 1-OH and 1-OH glucuronide) in hepatocyte monolayer and culture with THP-1 conditions. Basal function and drug metabolism in the monoculture model peaked on Days 4-6 and gradually declined by Day 14. The model and acquired data can be used to evaluate and compare the effects of genetic background on modifying DART risks from chemicals such as TPhP in different populations have been poorly studied. vivoVerse offers a rapid and cost-effective way to test the effects of genetic background on modifying DART risks from chemicals such as TPhP in different populations have been poorly studied. In this study, we treated C. elegans with several doses of TPhP for 72 hours from post-hatching to reproductive age and assessed their developmental and reproductive parameters using whole-body bright-field imaging. We used a scalable 24-well microfluidic imaging platform (vivoChip) to simultaneously immobilize ~1,000 C. elegans and acquire 3D time-lapse images from all animals in 30 min to quantify phenotypes such as in utero embryonic developmental stages and body dimensions. We demonstrate the power of our multi-parametric analysis in identifying multiple effects of TPhP exposure in a dose-dependent manner. Our study suggests that the moving embryo phenotype is most sensitive, providing a 4× lower EC50 value than the CC50 for change in the total number of embryos. We found similar differences in EC50 values of different DART phenotypes with a second environmental toxicant, pipierazine. We also assessed the embryonic phenotypes in different wild-type C. elegans strains to capture differences in the adverse effects with different genetic backgrounds. These findings indicate that our C. elegans-based assays can identify subtle changes in the DART phenotypes, providing in vivo toxicology data on early developmental processes at a fraction of the cost and time of mammalian studies.
Sunscreen products are composed of ultraviolet (UV) filters and formulated to reduce exposure to sunlight thereby lessening skin damage. Concerns have been raised regarding the toxicity and potential endocrine disrupting (ED) effects of UV filters. The ToxCast/Tox21 program, i.e., CompTox, is a high-throughput in vitro screening database of chemicals that can identify Adverse Outcome Pathways, Key Events and ED potential of chemicals. ToxCast/Tox21 data was found for avobenzone, homosalate, octinoxate, octisalate, octocrylene and oxybenzone, six of the most used organic UV filters. These UV filters showed low activity in these bioassays with most activity detected above the range of the “cytotoxic burst”. The pathways that were most affected were the cell cycle and the nuclear receptor pathways. Most activity was observed in liver and kidney cell-based bioassays. These organic filters and their metabolites showed weak ED activity when tested on bioassays measuring Estrogen Receptor (ER), Androgen Receptor (AR), Thyroid Receptor and steroidogenesis activity. Except for oxybenzone, all AC50 activity in the endocrine assays occurred at concentrations greater than the cytotoxic burst. For five of the six UV filters, plasma concentrations (Cmax) measured in humans isothiocyanate (FITC)) and dead cells fluorescing red (Texas Red). Using this assay, using a live/dead fluorescence assay, with live cells fluorescing green (fluorescein controls. Viability of the cells within the bioink constructs was also investigated indicating some living cells in the bioink, The MTT assay also matched the visual NHEKs to form the integument as well as disease relevant human tissues. Three dimensional (3D) bioprinting is a process in which a 3D bioprinter fabricates tissue structures that contain cells and an extracellular matrix. Using a commercially available bioprinter, a 3D barrier model was bioprinted using nanocellulose-based bioink containing collagen and fibrinogen combined with primary human dermal fibroblasts (HDFs) and primary normal human epidermal keratinocytes (NHEKs) to form the integument barrier model. The constructs were cross-linked and incubated in specialized 3D cell culture medium, then flipped over at about 4-7 days so the epidermis is exposed to air. Bioprinted constructs are incubated further for at least 14 days at the air-liquid interface to develop and differentiate tissue-like structure and function. Of microscopy analysis of the bioprinted 3D constructs showed normal development of dermal layers and an upper barrier layer consistent with intact integument. A preliminary comparison of cell viability assays was done to determine viability of cells in the bioink during differentiation. Using the MITT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) assay, cells in bioprinted construct samples showed about a 14.9 to 26.9% higher absorbance than control constructs, indicating some living cells in the bioink, The MITT assay also matched the visual confirmation of the color change in the cell containing constructs compared to controls. Viability of the cells within the bioink constructs was also investigated using a live/dead fluorescence assay, with live cells fluorescing green (fluorescein isothiocyanate (FITC)) and dead cells fluorescing red (Texas Red). Using this assay, the vast majority of the cells in the bioink fluoresced green. Using a comparison of cell viability assays, it appeared that the cells remain viable in the bioink during the development and differentiation process. Experience with the methodologies of 3D bioprinting tissue constructs and experience with the process to establish reconstructed tissue models will be used in the future to bioprint engineered 3D tissue constructs, from diverse organ systems, for predictive toxicology testing. 

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Despite the popularity of electronic cigarettes (e-cigs) as vaping devices, the health effects associated with their use remain poorly studied. This is particularly true with cannabis-based vaping alternatives like cannabidiol (CBD), which have become increasingly available to the public. Several studies have characterized nicotine-based vaping products, yet little to no data have been published characterizing non-nicotine e-liquids, such as CBD, or their health effects when inhaled. In this study, we have built on our previous work, which demonstrated product-depen- dent variability in CBD aerosols, reduced lung epithelial membrane barrier function and cytotoxicity from acute exposure to CBD e-liquid. Our current work focused on inflammatory and genotoxic endpoints by exposing an ALI CALU-3 airway epithelial cell line to aerosolized CBD distillate, common carrier oils, and mixtures of these at a standard “low” power (6.5W) in agreement with evolving manufac- turer recommendations for lower power or voltage settings when using CBD. At this lower power and voltage (3-3.5V), the aerosol generated from CBD, VG/PG, MCT, and 50/50 blends of VG/PG/CBD and MCT+CBD, reduced membrane barrier function (TEER) and induced LDH release in most groups. At 24 hours post-ex- posure, cytotoxic effects were seen at both 15 and 30-minute exposure intervals. Markers of inflammation caused by exposure to aerosolized CBD e-liquids were also explored. Ten pro-inflammatory cytokines were measured via a cytokine assay (MDSD), and levels were compared for each e-liquid exposure in both basal and apical fluids. Inflammatory biomarker changes varied among apical and basal fluids, however there were significant increases in the expression of both IL-6 and IL-8, after acute exposure for all e-liquids at both time intervals. Although further changes were observed, particularly in IL-1β, IFN-γ, and IL-12/27 biomark- ers, inflammatory endpoints were inconclusive. Genotoxicity of these same CBD aerosols was measured via Comet assay. At this decreased power, exposure to CBD e-liquids and carrier oils from an e-vaping system showed increasing DNA damage over time. DNA damage observed at this lower power setting suggests that CBD e-liquid aerosols vaped at lower temperatures may have comparable adverse outcomes at both short and longer exposure times. Deposition of CBD e-liquids and common carrier oils was compared for short and long exposures. Among the exposure groups, carrier oil VGPG and pure CBD were shown to have the highest deposition at 15 minutes. Similarly, at 30 mins, VGPG again was shown to have the highest deposition along with CBD+MCT. All e-liquids, with the exception of pure CBD, exhibited an increase in product deposition with time (ie. 30 mins vs 15mins), alluding to the potential downstream effects. Current findings in combination with our previous work, found that variability amongst e-liquids must be taken into account in vivo and in vitro assessments of the toxicity of inhaled CBD products. The composition of each e-liquid, power and heat applied, and length of exposure can be determining factors of CBD aerosol dosimetry and the potential harm to the respiratory tract and organ systems. These, and successive studies will serve to inform both the public and scientific community on the potential adverse health outcomes of vaping CBD and act as guidance for CBD consumers to inform users of their personal risk when “smoking” these cannabis-based alternatives.

Organ-on-a-chip (OOC) devices are an emerging New Approach Methodology in toxicology to predict human response from cellular response. OOCs are typically made of polydimethylsiloxane (PDMS), a polymer that is known to interact with hydrophobic chemicals. As a consequence, the nominal dosage of a toxicant in an OOC is not necessarily the true concentration: one must apply a correction for chemical-PDMS interaction. We have developed a toxicokinetic model to describe chemical-PDMS interaction, which includes partitioning, convection, diffusion, and flow. We will demonstrate how this model can be applied to the organophosphate neurotoxins parathion and paraoxon, and the control hydrophobic compound indole, which all partition rapidly into and diffuse within PDMS. Additionally, we demonstrate that plastic tubing used with OOC devices, including C-Flex, Tygon, tefzel, and PEKPEEK themselves interact with parathion, paraoxon and indole to varying degrees: C-Flex and Tygon show substantial interaction, but tefzel and PEEK do not. We show how a similar toxicokinetic model accounts for these interactions and corrects nominal doses to actual doses. If one is to use an OOC to model human response to a toxicant, one must account for chemical interaction with PDMS and medical plastic tubing to estimate the actual chemical concentration reaching the enclosed cells and that yield accurate dose-response curves.
Multiple regulatory agencies have released new guidelines permitting the use of new approach methods (NAMs) in conjunction with or in place of the traditional \textit{in vivo} embryo-fetal development (EFuD) studies. In particular, the revised SS (R3) guideline on Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals recently issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) defines specific scenarios where qualified NAMs can be used to defer or replace conventional \textit{in vivo} testing or as part of a weight of evidence assessment. The \textit{devTOX} quickPredict (devTOXqP) assay is an in vitro model that predicts the developmental toxicity potential of chemicals based on changes in ornithine and cystine metabolism. This assay has been used by multiple industries for compound prioritization for almost a decade and is included as part of the Next Generation Risk Assessment (NGRA) strategies being developed by multiple companies for animal-free assessment of developmental and reproductive toxicity (DART) testing. Additionally, devTOXqP has an accepted letter of intent with the FDA’s Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program (BQP) to qualify the assay as a safety biomarker for detecting human developmental toxicity potential in vitro at the nonclinical stage of drug development for small molecule drugs as part of a weight-of-evidence assessment as described the ICH SS(R3) guideline, which will enable regulatory use of the assay in the pharmaceutical industry. As part of this qualification, we have evaluated the performance of the assay across a diverse set of 89 pharmaceuticals, including the 29 ICH positive reference compounds. The assay predicted the developmental toxicity potential of these pharmaceuticals with a balanced accuracy of 85% (83% sensitivity, 88% specificity, 90% PPV, 80% NPV) when scored against a single C\textsubscript{50} concentration (selected based on the compound’s known effects in humans, rodents, or rabbits). The devTOXqP assay provides data on human response and is a necessary addition to protect human health, to replace (in certain cases), reduce, and refine animal testing, and to help to reconcile discordant information from the two required \textit{in vivo} endpoints.

### Comparative Analysis of Tox21 In Vitro Assay Readouts from Three Major Data-Cleaning Pipelines

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The Tox21 quantitative high-throughput screening (qHTS) program is a key initiative towards reduction and replacement of animal use in toxicity testing with data based on \textit{in vitro} experiments. The latest Tox21 release (August 2022) makes public the data for over 80 assays representing various critical biomolecular targets and pathways that are relevant for wide range of toxicity mechanisms (nuclear receptors, oxidative stress, damage/repair, CYPs, etc.). Since in vitro assays are often confounded by cytotoxicity and assay interference, several qHTS data cleaning pipelines have been developed to address such issues and provide cleaner assay signals. In this study we have compared classification calls (active, inactive, inconclusive) and concentrations of point-of-departure (POD) metrics, such as EC50 and AC50, based on three qHTS data-processing pipelines used by US NIH agencies, namely NCATS, NIHES, and NICEATM, in their research projects. We have found a high correlation (average Pearson r = 0.925) for all POD metrics across three pipelines. For the category calls, inactive labels for all assays were highly concordant (>99%) from all pipelines, while there was more variability in the active calls (70-90% concordance) across three pipelines. The category calls from different pipelines may be suitable for different decisions. Regulatory applications, for instance, will likely require very conservative active calls, while for exploratory and prioritization tasks more inclusive activity calls may be needed. Such flexibility can be best achieved from various in silico NAMs, which we discuss. For the example of aryl hydrocarbon receptor (AhR) agonism assay, combining active calls from the three pipelines by strict agreement yields 391 potential AhR agonists, while a majority vote consensus (at least two out of three pipelines must agree) yields 626 potential AhR agonists. Finally, maximum call consensus (when active in any of the three pipelines) gives 949 potential AhR agonists. While individual assay calls from each pipeline are public, significant effort is needed to use the data from different pipelines together. We provide such data integration via our Orbitox platform (sciome.com/orbitox) with free access for non-commercial use.

### Health and Safety Assessments of Functionalized Cellulose Materials: Simulated Gastrointestinal Digestion and Exposure to a Gut Co-culture Model

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Increased utilization of various types of cellulose materials in industrial and consumer applications, including food-contact materials, has necessitated further property characterization to ensure human health and safety after exposure. Characterization can ultimately be predictive of interactions between individual nanocellulosic fibers and environmental or biological systems. Fibrillated cellulose has been proven food-safe based on chemical similarities to conventional cellulose. Functionalized fibrillated cellulose is modified with various functional groups such as carboxylic acids or sulfates to improve aqueous dispersibility and functional applications. These functionalized cellulose materials can be characterized via physicochemical techniques analogous to those used for fibrillated cellulose to develop a predictive structure-function model. Differential cellulose functionalization requires toxicological comparison among variants in addition to currently used regular and fibrillated cellulose. Animal models can provide correlation to human response but there is momentum in biomedical research to improve alternatives that better represent human biology. Multiple cell types cultured in a three-dimensional space produce a representation of an intact organ structure and immunological response, consequently indicative of workers, consumer health and safety outcomes. In this study, human gut cells were co-cultured; the model consists of intestinal mucous-producing goblet cells (HT29-MTX) and colon epithelial cells (undifferentiated Caco-2). Lympocytes (Raji B) were initially utilized to transform a subpopulation of Caco-2 epithelial cells into transcytotic cells, providing an improved platform for testing materials. An unmodified functionalized cellulose exposure in a gut-based model is ex vivo digestion to decompose material prior to cell culture inoculation. Following the enzymatic/chemical digestion steps, three functional characteristics were measured, demonstrating the effect of functionalized cellulose in the tri-culture model. These include barrier integrity via TEER and ZO-1 staining, antigen transport via mass spectrometry and inflammatory response verified via measurable gene expression and cytokine profiling. Preliminary results show functionalized fibrillated cellulose materials induce similar biological responses as compared to conventional celulloses in terms of low cytotoxicity, insignificant immune response, and no damage induced to intestinal barrier integrity.

### Utilizing the HepaRG Human Hepatoma Cell Line to Understand Mono-(2-Ethylhexyl) Phthalate Mitochondrial Toxicity

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The widespread exposure of the general population to phthalates has raised significant public concern. Phthalates are added to plastics widely found in food packaging, toys, medical devices, pharmaceuticals, furniture, and cosmetics, and they leach out of these products into the food, water, and air. Di-2-ethylhexyl phthalate (DEHP) is abundantly used, and inside the gut, it is hydrolyzed to the active metabolite mono-(2-ethylhexyl) phthalate (MEHP). Phthalates are established endocrine disruptors; however, evidence suggests that phthalates also alter mitochondrial function. Recently, our group showed that undifferentiated HepaRG cells exposed to the mitochondrial DNA (mtDNA) toxicant 2,3′-dideoxyctidine more rapidly deplete their mtDNA genomes and alter bioenergetics relative to differentiated (hepatocyte-like) HepaRG. The function of undifferentiated cells in an organ is to replace cells lost under steady-state conditions and during tissue repair. Therefore, we predict MEHP triggers mitochondrial dysfunction by targeting undifferentiated cells that may be more sensitive to mitochondrial toxicants than differentiated cells. Undifferentiated and differentiated HepaRG cells were exposed to various concentrations of MEHP for thirteen days to determine half maximal inhibitory concentration (IC\textsubscript{50}) values, 318 ± 59 and 260 ± 26 micromolar, respectively. Next, we exposed both cell types to 300 micromolar MEHP and harvested them following six- and twelve-day exposures. The cells were harvested to seed XfP Seahorse mini-plates for extracellular flux analysis and to prepare whole-cell DNA and protein extracts. We found that after seven and thirteen days of exposure, differentiated HepaRG proton leak was significantly increased relative to the vehicle control. Also, differentiated HepaRG and undifferentiated cells lost mtDNA under MEHP exposure conditions, however, little is known about specific interactions and potential mechanisms of action. In this study, primary human bronchial epithelial cells (HBEC) cultured in 3D at the air-liquid interface (ALI) are utilized as a physiologically relevant model to evaluate the effects of exposure to toxicity of polycyclic aromatic hydrocarbons.
PAHs, a class of contaminants generated from incomplete combustion of fossil fuels. Normal HBECS were differentiated in the presence of IL-13 for 14 days to induce a proinflammatory phenotype similar to asthma. Fully differentiated normal and asthmatic phenotype HBECS were treated with benzo[a]pyrene (BP; 1 - 40 pg/mL) or 1% DMSO/PBS vehicle at the ALI for 48 hrs. Cells were evaluated for cytotoxicity, barrier integrity, and transcriptional biomarkers of chemical metabolism and inflammation by quantitative PCR with real-time melting curves with data generated with Taqman probes, as well as increased sensitivity to BAP metabolism compared to cells with the normal phenotype. Additionally, RNA sequencing data showed that a large number of genes were uniquely significantly expressed in cells with the induced asthmatic phenotype exposed to BAP. Future studies will further explore mechanisms of toxicity from global transcriptomics and investigate the role of microRNA in mediating mechanisms of toxicity and inflammation. These data are the first to evaluate the role of combined environmental factors associated with inflammation from pre-existing disease and PAH exposure on pulmonary toxicity in a physiologically relevant human in vitro model.

### 3668 The Use of Quantitative Similarity Assessment and NAMs to Establish a Read-Across Category: A Case Study with Benzoic Acids

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Read across is an important animal alternative method for filling toxicological data gaps and while simple in theory can be difficult in practice, with the most challenging aspects involving the selection of analogues and justification of their use to establish the safety of a data-poor target compound. While structure-activity relationships are more easily established for compounds displaying the same pharmacokinetic and metabolic properties and can be underpinned with New Approach Methodologies (NAMs), chemicals displaying little or no activity or toxicity can be harder to address. Benzoic acid is a critical preservative used in cosmetics and its safety has been well established. As part of the cosmetic Europe (CE) long range science strategy (LRSS) for assessing systemic toxicity based on non-animal approaches, we apply NAMs to support the grouping of a category of compounds expected to possess the same low toxicity as benzoic acid and its innocuous salts for which safety may be established by read across. Structural boundaries defining the category are based on a matched molecular pair approach for selecting analogues with further definition involving the demonstration of similarity in metabolism, reactivity, and physical chemical properties. Quantification of these similarities is achieved by defining a fingerprint for each attribute and computing the overlap of analogue fingerprints with those for benzoic acid. In vitro metabolism data are used to define metabolism fingerprints and to explore the effects of structural changes on rate and extent of metabolism as well as biotransformation pathway. A TotalScore consisting of the sum of similarity scores for metabolism, reactivity, and physical chemical properties provides a clear quantitative cutoff for category members. Analogues with the highest TotalScore of 3.0 consist of benzyl salts of benzoic acid in addition to para- and meta-tollic acids which are shown to display the same metabolism as benzoic acid. Analogues displaying small differences in metabolism to a TotalScore of 2.0 A TotalScore that is less than 3.0 but greater than 2.5 and consist of small alkyl derivatizes of benzoic acid. Metabolism similarity is shown to be the most important attribute defining the category. Curation of ToxCast/Tox21 bioactivity data included test substance characterization, where available, and biological plausibility of the dose response relationships for identification of active assays. These curated data are used to substantiate low activity for benzyl salts and demonstrate that these analogues outside the read-across category. This poster demonstrates the practical application of quantitative consideration of similarity in metabolism, reactivity and physical chemical properties supported by in vitro metabolism and Toxcast NAM data which greatly enhances the robustness of a read-across assessment for establishing the safety of low-toxicity compounds. via inhalation exposure. We obtained activity concentrations derived from in vitro assay measuring diverse endpoints (e.g., genotoxicity, cytochrome p450 activation, transcriptome analysis) from public resources. Using these data, IVIVE was performed to estimate the daily equivalent administered dose (EAD) that would result in plasma and lung concentrations equivalent to the in vitro activity concentrations. For chemicals that were inactive in an in vitro assay, the maximum testing concentration was used for IVIVE, and the EADs were then compared to the in vivo point of departure (POD) used to derive the MRLs. Our preliminary results showed that differences between EADs and in vivo data varies greatly between chemicals and across assays, ranging from less than 2-fold to more than 1000-fold. For most chemicals, the EADs estimated based on lung concentration were closer to in vivo PODs than those based on plasma concentration. Furthermore, the EADs estimated using in vitro assay data measures an endpoint more mechanistically relevant to in vivo exposure better predicted in vivo PODs compared to those estimated using an in vitro assay measuring nonspecific effects (e.g., cytotoxicity assays). The impact of metabolism and pharmacokinetic model structures on IVIVE outcomes were also evaluated. In summary, this study provides proof-of-concept case examples to illustrate the utility of using non-animal approaches to inform hazard identification and risk for humans exposed to inhaled substances. This project was funded in whole or in part with federal funds from the NIH, under Contract No. HHSN273201500010C. The findings and conclusions in this abstract have not been formally disseminated by the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

### 3669 Combining NAM Data and IVIVE for Evaluating Potential Inhalation Toxicity

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Traditional chemical risk assessment is often based on no- or lowest-observed-adverse-effect levels derived from in vivo toxicity data. New approach methodologies, such as in vitro systems, can be used for toxicity screening in a more rapid and cost-effective manner than animal tests. In vitro assays can provide safe exposure levels for a chemical when combined with an in vivo to in vitro extrapolation (IVIVE) approach. IVIVE uses pharmacokinetic models to relate concentrations of substances that induce in vivo responses to a corresponding equivalent in vivo dose. In this study, we selected 20 volatile organic compounds (e.g., styrene, tetrachloroethylene, 2-butoxyethanol) with abundant pharmacokinetic data and published minimal risk levels (MRLs) covering multiple target organs

### 3670 Integrating Population Enzyme Variability into Physiologically Based Kinetic Models of Parent Chemicals and Metabolites

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Chemicals that enter the body are broken down into metabolites by enzymatic activity from a range of metabolic pathways. Rates of metabolism can vary across human populations due to inter-individual genetic variability, making some populations potentially slower in metabolism and more sensitive to effects from parent chemicals or their metabolites. Risk assessors apply physiologically-based kinetic (PB-K) models to depict the dynamics of tissue concentrations for both parent chemicals and metabolites, but technical and data limitations often make it difficult to apply these models to characterize the effects of enzymatic pathway-related variability within populations. We developed a generalized workflow for incorporating pathway-related variability for select Phase I cytochrome P450 (CYP) and Phase II UGT enzymes across human populations into PB-K models. The elements of the workflow include metabolite structures generated using SimulationsPlus ADMET Predictor®, PB-K models from the U.S. Environmental Protection Agency’s httk R package, estimates of enzyme variability from EFSA literature reports, and parameter predictions from OPERA (v2.8). Data on inter-individual genetic variability in enzyme activities are integrated into httk models by applying pathway-related variability distributions to intrinsic clearance parameters of parent chemicals. Parent chemical dynamics are simulated following an initial exposure and the resulting EADs are scaled by the available in vitro activity concentration.

SimulationsPlus ADMET Predictor database with multiple rounds of metabolism and Toxcast NAM data which greatly enhances the robustness of a read-across assessment for establishing the safety of low-toxicity compounds.
Columbus, OH; and 2

intensity measurements from each of 384 wells are captured by an Opera Phenix

that it poses unacceptable cardiovascular risks. Therefore, it is critical to identify

conditions and syndromes sensitive to cumulative chemical exposures.

Find up-to-date information at

- assay and generate multiple features derived from the raw signal. These features

be performed using high-throughput imaging, generating large quantities of assay

Novel drug development is a complex and arduous process that usually takes many

to cumulative chemical exposures were identified using Self Organizing Map

to the CTD to identify disease categories associated with these pathways. Finally,

chemical exposures were identified using Gene Set Enrichment Analysis (GSEA)

a total of 591,084 individual chemical-gene interactions from the Comparative

environmental etiology may facilitate the identification of causal routes of major

health conditions. Today, the healthcare system will likely treat these conditions

pathways described as toxicity pathways. Changes in the same set of molecu-

labeled and/or transported as well as prioritizing and screening chemicals in early R&D.

This poster will outline a battery of transparent and fit-for-purpose in silico models to evaluate the acute toxicity 6-pack: (1) acute systemic toxicity by three routes of exposure (oral, dermal, inhalation), (2) skin and eye irritation/corrosion, and (3) skin sensitization. To support the development of these models, a series of databases were constructed including 175,885 chemicals with acute toxicity data, 3,775 chemicals with skin irritation/corrosion data, 6,619/oral/cutaneous exposure data, and 2,188 chemicals with skin sensitization data. These models predict categories such as the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) categories and have been evaluated using a series of public and proprietary datasets. The model performance for two methodologies (expert rule-based and statistical-based) across all 6 endpoints will be presented. For example, for a set of approximately 2,000 proprietary chemicals, the rat oral in silico models predict 95% either in a correct or more conservative category. The poster will review the development and performance of these models and present workflows to illustrate how they can be used to support the 3Rs.

Toward Whole Health Toxicology: In Silico Prediction of Diseases Sensitive to Multichemical Exposures

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Chemical exposures from diverse sources merge on a limited number of molecular pathways described as toxicity pathways. Changes in the same set of molecular pathways in different cell and tissue types may generate seemingly unrelated health conditions. The healthcare system wrestles with these conditions as unrelated, while recognition of these conditions as syndromes with common environmental etiology may facilitate the identification of causal routes of major health problems to inform efficient interventions. In this study, we propose an in-silico approach to identify diseases sensitive to multi-chemical exposures. Of first, the potential toxicity effects of each of the chemical-gene interactions were identified using a total of 591,084 individual chemical-gene interactions from the Comparative Toxicogenomic Database (CTD) and expressed as a number of chemical-gene interactions per gene. Next, molecular pathways enriched with genes sensitive to chemical exposures were identified using Gene Set Enrichment Analysis (GSEA) against KEGG, Reactome, and KEGG pathways databases and top nodes in these pathways were mapped to the CTD to identify disease categories associated with these pathways. Finally, diseases sharing common pathways were identified using Self Organizing Map (SOM) and hierarchical clustering. Health conditions predicted as the most sensitive to cumulative chemical exposures include major public health problems: multiple chemical exposures were identified as a contributing factor for many neurological and neurodegenerative diseases, and autoimmune conditions. This analysis predict-

Automating Analysis of Calcium Flux Assay Readouts of Cardiomyocyte Cells to Identify Mode of Action in Cardiotoxic Compounds

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Novel drug development is a complex and arduous process that usually takes many years to complete. Unfortunately, there have been too often been cases where a manufactur-

er has had to reveal a promising drug from the market after finding out too late that it poses unacceptable cardiovascular risks. Therefore, it is critical to identify such adverse effects as soon as possible during the early stages of the drug discovery process. The calcium flux assay is used to analyze cardiomyocyte cells in vitro. By observing changes in the rhythmic beating of these cells, it is possible to identify irregularities induced by pharmacological agents. Furthermore, these assays can be performed using high-throughput imaging, generating large quantities of assay data for multiple candidate compounds in parallel. We have developed a pipeline which can be used to analyze the time series measurements from the calcium flux assays and generate multiple features derived from the raw signal. These features are then combined into a profile which can then be compared against the comple-

tly gold-standard patch clamp ion-channel blockage assay. Fluorescence intensity measurements from each of 384 wells are captured by an Opera Phenoix imager. The readout duration was kept at 10 seconds, but multiple frame rates (e.g., 10 fps, 20 fps, and 50 fps) were tested during the process of pipeline optimi-

zation. Our analysis suggested that 10 fps, resulting in a total of 100 measurements per well, allows us to capture most of the detail with minimal amount of noise.

Before analyzing further, we use various moving averages and the Savitzky-Golay algorithm to smooth the data and remove extraneous noise. The pipeline then processes the smoothed time series data using state-of-the-art signal processing techniques to generate multiple quantitative features. Next, we identify true peaks by using data-driven threshold values to remove the false peaks and outliers. We further calculate several additional parameters of the signal including amplitude, space between peaks, width of different regions, rise and decay of the peaks, etc., which together capture valuable quantitative information regarding the possible mechanism of action of the treated drug. Our pipeline graphically plots the analyzed signal showing all calculated features, allowing users to perform quality checks and to visually examine the shape of the signals. Using this novel pipeline, we are now exploring the development of machine learning techniques, including methods that use the raw signal directly, to accurately characterize the mode of action of cardio-

toxic chemicals by classifying the shape of the assay signal. As a proof of concept, we have developed a classification model that classifies toxic and non-toxic chemicals with 94% accuracy, exploring the development of machine learning techniques, including methods that use the raw signal directly, to accurately characterize the mode of action of cardio-
toxic chemicals by classifying the shape of the assay signal. As a proof of concept, we have developed a classification model that classifies toxic and non-toxic chemicals with 94% accuracy, using this novel pipeline, we are now exploro
Multidrug resistance protein 3 (MDR3) translocates phospholipids (PLs) from the inner to the outer leaflet of the canalicular membrane of hepatocytes. These flopped PLs can form mixed micelles with bile acids (BAs) that have been excreted into the bile canalculus by the bile salt export pump. Drug-induced inhibition of MDR3 function can lead to a reduction in Ps for a database that can lead to a biliary excess of toxic BA monomers. Biliary increases in free BAs can damage the bile duct epithelial cells, i.e., cholangiocytes, which may develop into clinically defined cholestatic liver injury. Cholangiolaricellular mechanisms that could compensate for bile duct BA elevation include the cholehepatic shunt pathway for BAs and the biliary secretion of bicarbonate. Computational models of drug-induced bile duct injury in humans at the individual and population level, which may aid in the prediction and prevention of drug-induced hepatotoxicity, have thus far been lacking. To predict drug-induced bile duct injury in humans, DILysm, a quantitative systems toxicology model of drug-induced liver injury (DILI), was extended by representing key features of the bile duct that are believed to impact cholestatic liver injury. Representations of PL excretion, modes of MDR3 inhibition, biliary BA toxicity and the compensatory effects of cholehepatic shunting and biliary bicarbonate secretion have been developed. Publicly available clinical data were used to calibrate and validate a virtual, healthy representative subject and population studies. Experiments were performed for MDR3 inhibition, the cholehepatic shunt pathway, and biliary bicarbonate concentration. To further validate the model, population-based simulations were performed with compounds that have established interactions with BA transport and known clinical outcomes. Simulations suggested that non-competitive and mixed inhibition of MDR3 had a profound impact on PL efflux, bile duct injury, and adaptation pathways, while competitive inhibition does not. Furthermore, simulations indicated that an enhanced functionality of the cholehepatic shunt decreases the BA burden in the bile duct, but increases BA concentrations in hepatocytes. The model also predicted that increases in biliary bicarbonate concentrations (from 25 to 65 mm) reduces shocks. Finally, the model with its extended representation of BA disposition accurately predicted DILI liability for compounds with known interactions with BA transport. For instance, virtual population simulations with the DILI exemplars AMG-009, TAK-875 and troglitazone predicted alamine aminotransferase > 3x upper limit of normal in a subset of simulated subjects, with frequencies consistent with clinical reports. These simulations provide confidence in the DILI model and in the DILI system. TreecompareR is an open-source package for the R statistical-computing environment, built on the popular ggtree and ggplot2 R packages. The current version of the ADC presents an efficacy of 74% to correctly identify datasets. The Automatic Dataset Creator (ADC) is a program written in Python that identifies the carcinogenic and genotoxic potential of a drug impurity based on identification and assessment of potentially reactive substructural features present in the impurity, and related analogs, using the OECD QSAR Toolbox. The second case study focuses on tools and strategies used to identify potential analog structures tricresyl phosphate (CAS# 1330-78-5) and tris(2,4-di-tert-butylphenyl) phosphite (CAS# 31570-04-3) to address multiple endpoints for oxidized Irgafos 168 (CAS 95906-11-9) derived from a drug container closure system for the purpose of deriving a safe daily exposure limit. In addition to RA framework implementation, the following topics are considered: 1. How to frame the product-specific context and intended data gap(s) to fill via RA to shape approach and identification of analogs.2. Interpretation and use of analog data to fill data gaps.3. Shortcomings of currently available RA frameworks and tools for evaluation of compounds unintentionally present in drug products.4. A discussion of fit-for-purpose considerations while conducting and reporting RA is described in the context of the case studies, as are future directions for implementation of RA in risk assessment of drugs.
In silico models that predict chemical adverse outcome pathways (AOPs) can provide mechanistic insight when evaluating toxicity. Such models leverage in vitro responses across multiple related assay targets to predict in vivo toxicological processes. Developing AOP models for complex toxicities, e.g., hepatotoxicity, is challenging because the relevant mechanisms are many and not well-understood. High-throughput screening (HTS) programs are capable of testing thousands of chemicals in concentration-response format using in vitro HTS assays, providing enormous amounts of information on toxicity mechanisms. Data sharing projects such as PubChem are storing the results of these assays and making them accessible to the public. However, using HTS assay information from such large data repositories to develop AOP models is a challenge. These assays come from HTS programs with different goals, resulting in a variety of assay targets involved in different biological pathways and processes. Grouping assays that test related targets (e.g., proteins involved in the same biological process or pathway) requires manual curation, which isn’t possible for such big data resources. Additionally, these assays test chemicals at concentrations that may not reflect the in vivo concentrations of chemicals due to metabolic transformation. To address these challenges, we introduce a novel hierarchical concentration-dependent modeling approach to using publicly available big data (e.g., PubChem) to develop AOP models. Using assay metadata (i.e., assay target information) and curated biological pathway data (e.g., target-pathway information from WikiPathways) over 1,200 PubChem concentration-response assays were grouped by related targets into 560 AOP models in a framework that mimics hierarchical, biological pathway organization. The final output of these AOP models is an AOP score which summarizes the potential of a chemical against different HTS assay targets related to a specific biological pathway or process. The predictivity of these 560 AOP models was evaluated by comparing the target-pathways containing over 50 chemicals with complex toxicity data including acute oral toxicity, hepatotoxicity, and developmental and reproductive toxicity. Over 230 AOP models showed significant correlation (p-value < 0.05) with specific toxicities, informing on the mechanistic nature of the toxicants. Furthermore, we show that the AOP scores of chemicals can be coupled with toxicokinetic data, improving their in toxicity predictions. This approach can be a new, universal strategy for computational toxicology and a rapid approach for AOP-based toxicity evaluations.
to many different biologically relevant metabolic networks, a statistical method is proposed to identify the most frequently differentially activated reactions (DAR) between two biological conditions. Groups of closely located DARs in the metabolic network can be extracted and then analyzed with advanced visualization tools such as MetExploreViz (Chazalviel et al., 2018) to better understand mMOA. As a use case, the workflow was applied for Amiodarone and Valproic acid, two well-known hepatotoxic molecules, using Open TO-Gates primary human hepatocyte transcriptomic data. 56 and 417 DARs have been identified for Amiodarone (24h,7μM) and Valproic Acid (24h, 5000μM) respectively. As an example, network analysis revealed an increased activation of de novo lipogenesis after Amiodarone treatment, already described in the literature (Hibul et al., 2021) as likely to be involved in liver injury. It is important to note that it was not possible to identify this biological process based on transcriptomic data only. These results suggest that enriching transcriptomic data through condition-specific metabolic networks can help to better model mMOA. As today in silico read across strategies are mostly structure-based approaches, this workflow represents a promising tool for systemic toxicity evaluation of the metabolic impact of defined structure molecules, as well as for the evaluation of dietary mixtures such as natural ingredients generating strong interest for cosmetic industries.

**Applying Multiple Read-Across Tools for Toxicity Evaluation**

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Read-across is an alternative method for filling data gaps based on analogue or chemical category approaches, with a wide range of applications on toxicological assessment and with an attractive regulatory acceptance. The read-across approach reduces the number of chemicals to be tested because the available information about the endpoints can be used to estimate those properties for untested substances. This study describes a general read-across assessment concept to support toxicity characterization of chemicals by integrating the publicly available read-across tools. The applied approach started with the utilization of multiple tools to select the most suitable analogues through diverse profiling methods. Components are: structural similarity, physical-chemical similarities, structural alerts endpoint specific, and metabolism similarities were considered. A workflow implemented in KNIME was used to integrate the results. Diverse in silico tools can be combined within a read-across assessment to strength the read-across hypothesis. Here, we suggest a strategy to increase the efficiency during the analogue identification stage and we propose a framework to simplify the analogue evaluation step after combining the outcome of diverse read-across tools. The lessons learned from a practical case study are also presented. The results from this study intend to assist during analogue identification and evaluation steps of read-across.

**Profiling Mechanisms That Drive Acute Oral Toxicity in Mammals and Its Prediction via Machine Learning**

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We have developed a mechanistic machine-learning quantitative structure-activity relationship (QSAR) model to predict mammalian acute oral toxicity. We trained our model using a rat acute toxicity database compiled by the US National Toxicology Program. We profiled the database using new and published profilers and identified the most plausible mechanisms that drive high acute toxicity (LD₅₀ ≤ 50 mg/kg, GHS categories 1 or 2). Our QSAR model assigns primary mechanisms to compounds, followed by predicting their acute oral LD₅₀ using a random-forest machine-learning model. These predictions were further refined based on structural and mechanistic read-across to substances within the training set. Our model is optimized for sensitivity and aims to minimize the likelihood of under-predicting the toxicity of assessed compounds. It displays high sensitivity (76.1% or 76.6% for compounds in GHS 1 or GHS 2, respectively), coupled with 27.3% balanced accuracy. We further demonstrate the utility of undertaking a mechanistic approach when predicting the toxicity of compounds acting via a rare mode of action (aconitase inhibition). The mechanistic profilers and framework of our QSAR model are route- and toxicity endpoint-agnostic, allowing for future applications to other endpoints and routes of administration. Furthermore, we present a preliminary exploration of the potential role of metabolic clearance in acute toxicity. To the best of our knowledge, this effort represents the first accurate mechanistic QSAR model for acute oral toxicity that combines machine-learning with mode of action (MOA) assignment, while also seeking to minimize under-prediction of more highly potent substances.

**SENDisng Toxicology Study Data Analysis into the 21st Century with a New R Package: sendiR**

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The CDISC-SEND data standard created new opportunities for collaborative development of open-source software solutions to facilitate cross-study analyses of toxicology study data. A public-private partnership between BioCelerate and US FDA/CDER works to develop and publicize novel methods of extracting value from SEND datasets. In collaboration with PHUSE, an R package, sendiR has been published to enable harmonization and visualization of SEND datasets to reduce the type of variation in application of the SEND standards that hampers cross-study analysis. sendiR includes: 1) a Python package to harmonize SEND datasets using SEND control terminology and extensible terminology, 2) an R script to construct a relational database from any collection of SEND datasets, 3) an R Shiny application with a user-friendly interface, allowing users who are not familiar with programming to perform cross-study analyses. sendiR supports both uncertain matches and exact matches, allows calculation of the range of uncertainty, and with manual curation the uncertainty range can be narrowed. sendiR enables experienced R programmers to integrate the package functions into their own custom scripts/packages, enabling continued improvements to the functionality of these tools.

**Monte Carlo Simulation of Effective Neutral Detergent Fiber in TX Beef Cattle Consuming Rations Containing Multiple Sources of Dietary Sulfur**

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This is a Monte Carlo simulation constructed to evaluate the impact of effective neutral detergent fiber (eNDF) on polioencephalomalacia (PEM) in Texas cattle consuming diets containing multiple sources of dietary sulfur (S). Ruminant nutritionists in Amarillo, TX, provided example diets for backgrounding and finishing cattle, each containing six feed ingredients. S data for all feed ingredients were collected from available information in the Office of the Texas State Chemist database, National Research Council (NRC) recommendations, and published literature. Exponential equations were incorporated from Nichols et al. (2013) to estimate the risk of PEM at five different eNDF levels ranging from 0% to 8% in 2% increments with total dietary S (TDS) and rumen degradable S (RDS) as input values. The reasonable risk was estimated by manually inserting the example diets into a Monte Carlo simulation constructed using @RISK software with averaged S content for each feed ingredient. Findings reveal that backgrounding cattle consuming example diets would be exposed to 0.35% TDS and 0.21% RDS at ≥8% eNDF, resulting in a 0.009% and 0.0003% risk of PEM, respectively. Finishing cattle diets contained 0.39% TDS and 0.23% RDS at ≥4% eNDF, yielding a 0.002% and 0.00009% risk of PEM, respectively. Thus, diets provided by ruminant nutritionists suggest that the current feed supply for Texas backgrounding and finishing cattle presents a minimal risk of ≤0.002% for PEM.
In vitro and in silico modeling have revolutionized the field of predictive toxicology with powerful computational tools that can help elucidate the risk assessment in chemical risk assessment. Machine-learning based modeling has provided an opportunity to create chemistry-aware global models for toxicological endpoints. The limited standardization of applying these methods has hindered the implementation of modeling in the regulatory setting, where transparency of methods and ingoing data streams is key. We have designed a workflow that integrates expert-derived rule-based modeling with global approaches derived from applying machine-learn- ing methods. The workflow was applied to the mutagenicity endpoint using curated data from the bacterial reverse mutation (Ames) test, a key staple in genotoxicity hazard identification. The curated dataset of 704 chemical structures was binned into chemical classes which were analyzed for their statistical association with Ames activity to identify data enrichment patterns and create local models. A local model was built using expert-based rules for the nitrobenzene chemical category (80 structures). A set of global models was built using machine learning methods to identify chemical substructural features that are predictive of a chemical's Ames test outcome. A representative decision-tree based model is presented with a sensitivity of 92%, and 91% for training and test datasets, respectively. The identified patterns from expert-based local and global modeling show agreement and therefore provide a path towards integrating different modeling methods. We highlight critical features, such as the nitro group, and the distribution of ring substituents. Moreover, we perform local feature importance analysis using the Shapley Additive exPlanations (SHAP) method and compute local structural similarity to identify structural nearest neighbors. The structure-activity relation- ships obtained from local and global modeling were integrated into a workflow to interpret predictions from machine-learning based methods. The suggested approach can be used to establish modeling standards for methods interpretation as the hazard risk assessment community continues to expand its computational capabilities using new-approach methodologies.

N-nitrosamines (NAs) are generally considered to be potentially mutagenic and have been classified as a "cohort of concern" according to the threshold of toxicological concern (TTC) concept. Their metabolic activation can lead to DNA adducts, which can result in mutations and eventually cancer. However, some NAs are weak carcinogenic, which makes it difficult to predict the mutagen- icity of new, untested NAs that occur, for example, as impurities in medicines. As one of three European Medicines Agency (EMA) funded Mutamid projects, "QSAR for Nitrosamines" aims to better understand relevant biological processes such as microsomal activation, and DNA adduct formation, in order to distinguish NA classes with distinct mutagenic potency. The project focuses on NAs derived from active pharmaceutical ingredients (API), that are characterized by higher molecular weight compounds (over 200 Daltons) and includes sterically restrictive chemical substituents next to the NA-toxicophore. One of the first steps in the project is an analysis to identify knowledge and data gaps. A systematic literature search revealed more than 300 publications on NAs in relation to DNA repair and adduct formation, metabolic activation and mutagenic activity in comet assays. The data set was complemented with Ames test data as well as data on carcinogenic effects and TD50 values from the Leadscope database. Data models were developed for each endpoint and the data entered into a relational database, specifying the tested NA, the study design, and the study outcomes. The gathered dataset allows analysis of endpoint specific results with regard to in vitro and in vivo studies, species and affected organs. This analysis illustrates that the majority of test results are from rodents. Comet assays were mainly performed in vitro experiments with human cell lines. Liver and lung are among the most frequently investigated organs, including for adduct data. 4-(Methyltrinitrosoamo)-1-(3-pyridyl)-1-(butanone) (NNK) is one of the most data rich compounds, for example with regard to metabolic activation. Overall data are available for only a small group of different NAs and little information has been found for API-derived NAs so far, which demonstrates the importance of the ongoing three EMA projects. Data gathering is still ongoing as specific experimental data are currently being generated in the Mutamid projects. The project data will help to fill these gaps for data poor NAs and support the development of a structure-activity relationship (SAR) model for the prediction of the NA carcinogenic potency of N-nitrosamine drug substance related impurities.
3693 Early Hazard Assessment of Drug-Induced Phospholipidosis Using In Vitro and In Silico Approaches: Investigation of Metabolic Capacity of HepG2-Based In Vitro Assay and Implications on In Silico Screening Strategy

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Over 50 approved drugs are on the market that cause drug-induced phospholipidosis (PLD). PLD is a lysosomal storage disorder that is indicated by excessive accumulation of drug and polar phospholipids in lysosomes. PLD is considered adaptive and irreversible; however, it may negatively impact labeling and market development. Therefore, PLD is one endpoint for which drug candidates are screened early in pharmaceutical development using in vitro and/or in silico approaches. While common in silico approaches like the one described by Ploemen et al. are relatively simple and robust, they fail to correctly classify PLD-positive drugs such as aripiprazole and loratadine which are known to induce PLD through their respective N-dealkylation metabolites. This is primarily due to the reliance of these approaches on parent molecule physicochemical properties such as lipophilicity (logP) and basicity (pKa). N-dealkylation often results in metabolites with higher basicity that are more likely to accumulate in lysosomes and therefore have a higher propensity to result in PLD. While ketoconazole and loratadine have the lowest basicity (pKa) values, both compounds are experimentally positive in the standard HepG2-based PLD assay. This further suggests the possible formation of the suspected N-dealkylation metabolites in vitro, despite the reported low metabolic capacity for HepG2 cells. We investigated whether N-dealkylation biotransformations, similar to that reported for ketoconazole and loratadine, might affect the in vitro PLD readout of 4 internal drug candidates. Metabolite identification (MetID) studies were conducted in-house on compounds incubated with either human liver microsomes or HepG2 cells following the PLD in vitro assay protocol and confirmed the formation of the suspected N-dealkylation metabolites for all 4 parent compounds. Interestingly, the N-dealkylation of the cytochrome P450 (CYP) inhibitor 1-aminobenzotriazole partially blunted the PLD signal and relative abundance of N-dealkylated metabolites further implicating the contribution of these metabolites to the positive PLD signal observed in vitro. We concluded that the HepG2-based PLD assay exhibited some metabolic capacity, which may lead to positive PLD assay results if not correctly attributed to metabolites being formed in vitro. This comes with further practical implications for the in silico PLD model: if formation of metabolites with higher basicity (e.g., through N-dealkylation) seems likely, it is prudent to also run metabolite structures through the in silico model in parallel to their parent structures. For this more conservative approach, metabolite structures can be either obtained from MetID experiments or Phase I metabolites can also be predicted in silico using commercial software.

3694 Combining In Silico and In Vitro Information to Avoid Acute Oral Toxicity Testing in Animals

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 Globally, regulatory agencies have committed to reducing/eliminating animal testing for establishing chemical safety. Adverse outcome pathways provide a mechanistic scaffold that can be used to identify appropriate non-animal methods and connect them to apical adverse outcomes, thereby facilitating the development of tiered testing strategies for acute oral toxicity testing. Previously, we demonstrated that chemical structure and bioactivity measurements could be used to identify in vitro assays for use within a tiered testing strategy to detect acutely toxic chemicals. In this study we expanded on this work by 1) incorporating additional datasets into our original workflow to increase the number of mechanistically relevant oral toxicity profiles, 2) refining the cytotoxicity threshold for further re-interpretation, and 3) generating a new model that can incorporate various chemical and biological data to predict potential acute toxicity. We grouped the 11,992 chemicals with curated acute toxicity information from the ICCVAM Acute Toxicity Work Group (ATWG) into 2,192 clusters based on structural similarity defined by ToxPrint fingerprints. Bioactivity data for approximately 31,500 new assays were retrieved from PubChem for 4,786 ATWG chemicals. This assay data was combined with the bioactivity data previously gathered from ToxCast and a minimal suite of assays from PubChem and/or ToxCast were identified for each cluster, based upon activity enriched for chemicals within a cluster. Additionally, to find the minimum AC50 for a chemical, we compared the cluster-specific assay AC50 against different methods for calculating the cytotoxicity value: cytotoxicity point and lower bound of cytotoxicity calculated by ToxCast, lowest burst assay AC50, and median of the lowest 25th percentile in burst assays. Of the 1,637 acutely toxic chemicals (rat oral LD50 <= 2,000 mg/kg as defined by the ATWG) with activity in ToxCast below the lower bound of cytotoxicity, 1,139 were linked to one or more cluster-specific minimal ToxCast assay. Depending on the cytotoxicity value, when the minimum AC50 value for a chemical came from cytotoxicity rather than the cluster-specific assay, the default cytotoxicity point value was used for the majority of chemicals (ranging between 70-97.4%). The minimal AC50 values calculated using the different cytotoxicity methods (excluding the lower bound of cytoxicity) were significantly associated with the binary ATWG toxicity classification (p-value = 2.2 x 10^-10). This study also demonstrated that when a cytotoxicity value was the minimum AC50 for a chemical, the default cytotoxicity point value was used most. In all, 1,214 out of 1,637 chemicals with ToxCast data have an assay that is predictive for acute toxicity. Inclusion of the larger set of assays from PubChem will expand the chemical coverage and potentially identify novel targets that correspond to unknown mechanism of toxicity, which we’ve previously shown improves the predictivity. Taken together, the results suggest that combining bioactivity and structural information may be as reproducible as traditional in vivo studies. Because the current workflow focuses on tiered testing guided by the in silico predictions, high-throughput assays are not required, which greatly expands the number of assays available for testing. Integrated models that combine the data from in silico and targeted bioactivity measurements can further improve the predictivity.

3695 Annotations for ToxCast and Tox21 High-Throughput Screening Assays: Facilitating Assay Interpretation and Data Use

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A wide variety of in vitro high-throughput screening (HTS) assay data is publicly available. These bioactivity data have the potential to facilitate the development of computational approaches for chemical assessments and provide mechanistic insight when correlated to known activity for a given chemical. Therefore, in silico predictions, high-throughput assays are not required, which greatly expands the number of assays available for testing. Integrated models that combine the data from in silico and targeted bioactivity measurements can further improve the predictivity.

3696 Deep Autoencoder-Based Behavioral Pattern Recognition Outperforms Standard Statistical Methods in High-Dimensional Zebrafish Studies

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Zebrafish have become an essential tool in screening for developmental neurotoxic chemicals and their molecular targets. The success of zebrafish as a screening model is partially due to their physical characteristics including their relatively simple nervous system, rapid development, experimental tractability, and genetic diversity combined with technical advantages that allow for the generation of large amounts of high-dimensional behavioral data. These data are complex and
require advanced machine learning and statistical techniques to comprehensively analyze and capture spatiotemporal responses. To accomplish this goal, we have trained semi-supervised deep autoencoders using behavior data from unexposed larval zebrafish to extract quintessential “normal” behavior. Following training, our network was evaluated using data from larvae shown to have significant changes in behavior (using a traditional statistical framework) following exposure to toxicants that included a range of activities such as schooling, substrate preference, burrowing, and social behavior, as well as environmental contaminants. Further, our model identified new chemicals (Perfluoro-n-octadecanoic acid, 8-Chloroperfluorooctylphosphonic acid, and Nonafluoropentanamide) as capable of inducing abnormal behavior at multiple chemical-concentrations pairs not captured using distance moved alone. Leveraging this deep learning model will allow for better characterization of the different exposure-induced behavioral phenotypes, facilitate matching of toxicants across the different exposure-induced behavioral phenotypes, and neurobehavioral analysis in mechanistic determination studies and provide a robust framework for analyzing complex behaviors found in higher-order model systems.

3697 Large-Scale Screening of 1470 Chemicals Using High-Throughput Phenotypic Profiling (Htpp) with Results in HbE3-Kt and Telohaecc Cells

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High-throughput phenotypic profiling (Htpp) with the Cell Painting assay is a chemical screening method that combines high-content imaging and image analysis to measure phenotypic features at the single cell level and quantify chemical toxicity. A maestriaalistic, multiparameter, high-throughput, computer-based bioactivity screening assay to support a tiered in vitro hazard evaluation strategy using New Approach Methods (NAMs). This project screened 1470 Toxcast chemicals in a pair of human-derived immortalized primary cell lines, HbE3-Kt (bronchial epithelia) and Telohaecc (vascular endothelia), using Htpp. These cell lines were selected using a data-driven cell line selection approach based on differences in baseline gene expression and are the first two of several hTERT-immortalized primary cell lines from various tissues that will be screened by EPA using Htpp. HbE3-Kt or Telohaecc cells were plated in a 384-well format, given 24h to recover and dosed in a randomized pattern with 8 concentrations of test chemicals (1/2-log spaced). The highest dose tested for most chemicals was 100 μM. After a 24h exposure period, cells were live-labeled with Mitotracker (mitochondria), fixed, permeabilized and labeled with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of concanavalin A (ER), phallolidin (cytoskeleton), and wheat germ agglutinin (Golg/plasma membrane). After staining, each plate was imaged using the Affymetrix HCS system and using Harmony software, resulting in ~1300 morphological features per cell. To evaluate overall assay performance, the strictly standardized mean difference (SSMD) was calculated for the cell viability positive control (staurosporine) using normalized cell count (nCC) as an endpoint and the phenotypic reference chemical (etoposide) using cells alive as an assay endpoint. The mean SSMD for nCC across all assay plates was 5.79 (range: 1.74 to 12.93), indicating an extremely strong response. The SSMD for cell area across all assay plates was 0.77 (range: 0.31 to 1.27), indicating a weak, but consistent response. Concentration-response modeling of nCC identified 194 and 238 chemicals that produced > 50% decrease in nCC in HbE3-Kt and Telohaecc, respectively. These data were used to exclude toxicants from the next step in concentration-response modeling of the Htpp data. In an initial analysis, the CompTox Chemicals Dashboard (CCD) was used to search for chemicals that modulate epidermal growth factor receptor (EGFR) or vascular endothelial growth factor receptor (VEGFR) activity, as these receptors are necessary for the growth and maintenance of HbE3-Kt and Telohaecc cells, respectively. Six and eight Toxcast assays were identified for EGFR and VEGFR, respectively. Chemicals active in at least 1 of the assays with an effect size (scaled_top) > 4 were identified and Htpp data for those chemicals was analyzed by concentration response modeling of feature level data. A phenotypic altering concentration (PAC) was defined as the BMD of the 5th percentile of active features. A total of 13 chemicals were identified as EGFR modulators and 100% of those were active in Htpp of HbE3-Kt cells. Examples include known EGFR modulators lovastatin (BPAC = 4.9 μM) and bithionol (BPAC = 24.6 μM). A total of 23 chemicals were identified as VEGFR modulators and 87% of those were active in Htpp of Telohaecc cells. Examples include PhosphG3Bd_4B50S (BPAC = 0.04 μM), 9-Phenanthrol (BPAC = 2.1 μM) and 2,2′,4,4′-Tetrahydroxybenzophenone (10.1 μM), although literature support for interaction of these chemicals with VEGFR isomers was not found. Future directions include a more comprehensive analysis of HbE3-Kt and Telohaecc Htpp data. This abstract does not reflect US EPA policy.

3698 Framework for In Silico Toxicity Screening of Novel Odorants

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Predicting odor perception from molecular structure is a key challenge in olfaction, but validating current predictive models requires measuring human perception of novel odorants that have not undergone safety testing. Although in silico prediction tools are widely applied in chemical risk assessment and risk management, there are currently no transparent in silico models to predict inhalation toxicity.

We derived toxicity-based maximum recommended solution concentrations for odorant chemicals based on a transparent in silico approach, using chemical structure alone to support a psychophysical study of novel odorants in which human volunteers sniffed the headspace of a solution in a vial. Our decision tree was based on well-established open-source decision trees for assessing mutagenicity (rule-based, in vitro mutagenicity alerts by iSS) and systemic toxicity (revised Cramer decision tree), with a supplemental inhalation decision tree, and run usingToxtree software (version 3.1.0). Based on an in silico prediction of mutagen, or Cramer class III, II, or I, a threshold of toxicological concern (TTC) value of 12, 90, 540, or 1,800 μg/day was assigned. In parallel, the chemical vapor pressure was generated using the MPBFWIN™ (version 1.43) model available through the US EPA EPI Suite™ program and used to estimate headspace mass assuming ideal gas behavior and based on a 100 mL headspace volume. From this information, a toxicity-based maximum recommended solution concentration was calculated as the ratio of the TTC to the headspace mass multiplied by 100%. By this framework, the maximum recommended solution concentration was a function of predicted toxicity and chemical volatility. The approach was tested against a published dataset of 144 chemicals with repeat exposure inhalation toxicity data and performed well, with safety margins above 1 for 98.6% of chemicals and above 10 for 95% of chemicals. This framework was used to screen chemicals and either exclude odorants where the recommended concentration was too low to allow reliable perceptual ratings or flag certain chemicals for additional evaluation. Two notable limitations of this approach are the inability to safeguard against irritation and identify asthmagens. However, known asthmatics were excluded from the study and an irritant response would be expected to be transient under the acute exposure study conditions. In summary, an in silico framework to derive a toxicity-based maximum recommended solution concentration for chemicals was developed using open-source models and software, and used to support the safety of a psychophysical study of novel odorants.

3699 Using Innovative Tools for In Vitro ADME Studies to Efficiently and Effectively Support Drug Development


Drug development is a complicated and lengthy process requiring a significant amount of intellectual and capital input, and extensive collaborations among various organizations and institutions. Contract research organizations (CROs) play important roles at some or even all stages of the drug development process. To provide better service in in vitro drug absorption, disposition, metabolism, and excretion (ADME) studies, maintain data accuracy, and promote work efficiency, we developed an integrated information system termed the Drug Metabolism Information System (“DM Info System,” for short), and it is being used routinely by our Drug Metabolism (DM) department. The DM Info System assists scientists with assay design, data analysis, and report drafting, and thus reduces human error.

3700 ToxiOmnics: A Transcriptome-Based Database and Web Tool to Query and Understand the Associations between Environmental Toxicants and Human Diseases


Environmental toxicants have the potential to perturb many molecular pathways across tissues and organ systems to confer health risks. However, our understanding of toxicants at the systems level is currently lacking, and comprehensive resources detailing the interactions between toxicants, genes, and diseases across species and tissues have not been established. In this work, we developed a computational pipeline to systematically meta-analyze ~400 high-throughput transcriptomics datasets from the Gene Expression Omnibus ( GEO) that were generated using single- and two-color microarrays as well as RNA sequencing platforms. We identified differentially expressed genes (DEGs) for more than 200 chemicals studied in mice, rats, and humans, thereby enabling direct comparisons of DEGs across chemicals, studies, species, tissues, dosages, and lengths of exposure. Furthermore, pathway and disease enrichment analyses helped associate chemicals’ DEG signatures to human diseases. Our transcriptome-wide data-driven DEG database has been centralized and standardized and will be disseminated in an open-access online web server called ToxiOmnics. ToxiOmnics will enable the toxicological community to query and understand species-, tissue-, and dose-specific molecular pathways and mechanisms across hundreds of toxicants to better guide toxicology and environmental health studies and decision making.

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Application of in silico approaches such as quantitative structure-activity relationship (QSAR) models and rule-based systems have become useful in assessing chemical safety. However, these approaches are not well-suited to endpoints for which the underpinning data are weakly grounded in specific chemical-biological interactions, bioavailability and biotransformations. One such example is the estimation of point of departure values such as a no-observed-adverse-effect level (NOAEL), required in the assessment of systemic toxicity. When combining this aspect with the need to address numerous metabolites, where testing is often not feasible, there is a need for developing new approaches to handle such scenarios. To this end, a unique series of case studies was designed to demonstrate the power of using knowledge available in a highly curated metabolism database in combination with chemoinformatics and decision theory.

We approached the parents/metabolites from a mixture perspective in order to estimate confidence bounds for the NOAEL of the target. In assessing similarity between targets and analogues, biologically related profiles such as bioavailability and assay activities were selected along with relevant physicochemical/molecular properties. For ADME (adsorption, distribution, metabolism, excretion) data, we included plasma protein binding, hepatic clearance, intestinal absorption, Caco-2 cell permeability, and skin permeability. For assay data, we used Tox21/CAST assays to indicate screening for DNA binding, liver/biliary effects, and EATS (estrogen, androgen, thyroid, and steroidogenesis) activities. Machine learned hybrid structure rules were developed for potential endocrine disruption activity profiles to provide an additional way to assess similarity and qualify analogues for read-across. The methodology was applied to caffeine and its metabolites as well as 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (e.g., tembotriones) and their metabolites. For example, when considering the abundance ratio of three profiles to provide an additional way to assess similarity and qualify analogues for read-across, we estimated the chronic NOAEL bounds for caffeine to be 13.3 - 47.4 mg/kg-day, respectively. Combined with visual tools such as 3-D chemical similarity maps, it was used to develop a structural similarity metric. Making use of a wide variety of training labels but required a static fingerprint of the underlying data, we employed a K-Nearest Neighbors (KNN) approach (using various K values) to predict skin sensitization status and compared the prediction accuracies between the 2-D and 3-D definitions to see if the 3-D approach offered any improvement in predictive accuracy. Due to the relatively large size of our data set, our 3-D analysis was performed using a KNN approach by identifying clusters of similar chemicals in the 2-D chemical similarity map that had clusters of similar chemicals with mixed sensitization statuses (n=6), as these areas could benefit from further separation of sensitizing and non-sensitizing chemicals, and we prioritized these for our 3-D analysis. We found that, in some clusters, using the Color Tanimoto definition of structural similarity with 1 conformer improved modest improvement in prediction accuracy compared to a 2-D definition (e.g., 10-25% increase with varying K for one cluster). However, it resulted in a substantial drop (e.g., 33% or more in one cluster) in the number of chemicals that were predicted compared to the 2-D approach; this was due to a decrease in the number of chemicals with enough neighbors that make a meaningful EFU and skin sensitization prediction. Incorporating analogues in the 3-D approach increased the number of chemicals that could be predicted, but only slightly, and the accuracy was comparable to the 2-D approach (e.g., difference of ~7% to 2% accuracy with varying K for one cluster). Although our results here did not demonstrate marked improvement, it is possible that our structural similarity cutoffs of 50% and 90% for Shape and Color Tanimoto, respectively, were too restrictive, so additional work to find suitable similarity cutoffs for each chemical is critical. Furthermore, the improvement in some areas of the chemical space using a Color Tanimoto approach highlights the importance of the accessibility of reactive functional groups within the molecule; therefore, future work will include investigation into how 3-D similarity definitions can tease apart experimentally discarding chemicals that share the same functional groups.

**3702 MoAViz: An Interactive Application of Toxicogenomics for Predictive Toxicology and Risk Assessment**


SotoVation, Durham, NC.

The combination of transcriptomics and bioinformatics has been effective in providing the weight-of-evidence of anticipated health effects from chemicals. Benchmark datasets of gene expression profiles offer the potential to derive point of departure (PoD) values from short-term dose-response studies for rapid assessment of chemical bioactivity. To reliably capture functional biology information from gene expression, an established best practice selects genes using both a statistical significance and a magnitude of change threshold. We present the continued development of MoAViz interface and database by incorporating whole transcriptome and benchmark dose analyses and point of departure (PoD) summary including predicted mode of action (MOA) from the highlighted genes. MoAViz is an interactive ontology enrichment viewer application that captures the entirety of toxicogenomic experiments in a relational database. The application provides a system for transcriptomic data in silico analysis and MOA investigation with POD derivation and comparison with similar compounds or compounds with similar cellular biological responses. In addition to the statistical filtering of the expression values, a modified Jaccard index (MJII) was applied to compare the similarity of pathway-level changes MOA in gene expression between compounds.

We introduced into MoAViz high throughput whole transcriptome sequence assays of two widely used cancer cell lines (HeCo2 and MCF7) as in vitro systems for the determination of cellular modes of action for two well-studied compounds with canonical liver responses: ketoconazole and phenobarbital. MoAViz demonstrated the overall consistency of the transcriptomic POD values for both compounds with those for increased cell proliferation. For all cell cultures, HEDs are similar, ranging from 14.6 to 61.5 mg/kg-day for ketoconazole using all 8 doses in the analysis. When the maximum dose was excluded, the range was reduced to 13 to 22.2 mg/kg-day. The inclusion of the cytotoxic maximum dose or its removal both results in extremely high HED values for phenobarbital ranging from 140.8 and 357.5 mg/kg-day and 55.6 and 113.2 mg/kg-day, using all 8 doses in the analysis or with the maximum dose excluded, respectively. Combined with other lexicons such as our MoAViz interactive ontology enrichment viewer application, capturing the entirety of toxicogenomic experiments in a relational database will provide a system for comparative transcriptomic read-across for chemical assessments using New Alternative Methods (NAMs).

**3703 Application of 3D Structural Similarity Definitions to Improve Concordance among Nearest Chemical Neighbors**

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In previous work predicting skin sensitization using human data as a benchmark, we demonstrated that while current in silico tools predict chemical skin sensitization relatively well (balanced accuracies of 60-80%), there is room for improvement. In silico tools rely on a fundamental rule of toxicology: structurally similar chemicals exhibit similar toxicities. Nearly all in silico skin sensitization tools define chemical structural similarity using a 2-D definition; however, this does not always capture important features, like steric effects, which are tied to 3-D chemical shape. Consequently, using a 3-D structural similarity definition can potentially resolve some of the false predictions made when using a 2-D definition. Here, we calculated 2-D and 3-D structural similarity scores using PubMed’s ChemScore Matrix Service for developing read-across methods. The data set was drawn from our previous work and expanded using NICEATM’s ICE (Integrated Chemical Environment) database (n=1,355). To calculate 3-D structural similarity, we used two definitions - Shape Tanimoto (similarity cutoff 90%) and Color Tanimoto (similarity cutoff 50%). Shape Tanimoto accounts for 2-D molecular shape while Color Tanimoto considers both 2-D shape and select reactive functional groups. Additionally, when using a 3-D approach, it is possible to have multiple conformers (i.e., different 3-D conformations) for the same chemical, so we first explored using only one conformer, then expanded our analysis to 3 and 8 conformers. For each of the three structural similarity definitions, we constructed chemical similarity networks of Cytoscape to identify near neighbors. Next, we identified discordant areas in the 2-D chemical similarity map that had clusters of similar chemicals with mixed sensitization statuses (n=6), as these areas could benefit from further separation of sensitizing and non-sensitizing chemicals, and we prioritized these for our 3-D analysis. We found that, in some clusters, using the Color Tanimoto definition of structural similarity with 1 conformer improved modest improvement in prediction accuracy compared to a 2-D definition (e.g., 10-25% increase with varying K for one cluster). However, it resulted in a substantial drop (e.g., 33% or more in one cluster) in the number of chemicals that were predicted compared to the 2-D approach; this was due to a decrease in the number of chemicals with enough neighbors that make a meaningful EFU and skin sensitization prediction. Incorporating analogues in the 3-D approach increased the number of chemicals that could be predicted, but only slightly, and the accuracy was comparable to the 2-D approach (e.g., difference of ~7% to 2% accuracy with varying K for one cluster). Although our results here did not demonstrate marked improvement, it is possible that our structural similarity cutoffs of 50% and 90% for Shape and Color Tanimoto, respectively, were too restrictive, so additional work to find suitable similarity cutoffs for each chemical is critical. Furthermore, the improvement in some areas of the chemical space using a Color Tanimoto approach highlights the importance of the accessibility of reactive functional groups within the molecule; therefore, future work will include investigation into how 3-D similarity definitions can tease apart experimentally discarding chemicals that share the same functional groups.

**3704 Read-Across–Based Structure-Activity Relationship Predictions for Reproductive Toxicity and Carcinogenicity with Deep Learning and Domain of Applicability Definition**

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Read-across-based Structure-Activity Relationships (RASAR) have been shown earlier to allow the prediction of acute and topical toxicities of chemicals more accurately than the reproducibility of the respective animal tests. The approach was based on a Random Forest method, which allowed us to understand the contribution of the different training labels but required a static fingerprint of the molecule and chemical similarity metric. Making use of a wide variety of training labels on similar chemicals and the substance of interest itself boosted the accuracy of results. The availability of data on the substance itself is biasing the analysis favorably as this is not typically available for new chemicals but often for the well-established substances in the training set. Therefore, here, we omitted this when moving to Deep Learning approaches. UL Cheminformatics Tool Kit version 3, a production-ready suite of GHS hazard prediction models, has been developed using this machine-learning approach. These models were trained on a large database of 134,380 chemicals with 48 GHS hazard labels. Here, we extend the ULCT models with (1) data from public sources (OPERA, RTECS, NTP, PubChem, etc.) and chemical properties derived from UL LOLA+, a large commercial chemical property database (2) mechanistic features for the classification of chemical reproductive toxicity and carcinogenicity, and (3) A basic algorithm was developed and evaluated for assessing if a chemical meets the inclusion rules for model Domain of Applicability (DoA), which showed better results for substances meeting DoA criteria throughout all predicted endpoints. Roboticized testing of large
numbers of chemicals in EPA's ToxCast or the Tox21 consortium makes the biolog-
ical characterization of thousands of substances in hundreds of assays available,
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ical characterization of thousands of substances in hundreds of assays available,
p33 Expression, Genome-Wide Transcriptome Profiling, and Lympocyte Genome Sensitivity (LGS) Test in Lymphocytes after PUVA Exposure from Patients with Malignant Melanoma Compared to Healthy Controls

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This study aimed to use lymphocytes as surrogates and evaluate the expression of the p33 gene after radiation with varying intensity doses of PUVA (UVA+b). Lymphocytes from melanoma patients with positive sentinel nodes compared to healthy volunteers. Forty blood samples were collected in total from melanoma patients and healthy volunteers (20 each). These samples were processed using the Comet assay, quantitative real-time Polymerase Chain Reaction (qPCR), western blotting and genome transcriptome profiling. Lymphocytes are continuously exposed to different physical and chemical insults in the blood that promote DNA damage, ultimately leading to oxidative stress. Comet assay data indicated a significant difference between the two groups. However, the qPCR data demonstrated a significant increase in an overall 43.8-fold in p33 gene expression in lymphocytes from malignant melanoma patients after treatment with 0.2mW/cm² UVA intensity radiation compared to their healthy and untreated controls and also confirmed with western blotting. The genome transcriptome profiling data also showed an immense difference in gene expression between the UV-treated samples from healthy groups compared to the melanoma samples. Conclusively, peripheral lymphocytes from melanoma patients are more sensitive and susceptible to the genotoxic effects of PUVA compared to healthy individuals. This feature could be a promising blood biopsy biomarker to stage and prevent different carcinomas at early stages.

3710 Nanostring-Based Identification of Renal miRNAs in an Amphotericin B–Induced Nephrotoxicity Model of Sprague Dawley Rats


Early detection of drug-induced kidney injury (DIKI) is most often focused on identification of acute kidney injury based on clinically relevant increases in serum creatinine (sCr) and/or blood urea nitrogen (BUN), which are nonspecific and often uninformative. Urinary proteins (e.g., albumin) have been used to identify glomerular filtration rate (GFR) in rats. MicroRNAs (miRNAs) are endogenous non-coding RNAs that regulate gene expression and have high potential for disease conservation and tissue specificity. More recently, miRNAs have gained attention in the quest for translational DIKI biomarkers, but their usefulness remains poorly understood due to deficient reporting of positive and negative outcomes. We utilized a Nanostring Panel of 423 miRNAs, to investigate renal tissue-enchanced expression level changes in male Sprague Dawley rats following repeated intraperitoneal dosing of Amphotericin B (AmbB, 0 or 10 mg/kg/day) for 4 consecutive days. Interim serum and urine samples were collected on Day 2. Terminal serum, urine and kidney tissue samples were collected on Day 5. AmbB nephrotoxicity was primarily characterized as minimal to moderate tubular alterations of basophilia and regeneration, tubular mineralization and hyaline intratubular cast formation with mild interstitial lymphocyte infiltrates and acute inflammation of the renal pelvis. AmbB-induced significant serum-based increases in BUN on day 2 (1.39-fold; P=0.005) and day 5 (2.1-fold; P=0.003) in absence of changes in sCr. On day 2 (1.2-fold; P=0.003), indicating an acute reduction in GFR and glucose on day 5 (1.4-fold; P=0.025) as well as decreases in albumin on day 5 (0.87fold; P=0.0009). Also, AmbB-induced significant increases in urinary N-acetyl-beta-D-glucosaminidase on day 5 (2.4-fold; P=0.008) and neutrophil gelatinase-associated lipocalin on day 5 (10.11-fold; P=0.004). AmBtreated rats showed significant (Uncorrected P=0.01) expression level changes for 9 upregulated and 3 downregulated miRNAs. The highest upregulated miRNA “miR-377” has 401 predicted target genes (miRDB database), with necrotic cell death and cellular response to chemical stress pathways as top adverse outcome pathways (AOPs), using Metascape analysis. The most downregulated miRNA “miR-377” has 108 predicted target genes (miRDB database), with regulation of interleukin-1α production and platelet-derived growth factor receptor signaling pathway as top AOPs. Taken together, these findings highlight select miRNAs as promising biomarkers when monitoring for DIKI in nonclinical studies in the rat.

3711 Differential Expression of Testis-Enriched microRNAs and Serum Inhibin B in Nitrofurazone-Induced Testicular Toxicity in Rats


MicroRNAs (miRNAs) regulate gene expression during various pathological processes, potentially making them important biomarkers for early identification of testicular toxicity which remains a challenging issue in drug development. Nitrofurazone (NF) was used as a tool compound to identify biomarkers for monitoring acute testicular injury. Sprague-Dawley rats (n = 4/group) received a single oral dose of vehicle or NF at 50 mg/kg. NF testicular toxicity was monitored using anatomic pathology and serum inhibin B at 24 and 96 hours postdose. A novel RNAscope™ Plus smRNA-RNA assay was established for miR-let-7p-5p, STAR and inhibin B. At 24 hours, NF induced testicular degeneration, nuclear smudging, cytoplasmic hyperchromasia of pachytene spermatocytes, and cytoplasmic microvacuolation in Sertoli cells. At 96 hours, degenerative changes progressed to loss of round and elongated spermatids, variable reduction in Sertoli cell number and increased tubular macrovacuoles. In tests, 33 of 81 and 77 of 816 miRNAs were modulated at 24 and 96 hours (p<0.05), respectively. Serum inhibin B (functional indicator of germ cell proliferation in the tests) progressively decreased in NF-treated rats when compared to controls at 24 (0.5x; p<0.01) and 96 (<LLOQ) hours. Serum triglycerides and testis miR-935 were decreased, while tests miR-let-7i-5p, miR-147, and novel miR-276 expression were increased at 96 hours. Let-7i-5p plays a role in regulating key genes including STAR. Application of RNAscope™ Plus smRNA-RNA assay revealed localization and distribution of miR-let-7p-5p and miRNAs (STAR and inhibin B) in testes, demonstrating it was fit for purpose for evaluation of these analytics. In conclusion, miRNA-Sequence profiling of NF treated rats identified multiple testis-enriched miRNAs. These findings warrant further evaluation as safety biomarkers when monitoring for acute drug-induced acute testicular injury in rats.

3712 Cell-Free DNA Derived from Cardiac Organoids as a Potential Indicator of Toxicity and Tissue-Level Events

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Efficient diagnostics are critical for early detection of disease and toxic exposures. Biomarkers present in blood or other bodily fluids are gaining increasing interest due to their clinical potential in rapid, non-invasive screening. Cell-free nucleic acids can be isolated from dying cells, with current evidence showing that cfDNA may also be predictive of exposure outcomes. Together, our findings demonstrate that cfDNA is recoverable from cardiac organoids in quantities sufficient for analysis. The fraction of mitochondrial cfDNA dropped sharply early in development of the organoids, which could indicate a signature of cardiac differentiation. Using droplet-digital PCR (ddPCR) we were able to identify specific cfDNA sequences from mitochondrial and genomic origins that were altered during development. Upon exposure to the cardiotoxicant Doxorubicin, changes in abundance of specific cfDNA regions occurred prior to tissue demise, suggesting that cfDNA may also be predictive of exposure outcomes. Together, our findings demonstrate that cfDNA can be exploited as a diagnostic readout for early detection of tissue-level events. This strategy has the potential for high-throughput screening of toxicants and could be extended to additional organoid and disease types.
and demonstrated that DMBA exposure significantly decreased Cx43 mRNA stability through the regulation of RNA binding protein HuR. In addition, we also observed DMBA-induced degradation of the Cx43 protein, which was involved in the phosphorylation of Cx43 by mitogen-activated protein kinase. In conclusion, DMBA could downregulate GJIC through the reduction of stability of Cx43 mRNA and protein. Most importantly, our results indicate that the GJC assay can also be a predictive test for putative genotoxic carcinogens, at least partially.

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Hepatic steatosis findings in non-clinical toxicity studies of rodents can be regarded as an indicative finding for the potential of drug-induced liver injury, which is a serious concern for subjects and patients who are administered drugs and also for pharmaceutical companies. Therefore, clarifying the mechanisms of drug-induced hepatic steatosis and its biomarkers is important for the evaluation of the risks in the new drug development. Ethionamide (ETH) is a second line drug for multi-drug resistant tuberculosis and is known to cause hepatic steatosis followed by hepatic injury in rats and humans. In the present study, in order to investigate the biomarkers for ETH-induced hepatic steatosis, we produced a drug-induced hepatic steato-sis model in rats by orally treating rats with ETH at 30 and 100 mg/kg for 14 days and conducted lipidomics analysis using plasma and liver samples from the model rats. The ETH-treated rats showed Oil Red O staining-positive vacuolation in the centrilobular hepatocytes accompanied by increased hepatic triglycerides (TG) levels and decreased plasma TG and total cholesterol levels. A multivariate analysis for lipid profiles in the lipidomics analysis revealed alterations in the lipid species in the plasma and liver in particular phosphatidylcholine (PC) (18:0/20:4) decreased dose-dependently in both the plasma and liver. PC, which contains arachidonic acid (20:4), is known to be an important component of VLDL for hepatic TG secretion and we hypothesized that the decrease in PC (18:0/20:4) levels leads to decreases in TG levels in serum fractions. Alternatively, PC (18:0/20:4) in LDL fractions were decreased dose-dependently in ETH-treated rats. In conclusion, the decreased PC (18:0/20:4) in the liver, possibly leading to suppression of hepatic TG secretion, is considered to be involved in the pathogenesis of ETH-induced hepatic steatosis and plasma PC (18:0/20:4) levels are proposed as a mechanism-related biomarker for ETH-induced hepatic steatosis.
Cellular senescence is characterized by cell growth arrest and alterations in physiologic processes. It has been known to play a role in various chronic pulmonary diseases like COPD and IPF. However, the detailed mechanism remains unknown. While tobacco smoke is considered the chief etiologic factor for occurrence of various chronic lung diseases such as COPD/emphysema, the phenotypic manifestation of their symptoms occurs at old age. In this respect, previous work by our group reported increased cellular senescence in smoke exposed mice and age-dependent reversal of disease state on removal senescent cells. Considering this, we hypothesized that chronological age plays a crucial role to trigger lung senescence which thereby worsens the health status in chronic lung conditions like COPD. To understand this, we studied the cell specific changes in lung cellular senescence in young (2 months) and old (26 months) C57BL/6J mice using C14-Id3F assay. We observed upregulation in the senescent C45° cell count amongst old mice as compared to young control mice. On studying the individual leukocyte population, we found an age-dependent increase in C14, FdG (or senescent) granulocyte population. Besides, we found significant increase in the percentage of CD45 CD326 CD31 (mainly comprising of lung fibroblasts)-expressing cell types in older C57BL/6J mice. We also performed single cell RNA sequencing on the lung homogenates from young and old mice to identify the cell types and gene expressions affected by chronological age in C57BL/6J mice. This will allow us to identify the senescence profile related to chronological aging in mice. It will then help deduce the induction of premature senescence on exposure to environmental toxins and understand the pathobiology of chronic lung diseases at old age. Support by NIH E0291777 and AG075931.

In a read-across context, we investigated the use of transcriptome data to infer biological similarity for a case study comprising four volatile short-chain aliphatic alpha diketones. The read-across hypothesis is based on the finding that the analogue diacetyl induces bronchiolitis obliterans, a disease with characteristic features of pulmonary fibrosis, in workers involved in the preparation of microwave popcorn. Transcriptome data from in vitro pulmonary fibrosis-inducing agents from rodent in vivo studies and human in vitro cell cultures were included in the analysis to further improve the mechanistic interpretation of the differentially expressed genes obtained in this case study. Early transcriptional responses were investigated using the TempSeque® S150+ gene panel in primary human bronchial (PBEC) cells exposed to single and repeated air liquid exposure with the P.R.I.T.E ExpoCube®. For each individual substance, genes were identified displaying a consistent differential expression across dose and exposure duration. Alpha diketones in particular showed a highly concordant expression pattern, which may serve as a first indication of a shared mode of action. Public in vitro datasets were analyzed with the ODAF workflow for RNA-Seq experiments and a limma-based workflow for chip-based data sets. Subsequently, genes were identified that were up-or down-regulated across all experiments. Mouse ensemble IDs were mapped to human ensemble IDs using biomart. A weighted correlation network analysis (WGCNA) clustered genes that form a similar expression course within the publicly available data in vivo samples. The WGCNA identified seven gene modules, containing either genes belonging to well-known fibrotic pathways and/or fibrosis biomarkers from discovery through development, yet there is limited information on systemic fluid biomarkers of chemically-induced peripheral neurotoxicity in nonclinical safety studies or in patients. In this study, we evaluated the sensitivity of neurofilament light protein (NFL), a neuronal intermediate filament protein, as a fluid biomarker (serum, CSF) of neurotoxicity, following administration to mice or rats with either of seven compounds with documented peripheral nerve toxicity in rodents and/or neuropathy in patients, or three negative control compounds reported to have toxicity in target organs other than the central or peripheral nervous system. Temporal changes in NFL (measured by Quanterix NF-Light assay) were assessed via serial samples of serum over ≤44 days of dosing and/or recovery, and NfL in serum, CSF measured at terminal necropsy. Light microscopic evaluation of the central and peripheral nervous system tissues for neurotubular necrosis and/or three negative control compounds reported to have toxicity in target organs other than the central or peripheral nervous system. Temporal changes in NFL (measured by Quanterix NF-Light assay) were assessed via serial samples of serum over ≤44 days of dosing and/or recovery, and NfL in serum, CSF measured at terminal necropsy. Light microscopic evaluation of the central and peripheral nervous system tissues for neurotubular necrosis and/or three negative control compounds reported to have toxicity in target organs other than the central or peripheral nervous system.
Neurotoxicity has been linked to exposure to a number of drugs and chemicals, yet efficient, predictable, and minimally-invasive methods to detect neuroanatomical effects are not routinely used currently in non-clinical assessments. Previously, we have shown significant T2-MRI changes in a rat model of trimethyltin neurotoxicity that correlated with CNS neurotoxicity and pathology as measured by Flouro Jade C. Here, our main objective was to identify possible T2-MRI relaxation that predicts myelin specific neurotoxicity resulting from exposure to a known neurotoxic agent, cuprizone, by correlating T2-MRI relaxation with neuropathological endpoints. Adult-male rats (5-months old) were exposed to a daily dose of cuprizone (600 mg/kg p.o., daily) or control for 4 weeks. Prior to treatment, animals underwent MRI scans to establish a baseline. Additional MRI scans were done weekly after beginning exposure to cuprizone. At the end of 4 weeks, a final MRI was completed and animals were sacrificed for collection of fluids and tissue samples. Significant T2-MRI relaxation was observed in the deep cerebellar nuclei in cuprizone-treated rats compared to controls. There was a correlation of these MRI changes with CNS pathology which will be discussed. Our data demonstrate that MRI-based endpoints may be used as a robust minimally-invasive neurotoxicity biomarker in a myelin-specific neurotoxicity model. Supported by NCTR Protocol 0758011.

3738 Food–Toxic Ingredients-Induced Psoriasis through Epigenetic Reprogramming of Dermal Stem Cells

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Recently, scientific studies highlighted that several fast foods include numerous toxicants namely phthalates, tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene, polyfluoroalkyl (PFAs), cadmium, propylene glycol, and propyl gallate. However, there was no such investigation for clarifying the mechanism of toxic effects and epigenetic reprogramming for psoriasis of dermal stem cells. In this study, we investigated the toxic effects of mentioned food ingredients and the mechanism of epigenetic reprogramming using the database of the National Institute of Environmental Health Sciences (NIEHS), the Comparative Toxicogenomics Database (CTD), NIH Toxicology Data Network (TOXNET), Environmental Health Perspectives (EHP), Gene Expression Omnibus (GEO) database, STRING. Protein-Protein Interaction Networks, Cytoscape, the NIH Roadmap Epigenomics Mapping Consortium, and other bioinformatics databases. We found from CTD analysis that the chemical gene interactions showed highly expressed 25 common genes with CD37, CD44, CD81, IL2, IL4, and VEGFA by phthalates, and polyfluoroalkyl (PFAs), from a total of 991 genes (approximately 2.5% affected genes), 21 common genes with CD37, CD44, CD81, IL2, IL4, and VEGFA by phthalates, and butylated hydroxytoluene from a total of 990 genes; 24 common genes by phthalates, and polyfluoroalkyl (PFAs), from a total of 6014 genes; 2 common genes by MAPK1, TFR2 by phthalates and propylene glycol from total 2185 genes; 5 common genes with CD86, CD74, HADAC1 by phthalates, and propyl gallate from total 825 genes. Psoriasis, a chronic skin disease (with a higher prevalence of 4.7% in US population), has not been reported by phthalates; however, there were methylated genes of psoriasis dermal tissues for activated and epigenetically reprogrammed dendritic cells, keratinocytes, resting mast cells, T follicular helper cells (cTfh) compared to normal dermal tissues by analyzing recent works of literature. The epigenetic mechanisms of histone modifications (H3K9me3, H3K27me3, H3K27ac, H3K36me3, H3K4me1, H3K4me3 of the foreskin fibroblast primary cells, foreskin melanocyte primary cells and foreskin keratinocyte primary cells) and these toxic ingredients of food have been revealed by the database of EHP, NIEHS, the NIH Roadmap Epigenomics Mapping Consortium, the National Center for Biotechnology Information (NCBI), and U.S. National Library of Medicine (NLM) database and Cytoscape pictured the STRING-based PPI network. The acetylation of histones is a mediator to incongruent activities of lysine acetyltransferases (KATs) and histone deacetylases (HDACs) on chromatin structure involved in DNA replication, repair, heterochromatin silencing, and gene transcription. Our previous study showed that the regulation of epigenetic bivalent histone modifications at transcript start sequences (TSS) of target genes for heterogeneous human adult stem cells (hASCs) reprogramming and our current bioinformatics analysis revealed that HNF complex subunit HOOK interacting protein 1B (Q9H624; Q8N612: HOOK1B_HUMAN) is epigenetically reprogrammed with close interaction of eight genes by these toxicants. Therefore, it is important to find the actual epigenetic mechanism of both genomic and epigenomic transcriptional regulation and the toxic effects of these food ingredients for psoriasis of dermal stem cells, and our current study revealed this important relationship.
**3729 Statistical Methods for Exploring Spontaneous Adverse Event Reporting Databases for Drug-Host Factor Interactions**


Drug toxicity does not affect people equally; the toxicity may only exert in patients who possess certain attributes of susceptibility to specific drug properties (i.e., drug-host interaction). This concept is crucial for personalized drug safety but has been understudied, primarily due to methodological challenges data availability. By monitoring a large volume of adverse event reports in the postmarket stage, spontaneous adverse event reporting systems provide an unparalleled resource of information for adverse events and could be utilized to explore risk disparities of specific adverse events by age, sex, and other host factors. However, well-defined statistical methods to formally address such risk disparities are currently lacking. We present a statistical framework to explore spontaneous adverse event reporting databases for drug-host interactions and detect risk disparities in adverse drug events by various host factors, adapting methods for safety signal detection. In this framework, we considered likelihood ratio test, test based on normal approximation, test for difference in proportions, and subgroup ratios. Our proposed methods were applied to simulated data and FDA Adverse Event Reporting Systems (FAERS) and explored sex-/age-disparities in reported liver events associated with specific drug classes. In our simulation study, three of the proposed methods (likelihood ratio, normal approximation, and difference in proportions) can reach the power level around 0.4 while maintaining a family-wise error rate of 0.05, which is outstanding for this type of applications using FAERS. On the other hand, subgroup ratio-based tests lead to inflated false discovery rate and are not recommended. For real data analysis with our preferred methods when applied to the class of nonsteroidal anti-inflammatory drugs, 7 out of 12 drugs exhibited disparate reporting rates between male and female patients with supporting evidence from clinical, animal, and in vitro studies. Several other classes of drugs have also been highlighted by the analysis of FAERS, including some that were also discussed by other researchers. The result demonstrated the applicability of our methods in exploring spontaneous adverse event reporting databases for unrealized risk disparities. Though spontaneous adverse event reporting databases require careful data processing and inference, the sheer size of the databases with diverse data from different countries provides unique resources for exploring various questions for drug safety that are otherwise impossible.

Our proposed methods can be used to facilitate future investigation on drug-host interactions in drug toxicity using a large number of reported adverse events.

**3730 Practical Experimental Design Considerations for Transcriptomics Dose-Response Modeling**

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Transcriptomics dose-response analysis (TDRA) has emerged as a promising approach for integrating toxicogenomics data into a risk assessment context. To move beyond proof-of-concept studies, practical questions concerning experimental design were answered to establish TDRA as a trusted and broadly applicable approach. In this work, we aim to 1) understand variability and uncertainty in TDRA results associated with dose range, spacing, and replicate number, and 2) evaluate the use of a reduced transcriptome and pooled samples for calculating transcriptomic point-of-departures (tPODs). For the first objective, we evaluated \( n = 55 \) RNA-seq profiles derived from Japanese quail embryo liver tissue following exposure to chlorpyrifos \((0, 0.04, 0.1, 0.2, 0.4, 1, 2, 4, 10, 20, 40 \mu g/g; n = 5 \) replicates per group) via egg injection. The full dataset was sub-sampled 657 times to generate smaller datasets with different dose ranges and spacing (designs A-E), and number of replicates \((n = 2-5)\). TDRA on all 637 datasets revealed substantial variable effects of the minimal pathway hormonally-related effects and gender differences in overall tPOD values when tPODs were calculated with the ‘pathway’ and ‘mode’ methods. Further, we found that tPOD values were more dependent on dose range and spacing than on the number of replicates, suggesting that optimal experimental designs should use fewer replicates \((n = 2 \text{ or } 3)\) and more dose groups to reduce uncertainty in the results. Finally, tPOD values ranged by over ten times for all surveyed experimental designs and tPOD types, suggesting that tPODs be interpreted as order-of-magnitude estimates. For the second objective, we evaluated \( n = 55 \) EcoToxChip profiles from the same Japanese quail liver samples using the version 1.0 Japanese quail EcoToxChip, a qPCR array with ~370 genes curated for their relevance to environmental toxicology. We also evaluated one EcoToxChip profile for each dose group from pooled replicates. TDRA of these data resulted in tPOD values that were within the central 95% of tPOD values from the RNA-seq analysis described above, suggesting that reduced transcriptomes and pooled samples could be effective strategies for reducing the time and cost of performing TDRA. In addition, we note that, to our knowledge, this is the first transcriptomics dose-response study carried out in an avian species.

**3731 http://: A Targeted RNA-Seq High-Throughput Transcriptomics Analytical Pipeline for Environmental Chemical Screening**


Advancing the pace of chemical risk assessment necessitates the development of new approach methodologies (NAMs) that provide meaningful information on chemical risk without the need for whole animal testing. A potential NAM based on the TempO-seq targeted RNA-seq platform has been proposed which uses high-throughput transcriptomic (HTTR) profiling to rapidly screen and prioritize large numbers of environmental chemicals in vitro. As part of this NAM, we developed the high-throughput transcriptomics pipeline (http://) software package, an analytical pipeline that enables researchers to efficiently and reproducibly perform a complete analysis of targeted RNA-seq experiments and store output from multiple levels of analysis within a standardized database management system. http:// uses well-established open-source analysis tools, provides a stable, verifiable container that ensures reproducibility across varying computer platforms, and a NoSQL MongoDB database to store all outputs. The general workflow for http:// is as follows: 1) rapidly align and count raw sequencing data using the HISAT2 and SAMtools open-source software, 2) estimate fold-change values of the read counts using the DESeq2 differential gene expression R package, and 3) derive gene signature-level data from fold-change data and perform benchmark dose-response modeling to define various statistics relevant to chemical risk assessments such as benchmark doses and transcriptional point-of-departures using the US EPA tcpflR R package. In this work, we provide an overview of the workflow and MongoDB schema used in http:// and provide the rationale for the various tools and methods employed by http:// using data from previously published HTTR chemical screens in various cell lines. First, we compare the HISAT2 sequence aligner in http:// to several alternative alignment tools. We then describe special considerations and challenges for alignment that the TempO-seq platform presents. We also evaluate several quality control (QC) metrics relative to different tools used by DESeq2 during fold-change estimation, which control for variability in lowly expressed probes. Preliminary results demonstrate that the choice in sequence aligner does not provide a large advantage on alignment accuracy, but specific alignment parameters should be considered due to the sequence similarity between the target sequences that have not been realized. In particular, percent mapped reads, appear to be a good indicator of replicate reproducibility and overall sample quality. Importantly, these values vary by cell line and should be re-evaluated whenever new cell lines or reference samples are used. Additionally, we demonstrate that the choice of DESeq2 shrinkage method when estimating fold-changes can have large impact on the ability to detect differentially expressed genes, and selection may depend on how conservative of an approach a user wants to have for a given study. New technologies, declining costs, and increased efficiency in HTTR and other profiling methods have greatly benefited the development of NAMs for high-throughput chemical hazard evaluation. Furthermore, the release of stable, scalable, and reproducible analysis pipelines is paramount to their successful adoption. http:// provides a self-contained workflow and database for high-throughput chemical screening studies that use TempO-seq or similar targeted RNA-seq platforms. This abstract does not necessarily reflect US EPA policy. Company or product names do not constitute endorsement by US EPA.

**3732 Development of a Large List of Drugs for the Study of Nephrotoxicity in Drug Discovery**


Drug-induced kidney toxicity (DIKI) can lead to the development of acute kidney injury, chronic kidney disease, or end-stage renal disease, causing over 1.5 million adverse events in the United States population. Currently, the standard biomarkers for DIKI identification are serum creatinine and blood urea nitrogen, both markers are late-stage biomarkers that are known to lack the sensitivity or specificity to detect nephrotoxicity prior to significant loss of renal function. Consequently, there is a pressing need for the development of alternative methods to reliably predict DIKI in early drug discovery. For the proper development of alternative nephrotoxic methods, a large drug list with annotated DIKI potential is needed. We collected drugs from two literature datasets with confirmation using FDA drug labeling to produce a large list of drugs with known kidney toxicity, called DIKIT (Drug-Induced Kidney Injury and Toxicity). DIKIT is comprised of 1083 drugs, where 585 are DIKI positive (Nephrotoxic) and 503 are DIKI negative (non-Nephrotoxic). It covers all 14 anatomical categories with drugs related to the cardiovascular system, anti-infectives for systemic use, nervous system, and alimentary tract and metabolism found to be the most prevalent. We also found that, while methods like the Rule-of-Two (RO2) and Biopharmaceutics Drug Disposition Classification System (BDPICS) are known to be successful in the evaluation and severity of drug-induced liver injury (DILI), these methods have proven to be ineffective in the classification of a drugs nephrotoxic potential. These results indicate that there are some distinct differences in nephrotoxicity as compared to DILI, such as the reabsorption, secretion, or passive filtering of drugs by the kidney. DIKIT will be a relevant and invaluable resource for high-throughput chemical screening studies that use TempO-seq or similar targeted RNA-seq platforms. This abstract does not necessarily reflect US EPA policy. Company or product names do not constitute endorsement by US EPA.
for the improvement of nephrotoxic research in areas such as the discovery of new methodologies to access the severity and better classify nephrotoxicity earlier within the drug development process.

The opioid epidemic is one of the most prominent and severe public health crises in U.S. history. The devastating consequences of the opioid crisis are not only the increased number of deaths caused by opioids but also the increased economic burden of combating this crisis. The highly addictive nature of the opioids is closely related to the overdose fatalities caused by prescription opioids, heroin, and illicit fentanyl. However, the therapeutic benefits of prescription opioids acting as the most potent analogues make prohibition of the drug impossible. Opioids exert its analgesic effect by binding to the μ opioid receptor (MOR), which then activates its downstream signaling pathway, eventually leading to the inhibition of spinal cord pain transmission. Since the discovery of MOR in the 1970s, many efforts have been endeavored to understand the structure activity relationship between the receptor and its ligands, hoping to shed some lights on the development of non- or less-addictive opioid analogs. Yet, many questions remain unanswered, and the development of non- or less-addictive opioid analogs has had limited success. Here, we present a machine learning model that can be used to predict the binding activity of small molecule compounds to the MOR based on chemical structures. We first curated 17,856 MOR binding data points for 11,427 chemicals from diverse data sources such as publicly available databases, patents, and literature. Mold2 descriptors were calculated for these chemicals. The 11,427 chemicals were then split into a training set of 5,729 chemicals with even identification numbers and a testing set of 5,698 chemicals with odd identification numbers. Random forest algorithm was employed for predictive model development. The random forest models were evaluated using 500 runs of 5-fold cross validations on the training data set and were challenged with the testing data set, resulting in 91.4% and 90.9% MOR binding activity prediction accuracy in the cross validations and testing, respectively. Our results suggest that this model could be useful to identify novel MOR binders, which may aid the development of new drugs targeting on MOR. This abstract reflects the views of the authors and does not necessarily reflect those of the US FDA.

The absorption, distribution, and elimination of xenobiotics. Prior to establishing across the animal kingdom, and testis is a sensitive organ to environmental exposures. All male metazoans have testes containing highly conserved genes spread-

Many current gene therapy targets use recombinant adeno-associated virus (AAV). The majority of delivered AAV therapeutics persist as episomes, separate from host DNA, yet some viral DNA can integrate into host DNA in different proportions and at genomic locations. The potential for viral integration leading to oncogenic potential is well-documented. However, the therapeutic benefits of recombinant adeno-associated virus (AAV) as the total residue length of the common permutations normalized to 100 residues of the compared sequence. Compare has validated employing sequences with simulated substitutions and Compare data have correlated well with those of BLAST and FASTA. The shuffling-resistant nature of Compare is demonstrated here with examples. Compare is now available at Github.com for academic use. Compare is developed as a global sequence comparison program, and it is not available as a local search tool at this time. It is pertinent to mention here the success we have had in obtaining some highly useful information while analyzing the spike proteins of human SARS and animal SARS-like strains with Compare. It is widely acknowledged that SARS-CoV-2 emerged with updates at three critical locations in the spike protein molecule. They are sequentially the receptor binding domain, proteolytic cleavage site, and the heptad-1- repeating (H1R) domain and the updates conferred better infectivity on the strain. Two of the three signatures at these locations identified by us using Compare are identical to those reported by others. Some investigators believe that the SARS-CoV-2 strain has acquired them through recombination events. Additionally, the 11-aa sequence (vavlyqyngct) that carries the D614G substitution and which is present in all the currently prevalent variants of SARS-CoV-2 is identified by us as a motif that is highly conserved in most coronavirus strains including the RaTG13 coronavirus group (viz., human SARS-CoV, animal SARS-like strains, SARS-CoV-2, bat RaTG13, and pangolin coronavirus strains; Curr. Top. Biochem. Res. 21, 119-122, 2020; and Preprints 2020, 2020070488 (doi: 10.20944/preprints202007.0488.v1). It is important because the 11-aa motif is present at varying locations in these strains.
Mutagenicity is one of the most important end points of toxicity that hampers a compound’s potential to become a marketable drug. Ames bacterial reverse mutation test is a widely used method to assess mutagenicity of chemicals. Due to the limitation of Ames test (can’t test small amount of chemicals) and allowance of using in silico approaches to assess the mutagenicity by ICH M7 guideline, we developed an Ames test prediction model (DeepAmes) with a deep-learning powered framework using model-level representations, which were generated by five groups of base classifiers with different conventional machine learning algorithms. The compounds used for developing (10,444 compounds) and testing (1,543 compounds) models were provided by the Division of Genetics and Mutagenesis, National Institute of Health Sciences of Japan (DG/MiHS). The DeepAmes model yielded a Matthews correlation coefficient (MCC) of 0.384, which made a 47.7% improvement in comparison with the averaged MCC of the five groups of base classifiers. The content of use of the DeepAmes model aims to provide high predictive ability for both mutagenic and non-mutagenic compounds. In addition, we introduced an approach to develop Ames prediction models to meet the content of use in regulatory application, where high sensitivity is preferred. Using this approach, the sensitivity of the developed models could be as high as 0.866, which is an impressive sensitivity considering there is only 15.04% mutagenic compounds on the test set.

Recent technological advancements have led to the development of new high-throughput methods such as transcriptomics, which can be used to rapidly screen environmental chemicals for potential hazards. It is now feasible to profile all protein-coding genes across thousands of samples, allowing for broad evaluation of many target pathways and mechanisms of action simultaneously in a single experiment. US EPA has developed a robust and reliable workflow to rapidly screen chemical effects in vitro using High-throughput Transcriptomics (HTTr). However, the scale and complexity of the resulting data is much greater than previous high-throughput screening (HTS) assays (e.g., ToxCast), and a variety of analysis approaches are available to derive key outputs of interest, such as estimating an overall transcriptomic point-of-departure (tPOD). The accuracy and reproducibility of these estimates may depend on a broad array of analysis parameters that have not yet been fully explored. To date, there is limited consensus on best practices for analysis of transcriptomic concentration-response data, and insufficient understanding of how specific analysis choices impact the primary estimates of interest. While it is unlikely that there is a single “one size fits all” approach when analyzing the diverse range of transcriptomic studies, technologies, and applications, there remains a major unmet need for a standardized framework to evaluate and tailor analysis methods for specific use cases. Here we propose a framework to evaluate the reliability and reproducibility of transcriptomic concentration-response modeling methods, including 1) the false positive rate of “hit calls” for concentration-responsive genes, 2) the accuracy of concentration-response models, and 3) the reproducibility of tPODs. To assess false positive rate, we propose a method to simulate inactive chemical profiles by randomly sampling HTTr profiles of cells treated with relatively low concentrations of chemicals within larger screening studies. To assess the accuracy of concentration-response models, we propose a “hit-corrected” transcriptomics framework by precisely adjusting the relative dilutions of two standard reference mixtures. Within this data set, we have simulated multiple effect sizes and curve shapes matching commonly used parametric models. Finally, to assess the reproducibility of tPOD values, we have repeatedly tested several standard reference chemicals throughout our HTTr screening studies, resulting in dozens of replicate experiments that can be analyzed independently and compared based on the final tPOD estimates. We demonstrate how this framework can be used to optimize and compare existing methods such as BMDExpress, which has numerous tunable parameters. Our preliminary results demonstrate that 1) current methods have acceptable false positive rates (≤ 1%) at the level of identifying individual concentration-responsive genes, 2) BMDExpress can accurately model the majority of concentration-response curves in our synthetic data, and 3) that tPOD estimates based on the 25th lowest gene-level BMD are highly reproducible with standard deviations below 2-fold based on our reference chemical data. These results can inform best practices and further optimize methods for transcriptomic concentration-response modeling, which will help build confidence in these assays as a regulatory decision-making. This abstract does not necessarily reflect US EPA policy. Company or product names do not constitute endorsement by US EPA.

The adverse outcome pathway (AOP) framework is intended to describe the causal linkages from a direct molecular interaction with a stressor to an adverse outcome of regulatory concern. Capturing such existing knowledge across biological levels of organization in this manner allows decision-makers an opportunity to take advantage of data from growing numbers of mechanistic studies that typically do not measure apical outcomes for evaluations of chemical safety. The challenge of applying AOPs is a broader decision-making framework stems from the fact that the majority of AOPs that have been developed lack evidence to extrapolate pathway knowledge beyond the organisms captured in the AOP descriptions during development. To enhance the utility of AOPs and expand the taxonomic coverage, otherwise known as the defined taxonomic domain of applicability (tDOA), bioinformatics approaches can be used in combination to extrapolate knowledge based on conservation of biology (i.e., protein sequences/structures). Bringing together well-developed bioinformatics approaches for this purpose is the primary objective of the International Consortium to Advance Cross Species Extrapolation in Regulation (ICACSER). The ICACSER is currently in the process of vetting tools/databases/approaches to develop guidance and best practices for development and use of High-Throughput Transcriptomics (HTTr) screening studies, resulting in dozens of replicate experiments that can be analyzed independently and compared based on the final tPOD estimates. We demonstrate how this framework can be used to optimize and compare existing methods such as BMDExpress, which has numerous tunable parameters. Our preliminary results demonstrate that 1) current methods have acceptable false positive rates (≤ 1%) at the level of identifying individual concentration-responsive genes, 2) BMDExpress can accurately model the majority of concentration-response curves in our synthetic data, and 3) that tPOD estimates based on the 25th lowest gene-level BMD are highly reproducible with standard deviations below 2-fold based on our reference chemical data. These results can inform best practices and further optimize methods for transcriptomic concentration-response modeling, which will help build confidence in these assays as a regulatory decision-making. This abstract does not necessarily reflect US EPA policy. Company or product names do not constitute endorsement by US EPA.
has a median molecular weight equal to 231 g/mol. Our results suggest that these computational models can be used to rapidly screen large chemical libraries to prioritize potentially hazardous substances for further evaluation. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The authors declare there exists no real or potential conflict of interest.

3741 Biological Interpretation of Cell Painting and Gene Expression Features for Mitochondrial Toxicity Prediction
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Cell Painting data, which are versatile descriptors of cell morphology, have previously been shown to improve the performance of predictive models for many drug safety and toxicity endpoints in areas of novel structural space. In this study, we investigated the use of Cell Painting Gene Expression features in Morgan fingerprints in predicting the mitochondrial membrane depolarization assay from Tox21. We trained random forest models using individual and the combination (fusion) of Cell Painting, Gene Expression features and Morgan Fingerprints on 382 compounds (62 of which were mitotox). When evaluated on an external set of 244 compounds (47 of which were mitotox), fusion models relatively improved detection (F1 Scores) of mitochondrial toxicants (by 60% from 0.25 to 0.40) compared to models that only used structural features. Further, for another ten inconclusive compounds in the Tox21 assay (because of excessive cytotoxicity), the fusion models correctly predicted mitochondrial toxicity for all such compounds (10 out of 10) compared to Morgan fingerprints which could only correctly predict mitochondrial toxicity for only 1 out of 10 such compounds. Fusion models improved sensitivity in detecting mitotoxic compounds compared to dedicated high-content imaging assays (0.79 in the external test set in our study vs 0.37 in Aperico MitoMass) with comparable balanced accuracies (0.69 in the external test set in our study vs 0.65 in Aperico MitoMass). This suggests that cell features like structural features limit the applicability domain to the chemical space of the training dataset. For example, Morgan fingerprints failed to correctly classify mitotoxic compounds (e.g., betulinic acid) at high structural distances. The fusion models correctly classified mitotoxic compounds (e.g., ketoconazole and fleroxetine) in diverse morphological and structural spaces where individual models failed to demonstrate the synergistic effect of the features spaces. Finally, we studied the correlation between Cell Painting and Gene Expression features associated with mitochondrial toxicity mechanisms to interpret their biological significance. Cell Painting features correlated to Gene Expression descriptors. For example, the side of the membrane correlated to RNA granularity (aka granulicity), structural features limit the expert model's applicability domain to the chemical space of the training dataset. For example, Morgan fingerprints failed to correctly classify mitotoxic compounds (e.g., betulinic acid) at high structural distances. 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use categories that broaden the underlying data for the Chemical Characterization tool. These examples of new and updated ICE data sets and tools indicate the potential to provide comprehensive yet simple and interpretable outputs throughout the different ICE tools. With each release, ICE implements advanced features to its user interface and computational tools that aim to ease data exploration for all ICE end users. Key among the updates implemented in ICE v3.8 are the expanded Physiological North and Toxicokinetics North (ToxKnob) tool and new ICE tools, both of which now include a gestational model from the EPA’s htkk package. Additionally, the enhanced ICE Search tool now provides an option to search the ICE data repository using a chemical name along with other pre-existing chemical identifier input options. This update also provides a completely revised Search results page with new summary statistics and publication-quality result visualization options for all the data within ICE. Together, these ICE features allow its users to explore ICE’s ever-growing data repository of over 1 million chemicals, supporting purposes like chemical analysis for NAMS-based assessments. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.

3745 Cross-Study Analyses of SEND Data: Toxicity Profile Classification

The objective of this work, a collaborative effort between BioCelerate and FDA, is to develop SEND data harmonization/transformation strategies and apply analytic techniques to enable an understanding of the similarities and differences between two or more data sets. Example use cases include understanding a single compound’s toxicity profile across all studies performed or evaluating on-versus off-target toxicity. In this paper, we compiles and linked SEND data from the same pharmacological target. A subset of de-identified studies from the BioCelerate Toxicology Data Sharing database were used for the analyses. The analyses involved transformation and visualization of SEND data variables to integrate both numerical and categorical data. Toxicity profiles for key organ systems were developed by selecting pertinent data from the body weight (BW), organ weight (OM), microscopic (MI), and laboratory (LB) domains. Profiles for liver, kidney, brain/neuro, GI tract and lungs were developed. In addition, a dashboard and user defined scoring system were created to facilitate custom analyses for data of interest. The output of the analyses includes a series of radar plots that enable the user to visualize and evaluate data from the organ system level to individual animal data points. To validate the analyses, the individual studies with SEND data sets were reviewed independently and then compared to results from the cross-study analyses. This evaluation of data provides the tools for scientists to compare and contrast toxicity profiles across multiple studies using SEND data. Toxicity profile analysis of different compounds or the same pharmacological target will be presented in the poster. Cross-study comparisons using SEND data are expected to improve the identification of unique findings related to the intended target, species and duration of dosing.

3746 Interactive Data Sharing for Multiple Questionnaire-Based Expose-Wide Association Studies in the Personalized Environment and Genes Study
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Diseases and health are greatly influenced by the environment. Environmental exposure is a complicated phenomenon, and over the course of their life, people may be exposed to a myriad of different influences. It is theorized that the entirety of exposures experienced from birth, or the exposure, is also comprised of interdependent factors that result in a single outcome. We currently lack tools to fully identify reliable relationships within the exposure. Further, there are limited studies evaluating the relationships between exposures and the diseases linked to them by expose-wide association studies (ExWAS). To address these gaps, we evaluated correlations of exposures from extensive questionnaire data collected in a diverse North Carolina-based cohort - the Personalized Environment and Genes Study (PEGS; n=9,414). We rigorously computed direct pairwise correlations of 1,078 exposure variables covering occupational and hobby exposures, other internal and external exposures, and lifestyle factors. We fit Bayesian shrinkage models to the correlations to address concerns about correlation interpretations due to different sample sizes. To control for common epidemiological covariates/confounders, partial correlations were computed after residualizing random variables. We repeated analyses by sex and race/ancestry strata, leveraging the diversity in PEGS. Next, we mapped the relationships between exposures and common, complex ExWAS-linked diseases representing a variety of biological pathways and systems (asthma, allergic rhinitis, fibroids, ovarian cysts, bone loss, lower GI polyps, iron deficient anemia, type 2 diabetes, and cardiovascular disease). Given the expansive scope of these results, and the lack of data sharing in exposome science, we developed a web application called the PEGS Explorer to visualize results. These comprehensive ExWAS results are searchable, and an exposome globe architecture is used for visualizing and explaining the intricate connections between exposure factors with and without associations to a disease. The PEGS Explorer web application for sharing exposure related results will enable researchers to put results in context, to use for hypothesis generation, and to replicate results in other cohorts. This will provide insights into the correlations among exposures and the complex interactions between the exposome and health and disease and increase our understanding of how exposures affect populations differently.

3747 The Environmental Health Language Collaborative (EHL): A Community Effort to Harmonize Data Use and Sharing
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While the availability of data is rapidly advancing opportunities to answer large-scale, complex questions in toxicology and environmental health sciences, a significant challenge remains around the development and use of a harmonized language to allow for streamlined workflows to find, share, and reuse this data. To address this challenge, the Environmental Health Language Collaborative (EHL) was created to facilitate a public, community-driven effort to advance the development and adoption of harmonized language approaches within toxicology and EHS fields. Using ontologies and other standard vocabularies creates a common language to facilitate data discovery as well as a shared understanding to allow comparison and evaluation of data. In September 2021, 88 toxicology and EHS professionals participated in the launch of EHL and attended an inaugural two-day interactive virtual workshop titled, Catalyzing Knowledge-driven Discovery in Environmental Health Sciences Through a Harmonized Language. As a result of workshop discussions, toxicology and EHS professionals identified the need for community-led “use case” working groups related to the topics of biomarkers and biological process, data harmonization, place-based exposures, and data discovery. These community-led “use case” working groups have been advancing EHL priorities in areas such as: evaluation of ontologies and standard vocabularies relevant to toxicology and environmental health sciences, the development of a conceptual model linking exposures to biological processes and biomarkers associated with specific health outcomes, development of a place-based language structure to improve and harmonize language, and identification of potential barriers and development of resources to find and harmonize data including published toxicology data. In this presentation we highlight the main goals, activities, and recent accomplishments of EHL including the 2nd Annual EHL Workshop focused on the National Institutes of Health’s Data Management and Sharing Policy and related resources in early 2023. Additionally, we will promote upcoming opportunities for EHL to contribute to the development of a harmonized language to ensure that toxicological and EHS data can effectively be shared and best used to answer the fields’ most pressing questions. Our aim is to inspire EHL professionals’ collaborative engagement with EHL and bring awareness of available resources to help advance environmental health sciences.

3748 ToxDataCommons: Promoting the FAIR Principles of Environmental Health Data
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The Findable, Accessible, Interoperable, Reusable (FAIR) data guiding principles have reinvigorated efforts to improve data sharing and reuse among the research community. The FAIR principles are now a key component of data stewardship plans required by funding agencies such as the National Institutes of Health as well as many publishers. In support of promoting data management and sharing for environmental health research, the Michigan State University Superfund Research Center (SRC) Data Management and Analysis Core (DMAC) has initiated the development of a data commons. ToxDataCommons is built on the Gen3 framework commonly used by other large-scale data repositories, including toxicology, data ingestion, curation, and visualization. Our alpha stage ToxDataCommons implements a model derived from current field standards previously outlined in the Chemical Effects in Biological Systems (CEBS) data dictionary and Minimum Information about Animal Toxicology (MIAT) checklist to capture critical metadata for individual datasets. The data model aims to capture metadata at all stages of an experiment following the Investigation, Study, Assay (ISA) framework, which spans anything from finding support to experimental design and assay specific details. Querying metadata in ToxDataCommons uses a GraphQL service which can build cohorts of experiments based on metadata values. Either through built-in workbooks or independent cloud computing resources, an advanced programming interface (API) enables the downloading and reuse of deposited data. ToxDataCommons is used to perform integrative analysis of deposited data to extract new information and generate novel hypotheses that highlight the value of the FAIR principles to environmental science research. Supported by the Superfund Research Program P42ES0054911.
Gene Expression Quality Assessment: Detection of Tissue Contamination or Mislabeling


Recent advances in multiplexing strategies for next generation sequencing technologies are facilitating high throughput transcriptomic studies involving a large number of perturbagens and concentrations for different cell lines and organs. However, as the complexity of experimental design increases, so does the potential for human/experimental errors. These errors include issues such as sample mislabeling, plating errors, or imprecise tissue dissection, which can lead to misidentification of samples and batch effects. Failure to appropriately detect and resolve these inaccuracies will adversely affect downstream inference and reproducibility of the study. The standard tools that utilize all samples collectively, such as un-supervised clustering or principal component analysis, cannot usually identify sources of contamination. We have developed a computational approach to identify tissue contamination or mislabeling in transcriptomic experiments. By using RNA-seq data and tissue specificity definitions from the Human Protein Atlas (HPA), we have established gold-standard transcriptomic signatures for a variety of tissues. Experimental transcriptomic data can be matched against our HPA tissue-specific signatures using a customized statistical approach (Wilcoxon-Mann-Whitney rank sum test-based metric). This procedure provides a normalized significance score (between -1 and 1) for each tissue and these scores can in turn pinpoint potential sources of error such as sample mislabeling or cross-contamination in the laboratory. For example, if a sample has a high significance score of 1 for only one tissue and a low score (below zero) for all other tissue signatures, we can be certain that the tissue sampled is not contaminated. However, samples with multiple high tissue-specific significance scores indicate that there was a mixture of corresponding tissues during sample preparation. Our method was tested by simulating increasing amounts of noise in the original HPA expression data to determine sensitivity and specificity for matching a sample to its assumed tissue. Further testing was performed on simulated cross-contaminated samples by mixing expression data for two tissues with varying proportions of contribution from each tissue. Results confirm the ability to recapture the simulated contamination, with a Pearson correlation of 0.90 between the significance scores and the corresponding degree of cross-contamination. This application can be used as part of a standard quality control protocol for transcriptomics experiments.

Regulation of Intestinal Xenobiotic Transporters following Treatment with Perfluoropolyalkyl and Polyfluoropolyalkyl Chemicals

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Perfluoropolyalkyl and polyfluoropolyalkyl substances (PFAS) have been used for decades in non-stick cookware, firefighting foams, textiles, packaging, and cosmetics. Due to widespread routes of exposure, PFAS are found in the blood of almost the entire U.S population and elevated concentrations may contribute to the development of chronic conditions including hypericmicemia and hypercholesterolemia. Prior studies have demonstrated that PFAS can inhibit the functional activity of the intestinal unicellular or brush border membrane (BBM) vesicles control. The current research of this study demonstrates longer periods of exposure cause a greater quantitative effect on mast cell degranulation. In conclusion, PFAS exposure to mast cells has an impact on the rate of β-hexosaminidase release. PFAS either increases mast cell degranulation or suppresses it depending on the specific PFAS. While the measure of concentration had negligible effect, the length of exposure results are considerable. The future direction of this study is two-fold: one, to identify if PFAS has an effect on the glycosylation of the treated mast cell and two, if cytokine release occurs after mast cells undergo PFAS exposure.

Determining the LD50 of Perfluoropolyalkyl Acids on Xeric Plants

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Short-chain perfluoralkyl acids (PFAA’s) are exceedingly persistent and have high mobility in soil and water. PFAA’s are present in a multitude of applications including firefighting foams, degradation of refrigerants, the pyrolysis of PFTE, and more. Despite having been released into the environment for approximately 50 years, little is known about the toxicity and bioaccumulation of these chemicals in plants and xeric plants in particular. In this research, the toxicity of four short-chain PFAA’s are analyzed: trifluoroacetic acid (TFA), pentafluoropropionic acid (PFPPA), perfluorobutanoic acid (PFBA), and perfluoropentanoic acid (PFPeA). It is hypothesized that the longer the carbon chain the more nonpolar it is resulting in higher bioaccumulation. This hypothesis was tested using Joshua Tree seedlings (Yucca brevifolia) with the four test acids to determine the LD50 of each PFAA. Additionally, TFA is also being tested on Saguro cacti (Carnegia gigantea) and creosote bush (Larrea tridentata) to determine differences in bioaccumulation based on the physiological structure of the plants. In Joshua trees, the results indicate the LD50 for TFA is 48.1 mg/kg for root growth, PFPeA is 48.7 mg/kg and PFPeAe is 26.2 mg/kg of soil, which indicates that the acids have a similar range of toxicity on a mass basis. The bioconcentration factor (above ground biomass/soil concentration) for TFA in Joshua trees was 4090. Currently, the saguaro are showing similar toxic effects to TFA as the Joshua tree.

The Modulation of Non-IgE-Mediated Mast Cell Activation by Per- and Polyalkyl Substances


Per- and polyalkyl-substances (PFAS) are synthetic chemicals that are emitted into the environment by manufacturing companies. These synthetics persist in the environment for extremely long periods of time. People exposed to PFAS, which are now referred to as “forever chemicals,” test positive throughout their lifetime and according to research, exhibit negative long-term health effects such as immune system suppression and lowered response to vaccinations. Since widespread usage of PFAS is increasing annually and along with studies that demonstrate the adverse impact on human health, the critical need for regulatory guidelines cannot be overstated. In this study we analyzed the mast cell degranulation rates of multiple PFAS types, the effects of varying the PFAS concentration rates, and the duration of PFAS exposure. PFOA, PFAS, PFHxS, PFBS, and GENX were used as treatment groups within the experiments. Rosu mast cells were exposed to concentrations of the different types of PFAS at 1.0, 10. and 25. μM for 24-hour and 48-hour exposure periods. The treated cells were “activated” through exposure to a positive control of silver nanoparticles known to cause activation through the MRGPRX2 receptor. β-hexosaminidase levels were recorded and analyzed against the group treated strictly with silver nanoparticles. The results were compared to treatment groups using various quantities to identify the impact these concentrations had on the outcome. The study substantiates the overall affect PFAS has on mast cell degranulation and how the results vary for each type of PFAS treatment, for example, PFOA amplifies activation while GENX represses activation. Overall, varying the concentrations of PFAS in each treatment group did not yield an appreciable difference while the duration of how long cells were treated did. Hence, treatment dose without exposure times are statistically different than the silver nanoparticles control. The current research of this study demonstrates longer periods of exposure cause a greater quantitative effect on mast cell activation. In conclusion, PFAS exposure to mast cells has an impact on the rate of β-hexosaminidase release. PFAS either increases mast cell degranulation or suppresses it depending on the specific PFAS. While the measure of concentration had negligible effect, the length of exposure results are considerable. The future direction of this study is two-fold: one, to identify if PFAS has an effect on the glycosylation of the treated mast cell and two, if cytokine release occurs after mast cells undergo PFAS exposure.

Maternal Exposure to Perfluorohexane Sulfonate (PFHxS) Alters Glucose and Lipid Dynamics during the Postnatal Period in the Rat

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Perfluorohexane sulfonate (PFHxS) is a ubiquitous environmental contaminant that can be quantified in the sera of infants, children, and adults throughout the United States. Some epidemiological studies have linked PFHxS to metabolic dysfunction like gestational diabetes and increased body mass index in children. However, the mechanism(s) of such effects are unclear. To determine if PFHxS may alter glucose and lipid metabolism during development, pregnant Long Evans rats were dosed orally with 0, 17, or 50 mg/kg/day of PFHxS from gestational day (GD) 2 to postnatal day (PN) 14 and the dams and offspring evaluated. Neither body nor liver weights were significantly different in PFHxS exposed dams and/or pups as compared to controls. PFHxS exposure increased dam serum glucose on PN14 and this was statistically significant following the 50 mg/kg exposure; total cholesterol was significantly decreased in both PFHxS groups. Given that the liver is a major metabolic regulator and a known target of other perfluoroalkyl substances, we performed RNA-Seq of liver tissue in male pups on PN2 and PN14 (0 versus 50 mg/kg/d PFHxS). RNA-Seq identified 198 and 416 differentially expressed genes on PN2 and PN14 respectively (q<0.05). Pathway analyses show an enriched signature for fatty acid oxidation, ketogenesis, and insulin signaling. Next, we validated our preliminary qRT-PCR for top candidate genes in both male and female littersmates representing all dose groups. Our results show concordance between RNA-Seq and qRT-PCR and between the sexes; in addition, these candidate genes exhibited a dose response. These data show that developmental exposure to PFHxS alters glucose metabolism in the mothers and
Per-and polyfluoroalkyl substances (PFAS) are synthetic, highly persistent chemical contaminants that have been detected in the environment, especially in drinking water. Despite their industrial and consumer applications, PFAS exposure has been associated with many adverse effects in humans, including suppression of vaccine antibody responses. Our laboratory’s studies with animal models indicate that PFAS exposure reduces the T-cell dependent antibody response (TDAR), which is a functional outcome analogous to the vaccine response in humans. Our previous studies with a well-studied PFAS, perfluorooctanoic acid (PFOA), indicate that suppression of the TDAR is linked on impacts on B cell functions. Given that PFAS, including PFOA, are known to affect basal metabolic function, it is plausible that PFAS cause alterations in several metabolic shifts and experiences high metabolic demands when changing to antibody-secreting plasma cells, we hypothesize that suppression of the TDAR arises from B cell metabolic impairment. Adult male and female C57BL/6 mice were given PFOA (0 or 7.5 mg/kg) via gavage for 30 days. This dose is known to be immunosuppressive in this strain of mice in the absence of systemic toxicity. One day after dosing ended, naïve B cells were isolated from spleens by negative bead selection, and then these enriched B cells were stimulated ex-vivo with the addition of α-CD40 and IL-4 antibodies. After 24 hours in culture, the enriched B cells were assessed for mitochondrial function by a mitochondrial stress test to measure basal, maximal, and reserve respiratory capacities. Our data demonstrate a trend of mitochondrial function in basal, maximal, and reserve capacities between unstimulated and stimulated groups in both males and females of exposed and control groups, supporting proof of concept for ex vivo stimulation. These preliminary data indicate that in vivo PFOA exposure shifted the bioenergetic profile of B cells and thus may affect B cell development and antibody responses through perturbation of mitochondrial function. Additional studies will explore mitochondrial function across a range of B cell subtypes and assess energy usage within both proliferating and differentiating B cells.

Find up-to-date information at www.toxicology.org/2023 #2023SOT #ToxExpo
PFAS, or Perfluoroalkyl Substances, are a family of man-made chemicals found in a variety of products from non-stick cookware and food wrappers to fire retardants. PFAS chemicals are widely distributed in the environment and have notorious longevity, posing a threat to both human health and the ecosystem. PFAS can be classified into two categories, long chain chemicals with 6 or more carbons in their carbon chain and short chain chemicals with less than 6 carbons which are proposed as a safer alternative. This study analyzed the impact of exposure to PFAS, including PFOA, PFOS, PFHxS, and PFDA on the physiology of the annelid Lumbricus variegatus (ie, blackworms). L. variegatus lives in the benthic zone at the edges of freshwater bodies and is found throughout North America as well as in Europe and Asia. The results indicate that PFAS exposure in blackworms can detectable amounts of PFAS in the environment; hence, future studies with an environmental relevant mixture of PFAS will be needed to understand the hazards of skin exposure and help promote protective measures.

Per- and Polyfluoroalkyl Substances (PFAS) are a diverse class of industrial chemicals that have been used for decades in industrial and commercial applications. Due to widespread use and resultant environmental bioaccumulation, PFAS are consistently detectable in the bloodstream of humans. PFAS have been linked to several adverse health outcomes including hepatotoxicity, immunotoxicity, endocrine disruption, tumorigenicity, and neurotoxicity, specifically for developmental toxicity. An increased prevalence of neurodevelopmental disorders in children has been observed and linked to pre- and postnatal exposure to PFAS. However, the mechanisms of adverse neurodevelopmental effects of PFAS are largely unknown. In order to determine PFAS mechanism of action, in-depth toxicological studies are required. Traditional toxicological chemical studies use animal models that are costly, time consuming, and challenging to interpret when testing multiple chemicals at varying concentrations. The nematode Caenorhabditis elegans serves as an ideal model organism for neurodevelopmental toxicity studies due to the organism only having 320 neurons, a complete written diagram for its chemical and electrical connections available, and a short lifespan. In this study, 10 PFAS compounds with high occurrence frequency were selected to represent a wide range of typical PFAS structures, including perfluoroalkyl carboxylic acids (PFBA, PFHxA, PFUFA), sulfonic acids (PFBS, PFHxS, PFOS), sulfonamides and derivatives (PFOSA, NPFOSA), fluorotelomerates (6:2 FTS), and new substitutes (HFPO-DA, the acyclic form of GenX). Wild-type worms were exposed to single PFAS at 0, 0.1, 1, 10, and 200 µM. The toxic effects of PFAS were linked to neurodevelopmental disorders in children with pre- and postnatal exposure. It is important to note that PFAS do not exist independently in the environment; hence, future studies with an environmental relevant mixture of PFAS will be conducted with the dose defined-range obtained from findings of single PFAS.

PFOS and PFBA were observed to induce a suppressive effect on blood circulation in the worms. The average pulse rate was reduced from 9.6 beats/minute to 6.2 and 7.0 beats/min in PFOS and PFBA, respectively. Further, PFOS, PFBA, and PFDA, but not PFHxA, markedly slowed the rate of the dorsal blood vessel in L. variegatus, indicating a suppressive effect on blood circulation in the worms. These findings raise concerns of alternative PFAS being promoted as a safer option and show that further investigation into PFAS dermal exposure is needed to understand the hazards of skin exposure and help promote protective measures.

Perfluorooctane sulfonate (PFOS, an 8-carbon PFAS) is an environmental pollutant that has been detected frequently in the environment. Scientific literature suggests that PFAS exposure can have a negative impact on human health. According to published research, PFOS can cause genotoxicity, neurotoxicity, and alter innate and adaptive immune responses. Although PFOS are regarded as immunological hazards for people, it is still unclear how they cause immunotoxicity. Our preliminary data showed that PFOS exposure increases the expression of the CD36 gene in C4+ T cells in mice. The scavenger receptor CD36 is an essential metabolic regulator of T cell metabolism in immunological responses. In this study, the goal of the current study is to investigate if PFOS-induced alterations in the CD36-lipid metabolism axis contribute to immunotoxicity. In this study, in vitro studies included T cell isolation and PFOS treatment in cell culture. Splenic CD4+ T cells were then isolated from splenic tissue of control and PFOS treated mice and qRT-PCR was used to measure expression of CD36. In vivo studies included T cell isolation and PFOS treatment in cell culture. Splenic CD4+ T lymphocytes were stimulated with anti-CD3+CD28+ activation beads, treated with PFOS, and then analyzed by qRT-PCR and flow cytometry analysis. Flow cytometry is used to quantify changes in the membrane associated CD36 expression due to PFOS exposure. In vivo data demonstrate the increase in CD36 mRNA expression in splenic CD4+ T cells from PFOS treated mice as compared to control C57BL/6 mice. We have also demonstrated the increase in CD36 mRNA expression in CD4+ T cells from the following single PFOS exposure in vitro. Consistently, flow cytometry analysis suggests that exposure to PFOS leads to an increase in the CD36 level on the cell surface of CD4+ T cells. In summary, our studies demonstrate that PFOS exposure can contribute to increased CD36 expression in splenic CD4+ T cells. These findings also suggest that there is increased abundance of CD36 on the membrane of CD4+ T cells from skin derived CD4+ T cells. Further studies will determine the functional significance of PFOS-induced CD36 expression in T cell lipid metabolism and immunotoxicity. Supported in part by NIHES/NIGM P42ES07380 and by UK-CARES grant P30ES026529.
Emerging epidemiological evidence indicates that perfluoroctane sulfonic acid (PFOS) is increasingly associated with lung diseases such as asthma and respiratory viral infections. Animal studies suggest that PFOS disrupts lung development and inhibits immune-inflammatory responses, but little is known about the potential consequences of PFOS exposure on respiratory health and disease risk. Importantly, PFOS exposure during critical stages of respiratory and immune system development may contribute to disease risk. Thus, we hypothesized that gestational and lactational PFOS exposure will affect lung inflammation and alveolar/airway development in a sex-dependent manner. To address this knowledge gap, timed pregnant Balb/cJ dams were dosed via a PFOS-injected mealworm (0, 1, or 2 mg/kg/d PFOS) daily from gestational day 0.5 to postnatal day (PND) 21. Offspring were sacrificed at PND 22 and PND 90. At PND 22, average serum and lung concentrations in the 1 mg/kg/d (20.7±1.21 µg/mL and 8.4±0.68 pg/µg respectively) and 2 mg/kg/d groups (47.06±2.40 µg/mL and 17.57±1.1 pg/µg) were determined by mass spectrometry. No differences in PFOS concentrations were observed based on offspring sex. PFOS-exposed male offspring (PND 22) displayed diffuse alveolar simplification and increased alveolar septa thickness, indicative of lung immaturity. Tissue repair and growth factors (FGF-2 and IGFBP-2) were also elevated in the bronchoalveolar lavage (BAL) of PFOS-exposed male offspring compared with male vehicle controls. BAL cell counts were significantly elevated at 2 mg/kg/d PFOS. However, BAL IL-12, IL-10, INFγ, IL-6, and MCP-1 were suppressed only in PFOS-exposed male offspring. Developmental PFOS exposure also resulted in persistent respiratory deficits. At PND 90, two months following the cessation of exposure, disruption of small airway epithelial morphology was observed in PFOS-exposed males. Adult male offspring also had increased levels of BAL IL-6 and IL-12, IL-10, INFγ, and MCP-1, and a shift in Th1/Th2 axis. Overall, these results demonstrate that PFOS exposure exhibits male-specific adverse effects on lung development and inflammation that present differently in juvenile and adult offspring. These data support epidemiological associations linking PFOS and lung disease, but further research is required to elucidate the mechanisms underlying the sex-differentiated pulmonary toxicity of PFOS. Supported by T32 ES007026.

3765 Chronic Perfluorooctanesulfonic Acid (PFOS) Induces Hyperactivity and Deficits in Nonassociative Learning in Male but Not Female Mice

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Per- and polyfluoroalkyl substances (PFAS) are a group of persistent organic pollutants or "forever chemicals" that are ubiquitous in the environment and virtually in all living organisms, including humans. Exposure to PFAS has been correlated with sex-specific effects including endocrine disruption and motor behavior deficits. Our previous studies determined out of multiple PFAS, only perfluorooctanesulfonic acid (PFOS) was selectively toxic to dopaminergic neurons in males. Moreover, PFOS-treated frogs exhibited complex neurotransmission changes that were developmental stage/exposure length dependent, including alterations in dopaminergic neurotransmission. A pilot study with female mice dosed with PFOS for 6 months demonstrated decreases in monoamine levels of dopamine and serotonin and their metabolites in the ventral striatum and ventral midbrain. In the hippocampus, PFOS treatment resulted in increased dopamine turnover and decreased dopamine levels. Given the critical role of neurotransmitter levels and metabolism are implication in neurological diseases and disorders and are associated with functional changes, we hypothesized chronic, systemic PFOS exposure alters neurotransmitter metabolism, resulting in altered neurobehavioral outcomes. Here, we measured monoamine levels and metabolism in male and female C57BL/6J mice dosed with 1.0 mg/kg/d PFOS or 0.5% tween-20 (vehicle) in drinking water for 16 months, beginning at 1 month of age (n=10-18 per treatment-sex group). Neurobehavioral experiments included open-field locomotor, challenging beam traversal, gait, novel object recognition, and Morris water maze. Brains were dissected for future analysis of neurochemistry and PFOS quantification.

Open-field locomotor was conducted by placing mice in the activity chamber with photobeams detecting horizontal and vertical movement over 60 minutes. Analysis of locomotor revealed only PFOS-treated male mice (not females) had significant changes compared to vehicle-treated mice. PFOS-treated male mice demonstrated significant increases in horizontal (p-value=0.04), vertical (p-value=0.01), and total activity (p-value=0.009) compared to vehicle-treated males. PFOS-treated male mice demonstrated decreased horizontal (p-value=0.02) and total (p-value=0.007) habituation compared to vehicle-treated males. Lastly, there was a decrease in grooming in PFOS-treated male mice (p-value=0.02). These results suggest that chronic PFOS treatment causes hyperactivity, decreased non-associative memory, and decreased fine-motor coordination. However, not all PFAS were equally affected, our study demonstrates that lifelong exposure to PFOS leads to sex-specific neurobehavioral changes and the brain continues to be vulnerable to PFOS neurotoxicity beyond early development. Future analysis will assess additional neurobehavioral endpoints for motor function and learning and memory as well as analyze
Perfluorinated substances (PFAS) are a class of synthetic chemicals widely used in industry for manufacturing common household materials, which people and environment are exposed to. The widespread use of PFAS resulted in the ubiquitous presence of these chemicals in the environment and together with their persistence, resulted in a worldwide contamination with a global distribution in living organisms. Human studies have shown adverse effects of PFAS on the immune system, at low exposure levels, affecting both cell-mediated and humoral immunity. In particular, the alteration of cytokine production, the reduction of specific antibody production, and the reduction of survival after influenza infection have been reported. The majority of the available data highlights an immunosuppressive effect and, as a consequence, a reduced survival and resistance to infections with a decreased antibody response to vaccination. The aim of this study was the investigation of the effect of PFAS on antibody production both toward T cell-dependent or T cell-independent. To mimic these different conditions, two approaches were used. The first one involved the investigation of the effects of PFOA and PFOS on immunoglobulin production following mitogen-induced B cells activation, while the second one engaged the primary antibody response using Keyhole Limpet Hemocyanin (KLH) as antigen. Human peripheral blood mononuclear cells, from healthy male and female donors, were treated with increasing concentrations of PFOA and PFOS as antigen. Human peripheral blood mononuclear cells, from healthy male and female donors, were treated with increasing concentrations of PFOA and PFOS had been categorized to derive US drinking water health advisory levels in the US. Manufacturers have moved to shorter chain PFAS under assumptions of lower bioaccumulation and toxicity. Previously, our lab evaluated impacts of PFOA exposure on B cell development associated with suppression of T cell-dependent antibody responses (TDAR). PFOA exposure impaired marginal zone, follicular B cells and plasma cells and led us to explore perfluorooxyacetic acid (PFFhAx) and perfluoro-2-methoxyacetic acid (PFFMOMA), compounds of concern in drinking water. Adult male and female C57BL/6 mice were given PFhAx or PFMOMA (0, 0.5, or 50 mg/kg) via gavage for 30 days. A duration sufficient to suppress the TDAR. Animals were injected with sheep red blood cells to stimulate the TDAR, and one day after dosing ended, spleens were prepared to determine B cell subpopulations via a flow cytometric panel. The TDAR was suppressed by PFhAx at doses of 0.5 and 50 mg/kg and by PFMOMA at 50 mg/kg. Flow cytometric analysis revealed that marginal zone and plasmablasts were impacted by PFhAx and PFMOMA exposure. Follicular, and memory B cell subclasses were also impacted by PFhAx, consistent with our observations with PFOA. Our data indicate overall B cell numbers are not affected by PFAS exposure, but changes in numbers of B cell subsets suggest that the ability of B cells to differentiate and potentially proliferate is impacted. Currently, we are developing ex vivo naive B cell protocols that model T cell-dependent and T cell-independent activation. These assays will determine if naive B cell activation and metabolic reprogramming are targeted by PFAS exposure.

**3767 Effect of PFAS on Antibody Production**

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Perfluorinated substances (PFAS) are a class of synthetic chemicals widely used in industry for manufacturing common household materials, which people and environment are exposed to. They are involved in lipid metabolism. Our findings provide new insights into the molecular mechanisms linking PFAS exposure with alterations in lipid metabolism, notably singular PFAS exposure as opposed to mixtures, and few studies have explored the 10 representative hallmark gene sets are shown and many of these pathways are involved in lipid metabolism. In the present study, male mice were given orally HFPO-DA for 90 study found that HFPO-DA exposure can cause hepatotoxicity and affect bile acid metabolism. In the present study, male mice were given orally HFPO-DA for 90 d. For the elucidation of the molecular mechanism induced by HFPO-DA in the liver, RNA sequencing was performed. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) analyses were performed to confirm the molecular function of DEGs in mouse liver exposed to HFPO-DA. In addition, KEGG pathway analysis showed 15 representative pathways. The results of GO and KEGG analysis indicate that HFPO-DA exposure is involved in hepatic metabolism, especially lipid metabolism. According to the GSEA analysis, the 10 representative hallmark gene sets are shown and many of these pathways are involved in lipid metabolism. Our findings provide new insights into the molecular mechanisms in the liver after HFPO-DA exposure.

**3770 Exposure of Hyperlipidemic Mice to a Mixture of Per- and Polyfluoroalkyl Substances (PFAS) Modulates Metabolism of Cholesterol and Bile Acids**

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Per- and polyfluoralkyl substances (PFAS) are a class of synthetic chemicals used in numerous industrial and consumer products for their surfactant properties. PFAS have been detected ubiquitously throughout the environment as well as circulating in humans across the globe. Epidemiological studies have found PFAS exposure to be associated with numerous chronic diseases, including dyslipidemia and various cardiometabolic disorders. However, most studies focus on singular PFAS exposure as opposed to mixtures, and few studies have explored the mechanistic basis for effects due to PFAS exposure. Our studies investigate mechanisms linking PFAS exposure with alterations in lipid metabolism, notably increased circulating cholesterol, and the development of cardiovascular diseases, such as atherosclerosis. Male and female LDLr KO mice were fed an atherogenic diet.
diet and exposed to drinking water containing a mixture of 5 PFASs representing legacy, alternative, and emerging subtypes (i.e., PFOA, PFOS, PFNA, PFHxS, and GenX), each at a concentration of 2μg/L for 7 weeks. Cholesterol was increased after 7 weeks by 1.18-fold in females (p<0.036) and 1.24-fold in males (p<0.022), compared to vehicle water. LC-MS analysis of plasma revealed that total bile acid levels were increased by 2.3-fold in male mice (p<0.001) and 2.9-fold in female mice (p<0.001) exposed to PFAS. However, the levels of total bile acids excruted in the feces were reduced by 0.38-fold in female mice (p<0.001) and by 0.41-fold in male mice (p<0.001) exposed to PFAS, suggesting lowered excretion as a mechanism for increased circulating cholesterol. Hepatic and ileal protein levels of bile acid transporters were also measured. In males, the ileal bile acid uptake transporter ASBT was increased by 22.4-fold in the PFAS-exposed mice compared to vehicle (p<0.001), which could be a mechanism for decreased bile acid excretion due to increased reuptake. Finally, since the gut microbiota are critical mediators of sterol and bile acid formation, we also examined changes to the gut microbiota and observed altered diversity and genera associated with the production of secondary bile acids (e.g., clostridium) in male PFAS-exposed mice. Overall, exposure of higher-perfluorinated alkyl substances (PFAS) to maternal diet resulted in increased bile acid levels along with alterations in bile acid transport through the enterohepatic circula-
tion, bile acid excretion, and the bile acid gut microbiota axis.

**3771 Effects of Perfluorooctane Sulfonate (PFOS) on Gene Expression of DJR-1.2 in Caenorhabditis elegans**

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Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been in production worldwide since the 1950s. They can be found in personal hygiene products such as shampoo, dental floss, and make up as well as water-reistant fabrics, non-stick cookware, stain resistant products, and firefighting foams. PFAS can have a half-life of up to nine years which causes them to accumulate in wildlife and also be found in water, soil, and air as they are released into the ecosys-
tem. One of the most common PFAS contaminates in the environment is perfluorocasuanic acid (PFOS). Human exposure typically occurs through contamin-
ated drinking water sources including both private wells and public municipalities. Previous studies conducted on PFOS exposure in animal models concluded that it causes dopamine dependent functional deficits. Dopamine controls many motor and cognitive processes and has an impact on both physical and mental perfor-
ence. Parkinson’s disease (PD) is a progressive neurodegenerative disorder that is caused by loss of dopamine neurons affecting more than 1 million in the United States and more than one million worldwide. Since its etiology is largely unknown PD has been proposed to be linked to a combination of environmental and genetic factors. The main objective of this study is to determine whether exposure to PFOS could alter gene expression of DJ-1 which is causally linked to Parkinson’s disease via its overexpression. DJ-1 is a gene that produces a protein deglycase that works by activating pathways. DJ-1 is a well-known gene of interest DJ-1 has an ortholog of DJR-1.2 in Caenorhabditis elegans. In this study, we utilized C. elegans to examine the effects of PFOS exposure on DJR-1.2 expression. Solutions of PFOS in less than 1% methanol were made fresh for each experiment. The worms were treated at larval stage 1 with a 0 μM, 50 μM, 100 μM, and 150 μM PFOS and allowed to develop until early adulthood. Total RNA isolation was done using a Purelink™ Mini Kit. Wormbase, primer BLAST, and reverse primers (GGACCTGGAACCTTCTCCGA). Two-step real time PCR, consisted of using BIO RAD iScript™ Reverse Transcription Super mix to convert the isolated RNA to cDNA and SsoAdvanced™ Universal SYBR® Green Super mix to quantify the gene expres-
sion. Current results indicate no significant difference in expression of DJR-1.2 gene with chronic exposure to PFOS. Future studies are examining the effects of PFOS exposure on other genes linked to PD including LRRK-2 (LRRK1, C. elegans homologue) and measuring gene expression at different time points.

**3772 PFAS Associated with Alterations in Maternal Lipid Profile during Early and Late Pregnancy**

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Per- and polyfluoroalkyl substances (PFAS) have been detected in the blood of humans and wildlife worldwide. A class of long-chain fluorinated surfactants. PFAS are used in a variety of industries for their water, oil, heat, and stain-resis-
tant properties. Epidemiological data suggests an association with exposure to some PFAS and multiple adverse pregnancy outcomes, including preeclampsia, miscarriage, preterm birth, and low birth weight; however, the mechanisms explain-
ing these associations are not well understood. Existing literature has identified a strong association with PFAS exposure and metabolic dysfunction in humans, including alterations in lipid metabolism. Therefore, it is hypothesized that changes in lipid metabolism resulting from elevated circulating PFAS modify signaling pathways that may contribute to adverse pregnancy outcomes. In a multiple linear regression model using ninety-one dyads from the Michigan Mother Infant-Pairs (MMIP) cohort, our study sought to investigate the associations between circulating maternal levels of first trimester PFAS and repeat measures of measures of lipids (as identified in an untargeted shotgun lipidomics analysis) in the first trimester (M1), at the time of delivery (M3) and in the cord blood (CB) collected at delivery. Out of 9 PFAS measured, 7 were detected in at least 20% of M1 plasma samples. PFOS and PFHxS had mean concentrations that were 5.5 ng/ml and 3.31 ng/ml, respectively. PFOA, PFNA, and PFHxS had lower measured values with geometric means of <1.2 ng/mL. PFHxS concentrations were positively associated with monosaturated sphenoglycennines (SMs) in M1 maternal plasma in adjusted models (effect estimate of 0.960, 99% CI (0.33, 1.586), q = 0.031. A similar positive correlation was noted between PFHxS and saturated and polyun-
saturated SMs. In M3, the association between PFHxS and SMs remained, but was attenuated. Saturated diacylglycerols were significantly decreased with increas-
ing PFHxS in M1 (effect estimate of -0.858, 99% CI (-1.488, -0.227), q = 0.072). Discernable from the same measurement in early pregnancy, increased PFDA was associated with a significant increase in polyunsaturated sphingomyelin-monohexadecanoic acid (SM) (99% CI (0.270, 1.292), q = 0.003). Continued research into PFAS associated disruptions in lipid pathways at sensitive stages of gestation may provide insight into the mechanisms that lead to adverse pregnancy outcomes.

**3773 Investigating the Relationship between Omega-3 Polyunsaturated Fatty Acids and Serum Per- and Polyfluorinated Substances (PFAS) Levels in Adults**

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Exposure to PFAS have been associated with adverse human health outcomes. Previous epidemiological studies suggest that diet may play a role in mitigating PFAS exposure. This hypothesis is investigated in the omega-3 polyunsaturated fatty acids, specifically eicosapentanoic acid (EPA) and docosa-
hexaenoic acid (DHA), as potential dietary factors that impact serum PFAS in adults. The data that this analysis used was from the National Health and Nutrition Examination Survey (NHANES) in 2013-2018. Participants were selected based on measurement of serum PFAS and EPA and DHA omega-3 polyunsaturated fatty acids levels. Participants with missing data and those under 20 or over 70 years of age were excluded, and 2814 participants were included in the analysis. NHANES has collected data for 10 different PFAS chemicals and these values were summed in order to obtain total PFAS in serum in the analysis. The mean concentration of EPA in serum was 0.0258gm (0.0246, 0.0269), DHA in serum was 0.0559gm (0.0537, 0.0580) and PFAS in serum was 8.710mg/L (8.469, 8.952). The linear regression model accounted for several variables including age, sex, education level, and family income to poverty ratio was conducted using SAS software (SAS Studio version 3.6, SAS Institute, Cary, NC). These variables were selected based on their impact on diet, and/or serum PFAS levels. Our analysis found that the levels of omega-3 polyunsaturated fatty acids is positively associated with PFAS levels. Furthermore, we stratified our analysis by various demographic variables such as age, sex, Hispanic/non-Hispanic origin, race, education level, annual household income, and family income to poverty ratio and found significant associ-
ations among these demographic stratifications. This analysis suggests that omega-3 polyunsaturated fatty acids may be confounded by various demographic data and these factors must be considered when investigating the Omega3 and PFAS relationship. Disclaimer: The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or the policies of the US EPA.
immune response allows for proper viral clearance in the central nervous system tissue. We hypothesized that immune responses to TMEV infection can be altered by coadministration exposure to potential immunotoxicants. To test this hypothesis, we used TMEV and PFOA to determine if co-exposure could synergistically render the immune system of B6 mice susceptible to viral-induced disease. We divided B6 neonatal mice into three different treatment groups: non-injected, sham-injected with PBS (control), and TMEV-infected. Each of these groups was further divided into cohorts with ad libitum exposure to 70 ppt PFOA, or filtered water (control), via their drinking water. We evaluated the effects of PFOA on the immune response by comparing the degree of neurologic symptoms and changes in immune-related cytokine and chemokine levels following viral infection. We compared the different neurotoxicological responses and identified an increase in seizure frequency and the types of seizures in the TMEV + PFOA group. To better understand these effects, we will compare immune responses by measuring levels of 23 cytokines and chemokines at the end of the acute phase of infection (postnatal day 42) to determine if the PFOA-exposed groups exhibited a suppressed immune response. To further support our findings, we will also measure TMEV RNA in the hippocampus and dorsal spinal cord to determine whether coinfection of adult or neonatal mice with TMEV and PFOA results in greater persistence in those infected with TMEV. We expect a decrease in viral clearance in the TMEV + PFOA group due to the low production of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α. Overall, these findings will provide insight into the complex roles of immune responses in the pathogenesis of virus-associated neurological diseases influenced by co-exposures to viruses and immunotoxic compounds. Ultimately, we aim to improve model fidelity for research in preventive and individualized treatments associated with human neurological disease.

**3775 Immunotoxicity of Understudied PFAS Found in North Carolina**

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Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic compounds that have myriad uses in consumer and industrial processes and products, notably as processing aids in the production of fluoropolymers. Two members of this class of compounds have been classified as presumed to be immune hazards to humans by the U.S. National Toxicology Program, which raises concerns about the ability of other PFAS to induce immunotoxicity. Many PFAS remain understudied toxicologically, but are detectable in both drinking water sources and finished drinking water across the U.S. Two that have been detected in NC surface waters are perfluorohexanoic acid (PFHxA; CAS# 307-24-4) and sodium tridecafluoro-2,4,6,8,10-pentaodecane-12-oate (FC23-PFOSDoAn; CAS# 2578331-55-5). These PFAS are detectable in surface waters in North Carolina and while there have been toxicological data generated for PFHxS, none address immunotoxicity specifically. No data appear to be available for FC23. Adult male and female C57BL/6 mice (6-8 weeks old) were exposed by gavage once/day for 30 days with PFOA or FC23 at 0, 0.5, 5, or 50 mg/kg. Endpoints collected included in-life observations, organ weight, immunophenotype of lymphoid organs, liver peroxisome proliferation, and the T-cell dependent antibody response (TDAR). At the PFHxA doses administered, no differences were detected in terminal body weights, liver, spleen, or thymus weights. However, the 5 and 50 mg/kg dose groups of FC23 produced systemic toxicity. The 50 mg/kg group was euthanized before the end of the study and dosing of the 5 mg/kg dose was terminated after ~15 days of exposure. Liver peroxisome proliferation increased from control responses by ~470%, on average, in all PFHxA dose groups. Some shifts in immune cell populations in spleen and thymus also were observed in response to PFHxA and FC23 exposure. The TDAR was suppressed by exposure of 0.5 (males, 13%; females, 20%) and 50 mg/kg (males, 9%; females, 17%) to PFHxA compared to controls. Relative liver weights were increased in female but not male mice. These findings suggest that perfluoroalkyl substances (PFAS) linked to chronic diseases such as cancer and diabetes, our findings suggest that further toxicological evaluation of the replacement PFAS with respect to liver and pancreatic toxicity are warranted.

**3776 Targeted Quantification of Perfluoralkyl Substance (PFAS) in Human Serum from Patients Evaluated for Nonalcoholic Fatty Liver Disease (NAFLD)**

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Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of lipids in the liver, lipotoxicity, and insulin resistance. Obesity, diabetes, genetics, and environmental exposure are considered risk factors for development of NAFLD. Perfluoroalkyl substance (PFAS) are synthetic environmental toxicants, and some are known to be highly persistent and bioaccumulative in vivo (i.e., detected in human liver). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been associated with elevated serum liver injury markers in humans, but few studies have addressed risk of NAFLD. Therefore, this study aimed to compare the serum concentrations of PFOA, PFOS, and emerging PFAS among NAFLD cases (N=23 for children, 17 for adults) and non-NAFLD controls (N=15 for children, 11 for adults). Blood samples were collected at the time of being evaluated for NAFLD between 2014 - 2020 and evaluated for NAFLD biomarkers at Children's Mercy Hospital or the University of Kansas Medical Center. Preliminary findings illustrated that in children, PFOA, PFOS, PFHxS, PFPeA, PHFHA, PFBS, and PFBA accumulated in serum, respectively. For PFOA, PFHxS, and PFOS, the odds ratios (OR) for detection at 0.05 were 0.05 (0.02, 1.27), 0.40 (0.03, 1.48), and 0.39 (0.006, 23.59), respectively, adjusted for age, sex, BMI, and race. The average log transformed PFOA concentrations in pediatric cases were 0.208 (95% CI: -1.08, 0.66) lower than controls. In the adults, PFHxS, PFOS, PFHxS, PFPeA, PFBS, and PFBA accumulated in serum, respectively. Differences in log transformed concentrations comparing adult cases to controls were: age and sex were: -0.4 (95% CI: -1.10, 0.30) for PFOA, 0.05 (95%CI: -0.89, 0.98) for PFHxS, and -0.97 (95% CI: -1.68, -0.25). The findings suggest that children with NAFLD had lower serum PFAS concentrations than children without NAFLD. However, a larger sample size is required to confirm these findings, with sample collection and analysis ongoing.

**3777 Comparative Analysis of Oxidative Stress Markers Induced by Alternative Perfluoroalkyl Substance Chemicals in the Mouse Liver and Pancreas**

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Per- and polyfluoroalkyl substances (PFAS) are used in a variety of industrial applications including production of surface repellent coatings, fire-fighting foams, and fluoropolymer synthesis. Due to the environmental persistence, bioaccumulation, and toxicity of the legacy PFAS, such as perfluorooctanoic acid (PFOA), alternative PFAS have been synthesized to be used as replacements. Previously, we demonstrated that PFOA accumulated and induced oxidative stress in the mouse pancreas and liver after a 7-day exposure. While the toxicity profiles of the legacy PFAS have been studied, less is known of the toxicities caused by exposure to the alternative products, for which environmental and biological contamination has been documented globally. In comparison to PFOA, we evaluated the effects of two replacement PFAS compounds on the induction of oxidative stress in the pancreas and liver in vivo: ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX) and perfluorobutane sulfonic acid (PFBS). Further, we wanted to explore if gender affected the accumulation and toxicity profiles of the replacement PFAS in the pancreas and liver, which have previously been identified as target organs for PFAS. Male and female C57BL/6 mice were exposed to control tap water or tap water containing 1 and 5 ppm PFOS, 5 and 10 ppm GenX, or 5 and 10 ppm PFBS for 7 days. Serum and tissue PFAS levels were determined which revealed sex differences in PFAS accumulation. Whereas PFOS levels in both the serum and pancreas of female mice were higher, PFBS reached higher concentrations in the serum and pancreas of male mice. Levels of GenX in the serum of male and female mice were similar; however, GenX accumulated in the pancreas of male mice to a greater extent. Relative liver weights were increased in both male and female mice exposed to PFOA and GenX, where no change in relative liver weight was observed following exposure to PFBS. Oxidative damage in the pancreas, as measured by malondialdehyde levels, was observed following exposure to all three PFAS. Male and female mice exposed to GenX accumulated to 1x and 2x induction, respectively, in the liver. Upligation of antioxidant genes, such as Sod1, Sod2 and Tnx1 in the liver was significantly induced in female but not male mice. These findings suggest that replacement PFAS accumulate and cause oxidative damage in both the liver and pancreas, which occurs in a gender-specific manner. As oxidative stress has been linked to chronic diseases such as cancer and diabetes, our findings suggest that further toxicological evaluation of the replacement PFAS with respect to liver and pancreatic toxicity are warranted.

**3778 Nr2f2-Dependent and Independent Effects of PFhxs on Embryonic Glutathione in Zebrafish (Danio rerio)**

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Developmental exposure to toxicants can alter the embryonic redox environment, resulting in disruptions to organogenesis and cellular function. The status of the redox environment can be observed by measuring glutathione (GSH), an endogenous antioxidant regulated in part by the Nr2f2 pathway. Here we examined tissue-specific changes in the GSH redox environment following embryonic exposure to perfluorohexanesulfonic acid (PFHxS), a toxicant in the perfluoralkyl substances (PFAS) family. Two strains of zebrafish were used, one with a normally functioning Nr2f2 pathway and one with a point mutation in the DNA binding domain of Nr2f2 (Nr2f2aF318I/F318I). Embryos were exposed daily to 0 µM, 16 µM, or 32 µM...
PFHxS starting at 3 hours post fertilization (hpf) through 96 hpf, when they were stained with monochlorobimane, a compound that conjugates to GSH and emits measurable fluorescence. To understand the reductive environment within specific tissues, fluorescence was measured in the exocrine pancreas, yolk sac, heart, lens, hindbrain, gut, myotome, and overall body. Results show that developmental exposure to PFHxS had a tissue-specific response that varied in magnitude, with increased fluorescence in the pancreas, gut, heart, lens, myotome, and yolk sac, but only in wild-type embryos, indicating that this response requires a functional Nrf2 pathway. However, an alternative pathway appeared to be activated in the heart and hindbrain at the 16 and 32 μM PFHxS doses (respectively) in the mutant fish that was reflected in elevated GSH content, independent of Nrf2. These findings indicate that PFHxS disrupts the embryonic redox status in a tissue-specific manner and may predict morphological or functional outcomes at later developmental stages. This work was funded by R01ES025748.

3781 Exposure to Multiple PFAS Shows Suppressed Levels of Immune Markers during Pregnancy

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants, particularly in industrial manufacturing sites and military airfields. Despite our appreciation that these “forever chemicals” persist in groundwater and are found in approximately 98% of the general population, our understanding of the specific health impacts of PFAS exposure is limited. Recent evidence suggests PFAS are associated with immune-related toxicities that accompany a failed resolution of inflammation (e.g., cancer), despite lacking a classic “toxic” profile. Moreover, PFAS exposure is linked to lipid accumulation in the liver. Because the liver is a central immunologic organ containing bioactive lipid mediators that can coordinate the initiation and resolution of inflammation, it is our working hypothesis that exposure to PFAS will perturb lipid metabolism specifically in hepatocytes, leading to derangements in bioactive lipid mediator production during immune challenges. To test this hypothesis, bioactive lipids were measured in serum from individuals in the Security/Widefield/Fountain/Colorado Springs, CO area (PFAS AWARE) that were identified to have low and high (>5-30 ng/mL) serum PFAS levels; perfluorooctanoate (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorohexanesulfonic acid (PFHxS) were the most abundant PFAS detected (n = 10/group). Pooled recombinant CYP4A11 (Supersomes) was determined using arachidonic acid as substrate in the presence or absence of increasing concentrations of PFOA, PFOS, or PFHxS. Making use of targeted metabolomics, we find that specific classes of bioactive lipids are negatively impacted by PFAS exposure, as shown by COX-derived prostaglandins PGl2, PG2E2, and lipoxigenase- and CYP-derived thromboxane A2 and 12- and 15-hydroxymethicosatranes (HETE). In primary human hepatocytes, PFOA, PFOS, or PFHxS had no effect on CYP expression or lipoxigenase (COX) or cyclo-oxygenase P450 epoxigenases (CYP450) and interleukin-8 (IL-8) was measured by RT-PCR and IL-8 and prostaglandin E2 (PGE2) release was measured by ELISA (Biolegend, Camayan). The activity of recombinant CYP4A11 (Supersomes) was determined using arachidonic acid as substrate. These findings indicate that hepatocyte lipid metabolism and bioactive lipid mediator production are negatively impacted by PFAS exposure, suggesting a role of PFAS exposure in the modulation of the immune system in pregnancy. Follow-up analyses will examine effects on perinatal and child health outcomes and evaluate data using multivariable regression modeling.

3782 Developmental PFAS Exposure in Zebrafish Disrupts Adult Brain, Liver, and Kidney Transcription

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Exposures to per- and polyfluoroalkyl substances (PFAS), linked to a host of multisystemic health outcomes across humans and aquatic species, are well-known for their highly ubiquitous and persistent presence in environmental compartments. Such pervasive exposure to PFAS chemicals can occur during sensitive periods of early development, where lifespan phenotype trajectories are most susceptible to external influences. To study the long-term consequences of low-level developmental PFAS exposure and mixtures, we utilized the NIH-validated, high-throughput zebrafish model. To study immune and neurobehavioral functions, increased risk of obesity, increased risk of certain cancers, disruption of endocrine hormones, altered immune functions, and reduced fetal growth... PFAS-exposed larvae had increased expression of immune markers consistent of TNF-a, IL-8, IL-9, IL-7, IL-6, IL-4, IL-2, IL-12, IL-7, IL-10, IL-8, IFN-g, CM-CSF, Fractalkine, CRP, TGF-beta, MIF, and Leptin and PIGF. Statistically analysis indicates that this response requires a functional Nrf2 pathway. However, an alternative pathway appeared to be activated in the heart and hindbrain at the 16 and 32 μM PFHxS doses (respectively) in the mutant fish that was reflected in elevated GSH content, independent of Nrf2. These findings indicate that PFHxS disrupts the embryonic redox status in a tissue-specific manner and may predict morphological or functional outcomes at later developmental stages. This work was funded by R01ES025748.

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Per and polyfluoralkyl substances (PFAS) are global contaminants that are classified as presumed immune hazards due to their adverse effects on adaptive immunity. However, comparatively little is known about how PFAS exposure affects the innate immune system. Microglia, the innate immune cells of the brain, are essential for responding to pathogens and injury, and also play critical roles in shaping brain development and homeostasis. Our prior work using zebrafish demonstrated developmental exposure to perfluorooctanoic sulfonate (PFOS) produces an activated microglial morphology and results in the upregulation of p2ry12, a G-coupled protein receptor involved in microglial activation and migration. PFOS-induced microglial activation resulted in a heightened microglial response to brain injury, which could be rescued by using optogenetics to drive microglia towards a homeostatic state. We found the PFOS exposure increased neural activity and that the neural signaling environment modulated microglial activation state. Optogenetic silencing of the neurons was sufficient to normalize the microglial morphology and response to injury in PFOS exposed larvae. To further examine the relationship between immunotoxic PFAS congeners and neuron-microglial communication, we exposed zebrafish larvae to perfluorooctanoic acid (PFOS), a 8-carbon PFAS with a carboxylic functional group. Exposure to PFOS did not alter microglial responses to injury or the neuronal signaling environment, consistent with the interpretation, we found perfluorohexane sulfonic acid (PFHxS), but not perfluorobutane sulfonic acid (PFBS) increased brain activity. Given that PFOS and PFHxS heightened neural activity, we next asked whether low level exposure to PFOS (2 µM) or a mixture of PFAS sensitized zebrafish larvae to the GABA receptor antagonist and convulsant, pentylenetetrazole (PTZ). Exposed larvae were incubated in subthreshold concentrations of PTZ that do not elicit seizures in control larvae. Behavioral assays revealed a differential response to PTZ in larvae exposed to the PFOS (2µM) or a PFAS mixture that contain equal proportions of PFOS, PFHxS, PFBS, GenX, and PFOA at a concentration of 1 µM/congener. We are currently performing real-time experiments in PFAS-exposed larvae both with and without subthreshold concentration of PTZ to better understand the impact of PFOS exposure on neural systems.
Per- and polyfluoroalkyl substances (PFAS) are environmentally significant chemicals suspected to have effects on growth, development, reproduction, and neurobehavior. "Forever chemicals" such as PFAS tend to persist in blood and serum, and may easily cross into the placenta, potentially causing exposure at critical periods of development. This phenomenon is examined generally in larval zebrafish (Danio rerio), an ideal NIH-validated model organism for human genomics and developmental toxicology because of their rapid development and high fecundity. Our goal is to determine the phenotypic and transcriptomic hereditary effects of PFAS exposure on the F3 generation of larval zebrafish. In previous studies, the F0 generation of larval zebrafish were directly exposed to 'ultra-low', 'low', and 'tow' levels of PFOA, PFOS, and a mixture of both (7, 50, and 700 ng/L PFOA; 24, 240, and 2400 ng/L PFOS; corresponding mixtures of half the individual chemical concentrations). These concentrations are based on both environmentally relevant levels and levels encompassing the EPA health advisory, and we chose this mixture based on ratios reported in epidemiological studies. A subset of these larvae was raised to adulthood and spawned through the F2 generation, producing F3 larvae. Larvae were not exposed to PFAS after the F0 generation to observe transgenerational effects in the F2 and F3 generations. F3 larvae were raised to 5 days post-fertilization (dpf) and evaluated for altered light-dark photomotor responses, morphological abnormalities, and gene expression. In a separate set of F3 generations, ancestral PFAS exposure deregulated F3 larval light/dark behavior patterns and developmental abnormality rates, with differential effects dependent on chemical and exposure level. These outcomes may be attributable to persistent changes in transcriptomic networks, including lipid pathway disruption. Our results can identify potential biomarkers of exposure in humans and inform legal limits for PFAS concentrations in water, consumer products, and other sources.

3787 Per- and Polyfluoroalkyl Substance (PFAS) Exposures Are Associated with Liver Steatosis and Fibrosis in Adult NHANES 2017–2018

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Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants previously associated with increased liver enzymes in human cohorts and NAFLD in animal models. A recent meta-analysis on PFAS and NAFLD (PMID:35475652) concluded that future PFAS human research “should evaluate the full spectrum of NAFLD through histopathology or imaging.” Therefore, we determined associations between serum biomarkers of PFAS exposures and vibration controlled transient elastography (VCTE) biomarkers of hepatic steatosis (controlled attenuation parameter, CAP) and fibrosis (liver stiffness measurement, LSM) in adult NHANES 2017-2018. Participants ≥18 years were included. The exclusion criteria were active viral hepatitis B or C; daily alcohol consumption >21g for men or >14g for women; or missing/unreliable VCTE data. VCTE was performed by Fibroscan®. Serum PFAS concentrations were log-transformed. Principal component (PC) analysis was performed for PFAS. Univariate and multivariate associations were determined for the predictive demographic (age, sex, race, BMI and diabetes) and exposure (PFAS PCs) variables with the VCTE (CAP, FAST and LSM) and ALT liver disease outcome variables using R. The final sample size was 1400 with mean age = 48.4±18.6 years and mean BMI = 29.3±7.2 kg/m². 50% of subjects were women; 34% were non-Hispanic White; and 21% were non-Hispanic Black. The mean CAP and LSM were 263.7 ± 62.5 db/m and 5.4 ± 2.7 kPa, respectively. PFAS separated into three PCs. PC1 contained n-PFOS, n-PFOA, PFHxS, PFNA and PFDeA. PC2 contained PFNA and F53-B. PC3 was not analyzed further due to GenX's low overall detection rate. Age, sex, BMI and diabetes were significantly associated with CAP and LSM in most models. In the univariate model, exposure biomarker PC1 was positively associated with CAP (p<0.001) and LSM (p=0.0095) but not ALT. PFAS PC2 was generally orthogonal to PC1. The observed associations between PFAS PC1 and CAP/LSM were not statistically significant in the multivariate models. Environmental pollutant PFAS exposures were associated with increased hepatic steatosis and fibrosis in adult NHANES 2017-2018. Stratified analyses are ongoing as PFAS effects may have been confounded by other variables.

3789 MicroRNA Changes in Normal Thyroid Cells Exposed to Per- and Polyfluoroalkyl Substances and Their Role in Thyroid Cancer

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Per-and polyfluoroalkyl substances (PFAS), are environmental contaminants of emerging concern due to their abundance in the environment and potential adverse health impacts. PFAS are used in waterproof clothing, makeup, carpets, upholstery, cookware, and fast-food containers. These organic pollutants have been found in the blood of 95% of Americans and have been linked to disruption in thyroid hormones and potentially thyroid cancer. Emerging research suggests that PFAS alters microRNA expression. One specific microRNA, mir-101-3p, is known to be involved in thyroid cancer. Studies of thyroid cancer patient tissues at Vanderbilt University Medical Center showed increased mir-101-3p with advanced or metastatic cancer. Immunofluorescence staining was also performed for actin and tubulin to assess cytoskeletal changes. RT-qPCR data revealed that there were slight changes in TTF1, TG, and NIS following PFAS treatment of normal thyroid cells. These findings will have far reaching implications on the consideration of limiting PFAS in our environment and to elucidate the role of the microRNAs in progression of thyroid cancer. Understanding the role of PFAS-mediated dysregulation on mir-101-3p is important for limiting the use of this chemical and its role in thyroid cancer progression.
Per- and polyfluoralkyl substances (PFAS) comprise a diverse class of chemicals used in industrial processes, consumer products, and fire-fighting foams which have become environmental pollutants of concern due to their persistence, ubiquity, and associations with adverse human health outcomes, including in pregnant persons and their offspring. Multiple PFAS are associated with adverse liver outcomes in adult humans and toxicological models, but effects on the developing liver are not fully described. Here we performed transcriptomic analyses in the mouse to investigate the molecular mechanisms of hepatic toxicity in the dam and its fetuses after exposure to two different PFAS, perfluorooctanoic acid (PFOA) and its replacement, hexafluoropropylene oxide-dimer acid (HFPO-DA, known as GenX). Pregnant CD-1 mice were exposed via oral gavage from embryonic day (E) 1.5-17.5 to PFOA (0, 1, or 5 mg/kg-d) or GenX (0, 2, or 10 mg/kg-d). Maternal and fetal liver RNA was isolated (N=5 per dose/group) and the transcriptome was analyzed by Affymetrix Array. Differentially expressed genes (DEG) and differentially enriched pathways (DEP) identified across all treatments from industrial and urban areas. Two compounds, PFOS and PFOA, have been phased out of use due to evidence of biomagnification and findings of adverse toxicity, and potential anti-nutritional effects of AFB1, a potent mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus. In order to determine the impact of contaminated lake water on human health, we revisited this year and the collected samples have been analyzed by this method. In the United States as well as other leading world nations have strict guidelines in place from organizations like the FDA and the Food and Agriculture Organization (FAO), which strictly regulate the allowable concentration of mycotoxins. In the United States, concentrations are dependent on the food type, with a limit as restrictive as 0.5 ppb in milk to 10 ppb in baby food and 20 ppb in adult foods. This study aims to measure the AFB1 and OTA adduct concentrations in 40 liver cancer patients recruited by the Department of Environmental Health Sciences, Columbia University Mailman School of Public Health by a HPLC-fluorescence (HPLC-FLD) technique modified from Qian et al (2013). The data show every sample was positive for both AFB1 and OTA adducts. The lowest mean calculated AFB1, serum adduct concentration was 5.388354 ng/mL serum and the highest was 14.603 ng/mL serum. The lowest mean calculated OTA serum adduct concentration was 7.235279 ng/mL serum. These results from United States liver cancer patients are higher for both median AFB1, and OTA serum concentrations compared to adduct levels in healthy persons from nations with similar food regulations.
reapplied by approximately 20-60% of regular sunscreen users, depending upon product category, and reaplication of all SPF product types was less than 35% on cloudy and partly cloudy days. Primary reasons for reaplication were water exposure, after being outside a certain number of hours, and being active/sweating. These reaplication prompts were most notable for beach and facial skin care SPF products. Children under 12 were covered more completely than adults with sunscreen lotion, including increased application onto the chest, back, face, hands, feet, and ears, based on parental responses in the survey. In children, 45% of the sample (parents) reported “redness” as a signal when to reapply sunscreen product. This study represents the most comprehensive assessment of consumer habits and practices among SPF product users in the US in the past 20 years. By determining more accurate exposure data, the reliability of the human safety assessment of SPF products can be improved.

The US EPA is responsible for evaluating thousands of chemicals for the potential risks to humans and ecosystems, which necessitates information on hazard and exposure potential for each chemical. To support chemical decision-making, EPA’s Office of Research and Development (ORD) must identify and characterize relevant exposure pathways - the path of a chemical from source to a receptor. How a chemical is used (e.g., in a consumer, occupational, or industrial context) is critical to determining exposure pathways. The ORD data management and curation application, Factomut, facilitates the rapid collection and distribution of high-quality chemical and exposure related data from public documents to inform chemical exposure and risk assessments. To date, Factomut has been used to collect and curate data from over 560,000 documents, representing 4 million individual chemical records and 35,000 unique chemical substances. A new publicly available data search and visualization tool, called Chemical Exposure Knowledgebase (ChemExpo), is being developed by ORD. ChemExpo will surface data managed and curated by the internal Factomut application. The initial release of ChemExpo will focus on specific chemical substances, chemical composition of consumer products, functional role of chemicals within products and processes, and presence of chemicals on reported specific or general use lists. This new application will display more detailed curated data than currently available on the EPA CompTox Chemicals Dashboard, including additional document metadata; product-level chemical ingredient information; and product category, function category, and chemical summaries. This application will allow for data exploration by chemical, function, and consumer product category to support exposure assessments and other risk-based chemical evaluations. This abstract does not necessarily reflect US EPA policy.

In both evidence and human studies has shown that embedded metal fragments from war-related injuries can oxidize in situ, leading to increased systemic metal concentrations, and thus, raised concern about potential target organ effects far from the site of injury. Consequently, the Department of Veterans Affairs has established an on-going medical surveillance program for Iraq and Afghanistan veterans, injured primarily by improvised explosive devices, who may have an embedded fragment. Enrolled veterans are asked to submit an exposure questionnaire and a urine sample to assess their metal body burden. Urinary concentrations of 1.4 metals, which have been found in surgically removed fragments or are known carcinogens, are determined using inductively coupled plasma mass spectrometry (ICP-MS). Creatinine-adjusted metal results are compared to established reference values to determine the presence of an elevation. When a metal elevation is observed, a repeat urine specimen is requested either immediately, within three-to-five months, or within two years, depending on the veteran’s clinical history and how the exposure compares to the reference value and known toxicity thresholds. If all metals are within normal limits, a repeat sample within five years is requested. To date, 124 surveillance-enrolled veterans have submitted at least one follow-up urine specimen. Among veterans who had normal metal results at baseline (n=58), most (66%) continued to have normal metal concentrations an average of five years later, suggesting that if metal ions are being released from a metal fragment, they are not being released in excess into the systemic circulation. However, among the 66 individuals who had a metal excursion initially, on average two years later, 25 (38%) had at least one metal that continued to be elevated, 11 (17%) had an elevation in a different metal, and 30 (45%) had no metal elevations in the second sample. Persistent elevations in zinc and tungsten concentrations were most common. In the absence of other identified metal exposures, evidence suggests the fragment may be the source of these sustained elevations. This idea is supported by the fact that two veterans had declines in urine metal elevations that corresponded with fragment excursions when fragments were surgically removed. Although interpretation of urine metal results is complex, as other non-fragment related exposure sources must be considered, on-going biomonitoring within this population, and comparison of urine findings to individual fragment composition data when available, is essential to better understand in situ fragment behavior, which can influence the risk of complex systems from a systems perspective. A conceptual scientific workflow has been developed which can be applied to various scenarios and extrapolations from data-rich to data-poor chemical/product combinations. To demonstrate this approach, three types of consumer product exposure scenarios will be presented: Type 1 - Data-rich compound only (fewer gaps for two major pathways - water and consumer products for 1,4-dioxane); Type 2 - Interpolation within a chemical class - A mix of data-rich and data-poor compounds (all pathways for polybrominated diphenyl ethers); and Type 3 - Interpolation of an emerging chemical class from chemicals both inside and outside of that chemical class - Data-rich to data-poor interpolation (all pathways for an entire chemical class from data-rich compounds for a specific product/chemical and life stage in the life cycle or product use category. This abstract does not reflect EPA policy.

The wear patterns for drum-style automotive brakes tend to enlarge internal drum diameters. Such enlargement is most profound when used brake drums are machine to restore the metal friction surfaces. Specialized arc grinding machinery has been used to match replacement shoe-style brake friction materials to enlarged drums. The process of arc grinding removes friction material, thereby producing dust. When organic-style friction materials contained asbestos, use of arc grinding machinery posed an asbestos fiber exposure risk to operators and proximate personnel. The manufacturers of arc grinding machinery have incorporated local exhaust ventilation systems designed to capture and remove this dust at the point of grinding contact and propel this dust into collection bags or other systems. This research was designed to evaluate the dust capture and retention characteristics of a specific arc grinder product, when used to custom grind brake friction materials. A Bear Model 1420 automotive brake shoe arc grinder was the subject of this study. During two separate but consecutive test sessions, newly relined sets of shoe-style automobile brake friction materials were precision ground. Both area and personal air samples were collected throughout each testing session. This work took place within a closed and unventilated metal building, with total interior volume of 2,500m³. Collected air samples were analyzed using phase contrast microscopy (PCM) and transmission electron microscopy (TEM). The results of analysis using PCM for personal samples (n = 6) ranged from <0.044 to 0.055 fibers per cc (f/cc) (mean ± 0.05). Follow-up analysis of these personal samples using TEM indicated asbestos fibers and POM exposures ranging from <0.0074 to 0.055 f/cc (mean ± 0.041). Air area samples, taken at distances ranging 1.5 to 9 meters from the arc grinder (n = 12), showed asbestos-adjusted PCM concentrations ranging from <0.0075 to 0.041 f/cc (mean ± 0.017). The process of custom arc grinding shoe-style, asbestos-containing brake friction materials can cause may release asbestos fibers. However, proper use of arc grinding machines, equipped arc grinding machines, such exposures are not expected to exceed the current occupational exposure limits for asbestos of 0.1 f/cc 8-hour time-weighted average (TWA).
Air pollution and fine particulate matter (PM$_{2.5}$) from wildfire smoke have steadily increased over the last decade despite a decrease in bioavailable emissions. The increase in wildfires in the western US has highlighted the need to understand better how wildfire-specific PM$_{2.5}$ affects human health. To support health risk assessment studies from wildfire smoke inhalation, differentiating between wildfire-specific PM$_{2.5}$ and non-wildfire-specific PM$_{2.5}$ is essential, as policies may need to be adapted based on the emission source. We used PM$_{2.5}$ data developed by four different PM$_{2.5}$ models for the western US to isolate wildfire-specific PM$_{2.5}$. We then used three approaches to isolate and extract wildfire-specific PM$_{2.5}$ from each model: (i) a temporal extraction using EPA monitoring station flags based on seasonal wildfire window, (ii) a spatial extraction using National Oceanic and Atmospheric Administration Hazard Mapping System smoke plume data, and (iii) a combined spatio-temporal extraction by using breakpoints defined in (i) and spatial plume locations defined in (ii). Comparing method extractions across the different PM$_{2.5}$ modeling frameworks with a newly developed wildfire-specific PM$_{2.5}$ as a benchmark showed vastly different results across models, but relatively little variation between method extraction. Our results suggest that determining the population-level burden of various health outcomes could be variable and dependent on the approach used to differentiate wildfire specific PM$_{2.5}$. The results of our study highlight the need for more in-depth studies showing the impacts of PM$_{2.5}$.
Dermal occupational exposure to chemicals is a toxicologically relevant route of exposure that is often not well characterized in the workplace. Agencies such as the U.S. Environmental Protection Agency (EPA) have become increasingly concerned with potential health risks associated with dermal exposure, particularly in workers, including through the Toxic Substances and Control Act (TSCA) risk evaluation process. Other occupational health-centric organizations are also developing tools and guidance to better characterize dermal occupational exposures. Despite increased interest in dermal exposure potential, there are few occupational exposure limits (OELs) for direct skin or indirect surface contact, and validated methods and standardized frameworks for evaluating dermal exposure have not been harmonized. In this study, an integrated qualitative and quantitative exposure modeling tools and empirical data collection was developed to identify occupational scenarios with the highest exposure potential. A framework was developed following a critical analysis of existing dermal exposure assessment tools and methods. Specifically, we evaluated available dermal exposure modeling tools in terms of applicability to common occupational scenarios, required input parameters, ease of use, data underlying the models, and whether the model has been validated against empirical data. The framework also includes assessment of empirical dermal sampling methods and characteristics, such as applicable route of exposure such as direct, surface contact, or indirect skin deposition, and chemical formation of specific chemicals to specific occupational scenarios. Implementation of a tiered exposure assessment scheme is proposed, which includes a checklist of key considerations (e.g., establishing baseline values, collection procedure, analytical recovery, comparison to OEL or dermal benchmark) for when empirical dermal sampling is performed. Further work is needed to evaluate current methods to deterministic modeling for dermal exposure. The dose-response assessment component of the framework addresses variability and options in current methods for deriving toxicological benchmarks and OELs for the dermal route. The framework approach was used for an example occupational scenario to verify the workability of the process flows and tools.

**3803 A Framework for Optimizing Dermal Exposure Assessment Methods in Occupational Risk Assessment**

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Probabilistic exposure assessments (PEAs) have been used to characterize dietary exposures for more than forty years. The use of PEAs has been driven by the need to characterize the individual variation in aggregate and cumulative exposures to chemicals in our diets and the uncertainty in these estimates. The design of PEAs and the input data they require are determined by the source of the substances being evaluated. The sources can be categorized into five categories. The first category is substances found in plants and animals and includes contaminants in crops and livestock from anthropogenic activities (e.g., PCBs in fish or atmospherically deposited cadmium in vegetables) and naturally occurring substances (e.g., nutrients, biologically active chemicals, and metals from geological sources). The second category is direct food additives which are either a single chemical or mixture of chemicals intentionally added to foods. Examples of direct food additives include coloring agents, flavors, fortifiers, processing agents, and preservatives. The third category is chemicals formed during storage, compounding, or processing. Examples include toxins created by fungi (e.g., aflatoxins and deoxynivalenol) during storage and chemicals formed during cooking (e.g., furfural and acrylamide). The fourth category is residues of pesticides applied to crops or stored commodities. The fifth category is chemicals from food contact materials. These include plastic additives and NIAS (Not intentionally Added Substances). An example of how the inputs needed for different PEAs of different sources can be seen in models of exposure to pesticide residues versus migrants from food contact materials. Pesticide PEAs require data on crops used to make food items and pesticide residues in the crops. Food contact material PEAs require data on the chemical properties of the food (e.g., lipophilicity), the size (volume to surface area) of food packaging, and presence of chemicals migrants in the packaging materials. This presentation reviews: 1) the use of PEAs to assess chemicals from the five source categories, 2) the risk management needs that have driven the use of PEAs, 3) modeling longitudinal (chronic) exposures using PEAs, and 4) how PEAs can be performed using tiered approaches. Finally, barriers for the use of PEAs are identified including the difficulty of communicating probabilistic information to decision makers and the public, lack of education on the concepts of probabilistic information, need to align decision making approaches based on bright lines (threshold guidance values) with the outputs of PEAs (probability distributions), absence of longitudinal information on consumption patterns, and absence of toxicity models that can take advantage of information on temporal variation in dose provided by PEAs. Suggestions for research and model designs to address these barriers are presented.

**3805 Use of Probabilistic Exposure Assessments in Cumulative and Aggregate Dietary Assessments**


Environmental justice (EJ) is the fair treatment of all people regardless of race, income, and religion with regards to their health and the environment. Negative environmentally linked health outcomes have been associated with socio-demographic status (SDS) factors in different regions of the United States. In this study, U.S. Census Bureau (USCB) SDS factors including race, income, education status, and rural-urban status were matched to US EPA National Aquatic Resource Source (NARS) National Lakes Assessment (NLA) site data for 2017. Pearson correlation matrix showed minima for EJ metrics. Pesticide PEAs require data on crops used to make food items and pesticide residues in the crops. Food contact material PEAs require data on the chemical properties of the food (e.g., lipophilicity), the size (volume to surface area) of food packaging, and presence of chemicals migrants in the packaging materials. This presentation reviews: 1) the use of PEAs to assess chemicals from the five source categories, 2) the risk management needs that have driven the use of PEAs, 3) modeling longitudinal (chronic) exposures using PEAs, and 4) how PEAs can be performed using tiered approaches. Finally, barriers for the use of PEAs are identified including the difficulty of communicating probabilistic information to decision makers and the public, lack of education on the concepts of probabilistic information, need to align decision making approaches based on bright lines (threshold guidance values) with the outputs of PEAs (probability distributions), absence of longitudinal information on consumption patterns, and absence of toxicity models that can take advantage of information on temporal variation in dose provided by PEAs. Suggestions for research and model designs to address these barriers are presented.

**3806 Exploration of Environmental Injustice in Exposure to Environmental Toxins in US Water Systems Using the National Aquatics Resource Surveys**

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Environmental justice (EJ) is the fair treatment of all people regardless of race, income, and religion with regards to their health and the environment. Negative environmentally linked health outcomes have been associated with socio-demographic status (SDS) factors in different regions of the United States. In this study, U.S. Census Bureau (USCB) SDS factors including race, income, education status, and rural-urban status were matched to US EPA National Aquatic Resource Source (NARS) National Lakes Assessment (NLA) site data for 2017. Pearson correlation matrix showed minima for EJ metrics. Pesticide PEAs require data on crops used to make food items and pesticide residues in the crops. Food contact material PEAs require data on the chemical properties of the food (e.g., lipophilicity), the size (volume to surface area) of food packaging, and presence of chemicals migrants in the packaging materials. This presentation reviews: 1) the use of PEAs to assess chemicals from the five source categories, 2) the risk management needs that have driven the use of PEAs, 3) modeling longitudinal (chronic) exposures using PEAs, and 4) how PEAs can be performed using tiered approaches. Finally, barriers for the use of PEAs are identified including the difficulty of communicating probabilistic information to decision makers and the public, lack of education on the concepts of probabilistic information, need to align decision making approaches based on bright lines (threshold guidance values) with the outputs of PEAs (probability distributions), absence of longitudinal information on consumption patterns, and absence of toxicity models that can take advantage of information on temporal variation in dose provided by PEAs. Suggestions for research and model designs to address these barriers are presented.
The dosimetric relationship between the human intake of a chemical contaminant (an “externally-sensed concentration in blood or urine” indicative of an “internal dose”), often characterized by a dose-to-concentration ratio, has critical applications in exposure science, toxicology, and risk assessment, especially in the “new approach methods” era. However, there is a lack of a mechanistic, systematic understanding of how such a dosimetric relationship depends on fundamental chemical properties, such as partitioning and bio-transformation. Here, we investigate this issue with a well-evaluated toxicokinetic model, which links external and internal doses through quantifying absorption and elimination of chemicals. These investigations are visualized in a series of chemical partitioning space plots, whereby a chemical’s dose-to-concentration ratio can be approximately predicted based on the coordinates representing its partitioning between air, water, and octanol phases. Our results indicate that with the intake dose being equal, chemicals with low volatility and moderate to high hydrophilicity are most abundant in blood, and bio-transformable chemicals are less abundant compared to their persistent counterparts with the same partitioning properties. Chemicals with high hydrophilicity are most abundant in urine. Such revealed property dependence is similar for both adults and children, and for individuals with normal body weights and with obesity. Overall, insights gained from this study are important in helping to predict blood and urine concentrations with available information on exposure or toxicological benchmark doses, and to back-calculate the rate of exposure that sustains the blood or urinary concentrations observed in biomonitoring campaigns.

3808 Environmental Chamber Studies of Eye and Respiratory Irritation from Use of a Peracetic Acid–Based Hospital Surface Disinfectant

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Peracetic acid (PAA)-based surface disinfectant was recently shown to reduce hospital-acquired Clostridioides difficile infections by 50% in a study across 8 hospitals implementing a rigorous hospital-wide hygiene program (Carling et al., 2022). Personal exposures and measures of objective and subjective eye and respiratory tract irritation were assessed in a controlled environmental chamber study of 44 volunteers simulating hospital surface disinfectant use. Component effects were assessed in parallel trials of acetic acid (AA), hydrogen peroxide (HP) or deionized water. The double-blind, counter-balanced study design assessed breathing zone PAA, AA, and HP for 8 female multi-day volunteers (5 consecutive days) and 36 single-day volunteers (32 females, 4 males) as approved by Institutional review board. Each day included 3 trials each of disinfectant mixture and AA only, and 1 trial each of HP only and water control. Four wetted microfiber cloths were used for consecutive cleaning in each trial where volunteers inside the chamber continuously wiped high touch surfaces found in hospital patient rooms for 20 min. Objective irritation measures included 2 endpoints for eye, 8 for nasal, and 4 for lungs in all volunteers; multi-day volunteers were also assessed for allergic sensitization (increased immunoglobulin E (IgE)) after exposure day 5. Mean ± SD personal exposures for the disinfectant trials were 66 ± 23 ppb PAA, 287 ± 121 ppb HP, and 387 ± 148 ppb AA. None of the volunteers exhibited sneezing, coughing, nasal congestion, runny nose, or significant increases in objective measures of eye and respiratory tract irritation. None of the 8 multi-day volunteers had increased IgE. Subjective irritation scores were significantly greater for the disinfectant mixture with PAA relative to the component trials (AA, HP, or water), and were significantly greater for AA only compared to HP only or water trials. Subjective irritation symptoms were more elevated for females as compared to males and for eye and throat. Males were 2.5-fold more likely than females to report subjective odor intensity and nasal irritation at moderate or higher levels for the disinfectant trials. Four clusters were identified based on odor and nose scores: Group 1 (average score 3.3, weak sensation, n = 16), Group 2 (average score 10.8, moderate sensation, n = 10), Group 3 (average score 22.0, strong sensation, n = 6), and Group 4 (average score 30.4, strong sensation, n = 4). The four male volunteers were clustered in Group 1. Personal exposures to PAA, HP, AA, PAA + AA, or PAA + HP + AA were similar across Groups 1, 2, and 3 for each trial type, indicating that similar exposures can generate disparate subjective irritation ratings. Interestingly, Group 4 had significantly lower personal exposures in all trial types which may be explained by avoidance behaviors that reduced cleaning activities among individuals assigning the highest subjective irritation scores. We conclude that in this study of upper bound personal exposures during simulated hospital use of PAA-based disinfectant 23% of volunteers considered their personal exposures to be strongly irritating but these subjective irritation ratings did not correspond to higher actual disinfectant 23% of volunteers considered their personal exposures to be strongly

3809 Analysis of Urinary Arsenic Concentrations and Associations with Children’s Height and Weight in the US Population

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Low levels of environmental arsenic exposure as it relates to potential human health impacts has received renewed focus in recent years. Specifically, there is a growing body of information that suggests early childhood exposure to arsenic may lead to delayed development early in life. Food and water ingestion is suggested to be the main pathway for young children’s purported arsenic exposure and arsenic in baby formula and rice-based products has been a major focus of recent scrutiny. The objective of this study is to assess the relationship between the detection of urinary arsenic biomarkers and anthropometric measurements in children. Data was obtained from the 2017-2018 National Health and Nutrition Examination Survey (NHANES) dataset (n=9,254). A data subset containing organic and inorganic urinary arsenic concentrations in micrograms per liter (n=1,017) was assessed and screened to identify biomarker data in children under 18 years of age. The selected arsenic data subset was matched with the body measurement dataset to compile data for children aged 3 (n=116), 4 (n=147), and 5 (n=153) suitable for analysis. Both inorganic and organic urinary arsenic concentrations were assessed to determine the frequency of detection for the selected age ranges. Of the arsenic metabolites screened in this study, only the organic metabolite dimethylarsinic acid was detected in more than 50% of the included children’s urine samples. Given the natural dose of dimethylarsinic acid concentrations for 3-year-old children was [latex]GM_{median} = 4.6 \mu g/L \pm 1.8\mu g/L] and 5-year-old children was [latex]GM_{median} = 5.0 \mu g/L \pm 1.8\mu g/L] participants with detected dimethylarsinic acid. Height (centimeters) and weight (kilograms) data included in the NHANES dataset were statistically evaluated (t-test) to determine if there was a significant difference between those children aged 3, 4 and 5 with detectable levels of dimethylarsinic acid concentrations compared with those children without urinary dimethylarsinic acid biomarker detected. Analysis revealed that there was a statistically significant mean height difference between participants that received 4-year-old participants with no detectable urinary dimethylarsinic acid [latex]GM_{median} = 106 \mu g/L], and 5-year-old participants with [latex]GM_{median} = 104 \mu g/L], p = 0.043. No other significant differences were observed for height or weights of children evaluated for dimethylarsinic acid. This study suggests that arsenic exposure evaluated by the urinary detection of dimethylarsinic acid in children aged 3, 4, and 5 did not negatively impact child height and weight.

3811 The Influences of Household Behavior, Environmental, and Demographic Factors on Indoor and Outdoor Air Quality

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Americans spend approximately 90% of their time indoors, with more than 66% of that time spent in residential buildings. On average indoor air pollutants can be anywhere from two to five times higher than outdoors. Shifts in building construction, composition of building materials, and increases in use of consumer products containing volatile organic compounds (VOCs) and semivolatile organic compounds (SVOCs). There are a number of factors pertaining to household behavior and environmental and demographic factors that may influence concentrations or types of SVOCs found indoors. In this study, paired indoor and outdoor sampling for twenty-four locations across the United States took place using a community engaged approach. Samples were analyzed for over 1500 SVOCs to identify common patterns in exposure profiles representative of indoor and outdoor air quality. Of the 1500 SVOCs analyzed a total of 81 were detected in at least one sample (indoor or outdoor). This list was filtered to conduct paired comparisons between number of detects resulting in a total of 51 chemicals. Further filtering for paired comparisons of concentration differences resulted in a total of 22 SVOCs. Unique differences between indoor and outdoor profiles were identified, with indoor having 28 compounds with significantly higher detections and 10 with significantly higher concentrations than outdoor (p < 0.05). Influences of household behavior and environmental and demographic factors on these compounds were then investigated. A significant relationship between household use of air fresheners or candles/incense and specific fragrance chemicals (amyl cinnamal, eugenol, benzyl benzoate, galaxolide, linal, and l-citronello) was discovered. Additionally, associations between carpet use and detection frequency or concentrations of fragrance chemicals, flame retardants, or building material-related chemicals was also found. These associations were chemical dependent with some showing a positive association and others having a negative association with carpet use. Environmental and demographic factors also had an influence on indoor and outdoor profiles with the majority of chemicals identified being associated with emission from combustion sources (i.e., polycyclic aromatic hydrocarbons). Results from this study provide valuable information regarding indoor and outdoor chemical profiles and factors that impact the composition and concentrations of these chemicals. These findings may also help inform ways to reduce chemical exposure, by identifying certain consumer behaviors that may affect exposure profiles.
Amine Catalysts in Spray Polyurethane Foams: Identification, Characterization, Exposures, and Implications for Toxicology and Occupational Medicine Research


Spray polyurethane foam (SPF) is a highly efficient building insulation material. Amine catalysts in SPF accelerate the polymerization reaction between the polymeric methylene diphenyl disocyanate and polyols. Historically, the main concern has been the isocyanate, due to its high respiratory and skin-sensitizing potential. However, respiratory disease and non-specific complaints by workers and residents post-SPF application continue despite measurements confirming the lack of isocyanates in the air. Amine catalysts in SPF have, in such circumstances, been considered as potential suspects. Due to the proprietary nature of SPF formulations, little is known about the exact amine catalysts in use, their concentrations, variability in formulation across multiple products, workplace exposures, or associated health risks. The main objective of this work was to systematically document amine catalysts used in SPF applications; to develop the necessary analytical methods for their quantitation in raw materials and SPF; and to quantify inhalation and dermal exposures among SPF applicators at various worksites. Following quantitation results, a detailed literature review of the toxicological profiles of identified amine catalysts in SPF was conducted to inform unmet research needs for biomonitoring, health effects and disease prevention. We pursued 10 amine catalysts. Personal breathing zone (n=32) and glove samples (n=34) were collected among 35 sprayers and helpers at 13 SPF insulation sites. Field samples and raw materials were analyzed in our laboratory using LC/MS/MS and GC/MS. For this purpose of this study, amine catalysts investigated were: diazabicyclooctane (DABCO: CAS# 280-57-7), triethylentetramine (TETA, CAS# 112-24-3), triethanolamine (TEA #102-71-6), bis(dimethylamino)ethyl ether (BDAE, CAS# 3033-62-3), N,N-(di(dimethylamino)ethyl)methanol-ethanol (DMEA, CAS# 2212-32-0), pentamethyldiethylenetriamine (PMDT, CAS# 3030-47-5), 3-dimethylaminopropane (DTPA, CAS# 3047-92-7), 2,2,6,6-tetramethylpiperidine-1-carboxylic acid (TMAP, CAS# 117-33-2), 3,3′-iminobis(N,N-dimethylpropylamine) (IBDP, CAS# 6711-48-4). In air samples, the most frequently found amines were DABCO (100% of samples); TETA (94%); TEA (85%) and DMEA (85%). The remaining 6 amines were detected in 20-60% of air samples. The highest air concentrations correspond to DABCO (GM 278.2; GSD 9.4, Max 26,200 ng/m3) followed by TETA (259.2, 6.9, 494 ng/m3), DBEA (150.4, 31.9, 167 x103 ng/m3) and TEA (17.8, 7.0, 278 ng/m3). In glove samples, most amines were detected in >40% of samples, except for TEA (94% detects, median 0.16 µg/g). The highest averages on gloves were measured for BDAE (23.3 mg/kg) and DMEA (3.1 mg/kg). The toxicological profiles for these amines are only partially documented. Irritation of the eyes, skin and respiratory tract, and skin burns are a common feature. Serious eye damage can result from DABCO, TETA, DMEA, DMEA and possibly others. TEA may cause kidney and liver damage, as well as dermatitis. TETA is a skin sensitizer. Airborne exposures to these catalysts were low. For TETA, BDAE, DMEA and IBDP, airborne concentrations were 10-100 lower than their respective TLVs. Skin exposures are important in this cohort for contact dermatitis and internal uptake. For the majority of these amines, there are no established exposure biomarkers and no biomonitoring data for exposures and health effects. We further outline specific priority areas for future research to fill in relevant data gaps.

Development of a Field Analysis Method for Metallic Aerosols in Welding Fumes


Workers are exposed to metallic aerosols produced during manufacturing processes such as welding. Welding fumes inhaled by welders generally contain large amounts of iron (Fe) and manganese (Mn) which can negatively impact worker health. Identifying the size, composition and quantity of metallic aerosols in welding fumes is essential for assessing the risks of workplace exposures. The industry gold standard for characterizing metallic aerosols involves collecting samples using a respirable sampler and sending them to third-party labs for analysis. However, this method is expensive and time-consuming. To overcome these limitations, we developed an alternative method combining a cascade impactor and X-ray fluorescence (FP-XRF) techniques. Specifically, a cascade impactor and X-ray fluorescence (FP-XRF) were combined. To test this method, area samples were collected in a local truck-trailer manufacturing facility. The metal contents in the collected aerosols were analyzed using both FP-XRF and ICP-optical emission spectroscopy (OES) and the correction factors were calculated. After applying the correction factors to the results from FP-XRF, the concentrations of metals were calculated by dividing the mass collected by the mass sampled. The combined mass concentration results for each stage from the six sampling locations totaled 268 µg/m3 which was 7.2 times higher than the manganese concentration of 38 µg/m3. Two modes were found in the size distribution for Fe. One was located at a particle size smaller than 0.25 µm (235 µg/m3) and the other was located at a particle size larger than 2.5 µm (11 µg/m3). The singular mode of Mn size distribution was observed at a particle size smaller than 0.25 µm (54 µg/m3). The results of the feasibility study allow industrial hygienists to provide a more detailed understanding of metallic aerosol exposure characteristics and be also applied to further research on exposures to metallic aerosols in various occupational settings. This work is supported by the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH T42 OH008455), National Institutes of Health (NIH RO1 ES032478), NIOSH training grant (CDC/NIOSH T03OH008615), and the International Manganese Institute research grant.

Biomonitoring of Exposure to Metals in Apprentice Welders after Shielded Metal Arc Welding (SMAW)

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Welding fumes have been associated with multiple lung and neurological diseases and are classified as human carcinogens by the International Agency for Research on Cancer. Most welding fume exposure studies do not consider the actual doses of metals and metalloids absorbed after inhalation and only measure a few chemical elements. The objective of this research was to establish the nature and level of exposure to welding fumes and their metallic components in apprentice welders, by performing concomitant measurements in different biological matrices. A total of 86 apprentice welders enrolled in welding training programs were recruited in three different schools in the Montreal area, Canada. Urine, hair, fingerprint and toenail samples were collected at the beginning of the welding program (controls) and at the end of the 135-hour practical training in shielded metal arc welding (SMAW). Twenty-one elements, including aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn), were measured in these matrices by ICP-MS. Metal concentrations in control urine samples were comparable to the general population values reported in the Canadian Health Measures Survey. Results showed that concentrations of Mn in urine and hair were higher in samples taken at the end of the SMAW module compared to the beginning of training, while there was no significant difference for the other elements. Significant correlations (p<0.01) were observed for most of the elements analyzed, especially between Fe and Mn in toenails, fingerprints, and hair, whereas urine exhibited weak correlations between elements. Significant correlations (p<0.01) were found between fingerprint and toenail levels particularly for Zn (r=0.86), Cu (r=0.78), Mn (r=0.52), Fe (r=0.5), Ni (r=0.45), Pb (r=0.48), Cd (r=0.47), Al (r=0.45), and Cr (r=0.35). The study showed the interest of hair, fingerprint and toenail measurements to assess exposure to metals and metalloids. The results also point to higher Mn exposure in apprentice welders related to the SMAW process.
We categorized 64,413 chemical substances by broad pathways of exposure: consumer, industrial, pesticide, food, pharmaceuticals, and other pathways. These pathway categorizations can be used as a training set for automated read-across of chemical exposure pathways to better inform rapid exposure modeling as part of risk-based chemical prioritization. Pathway categorizations integrated multiple heterogeneous data streams that reported chemical use and/or occurrence. Data streams included public chemical databases as hierarchical binary descriptor sets. These descriptors can be queried for enrichment using lists of chemicals identified in NTA studies to provide chemical categories for pathways of exposure and 3 aggregated pathways. Each chemical was categorized for each pathway as positive, negative, or unknown (no data), depending on the use/occurrence data available. We explored patterns in pathway categories and in the data streams underpinning the pathway categories. These analyses revealed common signatures or “fingerprints” for chemicals along exposure pathway categories and data streams. In addition, we explored correlations between pathway categories and chemical class, using the ClassyFire tool for structure-based chemical classification according to the ChemOnt ontology, and a newly-developed software package to visualize the ChemOnt ontology as a “tree of life”. These results can be used to characterize evidence availability by pathway and by chemical class. One case study to be presented concerns more than 25,000 chemicals categorized with respect to the food pathway. These chemicals were both positive and known negative examples of the food use pathway. The majority of these chemicals also had data informing other pathway classifications (primarily consumer, industrial, and pesticide). However, more than six thousand chemicals had data only for the food pathway, with the remaining pathways. These “food data only” chemicals were identified primarily from two data sources: the VCF (Volatile Compounds in Food) database and a Food Packaging Forum database of intentionally-used food-contact chemicals. Chemical classes for these chemicals were primarily mixture/UVCB (unknown or variable composition, complex reaction product, or biological mixture), organoxygen compounds, and prenol lipids. The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.

There is a need to rapidly assess the potential risk of chemicals in our environment. Necessary toxicokinetics (TK) data, however, are most often unavailable for non-pharmaceutical environmentally relevant chemicals due to the lack of clinical trials. High Throughput TK (HTTK) methods provide the ability to characterize large numbers of chemicals by combining generic TK models with in vitro measurements and in silico predictions of chemicals-specific toxicokinetics. To support the number of chemicals, the U.S. Environmental Protection Agency (EPA) provides HTTK methods through an open-source R package “httk” with data and models. The CDC’s National Health and Nutrition Examination Survey (NHANES) provides biomonitoring data and biological exposure monitoring data that are statistically representative of the U.S. population. Previously, reverse dosimetry was used to infer the steady-state (SS) human exposure rates for the U.S. population with urine biomonitoring data from the 2009-10 NHANES cohort for 106 environmental chemicals. Inference from urine was drawn using a SS assumption and a parent-metabolite stoichiometric mapping. In vitro-in vivo extrapolation (IVIVE) was performed using the HTTK physiologically based PK (PBTK) model and TK (PBTK) model classes in the “httk” package with both ToxCast AC50 values and TK data. As a surrogate for risk, we used HTTK to convert ToxCast bioactivities into equivalent dose rates (mg/kg/day) and compared those doses to the exposures (mg/kg/day) inferred from NHANES. In this project, we expanded our analysis using two different approaches: 1) for semi- and non-volatile chemicals in urine, and 2) for volatile chemicals in blood/plasma. The median SS human exposure daily intake rate estimates (mg/kg/day) were updated with urine biomonitoring data from the 2015-16 cohort, expanding the inference to 179 parent chemicals associated with 118 biomarkers. Among the 179 chemicals investigated, we observed an overlap indicative of a high potential risk for 5 chemicals. Inference from blood/plasma was evaluated with a generic gas HTTK model, which includes gas inhalation/exhalation component with inhalation and oral routes of exposure. The inferences were compared to the results from a generic oral ingestion HTTK model to demonstrate the importance of high-throughput chemical risk prioritization addresses a key limitation of previous efforts. These nationally representative exposure inferences provide critical chemical risk information and benchmarks for exposure models. By placing volatile chemical risk into the context with semi- and non-volatile chemicals, priorities can be better identified. This abstract does not necessarily reflect US EPA policy.
women (the selected chemicals of interest were those not also found in pooled samples from men). A Fisher’s Exact test was used to identify 44 descriptors that were enriched for chemicals in the women’s blood. These descriptors spanned a variety of consumer product and chemical function categories. An un-directed chemical co-occurrence network analysis helped identify related patterns in chemical use across the enriched descriptors. Specifically, a co-occurrence threshold of more than four chemicals helped elucidate patterns of chemical use as a fragrance, deodorizer, or solvent in cosmetics (e.g., lip color, lip balm, eyeliner), hair care products (e.g., shampoo and styling products), and laundry products. Given these early findings, fingerprinting and network analysis approaches show strong potential to rapidly inform exposure sources for chemicals identified in NTA studies. Complementary approaches that define directed relationships among exposure pathway components, or define a priori related descriptor sets, are also being investigated. Understanding the origin of exposure sources for chemicals sharing common mechanisms of toxicity will potentially inform mitigation priorities and risk management strategies.

3820 PFAS Exposure Assessment Results and Findings

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The Agency for Toxic Substances and Disease Registry (ATSDR) conducted eight exposure assessments (EAs) in communities near military installations that have documented exposures to PFAS in drinking water. In all cases, exposure to PFAS in drinking water was mitigated prior to initiation of the biomonitoring efforts. These EAs built upon prior pilot investigations conducted at study sites, resulting in a total of ten EAs located in nine states. The objective of the EAs was to determine the distribution of PFAS serum concentrations in communities with recent or past exposure to PFAS in drinking water. A random sampling approach was conducted in communities where the size of the population made it appropriate for the distribution of participants to be generalizable to the community. PFAS were measured in urine samples from a subset of participants and in indoor dust and tap water from a subset of participants, to allow the results of the EAs to be generalizable to the community. PFAS were measured in urine samples from a subset of participants and in indoor dust and tap water from a subset of participants, to allow the results of the EAs to be generalizable to the community. PFAS were measured in urine samples from a subset of participants and in indoor dust and tap water from a subset of participants, to allow the results of the EAs to be generalizable to the community.

3821 Biomonitoring of Heat-Induced Food Contaminants: Biomarker-Based Approach to Dietary Exposure Assessment of Furan

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Furan is formed in a variety of heat-treated food items, including coffee, canned or jarred food, and processed baby food. Due to the high volatility of furan, human dietary exposure assessment based on consumption data and concentrations of furan in food does not provide reliable exposure estimates. Considering the low margin between human exposure and doses that cause adverse health effects in rodents, a more accurate approach for exposure assessment of furan is needed. Furan biotransformation via CYP2E1 yields the highly reactive diol-epoxide cis2butene1,4ral (BDA), which covalently binds to amino acids or glutathione (GSH), giving rise to a broad spectrum of furan metabolites. In rats, we recently demonstrated a close correlation between urinary excretion of several furan metabolites and external doses, supporting the potential of a biomarker-based approach to furan exposure assessment. However, metabolites generated from the reaction of BDA with lysine as well as GSH also were detected in urine of untreated animals, suggesting either background exposure via animal feed or endogenous formation. As understanding the contribution of potential endogenous formation to overall furan exposure is critical for biomarker-based exposure assessment, the present study was designed to discriminate between external dose and endogenous formation or background exposure through administration of isotopically labelled [13C4]furan to rats at doses relevant to human exposure (0.11000 µg/kg bw) and subsequent analysis of urinary furan metabolites using stable isotope dilution ESILCMS/MS. Specifically, urinary excretion of [12C]labeled and unlabelled GSHBDA, NAclysBDA, NAcCysBDANAClys and NAClysBDA-NAclys sulfoxide was monitored for up to 7 days following a single oral dose of 1 mg/kg bw [13C4]furan, which reflect external exposure and [12C]furan-dependent metabolites potential endogenous or background exposure. After a single oral dose of 1 mg/kg bw [13C4]furan, 2.2 % of the administered dose were rapidly excreted as GSH[12C]BDA within the first 24 h after application, whereas NAClys[12C]BDA, NAcCys[12C]BDANAClys and NAClys[12C]BDANAClys sulfoxide was excreted in a delayed manner, accounting for 0.59, 1.33 and 0.64 % of the external furan dose. With the exception of GSHBDA, which showed no background occurrence, unlabelled furan metabolites were also detected in rat urine. Excretion of unlabelled NAcCysBDANAClys and NAClysBDANAClys sulfoxide was estimated to correspond to an external furan dose of 60 µg/kg bw per day, whereas NAClysBDA [12C]BDA and NAcCysBDA-NAclys furan via feed, excretion via feed was estimated at 0.6 µg/kg bw per day, excluding feed as a significant source of background exposure and supporting endogenous formation of furan or BDA. Interestingly, background levels of NAClysBDA in rat urine by far exceeded the levels of the BDA-derived lysinencysteine crosslinks and were strongly correlated with the external furan dose (r = 0.67, p < 0.01). These results suggest that other exogenous sources such as diet may contribute to background exposure to this particular metabolite. Results obtained in rats and analyses of urine samples of humans exposed to a high furan diet, which indicated the presence of GSHBDA, NAclysBDA, NAcCysBDANAClys and its sulfoxide NAClysBDANAClys, support biomonitoring approaches to furan exposure assessment and highlight GSHBDA as a potential specific biomarker of dietary furan exposure.

3822 Comparison of X-Ray Fluorescence (XRF) with Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-DES) for the Measurement of Toenail Metal Levels

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Welders are often exposed to a high concentration of metals in welding fumes. Chronic overexposure to metals, such as manganese (Mn), is known to cause neurotoxic effects. However, the dose-response of different metals in a chronic exposure setting is not well understood. The deleterious impacts of these exposures is the utilization of biomarkers for chronic exposure and their measurements. Our previous study showed that Mn concentration in toenails is an excellent biomarker of chronic exposure to Mn in occupational settings. Toenail metal levels are often measured via inductively coupled plasma (ICP)-mass spectrometry (-MS), a relatively cost-intensive method. Our group validated the use of ICP-optical emission spectrometry (-OES), a more cost-effective and non-destructive method, as an alternative to analyzing metal levels in toenail clippings as a biomarker of metal exposure. However, both ICP-MS and -OES methods require time and labor-intensive processing including acid digestion, which leads to sample degradation and analysis. X-ray fluorescence (XRF) is a rapid, inexpensive, and non-destructive method to analyze metals. In this study, a benchtop XRF (b-XRF) and a portable XRF (p-XRF) were utilized to evaluate their applicability to measure toenail metals, in comparison with ICP-OES. Specifically, metal levels of toenail clippings collected from 13 welders and 13 non-welders were measured using b-XRF, p-XRF, and ICP-OES. Results from b-XRF and p-XRF were compared to ICP-OES results and Pearson correlations for six metals (Mn, iron (Fe), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn)) were calculated. Results of b-XRF and ICP-OES showed strong correlations for Mn and Fe (Pearson correlation coefficient, r = 0.96 and 0.72, respectively), moderate correlations for Cr, Cu, and Zn (r = 0.45, 0.45, and 0.48, respectively), and no correlation for Ni (r = 0.04). The p-values of the correlation for Fe, Cr, Cu, Zn, Ni were < 0.01, < 0.01, 0.01, 0.01, 0.01, and 0.83, respectively. Similarly, p-XRF results were strongly correlated with ICP-OES results for Mn and Fe (r = 0.70 and 0.80, respectively), moderately correlated for Zn (r = 0.62), and not correlated for Cr, Cu, and Ni (r = -0.33, -0.22, and 0.24, respectively). These results demonstrated that both XRFs can be used to measure Mn, Fe, and Zn in toenails but need to be adjusted using regression slopes. The b-XRF regression slopes for Mn, Fe, and Zn were 0.95, 0.29, and 0.79, respectively, while the p-XRF regression slopes for those metals were 0.37, 0.38, and 0.79, respectively. In addition, b-XRF can be used to measure Cr (slope < 0.01, and 0.18, respectively. These results demonstrate that both XRFs can be used to measure Mn, Fe, and Zn in toenails but need to be adjusted using regression slopes.
How the 70-kg Man Impacts NIOSH-Recommended Exposure Limits

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This study examines the impact of assuming a single human body weight value when developing occupational exposure limits. NIOSH uses quantitative risk assessment to evaluate the risks of chemical exposures in the workplace and as a basis for determining occupational exposure limits. Recommended Exposure Limits (RELs, Recommended Exposure Guidelines) are developed using fractionation to account for both gender and body weight.

Simplifying assumptions are a necessary part of quantitative risk assessment and there are several key assumptions that go into occupational risk assessments - one, of note, is the assumption that the typical human body weight is 70 kg (typically also assumed to be male). In this investigation, the public draft proposed NIOSH REL for 1-bromopropane (1-BP) was used as a case study to explore the impact of this assumption. In the public draft risk assessment, NIOSH used data from a National Toxicology Program mouse 2-year bioassay to estimate the risk of cancer in workers exposed to 1-BP over a working lifetime. The risks were calculated using Bayerian model averaging of Benchmark Dose estimates of the mouse dose-response curve to estimate the Benchmark Dose Limit (BMDL). The BMDL extrapolated to humans, assuming dose equivalence in units of mg/kg-day scaled according to body weight to the 0.75 power to find the human equivalent BMDL at 70 kg. This value was then converted to ppm and adjusted to account for an 8-hour workday 5 days per week to derive the proposed REL of 0.3 ppm. At the draft REL, the risk of cancer was estimated to be 1 excess case per 1000 workers exposed to 1-BP every workday over a working lifetime of 45 years. In this study, the NHANES data for body weights by gender in the United States were used to create a distribution of weights for both adult male and adult female populations. From data collected in 2021, the mean body weight in the female population was 77.5 kg ± 21.2. In the male population, mean body weight was 90.6 kg ± 20.8. A simulation was run on a randomly selected body weight from the distribution of body weights and the risk of 1-BP was recalculated based on this selection. This experiment was repeated 10,000 times, each time using different randomly selected body weights. The results show how risks vary depending on body weight. Instead of a risk of 1.0/1000, for a person with a BMDL of 0.91/1000 - a nearly 10% difference. Conversely, a lighter individual would have a higher risk when exposed to the same concentration of 1-BP. Since the mean body weights for males and females were higher than the assumption of 70 kg, the mean female risk was 0.99/1000 and the mean male risk was 0.87/1000. However, for more than 38% of the female population and 17% of the male population, the estimated risks were higher than the 1/1000 target risk level in the NIOSH public draft document. These results demonstrate the importance of understanding and explaining the impact of the assumptions used in quantitative risk assessment. The unbalanced impact of the assumption depending on gender also points out hidden gender bias in occupational risk assessment. Clearly identifying and discussing the assumptions used in a quantitative risk assessment is an important aspect of ensuring that the occupational exposure limit is appropriately used in the workplace.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

Developing the Internal Threshold of Toxicological Concern (ITTC): Generating In Vitro Caco-2 Permeability and Hepatocyte Metabolism Datasets Needed for PBPK Modeling

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The threshold of toxicological concern (ITTC) is a risk assessment tool developed to screen and prioritize low level exposures to chemicals with unknown toxicity. The ITTC limits define exposure thresholds below which there is a low probability of adverse health effects. Current TTC thresholds establish limits for external exposures. A project is underway to derive internal TTC (ITTC) values (i.e., a TTC based on plasma concentration) to permit TTC assessments based on internal exposures. This study aimed to evaluate how body weight could affect TTC values based on the human equivalent BMDLs at 70 kg. In this study, the NHANES database was used to estimate the mean body weight for males and females in the United States. Plasma concentrations were measured for 7 chemicals, and PBPK model simulations were performed to predict plasma concentrations. The results showed that body weight significantly affected the TTC values for all chemicals, with a 10% increase in TTC for a 10% increase in body weight. Additionally, the study found that body weight had a greater impact on TTC values for chemicals with a lower BMDL.

Stochastic Simulation of Tumor Growth to Support Carcinogen Risk Assessment: Formaldehyde Case Study


Clonal growth modeling in support of carcinogen risk assessment requires an estimate of the time needed for a single tumor cell to expand clonally into a clinically detectable tumor. We refer to this time interval as the delay (D). D cannot be measured directly, since the exact time at which a tumor cell arises by mutation of its precursor is unknown. However, D can be estimated by formal optimization against the best fit in vitro assay response or for risk assessment based on internal exposures (e.g., plasma concentrations). Physiologically based pharmacokinetic (PBPK) modeling is being used to convert the external No Observable Adverse Effect Levels (NOAELs) of chemicals in the existing TTC database to estimates of internal exposure. PBPK modeling requires chemical-specific input data, including body weight, gender, tissues, clearance pathways, and plasma concentration distribution. In this study, formaldehyde was used as a model carcinogen. The model was validated using in vivo data and PBPK simulations. The results showed that the model accurately predicted the time to clinical detection of tumors.

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317.2 hr, which differ by 72.6 hr (3 days). We used stochastic computational modeling to clarify the biological underpinnings of these different values of D. The value of D depends on the growth kinetics of the tumor cells. Stochastic simulation used pseudorandom numbers from binomial and Poisson distributions to predict the division and death of tumor cells. The probability of division per hour was set to an upper bound on the rate of cell division ($0.021$) in rats exposed chronically to 9.93 ppm formaldehyde. Cell volume, the minimum size of clinically detectable tumors (0.1 to 1.0 cm$^3$) was estimated from relevant literature. With these parameters fixed, the probability of cell death was varied to achieve different values of D. Some tumors became extinct, even when the probability of division was greater than the probability of death. Average values for D and its upper and lower bounds were obtained by running the model repeatedly, typically from 1,000 to 10,000 times. These analyses showed that the values of D used by Subramaniam et al. (2007) are associated with little or no cell death as the tumor grows and that the 3day difference in D between incidental and fatal tumors would probably not be identifiable, given the stochastic variability of the system. The value of D used by Conolly et al. (2003) was associated with a relatively large death rate, with many of the tumors becoming extinct, and is consistent with the cytotoxic environment created by ongoing exposure to 9.93 ppm formaldehyde. Furthermore, p53 mutation, which is associated with inactivation of the apoptotic pathway, is not a consistent feature of formaldehyde-induced tumors in the rat nose. This genomic observation is consistent with a larger death rate and with the larger value of D identified by Conolly et al. (2003). This analysis increases confidence in the value of D used by Conolly et al. (2003) and in their BBR modeling in support of cancer risk assessment for formaldehyde.

3827 Moving beyond Similarity Scores: Use of Toxicokinetic and Toxicodynamic Data to Inform Selection of a Suitable Analogue for δ-HCH in Read-Across Assessment
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Delta hexachlorocyclohexane (ΔHCH) has limited toxicological data that are not adequate for traditional derivation of toxicity values. Instead, a tiered analogue approach relying on assessment of structural, metabolic, and toxicological similarity between the target chemical and potential analogues was explored. This read-across case study highlights the utility of looking beyond similarity scores and examining all available data in selecting an analogue. Structural analogues identified for ΔHCH included three of its stereoisomers (α-, β-, and γ-HCH). Critical endpoints identified from subchronic and chronic oral studies of these stereoisomers were liver and immune system effects. The stereoisomers each scored 100% for structural similarity with the target compound (δ-HCH) and share the same structural alerts and molecular weight as the target. However, toxicokinetic data showed that β-HCH undergoes very little metabolism in mammals and has a much longer half-life in the body than δ-, α-, or γ-HCH. Furthermore, comparative toxicity data revealed that toxicity of the HCH stereoisomers revealed similarities between δ-HCH and γ-HCH and differences from the other two stereoisomers, Ito et al. (1973 and 1975) examined liver effects in mice and rats, respectively, after 24- to 48-week administration of δ-, α-, and γ-HCH. In these studies, dose-response relationships for liver weight and histopathology changes were similar between δ-HCH and γ-HCH, while α- and β-HCH showed decreased potency (larger magnitudes of effect at lower doses) for hepatotoxic effects in both species. In vitro data also suggested that of the HCH stereoisomers, δ-HCH is most similar to γ-HCH with respect to immune system effects. Both δ- and γ-HCH inhibited mitogenic response to phytohemagglutinin in bovine lymphocytes while α- and β-HCH did not. Based on structural similarity, physicochemical properties, toxicokinetics, and toxicodynamic comparisons for sensitivity endpoints, including liver toxicity and immune system effects, γ-HCH was selected as the most suitable analogue for δ-HCH. This case study demonstrates how toxicokinetic and toxicodynamic information can be used to refine selection of analogues in read-across assessment. The views expressed are those of the authors and do not necessarily reflect the views and policies of the US EPA.

3828 A Systematic Comparison of the Temporal Transcriptional Responses by Hepatotoxins in Primary Human Hepatocytes and HepaRG Cells Using Concentration Response Modeling of Gene Co-expression Networks
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Next generation risk assessment (NGRA) of chemicals revolves around the use of mechanistic information without animal experimentation. In this regard, toxicogenomics has proven to be a useful tool to elucidate underlying mechanisms of toxicological action of adverse effects of xenobiotics. In the present study, two widely used human hepatocyte culture systems, namely primary hepatocytes (PHH) and human hepatoma HepaRG cells, were exposed to chemical liver toxicants known to induce liver steatosis, cholestasis or necrosis. Benchmark dose (BMD) response modelling was applied to transcriptomics gene co-expression networks (modules) in order to derive benchmark concentrations (BMCs) and to gain mechanistic insight into the hepatotoxic effects. BMDs derived by benchmark dose modelling of gene co-expression modules recapitulated benchmark dose modelling of individual genes. PHH and HepaRG cells showed overlap in the deregulated genes and modules by the liver toxicants. However, PHH demonstrated more biological activity compared to HepaRG cells, based on the lower BMCs of co-regulated gene modules, which could be used as point of departure for the associated cellular (stress) pathways/processes. This approach could serve next generation risk assessment practice to identify early responsive modules at low benchmark concentrations. In turn, this can assist in delineating potential hazards of new test chemicals using in vitro systems. Benchmark concentrations may be paired with chemical exposure assessment and used in a subsequent risk assessment. This project has received funding from Cosmetics Europe and the European Chemical Industry Council (EPI) (project AIMTT10), the EC Horizon2020 EUToxRisk project (grant number 681002), the EC Horizon2020 RISK-HUNT3R project (grant number 964537; part of ASPIS cluster) and the EU-EFPIA Innovative Medicines Initiative 2 (IMI2) Joint Undertaking under the TransGUST project (grant number 116030). This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation program and EFPIA.

3829 Systematic Application of Mode-of-Action and Human Relevance Analysis: Styrene-Induced Lung Tumors in Mice
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Risk assessment of human health hazards often relies on observations from animal experiments. Although exposure studies in rats and mice are a major basis for determining risk in many cases, observations made in animals do not always reflect health hazards in humans due to differences in biology. In this critical review, the mode-of-action (MOA) human relevance framework was used to assess the likelihood that bronchiolar lung tumors observed in mice chronically exposed to styrene represent a plausible tumor risk in humans. Available datasets were used to determine the weight-of-evidence (i) that styrene-induced tumors in mice occur through a MOA initiated by metabolism of styrene by CytoP2F2; and (ii) whether the hypothesized key event relationships are plausible in other species. Analysis of data using five modified Hill causality considerations indicated that the hypothesized CytoP2F2-dependent MOA is active in mice, but only results in tumorigenicity in susceptible strains. Assessment of species concordance to determine whether analogous key event relationships do or could plausibly occur in other species concluded that while some of the proposed key events are biologically plausible in rats, the MOA is improbable in humans due to poor concordance of both early and late key events reflecting key differences in airway biology and physiology. This analysis serves as a rigorous demonstration of the framework’s utility in increasing transparency and concordance in evidence-based assessment of MOA hypotheses in toxicological models and determining relevance to human health.

3830 Interindividual Variability Assessments through Benchmark Dose-Response Modeling of Primary Human Bronchial Epithelial-Fibroblast Co-culture Responses to Acrolein
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Human health risks are known to significantly vary according to individuals across populations; however, this interindividual variability cannot be captured by traditional in vivo or in vitro studies that utilize inbred animal strains and isogenic cell lines, respectively. The use of in vitro systems that utilize cells isolated directly from a range of individual human donors (i.e., “primary” cells) provides a novel opportunity to subtype and potentially increase the sensitivity of endpoints including living cell toxicity and early key event endpoints. The use of in vitro new approach methodologies. Unfortunately, the utility of incorporating interindividual variability into in vitro testing has not yet been integrated into toxicity testing strategies due to a lack of computational methods and software infrastructure. To address this gap, we hypothesized that benchmark dose-response (BMD) modeling could be leveraged to evaluate human interindividual sensitivities to chemical exposures. Here, we performed BMD modeling on a dataset containing a broad range of endpoints reflective of in vivo tissue physiology. Endpoints were derived from an inhalation assay battery, including 6 phenotypic and 11 secreted cytokine/growth factor endpoints, to evaluate the effect of acute exposure to the ubiquitous reactive volatile organic gas acrolein (0-4 ppm) on primary human bronchial epithelial-fibroblast co-cultures (n=14). We then clustered observations using model curve fit parameters to assess whether dose-response was associated with cell donor demographic variables. This study identified the following: First, we found that benchmark doses varied greatly on a per-donor basis, with BMDs spreading up to 1,000 to 10,000 times. These analyses showed that the values of D used by Conolly et al. (2003) and in their BBR modeling in support of cancer risk assessment for formaldehyde.

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responses rather than aggregating responses at the population level. Third, we found that the high-throughput screening (HT) method of staining secreted cytokine-like functional culture products yielded similar BMDS, with potential sex-based differences captured through model parameter clustering. This study provides critical information towards the improvement and implementation of in vitro-based methods to capture human response variability in chemical risk assessments. This abstract does not reflect EPA policy.

### 3831

**Investigation of the Fate and Effects of Microplastics upon Ingestion Using In Vitro and Ex Vivo Models**

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Humans are perhaps the most exposed organism to micro- and nano-plastics (MPs, plastic particles ≤ 5 mm) since can be exposed to them through multiple routes (ingestion, inhalation, or even contact). There have been several studies showing the presence of MPs in food, particularly seafood, sea salt, and drinking water, but also in human samples such as stool. Exposure of humans to MPs is most prominent via the oral route (ingestion) and respiratory route (inhalation) and is only expected to rise in the future. However, little is known about the effects of microplastics on gut health. Recent findings indicate that it is highly likely that MP can cross the epithelial barrier in the intestine (as well as airways/lungs and thus may exert detrimental biological effects on the human body). For this reason, one of this project’s main goals is quantifying human body exposure to MPs through the ingestion of MPs. The other goal is to study the effects of environmentally relevant MPs and concentrations of MPs on gut health. In this study, an established in vitro model for the intestinal barrier (differentiated CaCo2 monocultures) as well as a more physiologically relevant ex vivo model representing the gut (IntTESTine™) were used to study translocation of MPs and their potency to induce cytotoxic effects (in terms of LDH release) on the barrier function (both in terms of TER, FDA and radioactively labeled compounds transportation) or pro-inflammatory cell activation (in terms of IL-6 and IL-8). Copper (II) Oxide (CuO) Titanium dioxide (TiO2) and commercial Polystyrene (PS), of the same size (50 nm) in a dose-response setup (1-100ug/mL) were used as reference particles to test and set up the exposure models. CaCo2 cells were seeded in 3 point inserts for acute and chronic component differentiation and formation of an intact monolayer, which was confirmed by high transepithelial resistance (TEER, accepted values > 300 ohm*cm²). The cells were subsequently exposed to different doses of MPs mentioned above for 48h. For the ex vivo setup the intestinal tissue (colon) was obtained from sacrificed pigs with comparable health status, age and exposure history. Hepatic and mucosal epithelial cell lines were used for a biopsy punch with an 8mm diameter and capped in inserts of 24 well plates, for a 6h exposure. None of the exposures, for both models, significantly affected cell viability or induced a high inflammatory response. However, in the cell culture model, exposure to CuO after 48h resulted in barrier disruption of the monolayer which was confirmed both by low TER and barrier function loss. This indicates the potential for MPs to influence the barrier properties of the GI tract. In the chronic exposure the morphology of the cells was comparable to the acute exposure. Moreover, the results showed a consistent increase in cytokine and chemokine levels in both cell culture models. These results can provide valuable insights into the potential impact of MPs on gut health and may have implications for the design of future studies.

### 3833

**Relevance of Lead Enrichment in Soil Particle Size Fractions for Assessing Exposure**

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Soil cleanup levels at contaminated sites may be adjusted to reflect site-specific relative bioavailability of soil lead as compared with soluble lead forms. Tools used to assess potential lead exposure and bioavailability are based on lead concentrations in the particle size fraction most likely to adhere to children’s hands or other objects, thus leading to lead ingestion. Historically the less than (<) 250-micron (μm) fraction has been used for bioavailability studies. Recent recommendations from the USEPA suggest a <250 μm particle size fraction should be considered. Directive 92/69/EEC issued by the USEPA in 2016 recommends sieving soil samples collected from lead-contaminated sites to <150 μm for assessment of exposure via ingestion, increasing the potential for dermal adherence of finer soil particles to children’s hands. While greater enrichment has been observed at very fine soil fractions (e.g., <63 μm), published data assessing lead exposure in the <150 μm fraction, compared with the previously-recommended fraction of <250 μm, are quite limited. The resulting uncertainty warrants further study. To investigate the potential for lead enrichment in the <150 μm fraction compared to the >250μm fraction, this study compared lead concentrations measured in soil collected from Butte, Montana, an area with a long history of mining-related lead impacts. Arsenic, another constituent of interest in Butte, was also assessed. A total of 121 soil samples collected from multiple depth intervals were sieved to obtain the fraction less than or equal to (≤) 250 μm, analyzed for lead and arsenic, then re-sieved to obtain the <150 μm sample. Samples were selected for this study to encompass a broad range of environmentally relevant lead concentrations, including samples representative of background concentrations with minimal anthropogenic influence. Concentrations measured in each fraction were statistically compared using a non-parametric variation of the paired t-test. Percent difference was also calculated as a measure of enrichment and compared between samples reflecting different ranges of lead and arsenic concentrations and depth intervals. Statistically significant differences were observed between lead and arsenic concentrations in the <250 μm and <150 μm fractions. Concentrations of both metals were higher in the <150 μm fraction. However, consistent with the other few studies that have compared lead and arsenic concentrations between similar particle size fractions, the <150 μm fraction exhibited greater magnitude of enrichment. The fraction of the two fractions was relatively low for both metals. Average percent differences observed for lead and arsenic among all samples (excluding outliers) were 8% and 13%, respectively. Enrichment was generally greater in samples with lower concentrations of both metals, and at the highest arsenic levels. Among samples with lead and arsenic concentrations indicative of anthropogenic influences, the average enrichment was 6% for lead and 11.5% for arsenic. Across three sampled depth intervals, lead and arsenic enrichment generally increased with sample depth. For both metals, greater enrichment was consistently observed in the 6-12-inch depth interval, with the lowest enrichment observed among samples collected from 0 to 2 inches. Overall, the results of this study, along with other comparable studies, indicate a small difference in reliance on the <250 μm versus ≤150 μm size range.
Risk assessments for HFPO-DA, a short-chain polyfluorinated alkyl substance (PFAS) used in the manufacture of some types of fluorinated polymers, have proposed reference dose (RfD) values ranging from 0.000003 to 0.01 mg/kg-day. These differences are due, in part, to mode of action (MOA) determinations influencing the selection of critical effects for risk assessment and the choice of uncertainty factors. Subsequent and concomitant to the development of these HFPO-DA risk assessments, new research on the mechanisms and adverse effects of HFPO-DA have been conducted. Herein, we review new data that inform the risk assessment of HFPO-DA and perhaps other PFAS. These data include newly published in vivo liver transcriptomic analyses in mice, unpublished in vitro transcriptomic analyses in human and rodent hepatocytes (including knockout mice), development of a new mode of action (MOA) for developmental effects in rodents, as well as published analyses on the applicability of the threshold of toxicological concern (TTC) to PFAS and metanalyses on how TTC and RfD values might inform the magnitude of RfD values. In the mouse liver, both molecular signatures and histopathological analyses do not support a cytotoxic MOA, but rather provide clear evidence of peroxisome proliferator-activated receptor-α (PPARα) activation. In vitro comparisons of transcriptomic signatures between HFPO-DA and cytotoxic agents (e.g., acetaminophen), rosiglitazone (PPARγ activator), and GW7647 (PPARα activator) indicate molecular overlap primarily with GW7647. Analyses on the impact of the addition of PFAS to the chemicals comprising Cr(VI) compounds indicate little effect on the Class III TTC value of 0.0015 mg/kg-day. Metanalyses comparing Class III TTC and respective RfD values indicate that TTC values are, on average, ~5-fold lower than RfD values, whereas some RfD values for HFPO-DA are ~400-fold lower than the Class III TTC value—perhaps indicating that such RfD values are overly conservative. Overall, these new data support that liver effects in mice are the result of a species-specific MOA with little human relevance, and that RfD values for HFPO-DA should not include liver effects that are the result of PPARα signaling.

Environmental injustice is the fair treatment of all people regardless of race, income, and religion with regards to their health and the environment. Negative environmentally linked health outcomes have been associated with socio-demographic status (SDS) factors in different regions of the United States. In this study, US Census Bureau SDS factors including race, income, education status, and rural-urban status were matched to US EPA National Air Toxics Assessment and Air Toxics Screening Assessment cancer risk estimates by census tract for years 2011, 2014, and 2018. Spearman correlations showed modest associations with various SDS factors and cancer risk estimates, both nationally and stratified by state. Multiple linear regressions were performed to relate Z-scored estimated cancer risks with multiple SDS factors. In multivariate regression analyses across the United States, increases in the number of Black, Hispanic, and Asian racial populations (relative to the number of White households) was positively associated with increased estimated cancer risk in urban areas (p < 0.0001). In suburban and rural areas however, such inequity is only seen for increasing Black populations while increasing proportions of mixed race, Hispanic and Asian populations were protective against estimated cancer risk (p > 0.0001). Areas with lower education attainment and income were also strongly associated with increased estimated cancer risk (p < 0.001). In addition, disproportionately high Black populations overlaps with the worst tracts of estimated cancer risk across the US. While inequity in environmental justice seems improved across the examined years, equity is far from achieved and future work should incorporate targeted exploration of extreme values in estimated risks to reveal the magnitude in disparity in estimated risk.

Recently, animal testing in the risk assessment of cosmetic products has been restricted, necessitating animal-free risk assessment methods called new approach methodologies (NAMs). To achieve the risk assessment with NAMs, detailed understanding of exposure is required, thus the physiologically based pharmacokinetic (PBPK) models have been considered to be a robust tool. To utilize PBPK models for risk assessment, it is necessary to evaluate the accuracy of model prediction. However, for many cosmetic ingredients, there is limited in
vivo pharmacokinetic data for model evaluation, limiting the model applicability. To address these issues, we attempted to establish an uncertainty factor (UF) for PBPK models to utilize the prediction results without model evaluation. In this study, we defined the deviation between predicted and measured values as the uncertainty of PBPK modeling and established modeling uncertainty factor (MUF) as a novel concept. As the prediction accuracy was calculated by building a PBPK model for various chemicals and comparing prediction results with the clinical area under the curve (AUC) or $C_{\text{max}}$, we built PBPK models for 150 compounds that have available clinical data. For input parameters, the intrinsic hepatic clearance and the fraction unbound in plasma were set to in vitro parameters. We used the Biopharmaceutics Classification System (BCS) and Extended Clearance Classification System (ECCS), well known for estimating the absorbability or major clearance pathways. By applying BCS and ECCS, the 97.5th percentile of AUC and $C_{\text{max}}$ ratio improved to 9.15 and 5.27, respectively and MUF was defined at 10 for the AUC and 6 for $C_{\text{max}}$. Finally, we conducted a case study of animal-free risk assessment using MUF using Bisphenol A. Although further investigation is needed, our concept will serve as a useful tool to predict plasma concentrations without using in vivo data. Regulatory Toxicology and Pharmacology 135 (2022) 105262.

3839 Risk Comparison of 1-Bromopropane in Production Enterprises and Use Enterprises with Two Toxicological Health Risk Assessment Methods

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To compare and analyze the risk of 1-bromopropane(1-BP) hazards in production enterprises and use enterprises through two risk assessment methods and to estimate the occupational health risk. Occupational health investigation and 1-BP detection for workplaces were carried out in three production enterprises in southern of China and in three use enterprises in northern of China. The risk ratings of different posts were assessed by US Environmental Protection Agency (EPA) inhalation risk (EPA assessment model) and Singapore Semi-quantitative Assessment Model (MOM assessment model). The risk classification results of the 2 risk assessment methods were compared and analyzed, based on occupational exposure limits and hazardous agents in the workplace (OSHA in the USA) and classification for hazards of occupational exposure to toxicant(GBZ 203-2010). The concentration of 1-BP on the positions of reaction, rectification, lavation and packaging were 0.1, 0.9, 1.6 and 0.5mg/ml respectively in production enterprises, while on the positions of clamping, cleaning 1 line, cleaning 2 line, and checking were 56.4±20.83, 63.4 and 5.7mg/ml respectively in use enterprises. Through the MOM assessment model, the four positions were negligible risk in production enterprises and all the positions were low risk in use enterprises. Current results suggested that 1-BP exposure levels were higher in use enterprises than production enterprises. The EPA assessment model could quantitatively and qualitatively assess the non-carcinogenic effects of chemicals, but the assessment results were low risk and relatively conservative for two positions where the exposure concentrations were above the limit. Based on the comprehensive considerations, hazard level and exposure level, the MOM model was assessed as more suitable for risk warning than the EPA model for 1-BP occupational health hazard risk assessment in China.

3840 Use of Physiologically Based Pharmacokinetic Modeling to Support Risk Assessment for Susceptible Populations

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Risk assessment is the characterization of the potential adverse effects in humans to exposures of environmental hazards. Traditional risk assessment methods include determining a point of departure (POD) from animal toxicity studies and calculating a human reference dose using uncertainty factors (UF) to account for data limitations and variability resulting from differences between and within test animals and humans. This traditional approach may not account for pharmacokinetic (PK) processes influencing the dose-response, PK processes that may vary across dose levels, dose routes, and species. In this project, to address the concern for age-related or disease-related sensitivity to chemicals we showed how a pharmacokinetic (PK)/pharmacodynamic (PD) PBPK model of chemical X metabolite could be used to predict internal concentrations of chemical X and its metabolite in different populations (adults, children and renally impaired population). Both in vitro metabolism parameters and in vivo kinetic data were developed for chemical X and its metabolite in rats. The model was used to recapitulate these in vivo pharmacokinetic results. The IVIVE approach was then similarly applied to the human model development. The blood area under the curve (AUC) was used as the dose metric to compare between species. The PBPK model was used to predict human internal dose metrics of various age groups (children and adult) including renally impaired adults. The resulting internal dose metrics were compared against equivalent rat (oral) POD metrics. The aggregate results suggested that more realistic as it considers differences in exposure (MOE) and margin of exposure (MOE). The MOEs for chemical X metabolite are higher compared to the usual minimal MOE value of 100. However, the traditional MOE approach does not consider physiological animal to human differences that influence toxicokinetic. The MOEs reported for chemical X metabolite are lower than the traditional MOE. This indicates that the use of the MOE is a more conservative approach. Traditional measures from the 2-year NTP pathology data. We observed a dose dependent increase in significant genes for the NiSO₄ exposure from 54 - 106 DEGs at 3.5 - 15 mg/kg with a slight drop at the highest treatment group whereas for CuSO₄ there was an increase in DEGs across the upper three treatments, which identified 31 - 458 DEGs at 4000-16000 ppm. A total of 26 and 8 DEGs were shared across treatment groups for NiSO₄ and CuSO₄ respectively representing consistency in response that was not likely a result of fixation related damage. When mapped to pathways, NiSO₄ DEGs indicated upregulation in inflammation and immune response, which has been linked to metal exposures while pathway analysis of CuSO₄ DEGs suggested disruption in sterol synthesis as the top impacted pathway across treatments, which is known to be modulated by copper levels. Dose response modeling of NiSO₄ genes resulted in median BMDs of 0.91 mg/m² and 0.99 mg/m² for female and male rats, respectively, when mapped to the most sensitive gene ontology biologic process. This was 8-fold higher than the

Since the onset of the pandemic, a remarkable number of drugs have been considered for clinical trials in the treatment of COVID-19. To combat the pandemic and better protect public health, it is important for physicians to select safe drugs for COVID-19 patients. This study evaluates the safety of drugs utilized for COVID-19 treatment by analyzing adverse events reported in post-market surveillance. Adverse events are undesirable experiences associated with drugs, and adverse events analysis can be used as a safety measurement to provide information regarding which adverse events are more likely to occur in patients. In this study, we examined 26 drugs that have been evaluated in clinical trials for COVID-19 and identified 28,597,464 associated adverse events at the System Organ Classes (SOCs) level in the FDA Adverse Events Reporting System (FAERS). We calculated the Z-score for each SOC to determine a statistically relative frequency of adverse events in the SOC. The Z-scores analysis revealed that most drugs have multiple significantly frequent adverse events. Physicians may need to be cautious when prescribing drugs with high z-scores to patients who are vulnerable to the associated adverse events. Our results suggest that this safety concern metric may serve as a tool to inform selection of drugs with favorable safety profiles for COVID-19 patients in clinical practice. Disclaimer: This abstract reflects the views of the authors and does not necessarily reflect those of the US FDA.

3842 Toxicogenomic Analysis of >25-Year-Old Archival Tissue Samples Indicate Metal Sulfate Mode of Action and Potency

L. Wehmas, and S. Hester, US EPA, Research Triangle Park, NC. Archival tissues provide an invaluable resource for retrospective molecular analyses; yet widespread use for toxicogenomics has been limited by formalin-fixation related damage to nucleic acids. Advances in targeted RNA-sequencing technologies have helped overcome this challenge by focusing reads on smaller expressed regions of the transcriptome; thereby opening archives for new studies into chemical modes of action and retrospective proof of principle research. In this study, formalin-fixed paraffin-embedded (FFPE) rat lung or liver tissue (>25-years-old) was obtained from early timepoints (15-16 days) of two chronic toxicity studies (niskel sulfate or cupric sulfate: CuSO₄ by ingestion) archived by the National Toxicology Program (NTP). Each study tested multiple dose levels (3.5 - 30 mg/m² NiSO₄ or 1000-16000 ppm CuSO₄ plus concurrent controls) in both male and female rats. Tissue type was selected based on the 2-year pathology reports and collected from the NTP archive for targeted, whole transcriptome sequencing (Illumina NextSeq). We analyzed each chemical for significant differentially expressed genes (DEGs, FDR < 0.05, fold change ≥ 2) across treatment groups and looked for consistencies in molecular pathway response using comparison analysis in Ingenuity (Qiagen). Dose responsive genes underwent benchmark dose (BMD) analysis in BMExpress (v 2) to compare short-term transcriptomic measures of chemical potency with traditional measures from the 2-year NTP pathology data. We observed a dose dependent increase in significant genes for the NiSO₄ exposure from 54 - 106 DEGs at 3.5 - 15 mg/m² with a slight drop at the highest treatment group whereas for CuSO₄ there was an increase in DEGs across the upper three treatments, which identified 31 - 458 DEGs at 4000-16000 ppm. A total of 26 and 8 DEGs were shared across treatment groups for NiSO₄ and CuSO₄ respectively representing consistency in response that was not likely a result of fixation related damage. When mapped to pathways, NiSO₄ DEGs indicated upregulation in inflammation and immune response, which has been linked to metal exposures while pathway analysis of CuSO₄ DEGs suggested disruption in sterol synthesis as the top impacted pathway across treatments, which is known to be modulated by copper levels. Dose response modeling of NiSO₄ genes resulted in median BMDs of 0.91 mg/m² and 0.99 mg/m² for female and male rats, respectively, when mapped to the most sensitive gene ontology biologic process. This was 8-fold higher than the...
most sensitive lung-related BMD (hyperplasia) at 2 years. For CuSO₄, the median gene set BMD values (2558 ppm for female and 4430 ppm for male rats) were also ~8-fold higher than the most sensitive 2-year BMD values of adverse health outcomes (liver inflammation and forestomach hyperplasia, respectively). These results show promise in obtaining potentially useful transcriptomic data from decades old archival tissue samples and suggest utility in using gene response to individual parent compounds and neglecting relevant metabolism processes. By focusing on the impact of parameter distributions, it was found that 1st and 99th percentiles of HED distributions, while uniform parameter distributions produced dramatically less lognormal HED distributions. Finally, it was found that 1st and 99th percentiles can be accurately predicted using distributional data for a relatively small subset of the model parameters (i.e., not considering variability in most of the model parameters), but that the list of important (influential) parameters depends on the chemical and the dose. In the future, these conclusions about HED distributions and the impact of parameter distributions may be generalized by investigating other PBPK models to better characterize uncertainty in reverse dosimetry calculations.

Characterization of the degree of susceptibility among vulnerable communities is an emerging need in health-based risk assessments with particular interest in the impact of non-chemical stressors. Further, publications have suggested that stress levels, or allostatic loads, are elevated among vulnerable populations compared to the general population which may impact health outcomes and chemical stressors. Recent agency and academic investigations have focused on historically disadvantaged communities likely impacted by non-chemical stressors with the development of geographical tools such as USEPA's EJScreen to locate such communities for additional consideration. The objective of this work is to investigate the degree of susceptibility within such populations. This was accomplished by leveraging publicly available National Health and Nutrition Examination Survey (NHANES) data (2015-2016 and 2017-2018 datasets) and nine biometric indicators of human health to estimate allostatic load for the general population and for defined subpopulations. Considering survey and demographic information, subpopulations within the NHANES cohort were subset by specific criteria highlighted in EJScreen and other investigatory tools. These criteria broadly relate to income, attained education, food availability, and healthcare access. A cumulative subpopulation which matched criteria from all of the aforementioned subsets was also included. The allostatic indicators (serum albumin, body mass index, serum C-reactive protein, serum creatinine, diastolic blood pressure, glycated hemoglobin, systolic blood pressure, total cholesterol, and serum triglycerides) were evaluated by percentile and summed to generate a score from 0-9, i.e., an allostatic load score. Medication use and declared health status were reviewed and adjustments to allostatic score were made for medications which may have potentially successful in treating the symptoms may not address the true basal stress of the respondents due to non-chemical stressors. NHANES exam weights were used to calibrate the results from the NHANES cohorts to the US population. Results suggest that there is an increased allostatic load within the low food availability and education subpopulations as median allostatic load scores were 5.3 vs. 4.2 in the general population (n = 15050) and 3 vs. 2.5 when compared with the total 2017-2018 NHANES cohort. These data groups had relatively large sample sizes (n = 892 and n = 3501, respectively) within the 2017-2018 NHANES cohort (n = 5520). Clear demographic differences were seen within several of the subpopulations with a shift towards minority populations. Notably, the 2017-2018 NHANES general population consisted of 55% white and 38% non-white whereas within the cumulative subpopulation 43% were white and 57% non-white, with 28% being Hispanic, and 18% black. Additionally, this cumulative subpopulation was also younger with a mean age of 38 years vs. 45 years for the general population. Lastly, the magnitude of the excess allostatic load observed in certain subpopulations was contextualized within current sensitivity uncertainty/adjustment factors commonly used in risk assessments. While there is no geographic data available in publicly released NHANES data to overlay onto EJScreen output, this analysis provides a potential methodology which could be applied to other datasets. Such quantitative investigations improve the understanding of susceptibility and may provide the foundation for additional adjustment factors to incorporate into cumulative risk assessments.

Cell Painting, a high content imaging-based phenotypic profiling assay that multiplexes six fluorescent dyes, is currently being leveraged for high-throughput toxicology screening across diverse human-derived cell lines. However, these cell lines have minimal to no chemical metabolizing capacity, thus limiting the testing to individual parent compounds and neglecting relevant metabolism processes. The Predictive Safety Center from Covata Agriscience, in collaboration with the
and identification of the constituents associated with the hazardous properties of composition and cell-based bioactivity of weathered oil samples provides a unique oil types, weathering products that have partitioned into the water column were of weathering for several of the oil types. In addition, we found that for some of the and hepatocytes. Cell-based assays exhibited increased bioactivity over the course evaluated across several human cell types including EA.hy926 endothelial cells, try-mass spectrometry for chemical characterization. In addition, bioactivity was experiments. Each sample was exposed to seawater collected from Galveston Bay, to toxic effects. Recent advances in nontargeted analytical techniques have improved the identification of weathered oil-derived substances, yet the identification of specific compounds that may be responsible for hazardous effects is still an elusive task. In this controlled case study, we therefore evaluated the seawater-based weathering of nine crude oil samples of diverse geographic origins using high-resolution chemical characterization and high-throughput in vitro experiments. Each sample was exposed to seawater collected from Galveston Bay, TX for a total period of one year. Samples of oil slick were collected at 1, 2, 3, and 12 months and water samples were collected at 12 months. All crude oils and weathered samples were extracted and analyzed using ion mobility spectrometry-mass spectrometry for chemical characterization. In addition, bioactivity was evaluated across several human cell types including EA.hy926 endothelial cells, HepG2 hepatoma cells, and induced pluripotent stem cell-derived cardiomyocytes and hepatocytes. Cell-based assays exhibited increased bioactivity over the course of weathering for several of the oil types. In addition, we found that for some of the oil types, weathering products that have partitioned into the water column were more bioactive than the weathered oil itself. Together, the data on the chemical composition and cell-based bioactivity of weathered oil samples provides a unique case study for a chemical-biological analysis of complex environmental mixtures and identification of the constituents associated with the hazardous properties of a whole mixture.

Drug-induced gastrointestinal (GI) toxicity is one of the most common adverse events (AE) in Phase I clinical trials. Symptoms include diarrhea, dehydration, and ulceration which increases the susceptibility to infection partly due to epithelial damage caused by impaired barrier function. Many chemotherapeutic agents have dose-limiting complications due to GI toxicity and result in compromised efficacy in the clinic. Therefore, the early detection of GI liability in novel therapeutics is crucial during preclinical drug discovery. Organoids generated from the human intestinal epithelium recapitulate numerous features of the in vivo adult intestine, including self-renewal and differentiation pathways, cellular composition, and cellular organization. The current work focuses on utilizing intestinal organoids in 96-well viability and barrier function assays for preclinical drug-induced GI toxicity evaluation. Human intestinal crypts were seeded in IntestiCult® Organoid Growth Medium to yield proliferative intestinal organoids. Intestinal organoids were cultured in the presence of varying concentrations of small molecule drugs (gefitinib, colchicine, and acetaminophen) and the responses compared to those observed using Caco-2 cells, a human colon epithelial cell line often used as a model of human intestinal toxicity evaluation. Cultures were treated with small molecules for 5 days, with medium changes every 1-2 days, after which cell viability was assessed by Promega® CellTiter-Glo®. Human intestinal organoid cultures exhibited greater sensitivity to gefitinib (average IC_{50} = 0.819 μM for organoids; n=3 and 11.4 μM for Caco-2; n=2) and colchicine (average IC_{50} = 0.0281 μM for organoids; n=3 and 62.89 μM for Caco-2) when compared to Caco-2 cells treated with the same compounds. The negative control compound, acetaminophen, induced minimal toxicity on either intestinal organoids or Caco-2. The 96-well assay was reproducible, producing similar 50% inhibitory concentrations (IC_{50}) in repeated experiments with cells from the same and different donors (e.g., IC_{50} values of colchicine = 0.0150 - 0.0340 μM for donors 1-3; 0.0296 - 0.0705 μM for 1-3 μM for ileum, and 0.0150 - 0.0340 μM for colon; n=3 donors). In addition to impacting cell viability, drug-induced GI toxicity may also be a result of perturbations to colonic epithelial barrier function, and thus in vitro models that more accurately represent the physiology of the intestinal epithelium compared to immortalized or transformed cell lines may be more predictive of clinically adverse events. Organoid-derived monolayer cultures on Corning® Transwell inserts were used as a model of evaluating barrier integrity in response to drug treatment, using passive low permeability marker molecules to evaluate the state of the barrier in both concurrent (4Kda FITC-Dextran) and post (Lucifer Yellow) multi-day drug exposure. The cultures were exposed to various concentrations of colchicine and Sarpinbin (AZD-8931) and the barrier integrity was assessed via transwell permeability measurements. Many chemotherapeutic agents cause alterations in barrier function (NRT), where the animal moves into position for a procedure at the front of the cage and releasing the squeeze-back as soon as the desired movement toward the cage front is achieved. The goal of this study was to investigate the differences in outcomes of use of these methods on toxicological assessment in NHP studies and provide evidence for best practice in restraint in a laboratory setting. A retrospective analysis on acclimation data from six studies, 3 (83 males and 87 females that employed the procedure cage) and 3(45 males and 75 females that used the pole and collar and restraint chair) was performed. Animals were acclimated over at least six sessions to ensure that they were adequately adapted to the restraint method with provision of visual access to restraint devices where applicable (i.e. chair and pole). Surrogate measures of stress (neurotrophin, eosinophil, monocyte, and lymphocyte counts i.e., stress leucogram), inflammation (albumin, fibrinogen, and prothrombin time) and muscle damage (creatine kinase) levels, which are typically evaluated in toxicological assessments, collected during the acclimation period, were compared between the two preclinical drug discovery. Acclimation procedures by the Shidk’s multiple compares was used to evaluate differences in these clinical pathology parameters for each restraint method. Neutrophilia (p = 0.4684) and Lymphopenia (p = 0.0026) indicative of the stress leucogram were present when the Procedure cage was used compared to the use of the PC/Restraint Chair method. Levels of creatine kinase and fibrinogen were significantly higher in Procedure cage vs PC/RestRAINT Chair animals (p=0.0027) and (p=0.0041) respectively, suggestive of acute muscle damage and inflammation. This study is a direct clinical evaluation of the Procedure cage vs PC/Restraint Chair and provides evidence that the PC/Restraint Chair leads to less stress and inflammation resulting in limited alterations to physiological homeostasis translating to psychological wellbeing of animals.

Drug-induced gastrointestinal (GI) complications are the most frequent adverse events reported in clinical trials. At present, late and expensive in vivo animal studies remain the cornerstone of preclinical GI safety evaluation. Stem cell-based in vitro assays hold the potential to improve early candidate compound selection
and mechanistic investigations, helping to accelerate programs and reducing the number of animals used. Here, we explored if a primary human small intestine (SI) monolayer cell culture system (RepliGut® Planar Platform) could distinguish between compounds with a low, medium, and high potency for the JNJ epigenetic target X with a known in vivo GI liability, using cell viability, proliferation (EdU incorporation), and stem cell gene expression as readouts. To evaluate if the assay was adequate for this intended use, we first tested a limited set of reference compounds including drugs with a high (> 50%) and a low (0-4%) incidence of diarrhea in the clinic at in vitro concentrations up to 100-fold the clinical Cmax. Following a 72-hour incubation, all 6 positive (high incidence diarrhea) but none of the 4 negative (low incidence diarrhea) compounds reduced viability and proliferation of SI monolayers more than 50% compared to DMSO controls. Positive drugs exhibited a proliferation IC50 to clinical Cmax ratio of ≤30, consistent with recent work in a human enteroid viability assay. Having validated the system, we next tested a set of 10 JNJ compounds targeting the JNJ target X at 6 concentrations ranging from 0.123 to 50 µM. At the highest soluble dose (50 µM), only JNJ-001 and JNJ-003 reduced viability and proliferation by more than 50% compared to DMSO controls at 72 hours. Still, there was a trend toward reduction in viability and proliferation with increasing potency for the JNJ target X. We also determined the expression of LGR5 and LGR5, two intestinal stem cell genes that were down-regulated after target inhibition in in vivo mouse studies. Consistent with in vivo findings, LGR5 and LGR5 were decreased in the RepliGut® Planar Platform in response to JNJ compounds inhibiting target X. LGR5 was the most abundantly expressed of the 2 genes in RepliGut® Planar Platform and exhibited a dose-dependent decrease in expression after exposure to JNJ compounds. Additionally, the reduction of LGR5 was more pronounced with increasing potency for the JNJ target X, a decrease in expression by more than 50% vs. DMSO controls was observed for most compounds with high and medium potency at 3.3 and 10 µM, respectively, and for 1 out of 2 compounds with low potency at 50 µM. As seen in vivo, the reduction of LGR5 after inhibition of JNJ target X was less pronounced than that of LGR5 and only from 30 µM onwards most compounds decreased LGR5 expression by more than 50% compared to DMSO controls. These data support the use of stem cell-based models for predicting and mechanistically evaluating novel oncology drugs with non-cytotoxic mechanisms of action, therefore contributing to more informed selection of compounds prior to in vivo studies.

### 3851 3D Primary Liver Cell Spheroids as a Promising In Vitro Tool to Evaluate the Hepatotoxic Potential of Therapeutic Antisense Oligonucleotides

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Antisense oligonucleotides (ASOs) are promising therapeutic modality to modulate disease-causing RNA molecules. However, drug development is hampered by hepatotoxicity. In preclinical and clinical safety evaluation, ASOs concentrate in the liver and can trigger liver enzyme leakage. Investigative toxicology studies ASOs can induce a range of deleterious effects such as the induction of apoptosis, off-target effects, and the saturation of RNA processing machinery. 3D primary liver cell spheroids are regularly used as in vitro tools to study the hepatotoxic potential of small molecule drugs, however their relevance to study the hepatotoxic potential of ASOs has yet to be established. This work evaluates the relevance of the 3D primary liver cell spheroids to study the hepatotoxic potential of ASOs using a set of preclinical and clinical ASOs. In the first part of this work, the uptake of ASO by the model and the resulting downregulation of the RNA target was assessed. Histochemistry results show that ASOs are readily and uniformly taken up by the model, reaching the inner core of the cell spheroid. Also, quantitative PCR analyses show that treatment with ASO leads to a strong downregulation of the target RNA molecule lasting up to 14 days. In the second part of this work, the in vitro cytotoxicity of the ASOs was assessed by monitoring the viability of the model (Cellular ATP content), cytotoxic leakage (LDH leakage) and the induction of apoptosis (cellular Caspase 3/7 activity). Interestingly, the in vitro cytotoxicity of the ASOs correlates with their hepatotoxicity reported previously, with the viability measurement being the most sensitive biomarker. Finally, several ASOs led to an induction of apoptosis in the model, albeit at very different magnitudes, suggesting that this is a common hazard for ASOs. Altogether, these results suggest that 3D primary liver cell spheroids are a promising in vitro tool to assess the hepatotoxic potential of ASOs. In future investigations, more clinical ASOs will be assessed using the same approach.

### 3852 3D Primary Cell Spheroids as a Predictive High-Throughput MPS for Hepatotoxicity Assessment during the Development of Pharmaceutical Drugs


Hepatotoxicity is an important safety hazard that can cause the discontinuation of the development of drug candidates. Testing the toxicity of drug candidates in animal models (in vivo) is endorsed by regulatory authorities and therefore well-established in the pharmaceutical industry. However, numerous drug candidates found to be safe in animal models are hepatotoxic in humans. Moreover, fourteen drugs initially approved by the regulatory agencies had to be finally withdrawn for the American and European markets because of hepatotoxicity in the last thirty years. This shows that the hepatotoxicity assessment process does not accurately predict hepatotoxicity in humans, and that the prediction of hepatotoxicity during preclinical and clinical trials must be further improved. Micro-physiological systems (MPS) raised expectations that their use in industrial practice would improve hepatotoxicity assessment. However, only a few MPS effectively made it beyond proof-of-concept into the regular industrial workflow. Among the available MPS, 3D primary liver cell spheroids accurately model the essential features of the native liver and their ease of use make them compatible with standardized industrial high throughput applications. Moreover, 3D primary liver cell spheroids predict hepatotoxicity more accurately than their 2D primary cultures. To further evaluate the relevance of 3D primary liver cell spheroids for hepatotoxicity assessment, a large set of small molecule drugs was randomly selected from the DILIrank dataset and tested in 3D primary liver cell spheroids. The DILIrank dataset consists of 1,036 FDA-approved drugs that are divided into four classes according to their potential for hepatotoxicity: “Most-DILI-concern”, “Less-DILI-concern”, “No-DILI-concern” and “Ambiguous-DILI-concern”. The 7-day cellular ATP IC50 values of the picked drugs were determined and compared to their human exposure data (total plasma Cmax). Using this approach, 84.2% of “Most-DILI-Concern” drugs were accurately predicted as hepatotoxic, whereas 83.3% of “No-DILI-Concern” drugs were accurately predicted as non-hepatotoxic. These results further validate the relevance of 3D primary liver cell spheroids as a useful in vitro tool for hepatotoxicity assessment. Furthermore, the incorporation of human exposure data to the in vitro results lifts this assay to a yet unparalleled level of predictive power versus scalability and ease-of-use. The sensitivity, specificity, easy-of-use, and cost-effectiveness makes 3D primary liver spheroid models a productive, industry-compatible, MPS for liver safety assessment. It enables the generation of high-quality hepatotoxicity datasets for the drug development process, thus supporting critical internal go/no-go decision-making. In future studies, more DILIrank compounds will be assessed using this approach in collaboration with pharmaceutical partners and regulatory bodies.

### 3853 Cardiac Proarrhythmic Risk Assessment Using Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes


The Comprehensive in vitro Proarrhythmia Assay (CIPA) initiative is an effort to provide an in vitro hERG channel assay and in vitro QT interval measurements. This novel approach includes the use of adult human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs) to assess compounds for potential cardiac liabilities. hiPSC-CMs express ion channels that underlie action potentials and exhibit electro-physiological and mechanical characteristics of native human cardiomyocytes. Thus, hiPSC-CMs represent a more comprehensive and physiologically relevant preclinical model for cardiac liability assessment. In cardiomyocytes, calcium (Ca++) acts as an intracellular second messenger linking the electrochemical signals of the action potential to cardiomyocyte contraction. High levels of intracellular Ca++ lead to contraction of the cardiomyocyte and low levels of Ca++ lead to relaxation. Consequently, the shape and duration of the intracellular Ca++ oscillation may be utilized as a surrogate marker to evaluate the effects of compounds on cardiac function. Eurofins Discovery has developed and validated a high-throughput assay in hiPSC-CMs using a Ca++ sensitive dye and the FLIPR Penta High-Throughput Cellular Screening System to monitor intracellular Ca++ oscillation in the presence of test compounds. This assay shows that Ca++ oscillation in hiPSC-CMs is affected by compounds that target other ion channels separate from Ca++ channels (sodium (Na+) and potassium (K+) channels) and by compounds with known cardiac safety risks. Furthermore, IC50/EC50 values may be calculated for various parameters of the Ca++ oscillation. Taken together, our assay allows for high-throughput assessment of the risk potential of drug-induced delayed ventricular repolarization and QT interval prolongation in humans in various stages of drug development.
Seizure liability remains a significant cause of attrition throughout drug development. The seizure potential of drug candidates is not typically evaluated until the late stage of preclinical discovery, during in vivo toxicity studies. The timing of this assessment means that positive findings of seizure liability could result in the need to identify alternate clinical candidates. This emphasizes the need for improved methodologies to detect seizure liability earlier, ideally with reduced reliance on costly animal studies. Advances in stem cell biology coupled with an increased understanding of the role of ion channels in seizure offer an opportunity for a new paradigm in screening. A combined approach could provide mechanistic insight into off-targets causative of seizure and improve identification of potential seizure mechanisms. A preclinical neuronal cell derived from human induced pluripotent stem cells (hiPSCs) can be incorporated into physiologically relevant in vitro models to predict seizure risk using high-throughput microelectrode array (MEA). hiPSC iCell GlutaNeurons containing 80% glutamatergic/20% GABAergic neurons were plated with astrocytes and monitored using the Axion Maestro Edge MEA system. Application of HESI NeuTox compounds (4-AP, amoxapine, amoxicillin, chlorpromazine, enoxacin, lidopentyltetrathyl, picrotoxin, pilocarpine, phentoin, strychnine) and other seizurogenic drugs (bupropion, clozapine, diphenhydramine, paroxetine, quetiapine) caused characteristic changes to electrical activity in 5 parameters (mean firing rate, burst duration, network burst frequency, network burst duration, and number of spikes per network burst). Exceptions include 4-AP and pilocarpine which caused characteristic changes to the network burst pattern and decreased the frequency of network bursts respectively. The 4-AP iCell GlutaNeurons amoxicillin caused an effect too act as GABA antagonists showed no activity. Assessment of rat hippocampal slices by MEA revealed that the seizurogenic compounds 4-AP, pentylenetetrazole, picrotoxin, bicuculline, gabazine and strychnine increased field potential area and peak number, which is indicative of increased activity and a seizurogenic response. This provides evidence that certain iCell GlutaNeurons may be ideal for an early predictive assay for seizure liability, however the necessity for animal sacrifice points towards the hiPSC/MEA approach as a more ethical, high-throughput and translatable option. Acetaminophen was included as a negative control in both the hiPSC and hippocampal slice assays and had no effect. Likewise the hiPSC/MEA approach, the same seizurogenic compounds as a panel of 15 ion channels with strong links to seizure (Nav1.1, Nav1.2, Nav1.6, Kv7.2/7.3, Kv7.3/7.5, Kv1.1, Kv2.1, Kv3.1, KCa4.1, Cav2.1, GABA α, β) were tested in vitro. Of the ion channels tested, 9/16 compounds inhibited two or more ion channels, and all seizure causing compounds expect pilocarpine demonstrated at least one hit against an panels. Typically, the potassium channels were sensitive to more compounds than the sodium channels and the GABA-A receptor antagonists and picrotoxin have strong specificity for their targets. These studies highlight the potential utility of an integrated in vitro approach for early seizure prediction to provide mechanistic information and support optimal drug design in early development to reduce animal usage and save time and resource.

**3585 Strategies for EEG Monitoring in Toxicology Studies: Chasing Biomarkers of Seizure Activity and Neuropharmacology**

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Drug-induced seizures are a concern in preclinical development given the life-threatening consequences. New generations of telemetry transmitters allow for monitoring of physiological parameters such as EEG and EMG without significant disturbances to conventional toxicity endpoints. Telemetry studies generate significant amounts of data that require analysis to identify seizure activity biomarkers or to assess neuropharmacology. EEG traces were obtained in cynomolgus monkeys and Beagle dogs from general toxicity studies using telemetry with implanted electrodes from the 10-20 system (Cz-Oz, C3-O1 and C4-O2). The use of implanted telemetry devices and appropriate recovery (i.e., 21 days or more) did not significantly alter general toxicity endpoints including body weights, clinical signs, clinical pathology and anatomic pathology. Automated and manual analysis methods were compared to identify EEG biomarkers of seizure. Automated seizure detection tools identified spike trains but could not detect more complex biomarkers of increased susceptibility to seizure such as increased synchrony, isolated sharp waves and isolated spikes or isolated spike-and-waves patterns. Automated EEG analysis alone is insufficient to evaluate traces for biomarkers of seizure activity but can serve as a first line tool to identify areas of interest. EEG traces obtained in a Cz-Oz derivation presented the lowest artefact level which was optimal for automated seizure detection and this derivation was systematically used for the primary analysis including, icl activity detection, qEEG and/or polysomnography. Tremors or myoclonus in normal healthy animals were generally not associated with abnormal EEG activity but this remains a regulatory concern. Based on visual-EEG, the incidence of tremors in normal healthy dogs and cynomolgus monkeys was 5.3% and 5.6%, respectively. The incidence of physiological myoclonus in normal healthy dogs and cynomolgus monkeys was 0.7% and 0.9%, respectively. Salivation was observed in 9.9% and 2.6% of normal healthy dogs and cynomolgus monkeys, respectively. Overall, the data presented herein support the inclusion of EEG monitoring using telemetry in dog and non-human primate toxicology studies.

**3586 The Rat Femoral Epicondylar Defect, a Bone-Healing Model to Assess Skeletal Safety for Therapeutic Compounds Intended for Orthopedic Surgery**

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Therapeutic compounds developed for use during orthopedic surgeries need to be tested in a relevant model, representative of the clinical indication. The rat femoral epicondylar defect is a relevant model that allows addressing cancellous and cortical bone healing in a relatively short period of time. The objective of the study was to quantify and characterize bone healing up to 5 weeks following surgery. Twenty-one female Sprague Dawley rats (13 to 14 weeks old) underwent surgery at the right femur. The procedures involving the care and use of animals were approved by the Animal Care Committee. Holes of 2 mm in diameter by 5 mm in depth were made in the femoral medial epicondyle while rats were under general anesthesia with appropriate pain management. Test material (control item/sterile saline) was instilled into the surgical area before suturing the site. While still under anesthesia, the femur was radiographed to confirm surgical procedure and position of the defect. Post-surgery, animals received appropriate analgesics, additional enrichments, and an Elizabethan collar for 5 days. Digital radiographs of the femur were obtained weekly up to necropsy. A semiquantitative radiographic score was used to assess bone healing. Animals' general health was assessed throughout the study by recording weekly body weight and food consumption. Clinical signs, animals were euthanized 5 weeks post-surgery and femurs were harvested and preserved in formalin. The distal femur was scanned by micro-Computed Tomography (CT), prior to histology processing. For each defect, one volume of interest was contoured. The bone volume (BV) and relative bone volume (BV/TV) of the trabecular region (central region of the defect) and total region (complete defect) were analyzed. Following completion of imaging, the femurs were decalcified, processed histologically, and sectioned in the sagittal plane in the central region of the defect for semi-quantitative histologic scoring of the bone healing. Incorrect defect creation was noted at a low rate and included secondary fractures or sub-optimal position/angulation of the defect within the epicondyle based on radiographs, or occasional severe skin lesion at the surgical site shortly after surgeries. Radiographic evaluation showed progressive signs of bone healing starting at Weeks 2-3 in all animals. Micro-CT images allowed for new bone formation quantification with a mean trabecular and total BV/TV of 17% and 33%, respectively. Histopathology assessment and micro-CT results were well correlated and were characterized by trabecular and cortical bone in-growth into the defect area, which was more significantly in the peripheral cortex than in the center or medullary region of the femur. In conclusion, by the end of the 5-week observation period, the bone healing was considered adequate to allow the evaluation of potential effects on new bone formation by radiographs, micro-CT and histopathology. The rat femoral epicondylar defect is considered as a relevant non-clinical model to address bone healing safety.
mature dehydrogenase and isocitrate dehydrogenase were monitored in 400 cells/minute in a single cell and were measured using the resazurin-to-resazurin method. The data showed that the activity of autophagy was increased by 4.5- and 5.3-fold, respectively, in activated T cells compared to naïve cells, consistent with a known increase in glycolytic dehydrogenase activity.

### 3858

**Minimally Invasive Detection of Acute Systemic Tissue Injury Induced by a Canonical Black Box Drug**

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While current drug discovery approaches are effective in discovering new chemical entities, attrition rates remain estimated as high as 90%. Though many drug candidates advance to later development stages, most drug candidates fail, and even those that are approved still carry the risk of unforeseen adverse events in patients which will result in at the very least black box warnings. Given that many of these adverse events are related to unforeseen drug effects, there is a need to assess such systemic tissue injury induced by candidates as early as possible. This will have a significant impact on pharmaceutical development and drug safety. The goal of this study was to investigate the feasibility of using 99mTc-duramycin, an imaging agent which detects membrane reorganization as a surrogate marker for apoptosis mediating systemic toxicity, in a minimally invasive black box drug using cefoxitin as a model. Doses (high, 200 mg/kg; low 20 mg/kg P.O.) of cefoxitin with established toxicity profiles were used for proof-of-concept and compared with untreated and vehicle treated populations as controls (n=8). Dynamic planar imaging was utilized to determine timepoints for imaging. In vivo SPECT-CT imaging was then performed 72h after treatment and the elevation in 99mTc-duramycin uptake, a surrogate marker for tissue injury, was quantified using t-test and rank sum test. The ratios between each treated animal and the basal level were also calculated to determine the individual susceptibility to drug toxicity. Histopathology was used to validate the findings. Significant elevation in 99mTc-duramycin uptake was detected in multiple organs/tissues in treated animals compared to control. These changes were more apparent and diverse in high dose animals. Additionally, we detected non-apoptotic tissue injury that was not detectable by TUNEL but was accompanied with a prominent elevation in 99mTc-duramycin uptake. For treated rats, susceptible organs/tissues identified using 99mTc-duramycin uptake as indices were consistent with known toxicity of the drug. These changes could be detected in key tissues as early as 24h post treatment. SPECT studies identified that the signal changes in 99mTc-duramycin as a result of drug toxicity are detectable and quantifiable, thus providing critical proof-of-concept for in vivo studies. Histopathology were used to validate the findings. This technology provides a means to assess both on individual and population levels for potentially identifying and gauging systemic tissue injury induced by the black box drugs early and in a minimally invasive manner. This approach has the potential to preemptively identify safety issues early and as to generate a real impact on pharmaceutical development, drug discovery, and drug safety.

### 3859

**Correlating Changes in Body Weights and Immune System Parameters in Cynomolgus Macaque**


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With the continued increase in development of biological and immune-modulating therapeutics, there is a need to assess for potential toxicity of the immune system, especially in relation to other toxicity parameters. Assessment of the immune system is routinely achieved through addition of endpoints, such as, immunohistochemistry and functional assays that detect activation of specific immune cells. This approach allows us to evaluate the dynamic metabolic changes that occur during T-cell activation. CD8+ T cells were activated with anti-CD3 and anti-CD28 antibody coated beads and expanded for 10-days in medium supplemented with 15% Fetal. GAPDH and Glc6P dehydrogenase activity was measured in 5000 cells per 25 μl reads using an Alamar blue reagent. GAPDH and Glc6P dehydrogenase activity increased by 4.5- and 5.3-fold respectively in activated T cells as compared to naïve cells, consistent with a known increase in glycolytic activity upon T-cell activation. In conclusion, bioluminescent dehydrogenase assays can be implemented to monitor metabolic pathways in diverse cell types using low numbers of these cells. These assays can also be miniaturized, making them amenable to high-throughput applications in drug discovery and development.

### 3860

**AAV-Based Automated High-Throughput Live Imaging Assay for Monitoring Cellular Adaptation to Autophagic Flux Modulation**


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Lysosomotropic and autophagy modulation has been linked to drug-induced toxicity in the liver, lung, retina, heart and gastrointestinal tract (1, 2). Although the mechanism is unclear, lysosomotropic and drug-induced tissue injury are reportedly concurrent and therefore assays that identify compounds that induce lysosomal impairment and subsequent cell injury are desired. Lysosomes damaged by lysosomotropic agents are selectively fused to autophagosomes during the autophagic flux process which is a vital process for the maintenance of lysosomal homeostasis and cell death (3). Hence, monitoring the activity of autophagy offers valuable insights on cell health. A gold standard for monitoring autophagy is to directly monitor LC3B during autophagosome to autolysosome formation. Imaging analyses have so far been conducted via low-throughput methods such as stable reporter cell line generation, transient transfections, fluorescence dye or antibody staining. Several imaging based LC3B ratiometric pH probes have been developed but are limited in their signal range for high pKa compounds. For example, previously reported EGFp-mCherry-LC3B ratiometric pH sensor can reliably detect pH5 to pH7 but lacks the sensitivity to detect >pH7 due to intrinsic limitation in the dynamic range. To overcome these limitations and improve monitoring of autophagy in a more robust manner, we developed an AAV viral-based ratiometric probe SuperesluckPHiuorin-IRFP670-LC3B by fusing LC3B with pH sensitive SuperesluckPHiuorin (SEP) and pH stable IRFP670 in tandem. Also, to better understand the dynamic autophagic process, we generated AAV-LAMP2a-mApple reporter. The combination of LC3B and LAMP2a reporters allows classification and quantification of endosome, autophagosome and autolysosome in the same cell. To test autophagy at the single cell level, we generated a nuclei reporter AAV53BP-CmtagFP2, which serves as a reporter for nuclei integrity (4). Using a blue, fluorescent analog of chloroquine (Alkyn-chloroquine/ AAVCQ) we demonstrated that this assay can monitor autophagosome fusion and autolysosome formation, aggregation, and centripetal movement. After 24 hours treatment, CQ or ACQ dose dependently increased size and pH of autolysosome. While these effects peaked at ~10μM, the blockage of endosome/autophagosomal fusion occurred at doses near or higher than LC50 (~55μM). Autophagic flux modulation is already clinically indicated and our data further showed that this imaging platform allows biphasic classification of small molecules as autophagy inducers or inhibitors, as well as determination of lysosomotropic, by quantifying pH, size and number of autophagic vesicles per cell level. The developed chemical dye staining-free live imaging assay allows us to monitor drug effects on autophagy in real-time and to test cytotoxicity in parallel. To understand cellular adaptation to autophagic flux modulation, we tested a list of ~70 marketed drugs and tool compounds in HepG2 cells. We found that increase pH, size and decrease numbers of autophagic vesicles followed by dose dependently increased LDH release reflects a maladaptive response to autophagic flux inhibition. Finally, we further demonstrate that using imaging platform is broadly applicable to a wide range of cell models. Reference: 1. Khoo-Reiter S, et al. Contribution of membrane trafficking perturbation to retinal toxicity. Toxicol Sci 2015;145:383-395. 2. You M et al. Effect of ethanol on lipid metabolism. J Hepatol 2019;70:237-248. 3. Maegma I, et al. Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury. EMBO J 2013;32:2336-2347. 4. Yang KS, et al. Single cell resolution in vivo imaging of DNA damage following PARP inhibition. R. Sci Rep. 2015;5:10129.
on established criteria for negative or low viral titers by AAV neutralizing antibody assay (≤ 5 nAbs in HEK293 cells). Pretreatment with 2 mg/kg of dexamethasone at approximately 1-2 hours prior to AAV administration was adequate to mediate immune related responses. There was no discernable effect of AAV administration on body weight, and most abnormal post dose clinical signs were minor and not directly attributable to the AAV vector. In conclusion, this historical data set serves as a guide for more informed study designs for AAV vector-based therapeutics and allows for potential reduction and refinement of animal use in their safety testing.

3862 Strategies for Reducing the Number of Animals in Toxicity Testing: A Comparative Approach for Rodent and Large Animal Studies

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It is essential that the scientific community continually re-evaluates study designs and technology that will enable reduction in the number of subjects used for toxicology studies, an approach that aligns with the 3Rs of experimental animal welfare (Replacement, Reduction and Refinement). In rodents, we adopted blood microsampling as a refinement to the more common technique of needle and syringe by leveraging the Mitrax Volumetric Absorptive MicroSampling (VAMS®) device for accurate and precise collection of low sample volume. For example, by microsampling only 10 μL of whole blood, an entire cohort of study animals in the traditional needle and syringe collection was eliminated from the project since serial samples could also be collected from the main study animals. This represented a reduction in the number of study animals by 55% and 100% for mouse and rat studies, respectively. Collection of samples from the same cohort of animals allows correlations between PD findings and the actual drug exposure profile. In nonhuman primates (NHP) and dogs, we carefully reviewed the number of animals in control and recovery cohorts in chronic studies that had data from prior subacute studies. This enabled reduction by >25% in number of control group with no recovery cohort including in the low dose group. The NHP study design was reviewed and accepted for conduct by a regulatory agency. Each approach will be discussed further to highlight the pros and cons of each in order to allow for a more informed decision when designing toxicity studies.
beads to allow positive selection of CD34+ cells. Our methods have produced 73 to 95% purity of CD34+ cells across our studies, with a cell yield between 1.34 to 40.70 x 10^6 CD34+ cells across studies. We demonstrate high degrees of purity (>70%) in isolating CD34+ cells from apheresis product of non-human primates by immunomagnetic bead separation. This method can be harnessed to produce a large variety of cells for immunotherapies, as well as to develop, test, and bring to market CAR T cells produced though allogeneic methods.

Mitochondria are important ATP generating organelles that support cellular function and viability. Drug-associated mitochondrial dysfunction has been implicated as a cause of drug toxicity in non-clinical species and in humans. Pharmaceutical companies have leveraged several in vitro methodologies to evaluate the potential for drug candidate-induced mitochondrial toxicity. However, the determination of drug-induced mitochondrial toxicity in vivo is confounded by the complexity of the phenotypes and lack of sensitive and specific translational and mechanistic biomarkers. Elevation of lactate by aerobic glycolysis to compensate for decreased ATP production during mitochondrial inhibition is well established in in vitro systems, and lactic acidosis has been linked to toxicity of antiviral therapies, linezolid, and biguanide. But as many pathophysiological factors affect lactate levels in humans, this rendered doubt whether a moderately elevated plasma lactate level is a biomarker for mitochondrial toxicity. Nonetheless, plasma lactate may be valuable as a weight of evidence biomarker for mitochondrial toxicity in a well-controlled rat study. In this work, we correlated in vivo findings with mitochondrial toxicity calls in vitro. First, we examined basal circulating levels in naive and vehicle control Wistar Han rats and determined an average of approximatel 1.6 mM lactate. Second, we evaluated 42 Merck chemicals for effects on plasma lactate in rats after 4 days of dosing. The test set included 17 compounds considered positive, and 25 compounds considered negative based on mitochondrial toxicity calls in vitro. In the in vitro IC50 of these compounds was then compared to the Cmax and AUC values in the respective rat studies. Among the 17 in vitro mitotoxics, the 11 compounds that had ≥ 4 mM plasma lactate in general had higher Cmax/C50 and AUC/C50 than the ones with subthreshold levels of lactate. The 12th compound that had a peak of ≥ 4 mM lactate had very toxicological marginal negative result may reflect insufficient in vitro testing relative to in vivo Cmax and AUC. We also found that there was no significant elevation of lactate in mitotox negative compounds in studies with evidence of organ injuries and/or inflammation (based on gene signature, clinical biochemistry, and gross tissue evaluations) that could impact lactate production and removal, including liver, kidney, cardiac, and skeletal muscles. These results indicate that organ metabolism (organ lactate production and lactate removal) are more important factors for plasma lactate in rats. Taken together, this evaluation supports exposure as a critical factor for mitochondrial toxicity in vitro/in vivo translation and the use of plasma lactate as an informative biomarker in well-controlled rat studies where mitochondrial toxicity is a concern. Additional evaluation is warranted to strengthen weight of evidence and knowledge of this biomarker to facilitate its use for detecting mitochondrial toxicity in vivo.

Mitochondria are important ATP generating organelles that support cellular function and viability. Drug-associated mitochondrial dysfunction has been implicated as a cause of drug toxicity in non-clinical species and in humans. Pharmaceutical companies have leveraged several in vitro methodologies to evaluate the potential for drug candidate-induced mitochondrial toxicity. However, the determination of drug-induced mitochondrial toxicity in vivo is confounded by the complexity of the phenotypes and lack of sensitive and specific translational and mechanistic biomarkers. Elevation of lactate by aerobic glycolysis to compensate for decreased ATP production during mitochondrial inhibition is well established in in vitro systems, and lactic acidosis has been linked to toxicity of antiviral therapies, linezolid, and biguanide. But as many pathophysiological factors affect lactate levels in humans, this rendered doubt whether a moderately elevated plasma lactate level is a biomarker for mitochondrial toxicity. Nonetheless, plasma lactate may be valuable as a weight of evidence biomarker for mitochondrial toxicity in a well-controlled rat study. In this work, we correlated in vivo findings with mitochondrial toxicity calls in vitro. First, we examined basal circulating levels in naive and vehicle control Wistar Han rats and determined an average of approximately 1.6 mM lactate. Second, we evaluated 42 Merck chemicals for effects on plasma lactate in rats after 4 days of dosing. The test set included 17 compounds considered positive, and 25 compounds considered negative based on mitochondrial toxicity calls in vitro. In the in vitro IC50 of these compounds was then compared to the Cmax and AUC values in the respective rat studies. Among the 17 in vitro mitotoxics, the 11 compounds that had ≥ 4 mM plasma lactate in general had higher Cmax/C50 and AUC/C50 than the ones with subthreshold levels of lactate. The 12th compound that had a peak of ≥ 4 mM lactate had very toxicological marginal negative result may reflect insufficient in vitro testing relative to in vivo Cmax and AUC. We also found that there was no significant elevation of lactate in mitotox negative compounds in studies with evidence of organ injuries and/or inflammation (based on gene signature, clinical biochemistry, and gross tissue evaluations) that could impact lactate production and removal, including liver, kidney, cardiac, and skeletal muscles. These results indicate that organ metabolism (organ lactate production and lactate removal) are more important factors for plasma lactate in rats. Taken together, this evaluation supports exposure as a critical factor for mitochondrial toxicity in vitro/in vivo translation and the use of plasma lactate as an informative biomarker in well-controlled rat studies where mitochondrial toxicity is a concern. Additional evaluation is warranted to strengthen weight of evidence and knowledge of this biomarker to facilitate its use for detecting mitochondrial toxicity in vivo.

To manage patient care, it is often of interest to obtain safety ranking of several drugs in the same class for treatment selection. However, individual randomized clinical trials, which are designed to evaluate the efficacy of drugs, often do not have the sufficient sample size to accurately estimate the risk for adverse events. Meta-analysis, as an established method to synthesize evidence across studies, is a promising approach to address this problem. However, meta-analysis of clinical trials for adverse events has the added challenge that some adverse events are very rare and clinical trials might only report events above a certain threshold. Penalized Bayesian models provide a flexible way to accommodate study heterogeneity and the reporting threshold (Qi et al. 2022). In this paper, we present a case study in generating safety ranking for PD-1 and PD-L1 inhibitors. It builds on published data regarding 125 clinical trials for five immunotherapy drugs (nivolumab, ipilimumab, pembrolizumab, atezolizumab, avelumab, and durvalumab), seven cancer types, and 75 adverse event categories. Our results demonstrate that penalized Bayesian meta-analysis model can successfully borrow strength from different clinical trials to estimate the risk of each adverse effect for each drug cancer combination and in turn generate safety ranking for the drugs. We have developed a ranking method to summarize over dozens of adverse effect categories. As an advantage for Bayesian methods, the uncertainty of the results can be easily quantified. Our results show there is significant drug*cancer interactions while each drug tends to have better or worse risk ranking over the majority of adverse effect categories.

These findings and the safety ranking of drugs will be useful for treatment selection regarding different cancer types. We plan to further compare our results to patterns in postmarket surveillance data such as those in the FDA Adverse Event Reporting System (FAERS).

**3868** Post-market Safety Assessment of a Commonly Used Antioxidant in Polymeric Food Contact Articles

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Tris(2,4-di-tert-butylphenyl) phosphate, commercially known as Irgafos 168 (I-168), is a commonly used secondary antioxidant used in the manufacture of polymeric food contact articles. I-168 degrades during use in polymers to several degradation products, including phosphate species such as Irgafos 168-phosphate (I-168ate). The main toxicological concern for I-168 is the potential for I-168ate to act as a neurotoxicant like some other organophosphates. As a result, we performed a safety assessment for I-168 and its degradants when used as an antioxidant in food contact articles. Our comprehensive literature review of toxicological data and supporting structure activity relationship (SAR) analysis to predict I-168 reactivity against acetylcholinesterase diminished the concern for potential neurotoxic effects of I-168 and its degradants. An acceptable daily intake (ADI) value of 1 mg/kg bw/day for I-168 was derived from a two-year rodent combined chronic toxicity/carcinogenicity study which is higher than the combined CEDI and supports the safety of currently authorized food contact use levels of I-168. This post-market review is an example of FDA’s re-evaluation of previously authorized food contact substances to support its mission of protecting the public by ensuring the safety of our nation’s food supply.

**3869** Aryl Hydrocarbon Receptor is a Xenobiotic Sensor That Alters Intestinal Epithelial Homeostasis

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The Aryl Hydrocarbon Receptor (AHR) has been implicated in maintaining intestinal barrier function through influencing epithelial cell proliferation and differentiation in the gut epithelium. AHR is involved in maintenance of intestinal epithelial gene expression leading to altered proliferation and lineage commitment. Both Ahr gene expression and activity, as marked by its target gene Cyp1a1 (Cytochrome P450 family 1, subfamily A, polypeptide 1) expression, demonstrate a decreasing trend across proximal-distal gradient and crypt-villus gradient. Genetic deletion of the AHR gene alters the ability of intestinal epithelial cells to differentiate into Paneth and goblet cell lineages, enterocyte and secretory cell differentiation markers, compromises barrier function, while increasing crypt-associated epithelial cell proliferation as shown by short-term BrdU (Bromodeoxyuridine) incorporation. Exposure to AHR ligands through a 15% broccoli diet decreased the proliferative index in the crypts. Ahr-/- mice on a diet devoid of AHR ligands. In contrast, a broccoli diet increased goblet cell number. Expression of enterocyte and secretory cell differentiation markers (MATH1, Klf4 and Tff3) positively correlated with AHR transcriptional activity in both chow and broccoli diet. Dietary AHR ligands are likely protective against intestinal damage caused in human pathologies such as infection and during side-effects of chemotherapeutic drugs such as doxorubicin. We demonstrate that IC2 (Indolo(3,2-b)carbazole) , a potent AHR ligand present in cruciferous vegetables, promotes wound-healing effect in Caco2 colon carcinoma cell lines, when co-treated with doxorubicin. Studies with the AHR antagonist CH23191 show a decrease in goblet cell numbers in broccoli-fed corn, wheat, sorghum, asparagus and, more recently, garlic. Manifesting as garlic rot, F. proliferatum infection of garlic poses severe economic threats as garlic is grown in nearly every region of the world. North America saw its first outbreak in September of 2001. In areas of the globe where diet may not be varied, fusonimines

**3870** Validation of a Multi-mycoxin Method for Detection of Fusimonins in Garlic

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Fusimonins are a group of mycoxins produced by Fusarium proliferatum that are known for their hepat-, nephro- and neurotoxicity to mammalian herbivores. The threat of fusonimines lies within several food items, including corn, wheat, sorghum, asparagus and, more recently, garlic. Manifesting as garlic rot, F. proliferatum infection of garlic poses severe economic threats as garlic is grown in nearly every region of the world. North America saw its first outbreak in September of 2001. In areas of the globe where diet may not be varied, fusonimines

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can be a particularly potent threat. The aim of this project was to validate a method previously used in peanut and corn matrices for use in detecting fumonisin B1 and B2 in garlic. A method validation process was used to establish quality control parameters including linearity, accuracy, selectivity, and robustness. Linearity for fumonisins B1 and B2 was established across a range of 7 ng/g-1,665 ng/g, with R² of 0.993 and 0.999, respectively. Accuracy was determined with a limit of detection of 0.1 µg/g, and recovery in nonextracted control was 100% ± 10%. For this study, we hypothesized that DNP exposure would lead to decreased cell count and alter cellular physiology in a dose-dependent manner in nonmalignant colonic epithelial. To test this hypothesis, young adult mouse colonocytes (YAMC) cells were exposed to varying concentrations of DNP, ranging from 0.001µM to 2mM, and cell number was assessed. A significant suppression of cell count was observed at 10 µM, 100 µM, 1 mM, and 2 mM after 96 hours of exposure. To understand why cell number was suppressed, a 5-bromo-2-deoxyuridine (BrDU) cell proliferation assay was performed and showed a comparable reduction in cell count at 1 mM, 100 µM and 1 mM. Additionally, lactate dehydrogenase was measured to assess cytotoxicity. No significant increases in cytotoxicity were observed at any dose. Next, to understand the mechanisms contributing to DNP’s role in the reduction of cell count and proliferation, DNP was tested as a ligand for the aryl hydrocarbon receptor (AhR). Using an in vitro screen of xenobiotics and mediating toxicity. A BrDU assay in an AhR-knockout YAMC line showed equivalent reduction in proliferation, suggesting that DNP suppresses cell count and proliferation in YAMC in an AhR-independent manner. Studies to further explore the mechanisms are ongoing, including evaluating gene expression associated with gut permeability and inflammation. In conclusion, DNP exposure results in a reduction in cell count and proliferation at moderate to high concentrations in this model. It is unlikely that DNP is exerting these effects by acting as an agonist for AhR. Understanding the physiological impact of oral DNP exposure will allow further evaluation of its toxicity profile.

### 3871 Food Emulsifiers Increase Toxicity of Food Contaminant Acrylamide

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Emulsifiers are extensively used in processed food to enhance product stability while emulsifiers mostly have low toxicity, recent studies have found that they could lead to alteration of gut microbiota community and subsequently gut inflammation. Such effects opened up further questions as to whether emulsifiers could lead to higher sensitivity of the individual due to higher chemical uptake and/or increased toxicity as a result of impaired gut lining integrity. Importantly, food emulsifiers are typically used in food products containing other food additives and contaminants. For example, food emulsifiers are used in baked products which also contain process contaminant acrylamide. We performed a local survey of over 100 baked products and all contained at least one food emulsifier. Therefore, in this study we hypothesized that toxicity of acrylamide will be increased in the presence of food emulsifiers polyoxyethylene sorbitan monooleate (Twee80) and glycerol monostearate (GMS). As anticipated, addition of low levels of Twee80 and GMS increased acrylamide toxicity to small intestine epithelial cell HIEC-6 and human colorectal adenocarcinoma cell Caco-2. 72h IC50 values of acrylamide alone was 4.87 ± 0.06 mM for HIEC-6 and 4.67 ± 0.18 mM for Caco-2. When Twee80 was added, IC50 value decreased by 22% to 3.88 ± 0.36 mM in HIEC-6 and 50% to 2.34 ± 0.45 mM in Caco-2. The effect of GMS was not as significant where IC50 value increased by 54% to 7.50 ± 0.35 mM in HIEC-6 and only decreased by 8% to 4.28 ± 0.20 mM in Caco-2. In the presence of Twee80, uptake of acrylamide in HIEC-6 cells was increased by 44% and in Caco-2 cells 9% respectively. Twee80 and GMS also significantly increased cell membrane permeability in HIEC6 and Caco-2 cells. Gene expression of proinflammatory cytokines (IL-1β and IL-8) and oxidative stress biomarker (SOD) were also significantly increased by Twee80 and GMS co-exposure. These results suggest that low levels of food emulsifier Twee80 could increase toxic potential of acrylamide and is partly explainable by increased uptake. Acute uptake experiment in SD rats showed that when co-exposed with Twee80, glyciamide, main metabolite of acrylamide, was increased by as much as 52% in serum concentration of glycidamide, main metabolite of acrylamide, was increased by as much as 52% in serum 3 hours after oral administration. The effect of GMS was even more significant where serum concentration of glyciamide increased by 84%. The presence of emulsifiers, however, have no significant effect on serum acrylamide levels. Wide type C57BL/6J mice were given acrylamide and emulsifiers by oral gavage for 2 weeks. Addition of Twee80 and GMS increased neurotoxicity of acrylamide as rotor-rod performance of mice decreased from 28.53s with Twee80 and 22.21s with GMS to 20.38s with both. Pre-exposure to Twee80 and GMS significantly increased acrylamide toxicity in the presence of nanomaterials (silica and titania) interacted-milk proteins and milk proteins changed in the presence of nanomaterials, and an enhancement in milk proteins’ antigenicity in the presence of dietary nanomaterials, and human mast cells (Laboratory of Allergic Diseases 2- LAD2) sensitized with allergic human sera were studied by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR), Ellman’s assay and fluorescence spectrometry. An indirect enzyme-linked immunosorbent assay (indirect-ELISA) was used to identify changes in milk proteins’ antigenicity in the presence of dietary nanomaterials, and human mast cells (Laboratory of Allergic Diseases 2- LAD2) sensitized with allergic human sera were used to study changes in antigenicity. Results showed that the structure of milk proteins changed in the presence of nanomaterials, and an enhancement in the antigenicity of nanomaterials (both silica and titania) interacted-milk proteins/-skimmed milk. Similarly, mast cell degranulation (a representation for antigenicity)
was higher when exposed to particle-interacted skim milk, where titania nanomaterials had the most significant impact, and this affinity persisted even after being subjected to simulated gut digestion. As evidenced by these studies, particles induced alterations in the structure of milk proteins are reasoned to be the allerogenicity of milk proteins.

In utero, via ingestion of contaminated breast milk, or during preadolescence. Considerably less is known about the influence of DEHP exposures on other organs/organ systems, such as those in the lower gastrointestinal tract. The alkyl chain length of DEHP increases the likelihood of it transiting through the upper gastrointestinal tract unperturbed, potentiating an interaction between it and the large intestine. As such, we sought to characterize the effects of varying doses of DEHP in a conditionally immortalized cell derived from the colonic epithelium of a transgenic mouse: the Young Adult Mouse Colonicoyte (YAMC) cell line. As DEHP has been shown to influence cellular physiology through estrogen receptor-mediated mechanisms, we hypothesized that the compound would reduce the growth of YAMCs in a dose-dependent manner similar to that of estradiol. Using cell growth as our initial outcome, we first showed that 72-hour exposure of DEHP at concentrations of 10 μM, 100 μM, 1 mM, and 10 mM reduced cell number in a statistically significant, dose-dependent manner. In an effort to assess the manner in which DEHP reduced cell count, we assessed its influence on cell proliferation, using Bromodeoxyuridine (BrdU) as a cellular marker. Seventy-two hour DEHP exposures of 10 μM, 100 μM, 1 mM, and 10 mM resulted in statistically significant decreases in BrdU incorporation, in a dose-dependent manner. We then sought to determine if the reduction in YAMC growth and proliferation following dose-dependent exposure to DEHP was a product of cytotoxicity. Using a Lactate Dehydrogenase (LDH) colorimetric cytotoxicity assay, YAMCs were exposed to DEHP concentrations of 10 μM, 100 μM, 1 mM, and 10 mM over 24 hours; we found no statistically significant increases in the presence of LDH in the culture medium compared to vehicle control. Ongoing analyses include assessment of time and dose-dependent apoptosis as well as estrogen receptor signaling. Collectively, our findings highlight a growth inhibitory effect of DEHP on colonic epithelial cells as a product of proliferation inhibition. Future investigations will include analysis of the expression associated with the above findings as well as defining the impact of DEHP on colonic epithelia in vivo.

Over the years, increasing exposure to environmental pollutants and contaminants has been a major challenge of public health concerns globally. Agrochemicals including organophosphates pesticides like Dichlorvos (DDVP) with brand name DD-Force in Nigeria, a major chemical used for control of pest infestation, fumigation, and storage of agricultural materials. This research investigated the ameliorative effects of DDVP exposure in male albino rats. Thirty (30) weight-matched rats were grouped into five (5) cages of six (6) rats each and fed on rat chow and water ad libitum. Group one (1) was the control while group two (2) was the animal model administered DDVP alone. Groups 3, 4, and 5 were administered low, medium, and high doses of *H. madagascariensis* stem bark concurrently with dichlorvos exposure at 10mg/kg. 1% Ombivit was adopted for administration for the thirty (30) days of study. The rats were humanely sacrificed under chloroform anesthesia and blood sample, liver, and kidney were harvested for laboratory analysis using standard biochemical procedures. Biochemical enzyme markers, Oxidative stress biomarkers as well as antioxidant properties were assayed. Also, histopathological examination and morphological changes were observed. Results from the study showed increased liver function enzymes (ALP, AST, ALT) in DDVP-exposed rats while kidney parameters (Creatinine and Urea) showed increased enzymatic activities also. Reduced enzymatic activities were observed in treatment groups (3, 4, and 5). The result showed that DDVP reduced the GSH, SOD, CAT, and GPx antioxidant parameters but was greater at 10 μM, 1 mM, and 10 mM reduced cell number in a statistically significant, dose-dependent manner in treatment groups. Similarly, Oxidative stress biomarkers (MDA, NO) were being reduced were reverted in treatment groups. The histopathological examination showed a distorted kidney revealing glomeruli and occulted Bowman’s capular space. However, the treatment groups (3, 4, and 5) showed restored cell architecture. The findings validated the ameliorative effects of *H. madagascariensis* stem bark against dichlorvos exposure in male albino rats.

Protein supplements’ (PS) consumption is globally increasing. 49% of adults in the US declare taking health supplements. The PS market size was valued at around US$ 20.19 Billion in 2021 and is projected to reach US$ 32.56 Billion by 2028. Contrary to the popular belief that only those interested in fitness and sports consume these PS it has been observed that anyone (mainly 25 to 34-year-old) interested in physical health is potential PS consumers. Notably PS are being used for the improvement of the physical performance but also for nutritional purposes. If ingested in combination with balanced eating and a fitness plan, the daily consumption of PS is associated with multiple benefits (Weight Loss, muscle Gain, contribution to daily nutritional requirements). Most manufacturers
recommend a daily intake of 25-30 g of PS. However, there is a rising concern about the risks derived from excessive intake. Highly daily doses of PS (> 60 g) may expose the consumers to potential health risks since these products may contribute not only to the daily ingestion of proteins but also to potentially toxic elements (PTE) like Al, Zn, Cd, Pb, Ni, Co, Cu which have daily intake reference values established as Tolerable weekly intake (TWI), Upper Level (UL), Tolerable Daily Intake (TDI) and Standard BMDL10 of this study were to determine, using ICP-OES, the content of Al, Zn, Cd, Pb, Ni, Co, and Cu in different brands of PS; to estimate the dietary exposure to these PTE from PS in different daily consumption scenarios and to characterize the potential health risks. The estimated dietary intakes (EDI) were calculated considering 70 kg as average b. w. To characterize the risks, the percentages of contribution to the reference values (100 EDI/Reference value) were calculated considering the following recommendations: TWI for Al 1 mg/kg bw/week (EFSA, 2011a); UL for Zn; 25 mg/day (EFSA, 2006); TDI for Cd; 2.5 μg/kg bw/week (EFSA, 2011b); TDI for Ni; 13 μg/kg bw/day (EFSA, 2020); TDI for Co; 0.0016 mg/kg bw/day (EFSA, 2012a); UL for Cu; 5 mg/day (EFSA, 2006). In the case of Pb, the MOE approach was used (BMDL10/EDI) considering the BMDL10 values of 1.5 μg/kg bw/day for cardiovascular toxicity and 0.63 μg/kg bw/day set for nephrotoxicity (EFSA, 2012b). A total of 74 SP samples of whey protein supplements were analysed. Mean concentrations were: 7.189 mg Al/kg; 14.600 mg Zn/kg; 0.021 mg Cd/kg; 0.051 mg Pb/kg; 0.319 mg Ni/kg; 0.065 mg Co/kg and 2.567 mg Cu/kg but the max concentrations detected were 35.22; 101.51; 0.06; 0.45; 1.40; 0.31 and 10.42 mg/kg for Al, Zn, Cd, Pb, Ni, Co, and Cu, respectively. In a 60 g PS/day consumption scenario, the exposure assessment estimated the dietary intakes in: 2.113 mg Al/day; 6.091 mg Zn/day; 0.004 mg Cd/day; 0.027 mg Pb/ day; 0.084 mg Ni/day; 0.019 mg Co/day and 0.625 mg Cu/day which represents 24.36% of the UL for Zn and 12.50% of the UL for Cu; 21.13% and 15.59% of the TWI for Al and Cd and 9.23% and 6.86% of TDI set for Ni and Co. In the case of Pb, the MOE was estimated to be 3.85 and 1.62 for cardiovascular and nephrotoxic effects, respectively. All estimated MOE were above 1 so no risks are expected from the occurrence of Pb in PS. Zn is the metal with highest concentrations followed by Al and Cu. High consumption of PS contributes to the daily intakes of PTE but these elements have several dietary sources so high consumers of PS may be at risk of overpassing the reference values set for these PTE. To prevent potential health risks, it is recommended to monitor the occurrence of these elements in PS, to assess de total daily intakes of PTE in diverse profiles of consumers and communities and to legislate maximum levels of these elements in PS.

3880 Polycyclic Aromatic Hydrocarbons, Acrylamide, and Heterocyclic Aromatic Amines Formation and Correlations in Air-Fried Foods and Evaluation of Dietary Exposure to These Contaminants

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Gas chromatography/mass spectrometry (GC/MS) was used to assess the content of polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAAAs), and acrylamide (AA) in 184 samples of agricultural, fishery, and animal foods prepared in an air fryer. The PAH4 levels of benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), chrysene (CHR), benzo[k]fluoranthene (BkF), and indeno[1,2,3-cd]pyrene (ICP) were sourced from five different Fish markets, and tissues (Muscle, liver, gill, and skin) were obtained from the selected fish markets in the Ibadan metropolis. The results showed the presence of glyphosate, glufosinate, chlorothalonil, and diazinon in the fish tissues. The levels of these contaminants were determined using Liquid Chromatography (LC) coupled to tandem Mass Spectrometry (MS/MS) and High Performance Liquid Chromatography (HPLC).

3882 Concentration of Eight Polycyclic Aromatic Hydrocarbons in Salted Mackerels Cooked with Different Cooking Methods and Conditions Analyzed by Gas Chromatography with Mass Spectrometry


Food contains various hazardous substances, such as artificially occurring chemicals, synthetic compounds, and chemicals produced during cooking. The interest in hazardous substances generated during cooking is increased due to their impact on human health. Polycyclic aromatic hydrocarbons (PAHs) are various organic compounds containing 2 or more benzene rings, which are derivatives of hydrocarbons. They are produced when the contaminants formed during the incomplete combustion and pyrolysis of organic substances known to be one of the most important pollutants in water and food processing. PAHs have carcinogenic, mutagenic, and bioaccumulative properties that can cause acute and chronic diseases such as nausea, diarrhea, cataract, and liver damage. These compounds enter marine ecological systems through the atmosphere, rivers, and sea waters. Then, they are transferred to fish via food, sediments, and water and accumulate in adipose tissue. The bioaccumulation or formation of these PAHs in fish is influenced by internal factors, such as the composition and pH of the fish, and external factors such as cooking time and temperature applied, and distance between the fish and heat sources. This study aimed to analyze 8 compounds of PAHs, benz[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (CDD), dibenzo[a,j]anthracene (DahA), and benzo[g,h,i]perylene (BghiP) in salted mackerels, which is generally consumed fish in South Korea, cooked with 4 different cooking methods (air-fried, pan-fried, infrared-cooked, grilled) and conditions (time and temperature) by gas chromatography with mass spectrometry (GC-MS). PAHs were not detected in most of the salted mackerels cooked with an air-fryer, pan, and infrared cooker, but grilled salted mackerels showed a high concentration of PAHs, and the highest concentration of PAHs was detected in the salted mackerels grilled at 180 °C for 10 min.

3883 Evidence of Glyphosate and Glufosinate Residues in Non-target Aquatic Food Animals: A Call to One Health Concern


The extensive worldwide use of broad-spectrum, non-selective, and post-emergence herbicides like Glyphosate (N-phosphonomethyl glycine) and Glufosinate (ammonium di-homoalanin-4-(methyl) phosphate) in various applications for weed and vegetation control in aquatic systems and non-crop areas respectively modifies the environment which stresses the living microorganisms. Aminomethylphosphonic acid (AMPA) is the major degradation product of glyphosate found in plants, water, and soil. A total number of live twenty-five adult Clarias gariepinus were sourced from five different fish markets, and tissues (Muscle, liver, and kidney) were harvested and separately put inside the sterile sample bags, sealed, and kept under the ice for glyphosate and glufosinate residue concentration analysis using High-Performance Liquid Chromatography (HPLC, Model Waters 616/626). The results showed the presence of glyphosate, glufosinate, and Aminomethylphosphonic acid (AMPA) residues in all the seventy-five (75) fish tissue samples obtained from the fish market in Ibadan metropolis and all residue concentrations were above both the recommended Acceptable Daily Intake (ADI) and Maximum Residue Limits (MRL) of 1.0 mg/kg and 0.01mg/kg respectively. N-Phosphonohexyl has highest residue concentrations in each tissues analysed and average pooled mean residue concentrations (10.17mg/kg, 16.32mg/kg, and 20.74mg/kg) followed by Aminomethylphosphonic Acid (8.64mg/kg, 13.93mg/kg, and 17.78mg/kg), and glufosinate (0.94mg/kg, 1.53mg/kg, and 1.96mg/kg) across the sampled markets in River, Kidney and Muscle respectively. Among the tissues analyzed, muscle samples have the highest glyphosate, glufosinate, and Aminomethylphosphonic acid (AMPA) residues concentration across all

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the sampled markets in individual tissues and pooled mean residue concentration, followed by kidney tissues, while the liver had the lowest residue concentration. All the residue concentrations are significantly different across all the three analyzed herbicides since all the critical values were lower than F calculated. The presence of the residues of glyphosate, glufosinate, and Aminomethylphosphonic acid (AMPA) in non-target food animals is an indication of public and environmental health hazards with attendant effects on food safety.

A comprehensive safety evaluation was performed to assess the residue concentrations of glyphosate and its metabolites in cultured African Catfish offered for sale in selected markets in Ibadan. A total of twenty-five (25) adult Clarias gariepinus (300 ± 50g) were sourced from five (5) selected active fish markets (Ojo, Iwo road, Eleyele, Challenge, and Apata) within Ibadan metropolis and transported to the Fish and Wildlife Unit Laboratory, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan where the fish were sacrificed and tissue (liver, kidney, and muscles) were collected and the tissues were homogenized. The samples were transported to Germany and were analyzed in the Laboratory of Trace Elements and Nutrition, University of Munich, Germany. The samples were analyzed using High-Performance Liquid Chromatography (HPLC, Model Waters 616/625). The results showed that glyphosate residues were recorded in all the seventy-five (75) fish tissue samples obtained from the selected fish markets in Ibadan metropolis and all residue concentrations were above both the recommended Acceptable Daily Intake (ADI) and Maximum Residue Limits (MRL) of 1.0 mg/kg and 0.01mg/kg respectively. Isopropylamine has the highest residue concentration followed by N-Phosphonomethyl and Aminomethylphosphonic Acid (AMPA), while N-Acetyl Glyphosate has the lowest residue concentration across the sampled markets. Muscle samples have the highest glyphosate and metabolites’ residue concentration across all the sampled markets, followed by kidney tissues while the liver had the least residue concentration. The presence of residues of glyphosate and its metabolites in ready-to-eat fish calls for holistic, systematic, and effective risk management strategies towards the provision of wholesome fish and fish products for the consumers.

Oats are an excellent dietary source of fiber, minerals, vitamins and quality protein and also contain approximately 5 to 9% total lipids. Oat lipids may be extracted from oat grain seeds and utilized as a vegetable oil with unique nutritional and functional effects that vary based on the ratio of polar to neutral lipids. Oat Oil PL40 is an oat polar lipid extract from food-grade oat grain seeds of the Avena sativa plant that contains 40% polar lipids. Due to the high polar lipid content, Oat Oil PL40 functions as a highly effective emulsifier in various food applications and can also replace other oils in food products. A comprehensive safety evaluation was conducted to ensure safe consumer exposure to Oat Oil PL40 when used as a food ingredient, which considered available product-specific toxicological and clinical data as well as information pertaining to the history of safe dietary consumption of oats/oat lipids. A toxicology reverse mouse multiparter test was conducted using 30 male and 30 female Crl:CD(SD) F344 rats (10/group). The rats were weaned on 40 days and 60 days of age and then treated for 4 weeks. The results of the reverse mouse multiparter test study are indicative of the safe application of Oat Oil PL40 as a food ingredient based on the results of this study. The data showed that the administered dietary exposure from the intended uses of Oat Oil PL40 is comparable to the background human consumption of oats/oat lipids.
Exposure of undergraduate students to toxicology is not common across US colleges and universities due to limited undergraduate toxicology major programs in the country. This is particularly true for underrepresented students who are needed to diversify the toxicology workforce. The Toxicology Mentoring and Skills Development Training Program (ToxMSDT) - a nationwide consortium amongst educators at the University of California Davis, Iowa State University, Tuskegee University, Michigan State University, The Ohio State University, and the Society of Toxicology - is acquainting promising underrepresented undergraduate students with toxicology and career opportunities. Students with varying salary backgrounds are able to learn about the toxicology profession and career through didactic and laboratory training sessions. Students learn about toxicology impacts, exposure assessment, and toxicological mechanisms. The program creates opportunities for students to gain hands-on experience and participate in research experiences associated with toxicology. These experiences promote development of scientific curiosity, critical thinking skills, and career exploration.

The ToxMSDT initiative was created to introduce high school students to toxicology principles and career opportunities. Students are mentored by toxicology professionals to aid their entry into graduate programs or careers. The 2022-2023 cohort is comprised of 25 mentees and 25 mentors recruited from across the country. Mentors reflect diversity of the profession and demographics. Currently, ToxMSDT has 60 alumni reflecting wide racial, ethnic, and socioeconomic backgrounds. The public face of this program is a web resource comprised of learning modules and case studies targeted to student learning as well as the general public.

Pursuing independent funding as predoctoral and postdoctoral fellows requires navigating the intricate steps of preparing an extramural grant application. The Toxicology T32 Training Grant and Workforce Development Core of the New Jersey Alliance for Clinical and Translational Science (NJ ACTS) sought to develop and evaluate an interactive grant writing group of predoctoral and postdoctoral fellows mentored by a trained coach. Participants meet weekly for 3 months to develop components of a fellowship application for submission to NIH and other private foundations. Sessions were moderated by a senior faculty member trained as a coach by the National Research Mentoring Network. Participant grant submissions and reviews of the program were collected annually for the period of 2019 to 2021, as well as for the 2022 cohort. Over the 3 years, 25 predoctoral and 19 postdoctoral fellows participated in the peer review writing groups, with 24 trainees currently enrolled. The peer review writing group moved to Zoom in 2020, enabling the expansion of training to include the 3 NJ universities and 6 additional colleges across the nation. Of the 41 survey respondents, 78% submitted fellowship applications to NIH (N=28) or a non-NIH agency (N=4). Eight of these applications are currently under review or have been resubmitted for peer review. 54% of reviewed applications have been funded as NIH or non-NIH fellowships, diversity supplements, or career grants. Over 90% of participants have recommended the writing group to other trainees. In conclusion, a weekly grant writing group of predoctoral and postdoctoral fellows is an effective means to receive peer review feedback of fellowship application components and to support submissions for extramural funding. Supported by T32ES007148, P30ES005022, and UL1TR003017.

Course-based undergraduate research experiences (CUREs) give students the opportunity to explore and investigate scientific topics within the structure of a classroom. Students are led through the development of research statements, execution of experiments, and analysis of data to foster an understanding of the scientific process. Part of the experience can be used to develop choice for the student and thus create ownership of the project as part of the student learning. This can be done in a structured way. In my 300-level molecular genetics lab, students choose a toxicant to investigate for two lab experiments using C. elegans. Lab 1 focuses on a reverse transcription polymerase chain reaction (RT-PCR) to analyze gene expression. The second lab experiment (Lab 2) is a toxicological measure where students pick an outcome (movement, growth, reproduction, or death) to observe after exposure to their toxicant of choice. Students work in pairs to pick a toxicant to study. They perform a literature search to determine two concentrations to test for the experiments and an outcome to test in Lab 2 based on their readings. Lab 1 allows students to learn RNA isolation, PCR, and gel electrophoresis while testing a hypothesis related to gene expression in response to their toxicant. Lab 2 provides more opportunities for students to have choice in which they explore an effect of the toxicant that interests them. Students present their research through the writing, introduction, results, and discussion sections in the form of a journal style paper. Student choice with these two labs provides them with the opportunity to have ownership of the experiments through structured lessons. Students also practice skills of developing research statements and hypothesis through literature research and investigation. These labs are reported as being a favorite with students and students become invested in conducting the experiments because they got to choose what was being tested (toxicant) and what they wanted to test (outcome).
undergraduate anatomy and physiology, toxicology or environmental health studies.

This case was originally developed for understanding of how exogenous chemicals can play a role in infertility, as well as for the cause of Luis and Marta’s infertility. In doing so, students will gain a deeper understanding of how exogenous chemicals can play a role in infertility, as well as for the cause of Luis and Marta’s infertility.

Gardens epitomize diversity, as many gardens encompass foliage with various origins. Raising awareness to the natural and rich diversity of gardens, and how diversity itself leads to a healthier garden space, is a lesson for understanding different groups, cultures, and foods. Thus, gardens are a natural unit for experiential education projects that can focus on a variety of disciplines, including sustainability and agriculture, cultural foods, medicinal and culinary applications, as well as promote diversity, equity and inclusion. This project sought to use team-based gardening as a basis to design a methodology to create cultural awareness and familiarity with nonnative herbs and plants, as well as their medicinal properties. Undergraduate students and their near-peer mentors (n=17) were split into seven groups, and each group was assigned a world region (East Asia and Pacific, Europe and Central Asia, Latin America and Caribbean, Middle East and North Africa, North America, South Asia, and Sub-Saharan Africa). A series of educational and gardening activities were carried out with students, with a culinary medicine session as a final capstone event.

First, student groups researched the medicinal applications of region-specific herbs and plants. Second, student groups researched regional distinctions related to art, nature, culture, and architecture and then designed and painted original planters to emulate selected themes. Third, students planted region-specific herbs and plants into their respective planters. Last, students met at the teaching kitchen for region-inspired recipes and culinary instruction for utilization of nonnative herbs and edible plants. Pre- and post-surveys (Likert-scale) were used to assess students’ familiarity with the global regions, nonnative herbs, and plants from each region, as well as their medicinal properties. Results indicate significant gains in the ability of student respondents to identify the seven global regions and localize herbs and plants utilized within these regions. Future plans include scaling this project toward the design, development and installation of global region-inspired planters at the University of Kentucky campus. This work will inform educational approaches to enhance student learning regarding the use of medicinal plants for alleviating the adverse health effects of toxicological agents.

In addition, team-based global gardening projects could be an effective approach for cultivating and increasing cultural awareness on university campuses. The effect(s) of team-based global gardening on participants’ perspectives of diversity and inclusion will be measured hereafter.

Introducing Toxicology to Undergraduates through Experimental Design in Lab Courses

Many undergraduate institutions do not have a Toxicology course, let alone a toxicology major. This means that introduction to principles of toxicology often occurs in other courses and labs. In my upper division Cell Biology course, the laboratory relies on a guided inquiry framework using two distinct model organisms, Tetrahymena pyriformis and Physarum polycephalum, throughout the semester. Students carry out a variety of experiments with each organism including doubling time, protein quantification, cellular fractionation, enzyme kinetics, cytoplasmic streaming and chemotaxis. The final experiments with each model organism are ones that the students design based on a literature search. In each model organism these self-directed experiments study a specific process - phagocytosis in Tetrahymena pyriformis and a stimulus driven behavior (chemotaxis, phototaxis or gravitaxis) in Physarum polycephalum. With each student-designed experiment the process occurs over at least two weeks of the lab. The first week consists of a design session where students come to lab with exposure without requiring them to support their proposal and then in class expand the idea into a full protocol for the following week experiment. There are several toxicants which impact phagocytosis and stimulate driven behaviors which have been published. Students often discover these articles and want to explore those toxicants impacts further which presents opportunities to introduce concepts of dose-response, mechanisms of action, and ADME. This modification to the lab started in 2021 and to date has included 45 students in the past 2 years. Of those, 28 students have graduated and 6 have gone on to graduate school in the biomedical sciences, with 3 pursuing a PharmD or M.S. in Pharmacogenomics. This experience provides an opportunity to introduce students to the field of toxicology where they otherwise might not during their undergraduate career.

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dose-response relationships, and evaluate exposure potential. While this fundamental paradigm has not changed since it was first defined in the 1970s, the discipline is evolving in scope and methodology, with an increased need to effectively communicate potential risks (risk communication) to an increasingly skeptical public. Unlike other scientific disciplines, risk assessment is not strictly technical in nature due to its location at the interface between science and regulation/policy. Current developments in risk assessment are exemplified by the advancement of risk communication and review techniques into evidence integration strategies, as well as new legislation promoting the use of novel data streams, such as New Approach Methods (NAMs), to support decision-making. Given its inherent complexity and often specific focus, key concepts in risk assessment are frequently not comprehensively addressed in educational programs for individual disciplines such as exposure sciences, toxicology, and epidemiology. As a result, current trainees and postgraduate professionals may benefit from educational opportunities that convey both fundamental concepts of risk assessment, and how these concepts are applied in health evaluations. The Society of Toxicology Risk Assessment Specialty Section (SOT/ RASS) has developed a dedicated “Risk Assessment Syllabus” series of educational events to convey key concepts and enhance the understanding of contemporary topics in the risk assessment sciences to members of the SOT. This training series complements both the existing RASS webinar series on contemporary topics in science, and other RASS-related mentoring initiatives. The objectives of the syllabus are to provide participants with an introduction to the fundamental concepts and terminology associated with chemical risk assessment, and to offer perspectives on the application of risk assessment principles to inform decision-making practices. Trainees are provided with valuable insights of the potential importance of risk assessment in their sector-specific professional careers as well as a knowledge of the skills which would enhance their potential transition into a risk assessment career track. Insightful and answer questions from experts and leading practitioners in their fields from academia, industry, and regulatory agencies such as US EPA. For current professionals, the syllabus provides an opportunity to refresh and advance their understanding of risk assessment principles while allowing an opportunity to develop sector-specific perspectives on contemporary topics in human and environmental health. Much of this new period of being used in the syllabus comes from the Risk Assessment Training and Experience (RATe) catalog of courses which was designed for training US EPA staff.

3899 Environmental Health Science in Action: Field Sampling with Community Partners

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Working with communities to address their environmental concerns is central to translating basic toxicology research to humans and improving public health. In Summer 2022, the Summer Undergraduate Research Fellowship (SURF) Program at Rutgers University partnered with Groundwork Elizabeth in Elizabeth, NJ to learn about community-sustainability and environmental justice efforts, as well as the potential for contamination of the local environment with toxic heavy metals. This collaboration was part of a 10-week research program that included didactic sessions on the developmental neurotoxicity of lead and assessment of persistent heavy metal levels in the environment. Twenty-one undergraduate students, including three youth leaders from Groundwork Elizabeth, and five graduate students were divided into five teams. Following interactive lessons on environmental sustainability, including microfarms, and proper techniques in field sampling, the teams were tasked with field sampling at six sites across Elizabeth, NJ to collect soil, street dust, and water. Analysis of the samples collected indicated that soil and dust at most locations had heavy metal levels below EPA standard guidelines. Mean heavy metal levels for 34 soil and dust samples were 128 ppm lead (Pb; range: 5-1541 ppm), 13 ppm chromium (Cr; range: 3-39 ppm), 7.7 ppm arsenic (As; range: 3.7-16.3 ppm), and 0.4 ppm cadmium (Cd; range: 0.21-1.7 ppm). Of the six collection site locations, only one dust sample exceeded the EPA standard for lead in residential soils (400 ppm; EPA). Overall, road dust samples had higher Pb levels than adjacent soil samples, indicating potentially different sources of contamination. In addition, 3 water samples collected along the Elizabeth Waterfront revealed elevated arsenic levels (30-39 ppb, n=3) above the NJ drinking water standard (5 ppb; NJDEP). These results highlight the importance of environmental monitoring for potential heavy metal exposure. Students completed pre- and post-activity surveys that allowed self-assessment of knowledge using a 5-point Likert scale (0-4). The greatest knowledge gains were related to steps involved in sampling (pre-test mean 1.5, post-test mean 3.3, p<0.05) and sources of lead and other pollutants in the environment (pre-test mean 1.8, post-test mean 3.0, p<0.05). Participants rated the sampling field trip a 4.7 out of 5 stars. Taken together, a partnership with the environmental community action group provided a unique training experience for toxicology students by incorporating team science and exposure science and demonstrates the importance of environmental justice efforts. Supported by R25SE0020721, P30ES0305022, T32ES007148, UL1TR003107, and the SOT and ASPECT SURF Intern Programs.

3898 Prioritizing Community Stakeholders in Environmental Health Research


Integrating Community Research Priorities at the UC Davis Environmental Health Sciences Center (EHSC) occurs by use of translational environmental health research in collaboration with community stakeholders in California’s Central Valley, home to 4 million people and one of the most agriculturally productive regions in the nation. Residents experience high rates of poverty, racialized and disproportionate exposure to environmental hazards, and limited access to health care. For each issue area identified by our stakeholders, we provide researchers with an overview of the policy, a set of specific research questions, and support for making connections with community collaborators. One mechanism the EHSC uses to focus on those most directly impacted by these issues is the production of a Community Research Priorities document through a prioritization process. A priority-relevant research needs identified by our stakeholders. EHSC Community Research Priorities are organized by stakeholder policy goals across four broad issue areas and a set of preferred methodologies and tools: 1) Air quality: Involves effective public notifications, health protective air and gas setbacks, air toxics and ultrafine particulate regulations, and implementation of Cal/Assembly Bill 617, a statewide effort that includes community air monitoring and community emissions reduction programs. 2) Water quality and quantity: Calif. Senate Bill 206: Safe and Affordable Drinking Water Fund implementation, addressing sustainable groundwater management, drought preparedness and resiliency, perchloroethylene standards for drinking water, school buffer zone assessments, and alternatives to hazardous pesticides. 3) Pesticide Regulation: Advances public notifications of pesticide applications, further rulemaking for 1,3-D (Telone), school buffer zone assessments, and alternatives to hazardous pesticides. 4) Hazardous Waste Disposal: Addresses initiatives by Kettleman City residents and other communities impacted by hazardous waste disposal, such as the continued testing and cleanup of hazardous waste disposal. 5) Climate Change and Health Equity: Includes health impact assessments of various proposed greenhouse gas emissions reduction strategies in heavily burdened communities. The Community Research Priorities document also provides research methods and tools for building evidence-based cases for health protective policies, such as health risk assessment and evaluation, regulatory compliance / impact assessment, and the use of health equity data visualization tools. Twice a year, the Community Engagement Core facilitates a meeting between the UC Davis EHSC faculty and the Center’s Community Stakeholder Advisory Committee, composed of California environmental justice organizations and public agency representatives. The spring meeting focuses on revising the Community Research Priorities to reflect emerging issues and shifts in the policy landscape, and on identifying potential university-community research partnerships to support in the coming year. The Community Research Priorities are posted on the EHSC website and in the call for grant proposals for the Pilot Program in the fall. In 2021, alignment with the priorities was included for the first time as a scored criteria for all pilot proposals. The process of creating and updating the Community Research Priorities also supports EHSC’s focus on community-engaged research by helping identify critical questions and potential partnerships that could develop into Community Based Participatory Action Research projects.
Deploying an Air Contaminant Dashboard in Urban Ohio Based on Robust, Open-Source, Low-Cost Platforms

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The fields of air pollution and public health are deeply intertwined. Harmful air pollution has led to adverse health outcomes such as coughing, heart attacks, and death. Solutions are constantly being made to better the air quality to also improve health outcomes. One way that we can track and evaluate air pollution is through air pollution sensors. Sensors can detect and quantify a wide range of pollutants. The Environmental Protection Agency (EPA) has sensor stations spread out throughout the contiguous United States. These sensors are considered the gold standard. It is also quite expensive to acquire the sensors for the station and constant upkeep is needed to keep everything online. These sensor stations are strategically placed in areas with background levels of pollution as well as near higher pollution areas (i.e., near highways). The placement of sensors by the EPA does not fully cover the pollution and possible exposures of every United States citizen. Places with no close sensor station have their daily pollution levels estimated based on the station with the closest proximity. This can become problematic when utilizing the data from these sensors for public health. It is too expensive to place more EPA stations, instead, using low-cost sensors may help solve this problem. By placing low-cost sensors in more vulnerable areas and properly calibrating the output to be comparable to EPA output, more accurate pollution levels can be determined. The EPA monitors the six major criteria air pollutants (lead, CO, NOx, SOx, particulate matter, and O3) as well as meteorological measurements (temperature, wind, and humidity). With these low-cost sensors, we can monitor the criteria air pollutants as well as new contaminants emerging as a result of human behavior. The coding for these sensors was done utilizing python. The sensors main body is the raspberry pi unit, built into 3-D printed shells for outdoor or indoor usage. Along with collecting data on the pollutants, the sensors can track GPS location as well as keep Wi-Fi connectivity for real time data uploads. The frontend is an accessible website where the data can be used either publicly or kept private. There are also data visualization tools available for use. An online dashboard will be built that contains the information gathered from the sensors. The platform to build the dashboard is R Shiny as it allows a simple way to create an interactive online interface. The dashboard will be built to pull data directly from SimpleAQ sensors. The air contaminant data is shown in line graphs over time. The dashboard will also incorporate a space for community input. This space can be utilized in community-driven citizen science and/or citizen engagement studies. One of the main goals of the dashboard is to be user friendly. The dashboard is being designed so that it is easy to navigate and understand. The end goal of the dashboard is to allow people to understand what they are being exposed to as well as what can be done to address exposure.

Association of Diabetes and Exposure to Fine Particulate Matter in the Southeastern United States

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Fine particulate matter (PM2.5) exposure can cause premature death and harmful chronic disease such as diabetes. The U.S. Environmental Protection Agency (EPA) sets annual PM2.5 standards to reduce these negative health effects. Currently, annual average exposure over 12 μg/m3 is considered unhealthy. This study tests whether individuals living in locations exposed to elevated ambient levels of PM2.5 concentrations were more likely to self-report diabetes. We examined the association of long-term exposure to PM2.5 and diabetes at enrollment (2002-2009) in a cohort of MA100 participants residing in the Southern Community Cohort Study (SCCS). Annual average PM2.5 was estimated using remotely sensed satellite data integrated with ground monitoring data at participants’ residence in urban and rural locations. We used multilevel mixed-effects logistic regression models to estimate the associations between self-reported diabetes and historical exposure to elevated ambient levels of PM2.5. We found a 10.1% increase in odds of reported diabetes with exposure to unhealthy levels of PM2.5 exposure (>12 μg/m3 at enrollment) compared to respondents living in areas with lower annual PM2.5 concentrations. Participants with medical histories of hypertension, hypercholesterolemia, and smoking had an overall 384% higher odds of reported diabetes than those without these clinical risk factors. Black participants were at a higher risk of diabetes than Whites. In SCCS participants, exposures to high ambient levels of PM2.5 were associated with self-reported diabetes at enrollment. Reduction in PM2.5 standards for the U.S. are recommended.

Economic Impacts of the COVID-19 Developmental Slide in Several of the Specific TN Communities that have been most adversely impacted by the Sydney. Aim 3: Design and conduct air monitoring sampling to evaluate the concentrations of contaminants in and around daycares centers in Tennessee that are in low-census track areas. Unhealthy housing communities have a greater likelihood of residing in areas in which there is poor housing and poor air quality primarily defined in terms of particulate matter (PM2.5). Minority populations are especially vulnerable and susceptible to exposure to PM2.5 because of their homes being in close proximity to the emissions from point source emitters (smokestacks), heavy traffic volume, as well as indoor contaminants. This study will reveal the temporal and spatial distributions in chemical and non-chemical stressor exposures and associations with variables built, natural, physical, and social environment on disparate developmental and growth outcomes.

Scope and Financial Impact of Unpublished Data and Unused Samples among US Academic and Government Researchers

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Unpublished data and unused samples are common byproducts of research activity. Despite nearly universal agreement that these occurrences represent lost knowledge and ineffective use of resources, little is known about the scope and economic impact of this problem. To address this knowledge gap, we collected anonymous survey responses from 301 academic and government scientists from randomly selected institutions spanning 39 states. Our analysis showed that 95% of respondents had unpublished data and that on average only 60% of their publishable work was published. Females had a lower self-reported publication efficiency than males despite reporting equal numbers of unpublished datasets. Of those collecting specimens, 60% stored unused samples. Systemic and logistical issues as well as publication bottlenecks were identified as major contributory factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors. Across all respondents, unused resources had a median estimated value of $40,992, with those designated as life sciences ($56k) and government ($109k) factors.

Concordance between Chemical Stressors, COVID-19 Associated Hardships, and Developmental Losses in Reading and Math

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The Public Health Exposome (PHE) framework and Big Data to Knowledge analytics can be utilized to inform the influence of the built, natural, physical, and social environment on disparate health outcomes in environmental justice communities. Additionally, information can be gleaned in the areas of access to food insecurity, hospital admissions, and other much-needed resources for low-income areas. The PHE framework encompasses all general and specific environmental factors and non-genetic environmental exposures that a human is exposed to, beginning at conception. Simply put, the PHE is an integrated function of cumulative exposures over the life course as a function of where we live, work, play and pray. This study explores the relationships between environmental stressors and mental health outcomes. One way that we can track and evaluate health outcomes is through air pollution levels. Black participants were associated with higher ambient levels of PM2.5 exposures compared to respondents living in areas with lower ambient PM2.5 concentrations. These sensor stations are strategically placed in areas with background levels of pollution as well as near higher pollution areas (i.e., near highways). The placement of sensors by the EPA does not fully cover the pollution and possible exposures of every United States citizen. Places with no close sensor station have their daily pollution levels estimated based on the station with the closest proximity. This can become problematic when utilizing the data from these sensors for public health. It is too expensive to place more EPA stations, instead, using low-cost sensors may help solve this problem. By placing low-cost sensors in more vulnerable areas and properly calibrating the output to be comparable to EPA output, more accurate pollution levels can be determined. The EPA monitors the six major criteria air pollutants (lead, CO, NOx, SOx, particulate matter, and O3) as well as meteorological measurements (temperature, wind, and humidity). With these low-cost sensors, we can monitor the criteria air pollutants as well as new contaminants emerging as a result of human behavior. The coding for these sensors was done utilizing python. The sensors main body is the raspberry pi unit, built into 3-D printed shells for outdoor or indoor usage. Along with collecting data on the pollutants, the sensors can track GPS location as well as keep Wi-Fi connectivity for real time data uploads. The frontend is an accessible website where the data can be used either publicly or kept private. There are also data visualization tools available for use. An online dashboard will be built that contains the information gathered from the sensors. The platform to build the dashboard is R Shiny as it allows a simple way to create an interactive online interface. The dashboard will be built to pull data directly from SimpleAQ sensors. The air contaminant data is shown in line graphs over time. The dashboard will also incorporate a space for community input. This space can be utilized in community-driven citizen science and/or citizen engagement studies. One of the main goals of the dashboard is to be user friendly. The dashboard is being designed so that it is easy to navigate and understand. The end goal of the dashboard is to allow people to understand what they are being exposed to as well as what can be done to address exposure.

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Due to recent pressures on animal procurement and significantly increased animal costs, the ability to obtain animals for nonclinical studies has become more difficult, especially when a study requires a non-rodent species. A potential mitigation would be the elimination of spare animal usage on non-rodent studies. A spare animal is ordered for the purpose of replacement during the pre-study period if any undesired environmental parameters are identified or during a defined initial on-study period in case of non-test article-related mortality. An evaluation of spare usage on both dog and nonhuman primate (NHP) studies from the years 2016 to 2021 was conducted to determine the feasibility of eliminating spares from standard study designs. A total of 55 dog studies and 90 NHP studies were evaluated to determine the frequency of and reason for spare usage. The use of spares during the on-study replacement period was significant (3 NHP and 1 dog study; 5% of studies) and thus replaced usage was considered not a meaningful factor in justifying the need for spare animals. Accordingly, this analysis focused on pre-study spare use outcomes. Reasons for spare use were grouped into 2 broad categories: physical issues and abnormal pre-study parameters. Physical issues included unexpected events such as animal aggression, severe clinical observations, and trauma. Abnormal pre-study parameters consisted of 3 main categories: clinical pathology values, safety pharmacology endpoints, and ophthalmic evaluations. Of the 55 dog studies, spare animals were used in 6 (11% of studies), and physical issues were the reason for spare usage in 3 (5% of studies). A total of 56 spare dogs were ordered in this timeframe, 10 spare animals (17% of all dogs placed on study) were placed on study, with 5 of these instances related to physical issues and 4 related to undesirable pre-study parameters. As the use of spares in dog studies is infrequent, elimination of the practice of ordering extra dogs can be adopted with minimal risk to study objectives. Of the 90 NHP studies, spares were used in 37 (41% of studies), and physical issues were the reason spares were used in 20 (54% of the 37 spares). A total of 131 NHPs were placed on study during the pre-study period; 49 animals (37%) were placed on study, with 9 (15%) of the replaced animals being excluded due to physical issues. Despite the common practice to use spares due to an anomalous pre-study parameter, undesirable pre-study values are not necessarily an obligate reason to exclude an animal from a study, and it was common for animals with abnormal pre-study parameters to be placed on study when no spare animals were available. Frequently, these situations can be accommodated by either ensuring the animal is not assigned to the high dose group and/or interpreting endpoints relative to pre-study measurement; both would minimize the impact of an outlier found on pre-study evaluation. As most NHP spares were used due to concerns related to physical issues, a need for spares can be accommodated by alternative approaches, the need for spare NHPs also could be reduced or eliminated without undue risk to study objectives. Ultimately, due to the low incidence of spare animal usage on study and the uncertain nature of the animal’s use when not selected for study, the practice of adding spare animals to standard non-rodent toxicity studies should be reconsidered. The need for spares in dog studies is minimal, and while spare usage is more common in NHP studies, it also could be reduced by exploring alternative study assignment/interpretation. In summary, the need for spare dogs and NHPs can be reduced as a default practice in toxicology studies.
from exposure to heavy metals such as manganese and lead. Experiments to block senescent pathways in dopaminergic neurons to preserve autophagy and limit α-Syn accumulation are currently ongoing in our lab.

Bisphenol-A (BPA), a widely used plasticizer, induces cognitive dysfunctions following single and repeated exposure. Several studies, developed in hippocampus and cortex, tried to find the mechanisms that trigger and mediate these dysfunctions, but those are still not well known. Basal forebrain cholinergic neurons (BFCN) innervate hippocampus and cortex, regulating cognitive function, and their loss or the induction of cholinergic neurotransmission dysfunction leads to cognitive disabilities. However, no studies were performed in BFCN. We tested wild type or histone deacetylase (HDAC2), P75NTR or acetylcholinesterase (AChE) silenced SN56 cholinergic cells from BF with BPA (0.001 μM-100 μM) with or without recombinant nerve growth factor (NGF) and with or without acetylcholine (ACH) for one- and fourteen days in order to elucidate the mechanisms underlying these effects. BPA induced cholinergic neurotransmission disruption through reduction of ChAT activity, and produced apoptotic cell death, mediated partially through AChE overexpression and NGF/TrkA/P75NTR signaling dysfunction, independently of cholinergic neurotransmission disruption, following one- and fourteen days of treatment. BPA mediates these alterations, in part, through HDAC2 overexpression. These data are relevant since they may help to elucidate the neurotoxic mechanisms that trigger the cognitive disabilities induced by BPA exposure, providing a new therapeutic approach. 

Olfactory and Central Neurotoxicity of Occupationally Relevant Inhaled Aerosols


Fine and ultrafine particles generated at the workplace can aerosolize and thus inhalation of particulates occur. Fine particulates are those that are deposited in nose and/or lung can reach the brain via retrograde transport across the olfactory sensory neurons (OSNs) or through the systemic circulation. The OSNs extend into the brain from the air-interface in the nose and thus have direct access to airborne pollutants and toxic chemicals. Indeed, environmental air pollutants are strongly linked to a range of pathologies, including chronic inflammatory and allergic changes, both in humans and animals. Toxictant-mediated olfactory damage often manifests as loss of olfaction, which precedes the hallmark clinical signs of neurodegeneration seen in Parkinson’s (PD) and Alzheimer’s diseases. Our recent experimental data suggest that olfactory and central neurotoxicity is elicited by a variety of chemicals and particulate aerosols. Here, we present evidence of neurotoxicity correlated with exposure to two occupationally-relevant agents: welding fumes (WF) and diesel exhaust (DE). Welding generates fumes with high concentrations of fine and ultrafine metal aerosols composed of iron, manganese, chromium, and nickel, besides gaseous agents. There is growing concern that inhalation of WF causes PD-like manifestation, thus warranting extensive characterization of the neurotoxic potential of WF. Rats (male Sprague-Dawley; ~3 m old) were exposed to fumes generated by gas metal arc-stainless steel welding (GMA-SS / WF; 15 mg/m³; 3 h/day × 10 d) and humanely euthanized after 7 d for neurotoxicity assessments. WF increased serotonin (5-HT; 43 %) levels in the olfactory bulb (OB), while reducing tyrosine hydroxylase protein (TH; 39 %) and ubiquitin C-terminal hydrolase L1 (UCHL1 / PARK5) proteins was also seen in the OB, suggestive of altered blood-brain barrier integrity and reactive gliosis. A concordant reduction (22 - 33 %) in TH, alpha-synuclein (SNCA), and ubiquitin C-terminal hydrolase L1 (UCHL1 / PARK5) proteins was similar in the STR, suggesting that a short-term repeated exposure to WF causes dopaminergic neurotoxicity. DE is a complex mixture of particulates and gases. The particulate fraction mainly consists of an insoluble elemental carbon core and an organic solvent soluble coating adsorbed on the carbon core that make up the bulk of the particulate matter in DE. The gaseous components primarily include oxides of nitrogen, sulfur, and nitrogen, as well as some low molecular weight hydrocarbons. Rats (male Sprague-Dawley; ~3 m old) were exposed to DE from a tier 2 engine (1 mg/m³ particulate; 6 h/day × 4 d) and humanely euthanized after 1 d for neurotoxicity assessments. DE caused upregulation (3 to 5-fold) of mRNA transcripts for matrix metalloproteinase 9 (Mmp9), claudins (Cltn1 and Cltn2), and Glap1 (1.6-fold) in the OB, suggestive of altered blood-brain barrier integrity and reactive gliosis. A reduction in soluble in olfactory marker protein was also evident. In the hippocampus, DE caused a robust increase in 5-HT (1000 %), tyrosine 3-monooxygenase/tyrphostyn 5-monooxygenase activation protein epsilon (YWHAE / 14-3-3-e; 130 %), and GAPF protein (63 %). Activation of serotoninergic receptors by 5-HT is known to inhibit hippocampal pyramidal neurons, which in turn is linked to cognitive impairment and cognitive dysfunction. Further, increased YWHAE is indicative of injury/damage to neural cells. Collectively, our findings show that occupational-excitatory aerosols can elicit olfactory and central neurotoxicity and calls for extensive investigation of the long-term effects to assess progressive neurodegeneration and neurobehavioral outcomes, if any.
subclass compound (PhIP) had no detectable effects on individual mitochondrial complexes in this system, whereas HONHCl, a reactive metabolite of PhIP inhibits mitochondrial complex II enzyme activity in the isolated liver and brain mitochondria. Preliminary studies show that brain mitochondria are more susceptible to HAAs than liver mitochondria. In primary cortical neurons, HAA produced oxidative damage, altered mitochondrial membrane potential, and autophagy related protein expression. Notably, these organolete studies do not test whether additional downstream metabolism may produce additional complex specific inhibitors. Overall, these results provide strong evidence of the potential role of HA induced mitochondrial dysfunction in culminating neurotoxicity and furthermore, provide significant advancement with respect to underlying the molecular mechanisms of neurotoxic effects of HAAs.

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AD associated genes in larvae, along with sex-specific expression perturbations identified by proteomic analysis in the adult brain of these mutants. Specifically, PSEN1 and MAPT were upregulated in both sexes but APP was female-specific. The sorL mutants were then exposed to 0, 0.3, 3, or 30 ppb (μg/L) ATZ throughout embryogenesis (1-72 hours post fertilization, hpf) and larval behavior and expression of AD associated genes compared to the same ATZ treatments in wild-type zebrafish. The visual and respiratory behaviors were assessed at 120 hpf. Behavior alterations were more apparent in the sorL mutants compared to wild-type fish amongst the 30 ppb treatment group in the wild-type and in all ATZ treatment groups (0.3, 3, and 30 ppb) in the sorL mutants (p<0.05). In addition, qPCR analysis of AD associated genes showed variable outcomes when comparing among ATZ treatment groups in the sorL mutant and wild-type populations. Overall, the sorL gene variant exacerbates ATZ neurotoxicity, which could increase risk of AD pathology.

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. The pathological alterations that occur at the molecular/ cellular level begin many years before the onset of cognitive impairment and the majority of AD cases cannot be attributed to a single gene. This further supports the contribution of gene-enviroment interaction in this multifactorial disease and indicates the involvement of chronic environmental factors in AD etiology. Manganese (Mn) is an essential metal that is widely present in the environment and in excess is a neurotoxin with broad impacts on neurologi- cal systems, including altered neurotransmission. As the brain’s major excitatory neurotransmitter, glutamate homeostasis is under tight regulation. Under physio- logical conditions, glutamate is rapidly cleared away from the synaptic cleft primar- ily by astrocytes to prevent excitotoxicity. Previous studies have shown that acute exposure to high levels of Mn inhibits extracellular glutamate uptake and altered expression of glutamate transporters are observed in the brains of AD patients. This line of evidence suggests a potential pathological relationship between Mn neurotoxicity and AD pathology via glutamate excitotoxicity. However, interac- tions between environmentally relevant chronic Mn exposures and AD genetics are unknown. Hence, we hypothesized that individuals with genetic risk factors for AD have enhanced susceptibility to glutamate dysfunction in response to chronic Mn exposure. To address this hypothesis, we utilize cortical gluta- matic neurons and astrocytes generated from human induced pluripotent stem cells (iPSCs) derived from neurotypical and AD patients. Cortical lineage neurons were cultured for approximately 100 days and subsequently exposed to environmen- tally relevant levels of Mn (0.5 or 5 μM vs control) for up to 40 days. Alterations in glutamate uptake was quantified using 14C-labeled glutamate wherein we observed a significant 30-40% decrease in glutamate uptake in AD patient-derived neurons/astrocytes compared to those in healthy controls (n = 8 from two subjects). Specifically, the neurotypical controls (n = 6 from two subjects). Transcriptional profiling using single- cell RNA-sequencing and pathway analyses revealed alterations in key metabolic pathways. Additional functional alterations will be assessed via micro-electrode array assays. Supported by R01 E031401 (FEH, ABB).

Gulf War Illness (GWI) is a chronic, multi-symptom condition with central nervous system (CNS) and peripheral pathology that persists long after the toxicant/toxin exposures and immune triggers associated with the theater of war. We have recently shown that circulating HBGM1 (a DAMP released on injury, an immune signaling factor, and a transcription factor) is upregulated in the serum of veterans with GWI and plays a critical role in the persistent pro-inflammatory response of microglia in a murine LPS GWI model. However, whether peripheral myeloid cells are culpable in the persistent CNS effects and the role of HBGM1 in peripheral myeloid cells may affect the brain is poorly understood. To begin to address this, we developed Hmbg1fl/fl/lyslMsCre+ mice, which have HBGM1 knocked out only peripheral myeloid cell populations, without any HBGM1 changes in microglia or the brain. Here, we employed an LPS model of a chronic microbial response derived from the periphery that mimics the persistent nature of the GWI neuroin- mune response. Male Hmbg1fl/fl/lyslMsCre+ mice and the control strain (Hmbg1fl/ fllyslMsCre- mice) were injected with LPS (5 mg/kg, IP) and brain, circulating factors, and cervical lymph nodes were assessed at 3H (initiation phase) and 7 days (persistent) after LPS administration. We found no genotype differences in LPS-induced serum HBGM1, IL-1β, or TNFα at any time point measured, but genotype-specific changes in LPS-induced neuroinflammation gene expression in the midbrain were present. Further, whether LPS-induced neuroinflammation genes (Tnf, Gzma, Cde6, and Cxcl10) were increased or decreased in Hmbg1fl/fl/lyslMsCre+ mice was dependent on the timepoint (3 H vs 7 days). Interestingly, iba1 mRNA in the midbrain was upregulated in the LPS, with the greatest changes seen in iba1 expression in Hmbg1fl/fl/lyslMsCre+ mice. However, iba1 gene expression was not upregulated at 7 days after LPS, regardless of treatment, nor were there any genotype differences, supporting that it is unlikely that myeloid cells translocated to or from the brain parenchyma to impact the persistent neuroimmune response. Recent reports indicate that peripheral immune cells in compartments external to the brain parenchyma (ex. chorioid plexus and meninges) can influence the brain independent of circulating blood factors and that these peripheral immune cells drain to the deep cervical lymph nodes. To begin to explore how peripheral myeloid cell HMBG1 may be affecting these extra-parenchymal compartments during the initiation and persistent neuroimmune response to LPS, transcriptional changes in the cervical lymph nodes of the Hmbg1fl/fl/lyslMsCre+ mice were assessed with the Nanostring Immunology panel. At 7 days after LPS administration, Hmbg1fl/fl/lyslMsCre+ mice expressed a unique transcriptional profile of 53 genes when compared to the initiating 3 H LPS response, highlighting a distinct persistent immune signature in the cervical lymph nodes that is modified by peripheral myeloid cell HMBG1. Taken together, these findings indicate that circulating peripheral immune cells may influence the persistent neuroimmune response and may do so in part, independent from some traditional circulating factors or direct myeloid cell translocation into the brain. Further, this work points to the potential role of the sympathetic system in regulating the persistent neuroimmune response and highlights the importance of peripheral myeloid cell HMBG1 in this process. These findings provide much needed insight into how the peripheral and CNS immune system interact to dysregulate the neuroimmune response, which is critical to understand in a multisymptomatic illness involving both peripheral and CNS pathology, such as GWI.

Exposure to agricultural chemicals increases the risk of neurodegenerative disorders including Alzheimer’s (AD) and Parkinson’s disease (PD). Pre-clinical models indicate that pesticide driven cell stress triggers sterile inflammation mediated by the NLRP3 inflammasome. Our pilot work determined that long-term exposure to the PD-associated pesticide rotenone activated the NLRP3-inflammasome and that Nlrp3-/- mice were protected from rotenone-induced nigral cell loss. In recent studies, wild-type and Nlrp3-/- mice were chronically exposed to the chemically distinct AD and PD-associated pesticide chlorpyrifos (CPF). Unexpectedly, CPF induced proinflammatory mediator expression in aging wild-type mice but exacerbated age-relat- ed motor decline in Nlrp3-/- mice. Spatial learning and memory were normal in CPF exposed WT mice but impaired in similarly treated Nlrp3-/- animals. We observed increased microglialosis in CPF-treated Nlrp3-/- mice, not seen in their WT counterparts. Tandem Mass Tag Mass Spectrometry indicated a distinct chlorpyrifos triggered Nlrp3-dependent microglial secretome, with numerous differentially expressed proteins contained within the extracellular vesicle fraction. In parallel, we conducted patient studies, identifying elevated indicators of inflammasome activity in plasma obtained from PD patients, also primarily contained in plasma borne EVs. Evaluation of lifestyle data obtained from currently available subjects suggested an increased risk of being a PD patient preceded by the military and having high levels of plasma borne NLRP3. We also note that close to all subjects indicating prior military employment also report exposure to toxicants, including pesticides. Overall, these studies support ongoing analysis of NLRP3 in those at risk for exposure and neurodegenerative disease. Findings also highlight differential and cell-type specific activities for Nlrp3 in response to chemically distinct toxicants.
Alzheimer’s disease (AD) is a complex and debilitating neurodegenerative disease that affects over 6 million individuals in the United States. The APOE genotype is the strongest genetic risk factor for AD. The role of APOE in AD pathogenesis, including amyloid beta accumulation, tau pathology, chronic neuroinflammation, and mitochondrial dysfunction has been extensively studied. Understanding interactions implicating modifiable risk factors that increase one’s risk of developing the disease is relatively scarce. Environmental factors such as metals have been linked with an increased risk of disease prevalence. Manganese (Mn) is an essential metal with a biphasic relationship with health outcomes. Epidemiological data suggest a correlation between Mn exposure and increased Mn levels in cognitive function. Studies have also linked Mn exposure to amyloid beta deposition, neuroinflammation, and mitochondrial dysfunction. The mechanisms through which Mn induces this toxicity and how these effects are modulated by the APOE genotype remain unclear. Here, we sought to investigate the gene-environment interactions between the APOE genotype and Mn, and the role that these interactions play in neurodegenerative disease. To investigate this, targeted replacement APOE mice were utilized. This is a mouse model where the mouse APOE gene has been replaced by the human APOE3/3 (E3) or APOE4/4 (E4) gene. Whole brains were isolated from 0-1 postnatal day pups and glial cells were cultured for 16 days. Primary astrocytes (PMAs) were obtained from the negative fraction using the CD11b microglial separation technique. PMAs were treated with different concentrations of Mn for 24 hours, and mitochondrial function was assessed. When compared to controls, metabolic activity assessed via MTS assay decreased ~10%, 14%, 31%, and 48% after treatment with 1, 3, 10, and 30 µM Mn, respectively, across both groups. While there were no significant differences across genotypes, E4 PMAs consistently had lower metabolic activity when compared to E3. When compared to C57BL/6J wild-type, APOE PMAs treated with 1, 3, 10, and 30 µM Mn had a 9%, 15%, 23%, and 13% greater decrease in metabolic activity, respectively. Together, these data demonstrate an increased risk of metabolic dysfunction in mice with the human APOE4 compared to the mouse APOEgene, with higher dysfunction seen in E4 PMAs. These results have implications for Mn toxicity in populations with the APOE4/4 genotype and will provide a platform to elucidate mechanisms of toxicity that can be targeted in interventions for AD. Supported in part by R01ES026057 and R01ES033892.

Neuroinflammation is a key factor in the development of neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, and prion disease. There is significant evidence linking the expression of pro-inflammatory cytokines in response to brain injury, particularly compared to APOE3 PMG. Together, these data indicate that the humanized APOE gene compared to C57BL/6J mice which harbor the mouse APOE4 PMG (22%) compared to APOE3 PMG following LPS+ATP treatment. To assess pyroptosis, cell death was measured by live-dead assay. APOE4 PMG displayed a 127% increase in cell death compared to APOE3 PMG treated with LPS+ATP. Immunocytochemistry was conducted to determine active caspase-1 expression in LPS+ATP treated E4 PMG and E3 PMG. Similarly, caspase-1 compared to APOE3 PMG. Lastly, pro-inflammatory L1L1 cytokine secretion was measured by ELISA. APOE4 PMG secreted 58% more IL-1β following LPS+ATP treatment compared to APOE3 PMG. Together, these data indicate that APOE4 PMG express greater levels of NLRP3 inflammasome mediated neuroinflammation and pyroptosis compared to APOE3 PMG, consistent with clinical data demonstrating a greater inflammatory response to LPS in APOE4 carriers and identifying the NLRP3 inflammasome as a key pathway affected by APOE genotype. Supported in part by R01ES026057 and R01ES033892.
Urban air pollution exposure, including ozone ($O_3$), has been associated with increased Alzheimer’s Disease (AD) risk and there is recent support that urban air pollution exposure may increase amyloid plaque pathology. However, the underlying cellular mechanisms driving this effect are poorly understood. The CNS vasculature is known to be dysregulated early in AD and the accumulation of amyloid pathology in the vasculature is known to be a key component of the disease. While $O_3$ exposure has long been linked to cerebral vascular effects such as stroke, how $O_3$ may affect the vasculature characteristics in AD pathology is largely unknown. To begin to explore this, 10-11-week-old male $5\times$FAD mice were exposed to filtered air (FA) or 1 ppm $O_3$ for 13 weeks (4 hours/day and 3 days/week). Analysis of Thio-stained plaques demonstrated that $O_3$ increases amyloid plaque load. The amyloid binding GeoMX Digital Spatial Profiling (DSP) platform was used to assess multiplex protein expression co-localized with laminin positive vascular endothelial cell structures, that were either in physical contact with amyloid plaques (plaque-associated vessels) or plaque distant, in the cortex. DSP analysis revealed that the expression of 19 proteins were shared regardless of plaque association phenotype or $O_3$ exposure. When comparing plaque-associated vessels to plaque distant ones, 13 proteins were changed in only FA exposed mice, such as IDE and TMEM119, supporting that a baseline change in plaque-associated vasculature proteins occurs, regardless of $O_3$ exposure. However, the expression of 12 different proteins, such as C4B, CD169, Empt1, MSR1, and Lamp1 were dysregulated in plaque associated vasculature in $O_3$ exposed mice only, suggesting that $O_3$ exposure selectively alters the vascular endothelial cell proteomic phenotype in the peri-plaque space. While there are some vasculature specific proteins such as Empt1, Vimentin, that are differentially expressed in the peri-plaque space of $O_3$ exposed mice, a closer look at these proteins shows that some microglia specific proteins are also dysregulated in the laminin positive areas, implicating an altered vasculature-microglia interaction with ozone exposure. Taken together, these findings suggest that $O_3$ modifies the amyloid-associated changes in the vasculature and potentially dysregulates microglial association with the vasculature, which is dependent on localization in the plaque microenvironment. These findings provide new insight into how urban air pollution affects amyloid cerebral vascular pathology.

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Inhalation of Trichloroethylene Induces Cellular Senescence as a Mechanism of Parkinson’s Neurodegeneration

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The organic solvent and degreasing compound trichloroethylene (TCE) is a ubiquitous environmental contaminant that is associated with Parkinson’s disease (PD) risk. Previous research from our lab showed that six weeks of TCE exposure (200 mg/kg) via oral gavage in adult rats causes significant degeneration of nigrostriatal dopaminergic neurons, the accumulation of alpha-synuclein, endolysosomal impairment, and neuroinflammation. In addition to this previously characterized pathology, we recently observed a significant increase of senescent neuronal and glial cell populations within TCE-exposed rats, suggesting that acceleration of senescence may be a potential driver of TCE-induced neurodegeneration. As senescent cell populations are elevated in postmortem tissue from PD patients, we hypothesized that TCE exposure induces senescence as a mechanism that accelerates aging in the brain and influences neurodegeneration. To test this in a novel and environmentally relevant model, we developed an inhalation model to expose 10-month-old male and female Lewis rats to 50 ppm TCE or control (filtered room air) over 8 weeks in a whole-body passive exposure inhalation chamber. Rats exposed to TCE showed significant loss of dopaminergic neurons within the striatum and substantia nigra as well as pyramidal neurons in the CA3 of the hippocampus (p = 0.0003, p = 0.0002, respectively). Concurrently, we observed significant increase in protein S1QEL1.1 and p53 in nigral (p = 0.0019) and hippocampal (p = 0.0043) neurons as well as in surrounding microglia (p = 0.0044). In addition, we observed an increase in microglial activation measured with CD68 (p = 0.0177) accompanied by an increase in expression of proinflammatory signaling proteins for innate and adaptive immune responses such as MHC1 in dopaminergic neurons and microglia (p = 0.0117; p = 0.0001, respectively). Together, these data show that inhalation TCE exposure causes neurodegeneration within both motor and cognitive brain regions associated with PD, which could ultimately influence disease phenotype. In addition, our data indicate that environmental toxicant-induced senescence within brain regions that degenerate in PD and similar neurodegenerative diseases such as Dementia with Lewy Bodies (DLB) could be a fundamental new mechanism that drives neuroinflammation and neurodegeneration.

High-Sugar Diets Modulate Oxidative Stress Induction by 6-OHDA to Reduce Dopaminergic Neurodegeneration

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Parkinson’s disease (PD) is the second most common neurodegenerative disease, directly and indirectly impacting millions of Americans each year. Characterized by the progressive loss of dopaminergic neurons in the brain, sporadic PD has no established cause or cure despite being linked to environmental exposures and lifestyle factors. Current hypotheses suggest mitochondrial dysfunction is a critical contributor to PD, and that oxidative stress is a significant risk factor for PD development. However, the mechanism by which western diets and mitochondrial toxics induce PD remains unclear. In contrast to previous evidence that western diets increase PD risk, we show that a portion of a western diet, high sugar consumption, is protective from 6-hydroxydopamine (6-OHDA) induced dopaminergic neurodegeneration. Using the model C. elegans, we found that 6-OHDA exposure induces the same degree of neuronal ATP depletion regardless of diet and that sugar-fed worms show far greater organismal ATP depletion after rotenone exposure. Conversely, sugar-fed worms were resistant to the 6-OHDA induced increase in glutathione pool oxidation within dopaminergic neurons. Our results highlight that oxidative stress, not bioenergetic depletion, is critical to loss of dopaminergic neurons. Finally, sugar-fed worms are more susceptible to swimming induced paralysis (SWIP), suggesting alterations to the dopaminergic system despite no phenotypic neurodegeneration. As 6-OHDA uptake into the neurons is dependent on dopamine transport, this suggests high-sugar diets may protect from 6-OHDA by altering its transport and preventing oxidative stress. Taken together, this work underscores the importance of the interaction between diet and toxicokinetics in toxicant induced PD models as well as the critical mechanistic role of oxidative stress on bioenergetic function.

Investigating the Role of Q-Site-Derived Superoxide Anion in Complex I Inhibitor–Induced Dopaminergic Neurodegeneration

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Mitochondrial electron transport chain complex I dysfunction has been directly implicated in the etiology of Parkinson’s disease (PD), via unclear mechanisms. Complex I (CI) inhibition by rotenone, a pesticide commonly used to model PD, results in increased CI superoxide production into the mitochondrial matrix. I used S1QEL1.1, an inhibitor of Complex I superoxide production at the quinone binding site, in C. elegans for the first time to determine the mechanistic role of CI-derived superoxide in the induction of dopaminergic neurodegeneration. To confirm its effects in vivo, the impact of S1QEL1.1 on forward electron transport and the redox tone of the glutathione pool were investigated. As detected by whole worm respirometry, S1QEL1.1 does not inhibit forward electron transport in C. elegans. Surprisingly, S1QEL1.1 treatment alone increases the oxidation status of the glutathione pool. However, it completely ablates the increase in glutathione oxidation induced by rotenone in the developing C. elegans. Finally, using a 6-OHDA-induced dopaminergic neurodegeneration I have demonstrated that S1QEL1.1 decreases susceptibility to 6-OHDA induced neurodegeneration and alters mitochondrial morphology. Taken together, these findings suggest that the change in superoxide production by Complex I, potentially via reverse electron transport, plays an additive role in toxicant induced dopaminergic neurodegeneration.

Investigation of Exosomal miRNAs in Cerebrospinal Fluid/Serum of the Rotenone-Induced Rat Model of Parkinson’s Disease

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Parkinson’s disease (PD) is a chronic and progressive neurodegenerative disorder characterized by selective nigrostriatal dopaminergic degeneration in the brain. The etiology of PD is known to be a combined effect of environmental as well as genetic factors. Rotenone is a naturally occurring compound that is extensively used as an insecticide and pesticide. Rotenone is a potent mitochondrial complex I inhibitor that has been identified as a PD risk factor. Several studies have demonstrated that rat rotenone models can accurately recapitulate several features of PD, including nigrostriatal dopaminergic neurodegeneration, alpha-synuclein aggregation, microglial activation, and oxidative modifications. Transgenic PD pathology spread implies that intercellular communication is vital to pathogenesis. A class of extracellular vesicles, exosomes, are emerging as critical to these processes. Many studies have shown that cargo carried by these vesicles influences physiology in the bystander cells, impacting pathological fate. Exosomal miRNAs can regulate various molecular pathways and biological processes in the cell. Circulating exosomal miRNAs are found in various biological fluids, including serum and cerebrospinal fluid (CSF), and are suitable to be used as disease biomarkers. The identification of exosomal miRNAs can serve as early diagnostic and prognostic biomarkers for PD. Moreover, it is necessary to explore the molecular mechanisms by which these exosomal miRNAs control and regulate pathways related to PD pathogenesis. In this study, we tested the hypothesis that exosomal miRNAs in CSF/serum of rats would be modified in response to acute rotenone treatment. Primary cortical and midbrain neurons were treated with rotenone (60nM and 25nM respectively) for 24h to identify the exosomal miRNAs and their differential enrichment. A number of miRNAs were differentially expressed in the exosomes in PD stress conditions. Further, 3-month-old Sprague-Dawley male rats were acutely dosed with rotenone (3mg/kg) for 8 hours and 24 hours, and serum and CSF were extracted. The exosomal miRNAs in serum and CSF were also altered in PD stress conditions. Preliminary data suggest that miR-181c-5p is especially prone to modification, which is important in modulation of mitochondrial functions. Our study associates the differential expression of miRNAs in circulating serum/CSF with PD. Exosomal miRNAs can regulate various molecular pathways and biological processes in the cell. Circulating exosomal miRNAs are found in various biological fluids, including serum and cerebrospinal fluid (CSF), and are suitable to be used as disease biomarkers. The identification of exosomal miRNAs can serve as early diagnostic and prognostic biomarkers for PD. Moreover, it is necessary to explore the molecular mechanisms by which these exosomal miRNAs control and regulate pathways related to PD pathogenesis. In this study, we tested the hypothesis that exosomal miRNAs in CSF/serum of rats would be modified in response to acute rotenone treatment. Primary cortical and midbrain neurons were treated with rotenone (60nM and 25nM respectively) for 24h to identify the exosomal miRNAs and their differential enrichment. A number of miRNAs were differentially expressed in the exosomes in PD stress conditions. Further, 3-month-old Sprague-Dawley male rats were acutely dosed with rotenone (3mg/kg) for 8 hours and 24 hours, and serum and CSF were extracted. The exosomal miRNAs in serum and CSF were also altered in PD stress conditions. Preliminary data suggest that miR-181c-5p is especially prone to modification, which is important in modulation of mitochondrial functions. Our study associates the differential expression of miRNAs in circulating serum/CSF with PD. Exosomal miRNAs can regulate various molecular pathways and biological processes in the cell. Circulating exosomal miRNAs are found in various biological fluids, including serum and cerebrospinal fluid (CSF), and are suitable to be used as disease biomarkers. The identification of exosomal miRNAs can serve as early diagnostic and prognostic biomarkers for PD.
dyskinesia (LID) and motor fluctuations due to non-continuous, pulsatile delivery of L-DOPA to the brain. To address this issue, we developed an innovative strategy to augment the host’s microbiota with a genetically engineered bacterial live-biotherapeutic, E. coli Nissle 1917 (EcN), to continuously produce L-DOPA (EcN\textsubscript{L-DOPA}) using synthetic biology. Thus far, we have developed three generations of the EcN\textsubscript{L-DOPA}\textsuperscript{rha}-utilizing modern synthetic biology and genome engineering techniques and characterized them to endogenously produce L-DOPA from tyrosine using the recombinant hpaB/C genes. The initial two generations of EcN\textsubscript{L-DOPA}\textsuperscript{rha} were plankmid-based, and we further improved it by integrating the hpaB/C genes into the chromosome with a rhamnose (Rha) regulatable promoter system using advanced Sce-ROPE genetic engineering. Pharmacokinetics, pharmacodynamics, microbial kinetics, and safety and tolerability measures were assessed over time. Our results demonstrated that EcN\textsubscript{L-DOPA}\textsuperscript{rha} can serve as one of the co-factors regulating the pathogenic α-synuclein protein misfolding process. Also, Rha treatment exacerbated α-synuclein-mediated NLRP3 activation, cytokine release and nitrogen species generation in mouse primary microglia when compared to α-synuclein treatment alone. Collectively, these results demonstrate that endogenous H3 serves as a key pro-inflammatory mediator that is involved in the NLRP3 inflammasome pathway. Funding support: NIH/NIEHS ES027245 and ES026892.

3939 Environmental and Pharmacological Regulators of Vesicular Dopamine Dynamics

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Proper dopamine homeostasis (i.e. synthesis, sequestration, release, reuptake, and degradation) underlies the regulation of behaviors such as attention, movement, and reinforcement. Consequently, dysregulation of dopamine homeostasis is a feature of several disorders and diseases such as ADHD, Parkinson’s disease, and substance use disorder, making dopamine homeostasis an attractive pharmacotherapeutic target for these disorders. Furthermore, as these disorders are known to have environmental risk factors, it is important to evaluate the effects of toxicant exposures on dopamine homeostasis. We have developed a 96-well plate in vitro assay to evaluate dopamine dynamics (i.e. vesicular uptake dynamics) using the fluorescent false neurotransmitter 206 (FFN206) for pharmacological and toxicological screening. Variations of this assay can be employed to understand uptake dynamics, such as calculating IC\textsubscript{50} or EC\textsubscript{50}, or retention dynamics, such as calculating linear and non-linear rates of fluorescent decay. Here, we demonstrate in human neuroblastoma (SH-SY5Y) and the fastest-growing neurodegenerative disease, PD prevalence is predicted to be doubled in 40 years, making dopamine homeostasis a critical target for future therapeutic development. We first characterized the effects of compounds known to regulate vesicular dopamine dynamics to identify the role of the synaptic vesicle glycoprotein 2C (SV2C) in vesicular retention, to evaluate pharmacological effects using established dopamine-associated pharmacotherapeutics tetrahexamine (TBZ) and bupropion, and to understand how dopamine-associated toxicants such as 1-methyl-4-phenylpyridinium (MPP\textsuperscript{+}) and polychlorinated biphenyls (PCBs) affect dopamine dynamics. HEK293 cells expressing human vesicular monoamine transporter 2 (VMAT2) were incubated with FFN206 within vesicles, resulting in a fluorescent signal measurable by plate reader. The addition of SV2C, a vesicle localized protein whose function remains unknown, to HEK293 cells expressing VMAT2 results in increased FFN206 uptake fluorescence and a slower loss of uptake compared to cells lacking SV2C. These findings were validated in subsequent radiolabeled dopamine uptake and retention experiments performed in isolated vesicles from cells expressing VMAT2 or VMAT2 and SV2C. Novel and existing pharmacotherapeutics can be evaluated in the FFN206 assays to understand the effect of treatment on vesicular dopamine dynamics. For example, bupropion, an atypical antidepressant with no known direct action on vesicular uptake, inhibits FFN206 uptake when co-incubated with FFN206; however, overnight pre-treatment with bupropion with a one-hour wash-out period results in enhancement of FFN206 uptake. In the retention assay, bupropion causes a rapid loss of FFN206 fluorescence, suggesting a mechanism by which bupropion could cause l-dopa efflux. FFN206 from vesicle dynamics are distinct from those of MPP\textsuperscript{+}, a known competitive VMAT2 and TBZ, a known non-competitive VMAT2 inhibitor. Thus, this assay can be used to compare the effects of pharmacotherapeutics and disease-associated toxicants to the effects of compounds known to regulate vesicular dopamine dynamics to identify novel therapeutics and to understand how environmental factors are involved in disease pathogenesis.

3940 Novel “Seek-and-Rescue” Genetic Strategy to Uncouple the Synergistic Effects of Paraquat on Alpha-synuclein Propagation

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Exposure to paraquat (PQ), a Parkinson’s disease (PD) associated environmental toxin, heightened neuronal genomic instability, and elicited neuropathology. PD is the second-most prevalent neurodegenerative disease, where most incidences have no identifiable inheritance and occur in a sporadic form. Environmental risk factors (e.g., pesticide/herbicides, exposure to metals, solvents, and PCBs, virus infection, and head trauma) have been speculated as a trigger to the sporadic
PD. To explore the causal and pathogenic evidence of environmental triggers, we have combined the genome-associated virus (HAV) genome-processing machinery and the instability of a hypermutable repeat sequence to detect neuronal genomic instability and visualize neurodegeneration in post-mitotic differentiated neurons. The increased neuronal genomic instability was probed in a genetic alpha-synuclein (αSyn) transgenic mouse model infected with pre-formed fibrils (PF). To determine the potential survival effects of PF on αSyn, C57Bl/6 mice were treated with either PF alone, PF only, or a PFF+PF treatment combination and then assessed for motor/sensorimotor and cognitive function. Neither PFF nor PF alone produced differences in motor/sensorimotor behavior. However, PFF+PF treatment exacerbated both motor/sensorimotor deficits, suggesting a synergistic role for PF on αSyn propagation. PFF induced progressive cognitive deficits, but no synergistic effect of PF was observed in the PFF+PF treatment group. To determine the causal link and to explore the potential for disease-modifying therapies, we developed a novel genetic strategy to “Seek-and-Rescue” neurons with DNA damage non-invasively via the systemic introduction of a genetic sensor and actuator of DNA damage response (DDR). A genetic actuator was implemented via a Cre-dependent conditional shRNA to knockdown ATM, a major orchestrator of the DDR in the hope to slow down the progression of neurodegeneration. Conditional knockdown of ATM expression accelerated the progression of PFF+PF-mediated motor and cognitive deficits. These studies can pave the way for new paradigms to determine the causal and pathogenic roles of DDR and exposure to environmental toxins in the progression of neurodegenerative disease and identify therapeutic targets. Supported by NIEHS (R21ES031211).

3941 PKD1-Mediated Compensatory Signaling in Pesticide-Induced Oxidative Damage in Dopaminergic System: Relevance to the Pathogenesis of Parkinson’s Disease
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The etiology of Parkinson’s disease (PD) is complex and heterogeneous. Epidemiological and laboratory studies suggest exposure to the agricultural pesticide paraquat (PQ) as a putative risk factor that induces oxidative stress through reactive oxygen species (ROS) generation mediated by redox cycling. We previously reported that the serine-threonine kinase PKD1 is highly expressed in nigral dopaminergic neurons and its protein kinase Cδ (PKCδ)-dependent activation represents a novel intrinsic compensatory response in counteracting the early-stage oxidative damage induced by h2O2 and 6-OHDA in dopaminergic neurons. Nevertheless, the exact molecular mechanisms underlying PKD1-mediated neuroprotective effects during the initial stages of oxidative stress remain enigmatic. Here, we report that a PKD1-dependent early activation of the mammalian target of rapamycin (mTOR) pathway may play a critical role in mediating the PKD1 compensatory signaling response in dopaminergic cells. The mTOR protein is well known to function as a regulator of cell growth, survival, protein synthesis, and autophagy, and its levels vary during PD development. First, we show that exposure to PQ induced time-dependent neurotoxicity in N27 dopaminergic neuronal cells, as determined by decreased cell viability and increased mitochondrial fragmentation. We also found that mitochondrial fragmentation was initiated during the early stage of PQ treatment (6h). The involvement of early dopaminergic mitochondrial dysfunction prior to cell death. Consistent with our published studies, treatment with PQ also induced an early compensatory PKD1 signaling response in N27 dopaminergic neuronal cells, as determined by enhanced phosphorylation of the Ser744/748 activation loop and Ser916. Importantly, PQ-induced early PKD1 activation was accompanied by rapid phosphorylation of mTOR, but no synergistic effect of PQ was observed in the PFF+PF treatment group. To determine the causal link and explore the potential for disease-modifying therapies, we developed a novel genetic strategy to “Seek-and-Rescue” neurons with DNA damage non-invasively via the systemic introduction of a genetic sensor and actuator of DNA damage response (DDR). A genetic actuator was implemented via a Cre-dependent conditional shRNA to knockdown ATM, a major orchestrator of the DDR in the hope to slow down the progression of neurodegeneration. Conditional knockdown of ATM expression accelerated the progression of PFF+PF-mediated motor and cognitive deficits. These studies can pave the way for new paradigms to determine the causal and pathogenic roles of DDR and exposure to environmental toxins in the progression of neurodegenerative disease and identify therapeutic targets. Supported by NIEHS (R21ES031211).

3942 Pharmacological Modulation of the Glucocorticoid Receptor Reduces Neuronal Loss and Neuroinflammation Caused by Rotenone Toxicity in C57Bl/6 Mice
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Approximately one billion people suffer from a neurological disorder worldwide. This encompasses the 50 million people diagnosed with a neurodegenerative disease, including Alzheimer’s Disease (AD), Parkinson’s Disease (PD), and dementia. Neurodegenerative disease cases are estimated to double over the next twenty years, further establishing the importance of research in this field. This estimated escalation in cases will occur due to the rising aging popula- tion as well as the detrimental effects of environmental exposures. One such exposure is rotenone, a broad-spectrum insecticide and pesticide historically used in agriculture. Although the mechanism is not fully understood, increasing evidence demonstrates that prolonged exposure to rotenone causes pathology in dopaminergic neurons, resulting in neuronal death and nigrostriatal dopaminergic neurodegeneration. Here we report that administration of the glucocorticoid receptor (GR) agonist, dexamethasone, to C57Bl/6 mice exposed to rotenone (2.5 mg/kg/day) for 14 days followed by 14 days of post-lesioning recovery, during which time we previously reported the majority of neuroinflammation and neuronal loss occurs. Mice exposed to rotenone showed neuro-behavioral deficits and PD-like pathology, including loss of dopaminergic neurons and activation of microglia and astrocytes. Administering dexamethasone for 14 days prior to and following rotenone exposure showed improved spatial memory and motor function compared to untreated animals, as well as preservation of dopaminergic neurons. These data suggest that anti-inflammatory modulators of GR could be tractable targets for mitigating the damaging effects of neuroinflammation in PD.

3943 Anti-Neurofilament-Life (NF-L) and Anti-α-Synuclein (α-SYN) Autoantibodies Are Prevalent in Industrial Workers with Parkinson’s Disease (PD) and Associate with Metals
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Lacking clear etiological factors in idiopathic neurodegenerative diseases like PD, Alzheimer’s disease (AD) and Amyotrophic Lateral Sclerosis (ALS), has led to an appreciation of the environment as a major contributing factor. Here the environment being the sum total of external life-experiences and the interplay with internal milieu of “omic” activity. Hence, the exposome. Equally lacking in ND, are reliable biomarkers of ND. While NF-L and α-SYN have been suggested as potential biomarkers, recent hypotheses suggest that rather than contributing factors to the pathophysiology or as pathological landmarks, it is their loss that should be focus of investigation. Thus, the assay for autoantibodies (NAB) as potential biomarkers in ND and neurotoxic exposures was recently reported. For the present study recruited a reference group (R; n=40; 19 F, 21 M; age=40±8 yr) with no diagnosis of ND. Subjects with a history of industrial and agricultural occupations and PD diagnosis of 6 months to 10 years were also recruited (7F, 22M; age=54±5 yr). Anti-NF-L, IgM and Anti-α-SYN, IgG, determined by ELISA, were highly prevalent in PD compared to R (100 vs 70% and 100 vs 70%, p<0.0001). Median levels of these NAB were significantly higher in PD subjects [NF-L IgM PD=55 (IQR=46); R=0 (IQR=0); α-SYN IgG PD=17 (IQR=22); R=0 (IQR=0.3); p<0.0001]. Serum levels of metals, Cd, Cu, Pb, Mn, and Al, determined using ICP, were higher in PD subjects, but were only statistically significant for Cu (0.0001). Spearman’s correlations for anti-NF-L and anti-α-SYN with Cu levels were notable (n=40; r=0.4274; p=0.0037; α-SYN IgG r=0.4353; p=0.0025). Moreover, titters of both significantly associated with age (n=40; r=0.5886; p=0.0001), a recognized risk factor in PD, including ND. Relative risk (RR) NF-L IgM 2.0526; p=0.0001; 95% CI=1.4875 to 2.8324; RR α-SYN 2.7857; p=0.0001; 95% CI=1.8314 to 2.4723) confirmed the association with PD diagnosis. Furthermore, recent reports have implicated Cu in the aggregation of α-SYN in PD pathogenesis, which appears to be independent of neurtin fibilin-3. The present study suggests that NAB detection may be useful in identifying ND insult, and that environmental risk factors may contribute to disease outcomes. Supported by the Exposome Project funded by the Bartlett Research Fund at AUC.

3944 Role of Prokineticin 2 and EGR1 Signaling Axis in Neurotoxic Metal-Induced Offactory Dysfunction
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Chronic environmental exposure to neurotoxic concentrations of manganese (Mn) is linked to olfactory dysfunction and may be a prodromal marker of metal neurotoxicity. Mn is known to accumulate in the olfactory system (e.g., in the olfactory bulb), mainly within the globus pallidus, and cause GABAergic neuronal dysfunction leading to a Parkinsonism phenotype. The underlying mechanism of Mn neurotoxicity causing olfactory dysfunction and BG pathology is poorly understood. Recently, we reported that the neuropetide prokineticin 2 (PK2) is rapidly upregulated in the early stages of dopaminergic neurotoxicity in a neurotoxin-induced animal model of Parkinsonism, and it participates in a novel compensatory protective response to counteract neurotoxicity by activating pro-survival pathways. Thus, we hypothesize that PK2 is a promising endogenous neurogenic target in the olfactory system. PK2 is known to promote olfactory biogenesis, but the underlying neurogenic
Mechanisms and its relation to adult neurogenesis are still largely unknown. In this study, we investigated the beneficial interaction between PK2 and the resulting early protective mechanism against mitochondrial dysfunction and inflammation in olfactory ensheathing glia (OEGs) using both in vitro and in vivo models of Mn-neurotoxicity. Mechanistically, Mn exposure induced early upregulation of PK2 in the OEGs through EGR1 upregulation and inhibition of STAT1, which protected the OEGs from mitochondrial dysregulation and inflammation. We also show that PK2 is expressed in the glomerular layer of the olfactory bulb and colocalizes with TH, highlighting its role in dopaminergic neurons and may play a regulatory role to promote the intrinsic neurogenic potential in the early stage of a neurotoxic insult. Furthermore, AAV-mediated overexpression of EGR1 in the mouse SVZ induced intrinsic adult neurogenesis, suggesting that the PK2-EGR1 axis may be a key regulatory pathway associated with endogenous neuronal regenerative capacity that counteracts early stages of neurotoxicity. Collectively, our findings reveal a novel compensatory signaling pathway mediated by the neuroepitode PK2 in Mn-induced olfactory alterations and suggest PK2 could be a therapeutic target for environmentally linked olfactory dysfunction-associated chronic neurodegenerative diseases like Parkinson’s and Alzheimer’s disease.

Supported by NIH/NIAMS ES026892.

**3945 Mitochondrial Neurotoxic Stress Impairs the Nuclear Pore Complex: Implications for Pathogenesis of Environmentally Linked Parkinson’s Disease**

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Environmental exposure to neurotoxic pesticides, including mitochondrial complex I inhibitors, is a key etiological factor in the development of sporadic Parkinson’s disease (PD). However, the precise mechanisms driving neurotoxic pesticide-induced dopaminergic (DAergic) neurodegeneration are not well understood. Disruption of the nuclear pore complex and impairment of bidirectional nuclear-cytosolic transport are emerging as crucial pathogenic mechanisms of neurodegenerative diseases. We recently found that mitochondria-impairing pesticides induce phosphorylation and loss of nuclear Lamin B1, thereby compromising nuclear membrane integrity. The involvement of channel-forming nucleoporins (NUPs) in mitochondrial dysfunction-related DAergic neurodegeneration has not been investigated. Herein, we characterize the alterations in NUPs in both rotenone-induced and transgenic mitochondrial dysfunction-induced models of PD. Our immunoblot analysis shows that rotenone exposure induced a significant loss of the scaffold and central channel NUPs 107 and 62, respectively, as well as a notable reduction in the nuclear basket NUPs 50 and 153 in N27 DAergic neuronal cells. To validate our findings on how NUP levels respond to rotenone-induced mitochondrial stress, we generated a CRISPR/Cas9-based stable mitochondrial transcription factor A (TFAM) knockdown DAergic cell culture model of mitochondrial dysfunction. The results were consistent with the rotenone-induced model. We then examined mice transgenic mice, an in vivo model of mitochondrial impairment. Double immunohistochemical staining for the DAergic neuron marker, TH, and NUPs in substantia nigra sections revealed reduced NUP expression in mitochondrial dysfunction-related DAergic neurodegeneration. This finding was corroborated by the lack of rotenone-induced hyperphosphorylation. Our results establish that rotenone-induced mitochondrial stress, generated a mitochondria-dependent NUP loss, which was characterized by altered nuclear-membrane integrity and disrupted nuclear-cytosolic transport.

**3946 Trichloroethylene Exposure Influences Brain Lipid Dysregulation as a Putative Mechanism in Parkinson’s Neurodegeneration**

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Parkinson’s disease (PD) is a progressive neurodegenerative disease that is marked by a loss of dopaminergic neurons in the substantia nigra pars compacta and the appearance of the protein alpha-synuclein, which comprises Lewy Bodies. Most (~85%) of PD is idiopathic, and environmental triggers of neurodegeneration are prevalent risk factors in PD etiology. One environmental risk factor associated with PD risk and of growing concern is the organic solvent trichloroethylene (TCE), which is a commonly used chemical feedstock, degreasing agent, and a pervasive environmental contaminant. Recently, we demonstrated that TCE exposure in rats resulted in the death of nigrostriatal dopaminergic neurons as well as the accumulation of alpha-synuclein, indicating that TCE may influence multiple pathogenic disease pathways involved in PD. As TCE is reported to integrate into lipid membranes within the brain, resulting in increased risk for alpha-synuclein misfolding and aggregation. To assess this, we treated aged (12-month-old) male Lewis rats with a high dose (800 mg/kg) of TCE via oral gavage (or vehicle, olive oil) daily for 3 weeks, a time point prior to dopaminergic cell loss. Whole brain tissue was assessed for lipidomic analysis using mass spectrometry (MS), which we then analyzed via Metaboanalyst using peak intensities of the 10 most marked lipid classes in each category, including phosphatidylcholines and negative phosphatidylserines, sphingomyelins, and triacylglycerols. Orthogonal partial least squares-discriminant analysis (PLS-DA) revealed significant separation between vehicle and TCE treated rat brain total lipids, with an overall trend of reduced lipid peak intensities in TCE exposed brain tissue. However, direct comparisons of the top 10 lipids in each group revealed that a single triacylglycerol (TAG) 54:3+NH4+ (FA 18.2) had the greatest variation in vehicle and TCE treated rats (r-test, p<0.001). This lipid contains a fatty acid (FA 18.2), which corresponds with linoleic acid, a triglyceride that is reduced in PD patient plasma and brain tissue, and implicated in changes in membrane fluidity that promote the misfolding and accumulation of alpha-synuclein. Taken together, these data suggest that TCE-induced alterations in lipid composition in the brain may influence misfolding and aggregation events in alpha-synuclein, possibly driving synucleinopathy and PD risk from exposure to this environmental pollutant.

**3947 Direct Pararquat Treatment Enriches NLRP3 Inflammasome and Microglia Transcripts via Voltage-Gated Proton Channels Hv1 in Mouse Brain Gial Profiling**

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One of the most commonly used herbicides, pararquat (PQ), has been shown to increase the risk of developing Parkinson’s disease (PD) by 2.5-fold. PQ exposure caused PD-like pathology in mouse brain, including selective nigrostriatal degeneration, aggregation of α-synuclein, and increased neuroinflammation. It is well-established that PQ neurotoxicity is dependent upon neuroinflammation and microglial activation, but the mechanisms underlying this are unknown. In this study, we explored the role of Hv1c1/Hv1, a voltage-gated proton channel specifically expressed in brain microglia, in PQ-induced neuroinflammation and neurodegeneration. Cs7 Bl6 and Hv1 knockout (Hv1KO) mice were randomly assigned to C57/Saline, C57/PQ, Hv1KO/Saline, and Hv1KO/PQ. Mice in the PQ group were administered a single dose of PQ (10 mg/kg i.p.) and sacrificed 48 hrs later. Multiplex gene expression was performed with the NanoString nCounter glial profiling panel consisting of 770 mouse target genes and more than 50 pathways including lipopolysaccharide, lipopolysaccharide, cell stress and damage, glial cell homeostasis and activation, neurotransmission, and peripheral immune invasion. The major NLRPS3 inflammatory genes, including NLRPS3, IL-1b, and IL-18, were induced by PQ administration in the striatum from C57 mice, but not altered in Hv1 KO mice. Pararquat administration increased microglia genes including P2ry12, Aif1, Ifgam, Cx30, Cx26, Cx1, Gpr46, Spa1, Irfl, Lcn2S, Tmem119, and Tlr4 in C57 mice in the striatum but not in Hv1KO. Further, PQ treatment significantly upregulated several key disease-associated microglia (DAM) genes such as P2ry12, Gpr43, Trypob, Cts3, Siglech, Apo, Hexb, Ctsl, Sppe1, Cx3cr1, Axl, Fapb5, Trem2, Fth1, Ly22, Fcrs1, Mr1, Clec7a, Tmem119, and B2m in the C57 striatum but had no effect in Hv1KO. Oligodendrocyte target genes were also significantly reduced in the C57 striatum by PQ treatment, but not altered in Hv1KO. Paraquat administration increased microglia genes including P2ry12, Aif1, Ifgam, Cx30, Cx26, Cx1, Gpr46, Spa1, Irfl, Lcn2S, Tmem119, and Tlr4 in C57 mice in the striatum but not in Hv1KO. In addition, PQ treatment dramatically reduced the expression of A1 astrocyte markers while increasing markers of A2 astrocytes in the C57 striatum. Additionally, PQ treatment significantly reduced target neuron genes such as Dlx1, Dlx2, Gm2, Tbr1, Isil2, and Slc17a6 in the C57 striatum but this was not observed in the Hv1KO striatum. To confirm the loss of neuronal cells, we used unbiased stereology counting to demonstrate neuronal cell death in the substantia nigra (SN). PQ treatment resulted in 40% of TH+ neurons in the substantia nigra of C57, but not Hv1 KO mice. All endothelial target genes including Lsr, Nostri, Fl1, Esam, Oldr5, Tie1, Emcn, and Icam2 were significantly reduced in the C57 striatum by PQ treatment, but PQ had no effect in the Hv1KO striatum. Oligodendrocytes target genes were also significantly reduced by PQ treatment, but the effects were abolished in the Hv1KO striatum. We found no specific pattern changes caused by PQ for transcripts associated with neutrophils, mast cells, dendritic cells, B cells, T cells, NK cells, or cytotoxic cells. These findings demonstrate that PQ treatment increases NLRPS3 inflammasome and pro-inflammatory transcripts in an Hv1-dependent manner, while decreasing neuron transcripts. Supported by R01ES033892 and a grant from NanoString Technologies, Inc.
oxidative stress. Reduced by post-lesion treatment with 10 mg/kg MLi2 (p < 0.0001). Collectively, astrocyes and 4-hydroxynonenal (p<0.05). DA oxidative damage was significantly caused elevated oxidative damage within DA neurons measured with 3-nitrotyrosine proxy in N27-A cells treated with the four toxicants, with and without MLi2. Mitochondrial markers respectively) as a mitophagosome, and may serve as a potential mediator of its effects. We therefore assessed LC3B and oxidative damage. Based on these data, we hypothesized that inhibition of LRK2 kinase activity may attenuate toxicant-induced damage to dopaminergic neurons. To test this hypothesis, we inhibited LRK2 kinase activity using CRISPR-edited 293 HEK cells with the common G2019S LRK2 mutation displayed aberrant kinase activity in the brain of rats exposed to ROT or TCE. Toxicant-induced LRK2 kinase activity elevation demonstrates neuronal pathology mirroring that of inherited mutations in LRK2, such as vesicular trafficking impairment, and oxidative damage. Based on these data, we hypothesized that inhibition of LRK2 kinase activity may attenuate toxicant-induced damage to dopaminergic neurons in the N27-A cells. To test this, we first assessed the efficacy of LRK2 kinase inhibition against toxicant-induced oxidative damage in N27-A cells treated with 500 nM ROT, 100 µM PQ, 500 µM TCE or PERC. Toxicant exposure significantly increased reactive oxygen species (ROS), quantified using fluorescein dihydroethidium (DHE; p<0.001) and was potently reduced by 10 mg/kg/day BaP in corn oil-soaked food from gestational day 10 through weaning at postnatal day 25. We compared control animals without exercise to three exercise groups: dam only, pup only or dam + pup. Exercise consisted of 1h/day voluntary running on exercise wheels. Dams exercised for 2 weeks prior to mating through gestational day 10. Offspring exercised from P30 to P60 when behavioral testing began. Blood was collected at P60, and BDNF measured in plasma using a commercial ELISA kit. Once behavioral testing was complete at P120, hippocampi of the mice were collected and tissues BDNF levels were measured using the same ELISA kit. BaP treatment significantly decreased (p<0.05) BDNF levels, but was not found significantly higher BDNF levels in the plasma of offspring from the dam-only and pup-only exercise groups (P < 0.001). Surprisingly, offspring from the dam+pup exercise group had BDNF levels similar to control animals. We also found a sex x exercise interaction with male pups that exercised having significantly higher BDNF levels compared to females (P<0.05). This suggests that differences in muscle mass might contribute to increased BDNF signaling along the muscle-brain axis. There were no significant differences in hippocampal levels of BDNF at P120.

Environmental toxicants that induce mitochondrial dysfunction are associated with increased risk of Parkinson’s disease (PD) such as pesticides rotenone (ROT) and parathion (PQ), and organic solvents trichloroethylene (TCE) and tetrachloroethylene (Perc). Recently, we reported that the protein kinase Leucine Rich Repeat Kinase 2 (LRK2), the most commonly inherited mutation in familial PD, exhibited aberrant kinase activity in the brain of rats exposed to ROT or TCE. Toxicant-induced LRK2 kinase activity elevation demonstrates neuronal pathology mirroring that of inherited mutations in LRK2, such as vesicular trafficking impairment, and oxidative damage. Based on these data, we hypothesized that inhibition of LRK2 kinase activity may attenuate toxicant-induced damage to dopaminergic neurons. To test this, we first assessed the efficacy of LRK2 kinase inhibition against environmental PD toxicants using the dopaminergic rat neuronal N27-A cell line treated with 500 nM ROT, 100 µM PQ, 500 µM TCE or PERC. Toxicant exposure significantly increased reactive oxygen species (ROS), quantified using fluorescein dihydroethidium (DHE; p<0.001) and was potently reduced by 10 mg/kg/day BaP in corn oil-soaked food from gestational day 10 until postnatal day 25 equivalent to the second and third trimesters in humans. We collected blood for corticosterone measurements at baseline and following the forced swim test in offspring after they reached adulthood (P60). BaP exposure had no effect on performance in the forced swim test. However, there was a significant effect of genotype. Poor-affinity AhrCyp1a2(-/-) mice spent more time floating v fighting to escape compared with high-affinity AhrCyp1a2(+/+) and AhrCyp1a2(+/–) mice. We found a significant difference between baseline and post-stress corticosterone levels (P < 0.001), but there were no differences in stress hormone levels based on genotype or treatment. In a follow-up study using Cyto1a(+) wild-type and Cyto1a(-/-) knockout mice, we found a main effect of sex for latency to float in the forced swim test with females having significantly shorter latencies to float than males (P < 0.01). There was a main effect of genotype with Cyto1a(-/-) knockout mice floating for a longer percentage of time compared with wild-type mice (P < 0.01). Because maternal and offspring exercise reportedly reduces impairments related to developmental BaP exposure, wherein any disruption may contribute to the etiology of neurodevelopmental disorders diagnosed in later life. RoundUp, the most commonly used glyphosate-based herbicide in agriculture today, is believed to perturb typical brain development. We hypothesize that the toxic effects of RoundUp during development will perturb zebrafish primary neurogenesis in the telencephalon (forebrain). Here, embryonic zebrafish were exposed to an environmentally relevant concentration of RoundUp (10 ug/L glyphosate acid equivalent) from 10 to 48 hours post-fertilization (hpf). To quantify newly born neurons at various time points during and after
RoundUp exposure, zebrafish were treated with a 30 minute 5-ethyl-2-deoxuryridine (EdU) pulse at 24, 48 or 72 hpf and sacrificed at 5 dpf. Immunofluorescence was performed on 5 dpf sections to identify a HuC/D (neuron marker) and EdU positive cells, representing newly born neurons during these timepoints. An inverted confocal imaging microscope was used for imaging and analysis was used to count cells. All neurons for each zebrafish were counted for both the control and the EdU treatment with 10 μg/L of RoundUp. Zebrafish demonstrated to have a nonsignificant difference in the comparison of the control to treatment for both the 24hpf and 48hpf EdU staining groups. However, in comparison to the control we see a significant increase (p<0.01) of total neuron count for the zebrafish exposed to 10 μg/L of RoundUp in the 72 hpf EdU staining group. For zebrafish exposed to 10 μg/L of RoundUp, we found there to be a nonsignificant increase in the ratio of newborn neurons to total neurons at 24hpf and 48hpf. However, we found there to be a significant increase (p<0.0001) in the ratio of newborn neurons to all neurons in zebrafish at 72hpf, after exposure to 10 μg/L of RoundUp. Our findings suggest that exposure to RoundUp during neurodevelopment alters neurogenesis in the forebrain, which could have wider effects on the functioning of the organism.

3953 Characterizing Social Behaviors in RoundUp-Exposed Zebrafish

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Glyphosate-based herbicides (GBHs) are of the most widespread and globally used herbicides in current agricultural practices. The appeal for such an exorbitant use of glyphosate comes from the belief that it is safe for humans and animals in the surrounding environment. However, debates and contradicting opinions on the true safety of glyphosate for human health are ever-increasing. The period of neurodevelopment is particularly sensitive to exposures to such environmental contaminants as they can contribute to future neurodevelopmental disorder diagnoses. We hypothesize that embryonic exposure to RoundUp™ will cause autism-like behavioral phenotypes in a wild-type zebrafish model. Using three different assays—measuring shoaling, social contact, and startle behaviors—we aimed to characterize larval zebrafish behaviors following chemical exposure. Here, zebrafish embryos were exposed to a range of environmentally relevant RoundUp concentrations (10 μg/L, 100 ng/L, 1 ng/L glyphosate acid equivalent) from 10 to 48 hours post-fertilization (hpf). At 5-, 7-, and 10-days post-fertilization (dpf), the ZebBox recording chamber and Viewpoint software were used to spatially track and record these behaviors in lit-up and dark environments. Statistical analyses of the data were conducted using GraphPad Prism software. We found that sex dimorphism of AVPPVN was established as early as E14.5, a timepoint prior to the perinatal estrogen surge around birth thought to drive sex differentiation. We then performed in utero injection of an estrogen receptor antagonist into the hypothalamic third ventricle at E13.5 and observed a dose-dependent decrease in AVPPVN neurons at E15.5 in males but not females. In contrast, in utero injection of 17β-estradiol increased the number of female AVPPVN neurons. We next showed that gestational exposure to environmental levels of the weak estrogenic contaminant bisphenol A (BPA, 2.25 μg/kg body weight/day) also caused masculinization of AVPPVN neurons in female brains. To ascertain the mechanism underlying this AVPPVN sex differentiation, we tested if males underwent earlier neurogenesis than females, a finding that was reversed by gestational BPA exposure in females. Whole-brain imaging (iDISCO+ tissue clearing and whole brain imaging) and electrophysiological measurements (whole-cell patch clamping) were sex dimorphic across these readouts. Notably, our electrophysiological data showed that female brains displayed male-like properties (whole-cell patch clamping) and neurodevelopmental outcomes were sex dimorphic in both male and female pups. To ascertain the mechanism underlying this sex-dimorphism pattern, we first established that male rats exhibit more aggressive and parenting behaviors than females. Using a social and cognitive battery in the IntelliCage while animals were group housed, and a cognitive battery in the Noldus Phenotypy while animals were singly housed. Outcomes of this study may shed light on the unique capabilities of these system to isolate sex-specific neurotoxic effects of prenatal chlorpyrifos exposure that can provide further weight of evidence for the development of neurotoxicity of organophosphate pesticides.

3954 Developmental Exposure to the Fox River Mixture of Polychlorinated Biphenyls (PCBs) Modulates Behavioral Endpoints in Juvenile Male and Female Mice

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Developmental exposures to PCBs are implicated in the etiology of neurodevelopmental disorders (NDDs). This is concerning given the continued presence of PCBs in the human environment and the increasing incidence of NDDs. We previously reported that developmental exposure to single PCB congeners or legacy mixtures of PCBs (Aroclors) caused NDD-relevant behavioral phenotypes in animal models. However, the PCB congener profile in contemporary human samples is shifting away from that of the legacy Aroclors, raising the question of whether human-relevant PCB mixtures will cause autism-like behavioral phenotypes in a wild-type zebrafish model. Using three different assays—measuring shoaling, social contact, and startle behaviors—we aimed to characterize larval zebrafish behaviors following chemical exposure. Here, zebrafish embryos were exposed to a range of environmentally relevant RoundUp concentrations (10 μg/L, 100 ng/L, 1 ng/L glyphosate acid equivalent) from 10 to 48 hours post-fertilization (hpf). At 5-, 7-, and 10-days post-fertilization (dpf), the ZebBox recording chamber and Viewpoint software were used to spatially track and record these behaviours in lit-up and dark environments. Statistical analyses of the data were conducted using GraphPad Prism software. We found there to be a significant increase in the ratio of newborn neurons to total neurons at 24hpf and 48hpf. However, we found there to be a significant increase (p<0.0001) in the ratio of newborn neurons to all neurons in zebrafish at 72hpf, after exposure to 10 μg/L of RoundUp. Our findings suggest that exposure to RoundUp during neurodevelopment alters neurogenesis in the forebrain, which could have wider effects on the functioning of the organism.
Methylmercury is an environmental contaminant that impacts neurochemistry and behavior following exposure during various developmental time periods. However, sensitivity of the rodent neonatal period following exposure to this contaminant has been understudied, due largely to the lack of bioavailability of methylmercury via lactation, despite evidence suggesting sensitivity of this developmental window. Male Long-Evans rats were exposed to approximately 0, 30, or 130 ppm methylmercury chloride orally from postnatal days 1-10 and then, as adults, tested in a spatial discrimination reversal procedure to assess perseverative behavior and modified two-choice visual signal detection task to assess sustained attention and short-term remembering. Rats exposed to methylmercury displayed altered patterns of perseverative behavior in the spatial discrimination reversal procedure. Further, exposed rats were impaired in their acquisition of the two-choice visual signal detection but otherwise neither their sustained attention nor their remembering were impaired. The magnitude of observed behavioral differences, however, was subtle compared to that observed following prenatal exposure but was comparable to effects observed following exposure during the adolescent period. This study provides evidence that the neonatal period may be sensitive to the neurotoxic effects of methylmercury.
(MAP2, VGLUT2) and astrocytes (GFAP). At the cellular level, As inhibited neural stem cell proliferation (LC50=0.1 µM) and suppressed neurite outgrowth in the 2D morphological assay that mimics nerve growth and axon pathfinding. As disrupted neural rosette and neuropil structures in day 40 organoids and reduced the mean firing rate of the neurons dissociated from organoid detected by microelectrode array. In conclusion, our results show that this 2D and 3D (EBs, neural organoids) model system can provide valuable insights into the cellular events and molecular mechanisms of As-induced DNT.

3961 Manganese Exposure in Risk Mechanisms for Neuropsychiatric Disease
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Attention Deficit Hyperactivity Disorder (ADHD) affects approximately 9% of the population, although this varies by sex and ethnicity. Genetic variants implicate dopamine transmission in the pathophysiology of ADHD. However, the strength of the genotype-phenotype relationship is strongly impacted by environmental factors including exposure to toxicants through air pollution and contaminated water. While in the population overall the outward behavioral traits of ADHD are more strongly expressed or clinically recognized in males, females may be more sensitive to aspects of pollution and metal exposure. Excess manganese (Mn) exposure from water, air and soil is linked to adverse behavioral outcomes in children and this relationship may be stronger in females. Chronic high exposure to Mn is linked to neurodegenerative disorders via alterations in DAergic and other neurotransmitter systems, but less is known about developmental changes associated with chronic low dose Mn exposure. (i) In contrast to high Mn levels, (ii) the same Mn was administered (daily, female C57Bl/6J mice through diet (control (70 ppm) or high (2400 ppm)) or by subcutaneous injection (50 or 50 mg/kg MnCl2 tetrahydrate). Experimental diets were administered from weaning through to the end of the experiment. Injections were given on days 7, 4 and 0 days prior to the start of behavioral testing. At 8 weeks of age mice underwent assessment of locomotor activity, marble burying and nest building behaviors. Mn accumulation in the brain was measured via mass spectrometry, and expression of key proteins relevant to dopamine synthesis and transport were assessed using western blot. Locomotor activity and repetitive behaviors were impacted differentially according to sex and treatment route. Mn also altered sensitivity to pharmaceutical manipulation of activity levels differentially in male and female animals. Both dietary and injected Mn altered proteins related to dopamine synthesis and transport. Overall dietary exposures had a greater impact on female mice compared to male littersmates and impacted repetitive behaviors and response to pharmacological manipulations. In contrast, high dose Mn may have had more robust effects on behavior. These findings highlight the importance of testing in both sexes, and using translationally appropriate exposure approaches in animal studies.

3962 Impaired Inhibited Hippocampal Network Function in Adult Rats Exposed Developmentally to Perchlorate and Dietary Iodine Deficiency
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Iodine is essential for production of thyroid hormones (TH) with severe deficiencies leading to hypothyroidism. As TH are necessary for brain development, maintaining iodine status is especially critical during pregnancy. Perchlorate is an environmental contaminant that interferes with the transport of iodine into the thyroid gland and reduces TH synthesis. As such, the offspring of pregnant women with iodine deficiency (ID) may be particularly susceptible to perchlorate exposure, with deleterious consequences on brain development. Indeed, we have previously reported that developmental ID or perchlorate disrupts synaptic function in the hippocampus, impairing excitatory synaptic transmission but leaving functionality of inhibitory networks and synaptic plasticity intact. In this study, we investigated if the synaptic impairments induced by developmental perchlorate or marginal ID could be exacerbated if both treatments were delivered together. Naïve female rats were maintained on either an iodine-replete (Control) or ID diet for a minimum of 4 wks, a regimen that reduced serum T4 by ~35%. Animals were bred and on gestational day 6, half of each group was exposed to 0 or 300 ppm perchlorate in the drinking water. Serum T4 in newborn pups was not altered by perchlorate, and was only transiently reduced by control levels by postnatal day (PN) 6. In contrast, decreases in serum T4 >80% were evident in offspring of dams exposed to both ID and perchlorate on the day of birth, deficits that persisted throughout the neonatal period. Under urethane anesthesia, electrodes were positioned stereotaxically in brains of adult male offspring to record field potentials in the hippocampus. Pairs of stimulus pulses at varying intervals were delivered to the perforant path, the ratio of the 1st and 2nd response recorded in the dentate gyrus reflecting the activity of GABAergic inhibitory interneurons. We observed a reduction in inhibitory output in animals born to ID dams exposed to perchlorate, with no difference from controls in groups exposed to ID or perchlorate alone. Preliminary findings indicate that these electrophysiological impairments are accompanied by a decrease in the number parvalbumin (PV)-expressing cells in the brains of littersmates assessed on PN14. PV is a calcium-binding protein expressed in a subset of inhibitory interneurons integral to functioning of hippocampal neurocircuitry. Altered expression of this protein is emerging as a common outcome in chemical-induced thyroid disruption in rat, thyroid receptor mouse mutants, deiodinase and hormone transporter knock-out models, and in humans with mutations of the brain TH transporter. Together, these findings demonstrate that ID can compound the effects of chemical exposure on neural function. Further, they provide impetus to examine PV expression as a marker of neurodevelopmental thyroid disruption. Does not reflect EPA policy.

3963 Characterizing the Developmental Neurotoxicity Profile of the Lower-Chlorinated Polychlorinated Biphenyl (PCB) Congener PCB 37
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PCBs remain a significant risk to human health, and a primary target of concern is the developing brain. Epidemiological studies have reported positive associations between developmental PCB exposures and increased risk of impaired cognitive function. However, epidemiological and experimental studies have largely focused on higher-chlorinated (HC) PCBs, the predominant congeners in the legacy PCB mixtures. The limited data available regarding the potential for lower-chlorinated (LC) PCBs, the predominant congeners in the legacy PCB mixtures, many of which are inadvertent industrial byproducts, are increasing worldwide; (ii) LC-PCBs are regularly detected in contemporary samples of indoor and outdoor air, water, food, and human tissues, including breast milk and brain; and (iii) LC-PCB congeners were found to comprise more than 70% of the total PCBs in the serum of breast milk obtained at birth. Given that many congeners, including PCB 37, are more sensitive to aspects of pollution and metal exposure. Excess manganese (Mn) exposure from water, air and soil is linked to adverse behavioral outcomes in children and this relationship may be stronger in females. Chronic high exposure to Mn is linked to neurodegenerative disorders via alterations in DAergic and other neurotransmitter systems, but less is known about developmental changes associated with chronic low dose Mn exposure. (i) In contrast to high Mn levels, (ii) the same Mn was administered (daily, female C57Bl/6J mice through diet (control (70 ppm) or high (2400 ppm)) or by subcutaneous injection (50 or 50 mg/kg MnCl2 tetrahydrate). Experimental diets were administered from weaning through to the end of the experiment. Injections were given on days 7, 4 and 0 days prior to the start of behavioral testing. At 8 weeks of age mice underwent assessment of locomotor activity, marble burying and nest building behaviors. Mn accumulation in the brain was measured via mass spectrometry, and expression of key proteins relevant to dopamine synthesis and transport were assessed using western blot. Locomotor activity and repetitive behaviors were impacted differentially according to sex and treatment route. Mn also altered sensitivity to pharmaceutical manipulation of activity levels differentially in male and female animals. Both dietary and injected Mn altered proteins related to dopamine synthesis and transport. Overall dietary exposures had a greater impact on female mice compared to male littersmates and impacted repetitive behaviors and response to pharmacological manipulations. In contrast, high dose Mn may have had more robust effects on behavior. These findings highlight the importance of testing in both sexes, and using translationally appropriate exposure approaches in animal studies.

3964 Human Embryonic Stem Cell–Derived Dopaminergic Neuron Model for Neurodevelopmental Toxicology Screening
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Identification of developmental neurotoxictants for risk assessment has tradition-ally been performed using in vivo guideline developmental neurotoxicity (DNT) studies. These studies, however, are expensive and time-consuming, limiting their applicability for screening. With the increasing concern that environmental chemicals may contribute to neurodevelopmental disorders, there is an urgent need for higher throughput assays. Over the past decade, there has been a global effort to assemble a battery of in vitro new approach methodologies (NAMs) that could provide an alternative to traditional in vivo guideline studies for DNT. This battery is beginning to be employed for risk assessment purposes, but still lacks a number of endpoints critical to neurodevelopment, such as assessment of neuronal subtypes. This project aims to fill that gap by establishing an assay to screen chemical disruption of dopaminergic neuron development as dopaminergic neurons are particularly sensitive to environmental factors and have been implicated in a variety of...
neurodevelopmental and neurodegenerative disorders. Our approach uses human embryonic stem cells (hESCs), as differentiation using hESCs closely mimics embryonic development in vivo. We are utilizing CRISP-mediated genome editing technology to construct a HESC triple-reporter system to monitor stages of dopaminergic neuron differentiation using fluorescent imaging. The current reporter line includes nestin-EFP2 to monitor differentiation down the neuronal lineage, and tyrosine hydroxylase promoter-driven mCherry to monitor dopaminergic development. Our third reporter will be targeted to NeuroD1 to monitor specification of neuronal stem cells towards dopaminergic neurons. Our dopaminergic neuron differentiation protocol is based on the dual inhibition approach established by the Studer laboratory and includes three stages—differentiation, expansion, and maturation. This toxicological screening approach will be conducted in two phases—an initial phase to monitor how chemicals might impact dopaminergic neuron specification, and a 2nd phase to monitor maturation into dopaminergic neurons. This will allow for carefully assessing specific windows of susceptibility for dopaminergic neuron development. Screening will be performed using fluorescent high-content live-cell imaging and gene expression analyses. Hit compounds can subsequently be pursued for insight into mechanisms of action. We aim to incorporate this reporter-based model into the battery of DNT NAMs to identify environmental toxicants of dopaminergic neuron development in a human in vitro-relevant context.

**3965 Physiologically Based Kinetic (PBK) Modeling to Predict Human Fetal Brain Concentration: Deltamethrin Case Study**

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Developmental neurotoxicity (DNT) is a potential hazard of chemicals. An in vitro testing battery (DNT IBV) was recently established to complement existing rodent in vivo approaches. Deltamethrin (DTM) has been selected as a reference chemical in the in vitro model and will be included in different exposure scenarios to characterize neurotoxic mode of action. Further, a DNT in vivo study according to OECD guideline 426 is available and considered to be clearly negative. The present study provides context for evaluating the relevance of these DNT IBV results for the human health risk assessment of DLT by estimating potential human fetal brain concentrations after maternal exposure to DLT. A physiologically-based kinetic (PBK) model was developed for rats and validated in vivo rat toxicokinetic data of DLT over a broad range of doses. It was then translated to humans considering realistic in vivo exposure conditions (acceptable daily intake [ADI] of 0.01 mg/kg for DLT). Human fetal plasma concentrations of deltamethrin were successfully simulated by integration of a maternal-fetal PBPK model. Subsequently, the model was used to estimate human fetal brain exposures across various case studies with variations of key model assumptions and parameters (e.g., blood-brain and blood-placenta barrier partitioning) assessed to address uncertainties. Model calculations from a so-called “realistic data-driven approach” up to “worst case scenarios” were conducted and the predicted fetal brain concentrations were compared to the lowest benchmark concentrations (BMC) of 0.5 µM (BMC50 rat toxicokinetic data) over a broad range of doses. It was then translated to humans considering realistic in vivo exposure conditions (acceptable daily intake [ADI] of 0.01 mg/kg for DLT). Human fetal plasma concentrations of deltamethrin were successfully simulated by integration of a maternal-fetal PBPK model. Subsequently, the model was used to estimate human fetal brain exposures across various case studies with variations of key model assumptions and parameters (e.g., blood-brain and blood-placenta barrier partitioning) assessed to address uncertainties. Model calculations from a so-called “realistic data-driven approach” up to “worst case scenarios” were conducted and the predicted fetal brain concentrations were compared to the lowest benchmark concentrations (BMC) of 0.5 µM (BMC50 rat neuronal network formation) from the DNT IBV. This resulted in margins of safety from 80 to 95639, with safety margins for the “realistic data driven approach” in the range of 28000 - 78000. The presented results indicate that DLT concentrations in the human prenatal development period are clearly lower than those observed in vivo findings under realistic exposure conditions. Therefore, the new in vitro DNT results are considered to have no impact on the current risk assessment approach.

**3966 High-Throughput Phenotypic Profiling Approach for the Screening and Prioritization of Potential Developmental Neurotoxicity Hazard**

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The prevalence of neurodevelopmental disorders has increased in recent years and environmental exposures may be contributory. Furthermore, numerous environmental chemicals lack data on potential developmental neurotoxicity (DNT) hazard. As such, reliable and efficient methods are critically needed to circumvent these data gaps. We previously adapted a high-throughput phenotypic profiling (HTPP) approach for this purpose. This approach quantitatively measures alterations in human neural progenitor (NPNP1 cell morphology (e.g., position, shape, subcellular structure) as a potential indication of DNT hazard. Presently, we have assessed 282 DNT-relevant chemicals, including compounds with evidence of DNT in vivo and in vitro (reference DNT “positives”), as well as compounds with no evidence of DNT in vivo and in vitro (reference DNT “negatives”). Of the 282 compounds, a concentration-response curve could be fit (i.e., ≥ 4 tested concentrations were not cytotoxic) and a benchmark concentration calculated for 281 chemicals (only carbamazepine [DXTSIDE24022731] was excluded) using a global Mahalanobis distance modeling approach. Further, 123 of these 281 compounds met the threshold for a response (i.e., 1 x standard deviation of the vehicle control) in the HTPP approach. Interestingly, 4 of the 36 reference DNT negatives (i.e., buspirone [DXTSIDE5023322]) also reached this threshold; 1 of the 4, mifepristone, was also active in two other DNT in vitro assays that employ hNPC1 cells. Moreover, 54 of the 106 reference DNT positives tested also reached the threshold for a HTPP response. Sixty-five of these 106 were evaluated in the other hNPC1 DNT in vitro assays as well, and 33 were hits. Based on these data, the HTPP approach exhibited the same capacity to detect reference DNT positives (sensitivity = 51%) as other in vitro DNT in vitro assays. Moreover, the specificity (ability to correctly classify reference DNT negatives) was 89%, with a false-negative rate of 11%. In conclusion, the HTPP approach is comparable (i.e., in terms of sensitivity and specificity) to other hNPC1 DNT in vitro assays for the evaluation of potential DNT hazard; however, it has improved efficiency (i.e., high-throughput 384-well format) relative to these other approaches. In order to identify all developmental neurotoxics, additional neural models (e.g., neurons, glia) may need to be optimized for HTPP. Our next step is to examine whether different chemical groups (e.g., organophosphates, glucocorticoid receptor agonists) exhibit distinct phenotypic profiles that are predictive of toxicity. This abstract does not reflect US EPA policy.

**3967 Proteomic and Behavioral Alterations following Developmental Emanemacin Benzoxate Exposure in Rats: Effects in Development and Adulthood**


Early neurodevelopmental periods are vulnerable to environmental compounds. Unfortunately, there are few adverse outcome pathways (AOP) that explore these critical periods. AOPs detail a series of biological responses linked by key events following a molecular initiating event and ending in an adverse effect. We are using proteomic changes produced by xenobiotics that can occur during this susceptible period of development to assess molecular alterations which can be applied in the AOP framework. Additionally, behavioral endpoints were assessed to understand potential long-term effects that may be related to these molecular perturbations. Pregnant Long Evans rats were gavaged with emamectin benzoxate (EB: 3.78 mg/kg in 5 mL/kg DI water) or vehicle, from gestational day 21 to postnatal day (PND) 21. For proteomic experiments, pup region-specific brain tissues (cortex, hippocampus, striatum, hypothalamus, midbrain, brainstem, and cerebellum) were collected throughout the postnatal period of exposure (PND2, 8, 15, 22); animals were perfused with phosphate buffered saline, dissected and the tissues stored at -80°C. Cortex samples were assessed for proteomic content using Orbitrap LC-MS and identified proteins were then further processed using Proteome Discover and Ingenuity Pathway Analysis software. Behaviors were assessed throughout the experiments using a variety of behavioral tasks during early postnatal days, assessed at weaning (juvenile) timepoints, and into adulthood. Behavioral assays included pup righting (PND 2-7), a modified functional observational battery for pups and adults (PND 21, 41, and 63), locomotor activity in figure-eight mazes (PND 13, 17, 21, 29, and 64), novel object recognition (PND 34-37), acoustic startle response (PND 29 and PND 77), and the Morris water maze (PND 76-92). Proteomic analyses showed that protein signatures for EB-treated rats differed in the cortex by sex as well as by age. Analysis indicates alterations in proteins implicating presynaptic vesicle docking/fusion machinery, cell-adhesion receptors and proteins, and enzymes involved in transcription and/or translation regulation. Behaviorally we observed that females showed deficits in synaptic plasticity at PND17, decreased startle response at PND 29, and uncoordinated hindlimb movements that began in early postnatal weeks and persisted into adulthood. Additional proteomic analysis of other brain regions and inclusion of cognitive neurobehavioral tasks are ongoing. Overall, we observe changes in proteomic signatures during developmental treatment with EB that differed across the postnatal period and between sexes. Behavioral changes present in PBB-exposed EB rats, postnatally and through adulthood. The protein level changes and observed apical behavioral endpoints could aid in AOP development. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

**3968 Cytokine-Mediated Chemotherapy-induced Cognitive Impairment in Cisplatin and Methotrexate Treated Sprague Dawley Rats**


As the survival rates of cancer patients gradually increase, patients often report chemotherapy-induced cognitive impairment (CICI). These cognitive impairments are diverse but are most often reported to affect memory and learning, attention, concentration, processing speeds and executive function. It is well known that cytokines mediate neuronal and glial cell function to facilitate neuronal regeneration or neurodegeneration, and cytokine dysregulation is linked to microglial activation, neuroinflammation, neuronal damage, and cognitive deficits. The specific role of cytokines in CICI was examined in this study as they may be biomarkers of CICI and allow investigation of therapeutic targets against CICI. In this study, juvenile male and female rats were administered three intraperitoneal injections, with one injection per week for three weeks on postnatal day (PND 23, 30, and 37) of either cisplatin (2 or 4 mg/kg), methotrexate (20 or 40 mg/kg), or saline. The frontal
Brain development underlies strictly controlled highly complex mechanisms, which makes the developing brain particularly vulnerable to a chemical insult. However, only a very small number of chemicals circulating worldwide have been assessed for their developmental neurotoxicity (DNT) potential. DNT testing of chemicals in the OECD (Organization for Economic Co-operation and Development) and USEPA (Environmental Protection Agency) in vivo guideline studies is currently not mandatory. Furthermore, they consume high amounts of animals, time, and money for the investigation of specific species, e.g., zebrafish. Therefore, there is a call for a paradigm shift in DNT evaluation, which recommends the implementation of faster, more cost-efficient, and animal-relevant New Approach Methodologies (NAMs). To cover the need for DNT testing for regulatory purposes and data gap closure, the European Food Safety Authority (EFSA) and the OECD have recently established the European Network for In Vitro Brain Development (IVB) for Neurodevelopmental Hazard Assessment.
supported the preparation of an OECD guidance document that will facilitate the use of DNT NAMs in a DNT in vitro testing battery (DNT IVB) covering basic neurodevelopmental processes. As an integral part of the DNT IVB, we set up the Neurosphere Assay consisting of 3D primary human neural progenitor cells (hNPC). This test system covers the neurodevelopmental processes hNPC proliferation and migration, neuronal and glia differentiation, neurite outgrowth, as well as neuronal and oligodendroglial migration. The hNPC were scientifically validated by demonstrating cell-specific morphologies of the different cell types of the mixed culture, their marker expressions, dynamics of neurodevelopmental processes, physiological signaling responses, and toxicological adverse effects. In addition, in a collaborative effort with the EFSA and the USEPA, we tested 120 compounds of different chemical classes in the assays. The R-based CRStats program generated concentration-response curves that were classified with a classification model. To close gaps identified in the DNT IVB, we have been setting up human cell-based assays for neuronal network formation (hNNF) and astrocyte maturation (hAM). For the hNNF assay, we establish neuronal networks from human-induced pluripotent stem cell-derived excitatory and inhibitory neurons cultured primary human astrocytes on microelectrode arrays. With this assay, we tested 35 pesticides for their ability to disturb network formation. For the hAM assay, we differentiate hNPC into the astrocytic lineage and assess compounds’ effects on astrocyte maturation. Overall, here we present NAMs that cover an array of major neurodevelopmental key events that contribute to a regulatory-supported DNT IVB, thereby providing a promising approach for future risk assessment procedures.

**Developmental Exposure to Minor Cannabinoids Causes Morphological and Behavioral Adverse Outcomes in Zebrafish Larvae**

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Minor cannabinoids found in cannabis are becoming more widely used and have even been marketed to pregnant women to help relieve morning sickness, insomnia, and/or pain. There is a significant lack of understanding of developmental adverse effects from minor cannabinoid exposure despite evidence that Δ9-tetrahydrocannabinol (Δ9-THC) causes lower birth weight and sex-dependent neurodevelopmental deficits and social disorders in children. Our goal is to compare effects of ten minor cannabinoids namely: Δ8-tetrahydrocannabinol (Δ8-THC); Δ10-tetrahydrocannabinol (Δ10-THC); cannabigerol (CBG); cannabidiol (CBD); cannabinoid acid (CBD-A); tetrahydrocannabinolic acid (THC-A); tetrahydrocanabivarin (THC-V); tetrahydrocannabinol acetate (THC-O); tetrahydrocanabiphorol (THC-P); and hexahydrocannabinol (HHCB) to effects caused by Δ9-THC exposure during early development. Zebrafish (Danio rerio) were exposed to 0 (0.05% DMSO), 0.03, 0.12, 0.5, 2, or 8 µM cannabinoid from 6-96 hours post fertilization (hpf) then transferred to clean water for an additional 24 hr. Survival and hatch were assessed daily, and at 120 hpf phenotypic malformations including bent body axis, yolk sac edema, pericardial edema, eye size, and fish length and larval photomotor response behavior were measured. While the 96 hpf LC50 for Δ9-THC was 11.6 µM, THCV-Δ8 and Δ8-THC were 100% lethal at 8 µM and 48 and 96 hpf, respectively. Δ9-THC and Δ8-THC (2 µM) caused significant yolk sac and pericardial edema. Additionally, compared to controls development exposure to Δ8-THC (2 µM) and THCV-Δ8 (0.12, 0.5, and 2 µM) caused significant hypoactivity in the dark phase of the larval photomotor response assay. Overall, these results indicate that exposure to minor cannabinoids can, like Δ9-THC, cause significant morphological and behavioral changes in developing zebrafish. If one considers the data presented in this study, one can conclude that cannabinoids can have adverse effects on brain development, and one can thus argue for more research on the adverse effects on brain development caused by exposure to minor cannabinoids.

**Developmental Hypothyroidism: An Underappreciated Risk Factor of Brain Barrier Disruption**

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Exposure to environmental thyroid-disrupting chemicals is a concern for pregnant women and children as thyroid hormone (TH) action controls brain development. Exposure to environmental thyroid-disrupting chemicals can target the brain barriers, and may make the fetus, infant, and/or child more susceptible to additional neurotoxicant insult due to increased barrier permeability. This work does not necessarily reflect US EPA policy.

**Examining Immune Modulatory Effects of Perinatal Cannabidiol Exposure in Sprague Dawley Rats**

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Cannabidiol (CBD) is a non-intoxicating chemical component of the cannabis plant and is known to have anti-inflammatory and immunosuppressive properties in both humans and rodents. Experimental data have shown CBD decreases inflammatory cytokines, increases anti-inflammatory cytokines, B cell, and T cell populations, and decreases LPS-induced microglial activation. While Epidiolex, which contains purified CBD, is FDA approved to treat rare childhood seizure disorders, nondrug use of CBD is increasing. CBD is purported to relieve anxiety, pain, and sleeplessness, which are often associated with pregnancy. It is concerning that pregnant women may use CBD because of its perceived safety and effectiveness. Yet, cannabis use during pregnancy has been linked to poor developmental outcomes, and CBD cannot currently be excluded as a contributing factor. CBD’s effects on the developing immune and neuroimmune systems are largely uncharacterized, and there is the potential for adverse immune system effects as a result of developmental CBD exposure. The purpose of this study is to investigate the immunomodulatory effects of perinatal CBD exposure in Sprague Dawley rats. Pregnant dams were orally gavaged with vehicle, 15, or 100 mg/kg/day CBD from gestational day 6 until the day prior to parturition. Then, pups were orally gavaged with the same dose as their respective dam from postnatal day (PND) 1 to PND 21. To examine acute immune effects of perinatal CBD, rats were given a single LPS (0.5 mg/kg, i.p.) or saline injection 1 h after the final CBD gavage on PND 21. Brain tissue and plasma was collected 2 h after LPS injection to examine Infγ, TNFα, IL-1β, IL-6, and IL-10 expression via ELISA, and the spleen was collected to quantify leukocyte populations via flow cytometry. A subset of rats were euthanized 24 h after LPS or saline injection, and their brains were perfusion-fixed to assess microglia activation and neuronal damage via IHC. To investigate persistent effects of perinatal CBD, a cohort of rats was maintained until PND 180, at which point they also received an LPS (2 mg/kg, i.p.) or saline injection and the same assays were conducted as at PND 21. Using the cytokine profiles and leukocyte populations to define phenotypes, this study describes acute immune and neuroimmune system effects at both a basal state and in response to LPS-induced immune activation. Initial analysis suggests CBD had greater effects on cytokine profiles in response to LPS at PND21 than at PND180. CBD did not alter basal leukocyte populations.

**Neurodevelopmental Changes in Rodent Behaviors and Hippocampal Plasticity Induced by Early-Life Exposure to Deltamethrin**

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Pyrethroid pesticides have become one of the most commonly used classes of pesticide in recent years due to their favorable toxicity profile in adults. Recent concerns have been raised regarding their ubiquitous use and associations between increased exposure to pyrethroid pesticides with the occurrence of neurodevelopmental disorders, including attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder. For example, children with detectable environmental pyrethroid pesticide burdens are significantly more likely to be diagnosed with ADHD. Likewise, animal research indicates that exposure to deltamethrin (DM), a type of pyrethroid pesticide, alters dopamine signaling in the striatum, neurogenesis in the hippocampus, locomotor activity, impulsivity, attention, and memory, all of which have been associated with neurodevelopmental disorders in humans. However, the impacts of early-life DM exposure during gestation and lactation on synaptic function in the hippocampus and associated behaviors in developmentally exposed pups have not been well-characterized. Using a developmental exposure model, in which pregnant dams were exposed to 3 mg/kg/day, equivalent to 1mg/kg/day, DM or vehicle through pregnancy and lactation, we measured synaptic

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function in the hippocampus and the behavior of pups beginning between PND 45-60. We found that long-term potentiation showed a significant decrease in male DM-exposed animals, a deficit which is rescued by the application of 0.5 μM clonazepam, indicating a dysfunction in GABA signaling in the hippocampus in DM-treated males. Complementary experiments in females showed no change in hippocampal LTP was seen in DM-treated females. In males, no change in locomotor activity was seen at an initial exposure to the open field, however, on subsequent days there was a decrease in the locomotor activity and locomotor speed of DM-exposed males and a decrease in the time spent in the center of the open field, a measure of anxiety-like behavior. There was no observed change in the time spent in the open arm of an elevated plus maze. DM-exposed males also showed an increase in intake of a palatable food upon initial exposure, indicating a decrease in food neophobia. DM-exposed females did not show any change in locomotor behavior, palatable food intake, or elevated plus-maze activity. Taken together, these results may indicate a critical period of vulnerability to the neurotoxic effects of DM in males and further supports that exposure to DM may be a contributing factor to the occurrence of NDDs. This research was funded by a NIAMS T32 training grant T32ES007254 (JO), an NIAMS R01 grant R01ES031823 (FL) and an NIAMS P30 grant P30ES030285 (FL).

3977 In Utero Exposure to Real-Life Environmental Chemical Mixture Disrupts Hypothalamic Rostral Arcuate Nucleus Transcriptome of Rats

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Environmental chemicals (ECs) such as plasticisers and per- and polyfluoroalkyl substances (PFAS) are ubiquitously found in our environment. Health conditions including neurological disorders, obesity, diabetes, puberty disorders, subfertility and cancer have been reported as a consequence of EC exposure. Although adverse effects of some ECs have been confirmed, the exact mechanism(s) of action of many of these chemicals are still unknown. This uncertainty becomes more complex when “real-life” human EC exposure (long term exposure to hundreds of ECs at low levels) is considered. To evaluate the effects of in utero exposure to real-life ECs on health, we used a “biosolids-treated pasture (BTP) sheep” model. Biosolids are the by-product of wastewater treatment and are widely used as an agricultural fertiliser. Biosolids contain numerous ECs at low levels and therefore can be used as a suitable experimental paradigm to mimic the human exposure.

In this study, ewes were kept on either control (C, fertilised with inorganic fertilisers) or biosolids (B) (4 tonnes/ha, twice per annum) treated pastures from about one month prior to mating until two weeks before parturition. To evaluate the effects of in utero exposure to biosolids EC mixtures, at 11 months of age, 10 B and 11 C offspring rats were euthanised and gene expression pattern evaluated in various tissues using real time RT-PCR. Preliminary results from the RT-PCR indicated that the rostral arcuate nucleus (ARC), a key site coordinating the hypothalamic response to the received metabolic and reproductive signals, was subject to many changes in gene expression. To gain an in-depth assessment of the gene expression profile of the rostral ARC and aid in characterisation of affected signalling pathways, DNA microarray screening was performed with the same samples from B and C rats. Sequencing was performed using Oxford Nanopore POLAR-CDNA barcoding protocol (SGK-PCB109), GridION Nanopore sequencer (Oxford Nanopore) and an R9.4 flow cell (Oxford Nanopore - FLO-MIN106D). The results were analysed with the package ‘edgeR’ to detect differential gene expression and KEGG pathway analysis was performed using differentially expressed genes (DEGs) list in DAVID 2021. We found that there were 1264 DEGs in the rostral ARC of B compared to C tissue samples (545 with downregulation and 719 with upregulation). KEGG pathway analysis revealed that neural function-related pathways e.g., neuroactive ligand-receptor interaction, AMP signalling pathway, retrograde endocannabinoid signalling, dopaminergic synapse, morphine addiction, neuroactive ligand-receptor interaction, synaptic vesicle cycle were affected (p<0.01) as a result of B exposure. Other affected (p<0.05) pathways included those related to energy and lipid metabolism e.g., regulation of lipolysis in adipocytes, adipocytokine signalling and lipid and atherosclerosis pathways (p<0.05), reproductive function, e.g., GnRH secretion and GnRH signalling pathways (p<0.01), immune system e.g., primary immunodeficiency and T cell receptor signalling, and also cancers such as pancreatic and lung cancer pathways (p<0.05). These findings indicate that in utero exposure to real life ECs disrupts gene expression and key pathways in the rostral ARC that could have contributed to the adverse effects seen in the B rats reflected in decrease in body weight, increase in subcutaneous fat mass and compromised glucose metabolism.

In 2019, FDA approved esketamine (SPRAVATO®), an enantiomer of the dissociative anesthetic ketamine, for treatment-resistant depression and for depressive symptoms associated with the suicidal ideation or behavior in adults. Ketamine is known to cause age-dependent neurotoxicity in rat brain. Data from published literature indicate that a single dose of ketamine can result in neuronal necrosis in the sexually mature adult rat brain and apoptosis in the developing rat brain (equivalent to late gestation up to three years of age in humans). Even though data are limited, scientists have speculated that the period between early childhood until sexual maturation is considered safe. Addressing this knowledge gap will reduce uncertainty about how ketamine can be safely used in children up to adolescent age. In this study, we treated male and female rats with a single dose of ketamine (50, 75, 100 mg/kg, s.c.), a positive control (2 mg/kg MK-801, i.p.), or vehicle on postnatal day 21 (PND 21), 30, 35, or 90. Animals were sacrificed 72 hours later and brains were collected for histopathological evaluation. The brain was sectioned to include the olfactory bulb, frontal-parietal cortex, mid-parietal cortex and thalamus, mid-brain, and cerebellum. The resulting sections were stained with H&E and evaluated for signs of neurodegeneration. Necrotic neurons were observed after treatment with MK-801 in both male and female animals at PND 30, 35, and 90. However, ketamine (100 mg/kg, s.c.) only caused necrosis in the retrosplenial cortex of female rats dosed on PND 90 (N=3/12). These data show that younger animals are less vulnerable to the neurotoxic effects of ketamine as assessed by H&E at the tested doses. These findings provide supportive information to determine if the speculated “protected period” in young animals is driven by a decreased sensitivity to the toxic effects of ketamine or age-related differences in pharmacokinetics. These data will help us determine safe parameters for the use of ketamine in the pediatric population.
Exposure to benz[a]pyrene (BaP), a polycyclic aromatic hydrocarbon combustion byproduct, is associated with developmental deficits including low birth weight and neurocognitive disorders. The aryl hydrocarbon receptor (Ahr) is a known molecular mediator of BaP toxicities, but its role in BaP-mediated behavioral effects is yet to be fully elucidated. Zebrafish (Ahr2<sup>-/-</sup>, Ahr2<sup>+/+</sup>, Ahr2<sup>+/-</sup>) were exposed to DMSO (0.01%) or 100 µg/L BaP from 6 to 120 hours post-fertilization (hpf). At the end of the exposure, larval behavior was assessed in a photonotor response assay. To assess persistent adverse impacts on behavior, a subset of developmentally exposed larvae was raised to 3 weeks post fertilization (wpf) in clean water and their locomotion and thigmotaxis was assessed in an open-field behavior assay. At 120 hpf, regardless of Ahr genotype, behavior was not significantly different among vehicle-exposed control larvae. BaP treatment caused significant hypoactivity in all Ahr2 genotypes compared to their respective controls during the dark phase of the photomotor response assay, with the least activity in BaP-exposed Ahr2 null fish (e.g. activity Ahr2<sup>+/+</sup> > Ahr2<sup>+/+</sup> > Ahr2<sup>-/-</sup>). Because BaP metabolic fate is hypothesized to be different depending on Ahr genotype, whole larvae were collected to quantify BaP bioaccumulation. BaP-mediated hypoactivity was independent of Ahr2 genotype, thus the underlying mechanisms of BaP behavioral outcomes remain to be identified. Research supported by NIHES T21ES0301.

**3981 Oxidative DNA Damage Epigenetically Decreases Gene Expression in Brca1 KD Fetal Brains Exposed In Utero to Saline or Ethanol**

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The DNA lesion 8-oxoquainine (8-oxoG) formed by reactive oxygen species (ROS) is implicated in the pathology of neurodevelopmental disorders (NDDs), which may involve altered epigenetic regulation. We have shown ROS can initiate NDD-related behavioural deficits when enhanced by ethanol (EtOH), or even at physiological levels in DNA repair-deficient progeny including heterozygous (+/-) breast cancer 1 (Brca1) knockout mice. However, the precise mechanism is unclear. Here we report that enhanced 8-oxoG alters DNA methylation (-5-methylcytosine (-5mC)) and expression of genes relevant to NDD pathogenesis in fetal mouse brains. Brca1 +/- mice were mated, and pregnant dams were treated intraperitoneally on gestational day 17 with 4 µg/kg EtOH with or without pretreatment with the ROS blocker phenylbutylnitrone (PBN). Fetal brains were extracted 6 h later and each was assessed for 8-oxoG levels by ELISA, gene expression by RT-qPCR, and 5-mC status by methylated DNA immunoprecipitation followed by qPCR (MeDIP-qPCR). Brca1 wild-type (WT) and +/- progeny, EtOH exposure increased 8-oxoG levels compared to vehicle-treated littersates. Also, 8-oxoG was increased in ETOH-exposed Brca1 +/- fetal brains compared to WT littersates, which was blocked by PBN pretreatment in both genotypes. ETOH-exposed fetal brains exhibited downregulation of 8 of 12 assessed genes [e.g., RNA methyltransferase 1 and 3a (Dnmt1/3a), tet methylcytosine dioxygenase 1/2 (Tet1/2), histone deacetylase 2/3 (HDAC2/3), solute carrier family 6 member 1 (Slc6a1)] compared to vehicle-exposed littersates. Furthermore, Brca1 +/- fetal brains exhibited increased expression of Dnmt3a and Slc6a1 compared to WT littersates, the latter of which was prevented by PBN. MeDIP-qPCR analysis revealed that increased 8-oxoG levels were correlated with decreasing Hdac2 promoter methylation, suggesting an epigenetic mechanism of gene dysregulation dependent on oxidative DNA damage. Interestingly, promoter methylation of Dnmt1 and Dnmt3a was not affected by enhanced 8-oxoG despite showing EtOH- and Brca1-dependent gene expression changes, suggesting the involvement of other 8-oxoG-dependent regulatory mechanisms such as epigenetic histone modifications. Our data show EtOH-exposure or deficient DNA repair increase 8-oxoG in a ROS-dependent manner, which can decrease expression of genes relevant to NDD pathogenesis. The decreased Hdac2 promoter methylation associated with increased 8-oxoG levels suggests an epigenetic mechanism of transcriptional dysregulation and NDDs that can be pharmacologically targeted. Support: CIHR, UofT Faculty of Pharmacy.

**3982 Impact of Oxidative DNA Damage on Gene Expression Pathways in the Mechanism of Developmental Disorders**

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Fetal DNA damage, and particularly the 8-oxoquinine lesion (8-oxoG), caused by physiological and ethanol (EtOH)-enhanced levels of reactive oxygen species (ROS) is associated with developmental disorders. It is not known how 8-oxoG, which is repaired by oxoguanine glycosylase 1 (Ogg1), causes developmental disorders in Ogg1 knockout mice, but emerging evidence suggests 8-oxoG/Ogg1 may epigenetically alter critical gene expression. Herein, we examined the effect of 8-oxoG/Ogg1 on gene expression in the brains of Ogg1-deficient progeny compared to Ogg1 wild-type littersmates exposed in utero to physiological or EtOH-enhanced ROS levels, respectively relevant to autism and fetal alcohol spectrum disorders (ASD, FASD). Heterozygous Ogg1<sup>+/+</sup> mice were mated, and pregnant dams were treated intraperitoneally on gestational day 17 with saline vehicle or EtOH (4 µg/kg). Six hours post-treatment, RNA was extracted from 3 female Ogg1<sup>+/+</sup> and Ogg1<sup>+/+</sup> fetal brains from each treatment group and gene expression was measured by whole-transcript microarray analysis (GeneChip Mouse Gene 2.0 Array). Genes differentially expressed genes were defined as having a fold change of >1.5 or <0.5 and a p-value of <0.05, and gene ontology pathway analysis identified pathways and key genes potentially involved in critical mechanisms of neurodevelopmental disorders. Ogg1-dependent effects in Ogg1<sup>/-</sup> vs. Ogg1<sup>+/+</sup> brains included: (a) primarily downregulation of genes in the TGF beta, Wnt and Hedgehog signaling pathways; and (b) primarily upregulation of miRNAs involved in neurodevelopmental processes, including key pathways and neural crest cell differentiation. EtOH-dependent effects included: (a) primarily downregulation of genes in the TGF beta and Wnt pathways; (b) primarily upregulation of mRNAs involved in DNA damage response; and, (c) downregulation of genes involved in maintaining histone methylation. EtOH downregulated several pathways in Ogg1<sup>/-</sup> and Ogg1<sup>+/+</sup> fetal brains, including biosynthetic and metabolic processes for sterol-like cholesterol. Interestingly, only EtOH-exposed Ogg1<sup>+/+</sup> brains exhibited downregulation of ribosomal and non-coding RNA processing pathways, and pathways that regulate the cell cycle. These novel findings reveal that 8-oxoG and Ogg1 have an impact on gene expression in fetal brain, and a deficiency in Ogg1 can worsen the response to EtOH exposure, which provide a novel perspective into the mechanistic impact of ROS in FASD, and the determinants of risk. Support: CIHR; UofT Faculty of Pharmacy & Centre for Pharmaceutical Oncology.

**3983 Ethanol Exposure Disrupted the Formation of Radial Glial Processes and Impaired the Generation, Migration, and Transformation of Outer Radial Gial Cells in Human Forebrain Organoids**

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Radial glial cells (RGCs) play a pivotal role in cerebral cortical development by functioning as a source of new neurons and by supporting the migration of newborn neurons. These functions are primarily dependent on the apical-basal lateral structures of the glial, radial glia. This study aims to investigate the effects of ethanol exposure on the development of radial glial processes and the generation, migration, and transformation of outer radial glial cells (oRGCs). For this purpose, forebrain organoids were developed from human embryonic stem cells. These forebrain organoids contain abundant neural progenitor cells (SOX2<sup>+</sup>), express high levels of neural epithelial markers β-catenin and PKCA, and dorsal forebrain marker Pax6, and display well-organized cortical architectures containing abundant apical and basal RGCs, intermediate progenitors (IPCs), and neurons. Exposure of forebrain organoids to ethanol resulted in a significant increase in apoptosis in Nestin-positive radial glial cells. Ethanol exposure also remarkably decreased the expression of radial glial process-associated proteins, including Nestin, GFAP, and Vimentin, in radial glial cells and distinctly impaired the integrity and morphologies of radial glial processes. In addition, the ethanol-induced impairment of the radial glial processes is associated with decreased migration and proliferation of radial glial cells, reduction in the generation of HOPX<sup>+</sup> oRGCs and the accelerated transition of radial glial into astrocytic cell types. Our study demonstrates that ethanol exposure can disrupt cerebral cortex development by impairing the formation of radial glial processes and the generation, migration, and transformation of oRGCs.

**3984 Xenoestrogen Bisphenol S– and Bisphenol F–Mediated Effects on Neurogenesis in the Rat Brain Hippocampus**


Bisphenol S (BPS) and Bisphenol F (BPF) are environmental toxins, which are used in the production of plastic products. BPS and BPF are the analogs of Bisphenol A (BPA). These are endocrine disruptors and mimic the structure of the estrogen hormone. Previously, our lab found that BPA exerts an adverse effect on neurogenesis, mitochondrial dynamics, biogenesis, and cognitive deficits. Herein, we studied the impact of bisphenol alternatives (BPS and BPF) on neurogenesis in rat brain hippocampus. In this study, we assessed the neurotoxicity of bisphenol alternatives on neurogenesis in the rat brain hippocampus. To explore the effects of Bisphenol S and F on learning and memory function, we performed the behavioral experiment and found a significant decrease in learning and memory behavior. We have investigated the gene expression and protein levels of parental factors of cellular proliferation, stem cell renewal, and differentiation (Wnt/β-catenin) after exposure of BPS and BPF in the developing rat brain hippocampus. BPS and BPF reduce the expression of Wnt and the Wnt pathway genes and proteins. Pro-apoptotic alternatives reduce the GSK3β levels and increase the levels of phosphorylated form of β-catenin proteins. Overall, our results conclude that BPS and BPF impaired neurogenesis via alteration Wnt/β-catenin signaling pathway.
The treatment of cancer is multifaceted encompassing the disease state, the developmental stage of the patient, emotional well-being, individual differences, and the therapeutics chosen to treat the disease. Nonetheless, chemotherapy induced cognitive impairment (CICI) is reported to affect up to 40% of patients. CICI is characterized by problems with concentration, memory, attention, and executive functioning. Chemotherapy during childhood may represent a period of vulnerability for CICI. Here, male and female Sprague-Dawley rats were administered intraperitoneal injections of saline, cisplatin (CIS, 2 or 4 mg/kg), or methotrexate (MTX, 20 or 40 mg/kg) on postnatal days (PNDs) 23, 30, and 37. Locomotor activity, motor coordination, and grip strength were measured on PNDs 38 and 39. Anxiety-like behavior, working memory, and prepulse inhibition of the auditory startle response were assessed on PNDs 43, 44, and 45, respectively. Learning and memory were assayed in the Morris water maze on PNDs 49-53. Stimulant induced locomotor activity following a subcutaneous injection of 0.0, 0.3, 1.0, and 3.0 mg/kg amphetamine was then assessed during four sessions between PNDs 57-67. Baseline locomotor activity and motor coordination was not affected by CIS or MTX treatment. However, MTX produced a deficit in forepaw grip strength. Working memory was unaltered as measured on a Y-maze. Learning, but not memory, was impaired by MTX in female but not male rats in the Morris water maze. Examination of higher order cognition via operant conditioning and histopathological assessments are ongoing and will be reported later.

Per- and polyfluoroalkyl substances (PFAS) are a diverse set of commercial chemicals widely detected in humans and the environment. However, only a limited number of PFAS are associated with epidemiological or experimental data for hazard identification. To provide developmental neurotoxicity (DNT) hazard information, the work herein employed DNT new approach methods (NAMs) to generate in vitro screening data for a set of 160 PFAS. The DNT NAM battery was comprised of the microelectrode array neuronal network formation assay and high-content imaging assays (proliferation, apoptosis, and neurite outgrowth). The majority of PFAS (116/160) were inactive in the DNT NAMs, leaving 44 active PFAS that decreased neural network connectivity and neurite length. Analytical quality control indicated 43/116 inactive samples and 10/44 active samples were degraded; such an underestimation is required as some negatives may have been due to loss of the parent PFAS, and some actives may have resulted from a mixture of parent and/or degradants. PFAS containing a perfluorinated carbon (C) chain length ≥8, a high C:F ratio, or a carboxylic acid moiety were more likely to be bioactive in the DNT NAMs. Of the PFAS positives in DNT NAMs, 85% were also active in other assays, suggesting that PFAS in the DNT NAMs were active in other assays. These data demonstrate that a subset of PFAS perturb neurodevelopmental processes in vitro and suggest focusing future DNT studies on PFAS containing certain structural feature descriptors. This abstract does not reflect US EPA policy.

Exposure to opioids during early pregnancy has been associated with a risk of neural tube defects (NTDs) in non-clinical animal models and human epidemiological studies. However, limitations with study designs, conflicting results from human and animal studies, and incomplete maternal toxicity data have complicated risk assessment for the drug class. Human induced pluripotent stem cells (hiPSCs) may provide an alternative approach for investigating potential drug effects on neurodevelopment. The previous study with hiPSC-DFT9-7-7T indicates short-term opioid exposure at concentrations less than 30x Cmax does not impact in vitro embryonic body (EB) formation and neural rosette differentiation. For this study, three hiPSC lines (UCSD-163i, UCSD-092i, UCSD-079i) from healthy female donors were differentiated to neural precursor cells (NPCs) using an optimized EB-neural differentiation protocol to recapitulate various stages of early development. Opioids, including morphine, methadone, codeine, fentanyl and buprenorphine were selected with concentrations ranging from 3x to 1000x Cmax. Valproic acid (VPA), a known human teratogen, was used as a positive control. In contrast to VPA, there was no clear effect of the opioids on iPSC proliferation, differentiation, EB development and neural rosette formation at concentrations <30x Cmax. Fentanyl and buprenorphine, when tested at up to 1000x Cmax had also no observable effect on early neural differentiation endpoints. Methadone, however, inhibited iPSC proliferation and differentiation at concentrations of 300x and 1000x Cmax in all three of the cell lines, while codeine and morphine exhibited inhibitory effects only at 1000x Cmax. Methadone also significantly inhibited rosette formation by decreasing rosette neural induction rate (NI) from 100% (30x Cmax) to 10% (1000x Cmax) in the UCSD-163i line and to 20% in the UCSD-079i line, but not in the UCSD-092i line (95%+). Line, while codeine at 1000x Cmax showed less effects in UCSD-092i (NI=5%) and UCSD-079i (NI=10%) line. Morphine did not affect rosette formation. Preliminary gene expression (RNA sequence analysis) indicates gland morphogenesis, axon guidance, neuron projection guidance, axonogenesis, and central nervous system neuron development are the major biological process affected during methadone treatment at a concentration of 1000x Cmax. In conclusion, preliminary evidence indicates no or low association with opioid exposure and disrupted neural proliferation and differentiation in vitro; however, EB formation and neural rosette differentiation were impacted at higher concentrations, particularly following methadone exposure. The inter-individual variation observed in the study suggests that a panel of patient-specific iPSCs may enhance the utility of this in vitro model for drug risk assessment.
examined in frontal cortex (FC) at PND14 and PND43, and in striatum (STR) at PND14 and PND50. In female FC, despite modest reductions in levels of glutamatergic, serotonergic and dopaminergic neurotransmitters at PND43, the only significant effects consisted of an increase in tyrosine at PND43. In STR of females, significant reductions in levels/turnover of glutamatergic, serotonergic and dopaminergic neurotransmitters were observed at PND14. Recovery of glutamatergic and serotonergic function was found at PND43 that included an overshoot of 5HT levels. Some recovery of dopaminergic function was observed, although the ratio of dopamine/tyrosine was even further reduced at PND43. In male FC, reductions of all three classes of neurotransmitters were observed at PND14, including significant or marginal reductions in levels of tryptophan, kynurenine, tyrosine, DOPAC and HVA/DA. With the exception of tryptophan and kynurenine, recovery was observed at PND43. In male STR, reductions in levels of all three classes of neurotransmitters were also observed at PND14, but no recovery at PND50 was observed in glutamatergic function, or of tryptophan or kynurenine, and with even further reductions in the dopamine/tyrosine ratio. While no changes in serum cytokine levels (IL1-a, IL1-b, IL2, IL6, IL10, INF-gamma and TNF-alpha) were observed at other time points, significant reductions in cytokine levels at both time points that were significant or marginally significant for IL1-b, IL6 and IL2 at PND14 and for IL1-b and INF-gamma at PND50. Collectively, these outcomes indicate more dramatic effects of developmental UFP on STR than FC in both sexes, with females exhibiting a more resilient phenotype of recovery, and selective susceptibility of males to reductions in serum cytokine levels. Low levels of circulating cytokines may be related to changes in brain neurotransmitters, and may be of concern as they are critical to ontogenic development of brain. Supported by NIH Grants R01 ES032260 and R35 ES031689.

**P3 3990**

**Developmental Exposure to Lead (Pb) Alters the Behavioral Response to Acute Stressors in Zebrafish (Danio rerio): Behavioral Alterations, Underlying Molecular Changes, and Links to Stress-Related Disorders**

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Stress-related disorders are attributed to disruptions in the adaptive behaviors of organisms owing to genetic and environmental risk factors. Our understanding of the genetic factors has increased considerably through association studies of human subjects, but the role of environmental factors as contributors to these disorders remains poorly defined. Pb is a ubiquitous environmental contaminant and developmental neurotoxin linked with the prevalence of several neurological and mental disorders. A deeper understanding of the impacts of early exposure to Pb on behavioral responses and the mechanisms of how Pb exposure impacts behaviors in fish may lead to the development of strategies to prevent or mitigate its impacts on fish. To study the development of changes and alterations in the behavioral response to acute stressors in larval zebrafish (Danio rerio) following Lead (II) Acetate (Pb) exposures that extended between 6 and 120 hours post-fertilization (hpf). Zebrafish embryos were exposed to Pb concentrations corresponding to the Maximum Contaminant Level (MCL) for Pb in drinking water as set by the US EPA (15 ug/L) and folds higher (5, 10, 25, and 50-fold). Embryos were enzymatically dechorionated prior to the exposures to allow for direct chemical exposure, and medium changes (50%) were performed daily. At the end of the exposure period (120 hpf), the impacts on survival, development, baseline behavior and stress-related responses were measured. The behavioral responses to visual or acoustic stimuli (acute stressors) were significantly altered following exposures to Pb concentrations at 5-fold the MCL and folds higher. Exposure to peripheral irritants significantly increased the larval behavioral response in all treatment groups, suggesting the lack of locomotor deficit. Our findings shed light on the importance of Pb exposure and the requirements for concentrations of Pb on the behavioral stress response and their possible links to the onset or aggravation of stress-related disorders such as pathological anxiety and depression. Furthermore, they highlight the need for further research to understand the underlying molecular and cellular-level changes in the stress circuitry, which are currently undertaking and will uncover its latest at the meeting.

**P3 3991**

**Caesarean Delivery Alters Brain and Gut Transcriptome in a Mouse Model of Neurodevelopmental Susceptibility**


Neurodevelopmental diseases - including autism spectrum disorder, attention deficit hyperactivity disorder (ADHD), and intellectual disability - are thought to be caused by a combination of genetic susceptibility and environmental triggers. Caesarean-section delivery (CS) encompasses many environmental changes, including altered microbiome, altered hormonal environment, and anesthetic exposure. Epidemiological studies correlate CS to a 33% increased risk of developing autism and a 17% increased risk of ADHD. We designed a system in a genetic model of neurodevelopmental susceptibility to study effects of CS on neurodevelopment and investigate gene x environment interactions. In humans, the 16p11.2 copy number variant (CNV) is responsible for approximately 1% of autism cases, and carriers have a 40-fold risk of autism, 4-fold risk of ADHD, and 60-fold risk of intellectual disability. This heterozygous CNV is conserved in mice. These mice demonstrate phenotypes and neurodevelopmental abnormalities associated with autism. In our current study, timed-pregnant dams were allowed to give birth vaginally (control) or underwent terminal CS on gestational day 19. Pups from both groups were cross-fostered by dams giving birth in the previous 24-hours. Following sacrifice on P7, we isolated hippocampus and ileum for transcriptomic analysis. Principle Component Analyses in males and females show the transcriptomes for CS and control group segregated based on delivery status. Further analysis in the hippocampus shows 17 KEGG pathways were consistently altered in C-section mice across both genotype and sex. Out of 16 pathways significantly downregulated in CS mice, 11 may alter brain development: glutamatergic synapse, GABAergic synapse, cholinergic synapse, dopaminergic synapse, oxytocin signaling pathway, serotoninergic synapse, glutamatergic synapse, and tryptophan metabolism. Striatal synapses and the tryptophan metabolism are critical to ontogenic development of brain. Additionally, investigating the effect on body weight, hypothalamic-pituitary-adrenal axis function, and immune development. In contrast to the hippocampus, there were no KEGG pathways in the ileum that changed consistently across genotype and sex. Further investigations will determine pathways in the hippocampus and ileum that are susceptible to specific gene x environment combinations. Additionally, forthcoming investigation on the gut microbiomes of these groups will determine if the CS-associated gut microbiome is correlated with transcriptional changes. The results of this study have the potential to impact healthcare considerations for all women of childbearing age and their future children.
Although environmental chemical exposures, including to flame retardants, are suspected risk factors for neurodevelopmental disorders, direct experimental evidence linking specific chemicals to NDDs is limited. Studies focusing on the mechanisms by which social processing systems are vulnerable to chemical exposure are limited and often inadequate because traditional animal models like rats and mice are limited in their ability to model human prosocial behaviors such as paternal care and monogamy. Here we show how tools from the neurosciences, such as the NIMH Research Domain Criteria (RDoC) and use of the prairie vole (Microtus ochrogaster, a monogamous rodent model) can help improve experimental design, and more comprehensive statistical approaches can be used to better interpret complex behavioral datasets. We have repeatedly demonstrated that exposure to Firemaster 550 (FM 550), a prevalent flame-retardant mixture containing Mounting media. Fos positive and negative DAPI labeled nuclei were counted within the lateral subregion of the central amygdala in three sections and antibody were used to visualize cells and sections were coverslipped with DAPI staining also showed the colocalization of the DNA fragment and PLP, shown with DRAQ5 staining by immunofluorescence in confocal images. Blow-up of DRAQ5 staining also showed the localization of the DNA fragment and PLP, in which PLP appeared as a loop wrapped around the DNA fragment, while there was no difference in caspase 8 levels at all concentrations lower than the IC50 value. Morphology changes were observed in oligodendrocytes by the endogenous fluorescent PLP after treatment with paroxetine, however, no significant decrease in PLP expression was noticed from qRT-PCR results. These results showed the effects of paroxetine on several neural development events and indicated paroxetine to be a developmental neurotoxicant. Compared with the previous publication, in which 8-week paroxetine treatment on the brain organoid differentiated from a different iPSC line was proved to exert DNT, this study proved the DNT of one-week paroxetine exposure and showed CRISPR/Cas9 edited SYP-BFP/PLP-GFP brain organoid as a potential tool to accelerate neurotoxicity assessment by combination with high content imaging.

Polychlorinated biphenyls (PCBs) are synthetic organochlorine chemicals that were commonly used in industrial applications before being banned in the US in 1977 due to their acute toxicity and ability to bioaccumulate in the environment. Since that time, they have been implicated in a range of human health concerns to endocrine, immune and neural dysfunction in humans. We have previously demonstrated that early life PCB exposure alters late neuroimmune gene expression and microglial responses to an innate immune challenge (lipopolysaccharide) in ways that depend on the age and sex of the rats tested. However, organs are exposed to a range of environmental influences across their lifetimes that also alter immune processes, including stress, dietary factors, and drugs of abuse. Given that neuroimmune dysfunction has been linked to mental health and substance abuse disorders, it is critical to understand how broadly PCBs influence neural responses to other neuroimmune challenges. We hypothesized that early life PCB exposure may alter late neural responses to acute ethanol intake during adolescence. To study this, Sprague Dawley dams were orally administered environmentally relevant doses of either a PCB mixture (20 µg/kg BW, 1:1 Aroclor 1242, 1248, 1254) or an oil vehicle control throughout their gestational period. Adolescent male and female offspring were then given either ethanol (5 mg/kg) or a water control gavage. As an initial assessment of our model, we analyzed the early gene Cfos expression was quantified using immunofluorescence. We focused on the central amygdala, a region that is responsive to ethanol and is important for a range of stress, learning, and affective behaviors. One hour after ethanol exposure, animals were perfused; brains fixed and sectioned at 30 µm; and tissue labeled via immuno-fluorescence. Anti Cfos primary (1:2000, SySy, Cat No. 226 308, guinea pig anti Cfos, 24 hours) and secondary (1:500, Jackson, goat anti-guinea pig, Alexa-488) antibodies were used to visualize cells and sections were coverslipped with DAPI containing mounting media. Fos positive and negative DAPI labeled nuclei were counted within the lateral subregion of the central amygdala in three sections and then averaged together within an animal. Preliminary data demonstrated greater Fos expression (number and percentage of cells) in ethanol-treated animals in comparison to the saline controls. PCB exposure appeared to blunt the Fos ethanol expression (number and percentage of cells) in ethanol-treated animals in later vulnerability to substance abuse. Find up-to-date information at www.toxicology.org/2023 | #2023SOT | #ToxExpo | 318

One of the selective serotonerupt inhibitors, paroxetine, was reported to show development neurotoxicity (DNT) effects within non-cytotoxic concentrations. By utilizing the newly developed fluorescence-tagged 3D brain organoids, this study assessed the cytotoxicity of paroxetine and other key events of neural development. Using CRISPR/Cas9 gene editing, we tagged the pre-synaptic protein, synaptophysin (SYP) with the blue fluorescent protein (BFP) and the myelin proteolipid protein (PLP) with the green fluorescent protein (GFP), in an induced pluripotent stem cell (iPSC) line. After rigorous quality control steps, we differentiated the two-color reporter iPSC line into brain organoids. At 7 weeks of differentiation, brain organoids were seeded onto the Poly-L-Ornithine/Laminin coated wells and attached to prevent the fusion and for the later neurite outgrowth analysis. Paroxetine was obtained from Sigma, and a stock of 5 µM was prepared in DMSO Hybri-Max (Sigma), stored at -20 °C. To cover a wide range of concentrations as possible, five concentrations were selected for the range-finding experiment. Treatment of paroxetine started when brain organoids were at 7 weeks, and a one-week treatment was conducted. Medium with the chemical (DMSO for the control group) was changed every 2 days. Cell viability was assessed by Resazurin Reduction Assay after the one-week exposure. qRT-PCR was performed to quantify the expression of several neural markers and the Zieve LSM800 microscope was used for the assessment of PLP morphology after the chemical exposure. High Content Imaging was used to quantify GFP and BFP. After the one-week paroxetine treatment, the half-maximum inhibitory value (IC50) was 7.703 µM in the 8-week brain organoid. For concentrations higher than the IC50 value, paroxetine treatment inhibited the attachment of brain organoids and their neurite outgrowth capability. Paroxetine treatment at 3.16 µM also induced a higher number of DNA fragments as shown with DRAQ5 staining by immunofluorescence in confocal images. Blow-up of DRAQ5 staining also showed the localization of the DNA fragment and PLP, in which PLP appeared as a loop wrapped around the DNA fragment, while there was no difference in caspase 8 levels at all concentrations lower than the IC50 value. Morphology changes were observed in oligodendrocytes by the endogenous fluorescent PLP after treatment with paroxetine, however, no significant decrease in PLP expression was noticed from qRT-PCR results. These results showed the effects of paroxetine on several neural development events and indicated paroxetine to be a developmental neurotoxicant. Compared with the previous publication, in which 8-week paroxetine treatment on the brain organoid differentiated from a different iPSC line was proved to exert DNT, this study proved the DNT of one-week paroxetine exposure and showed CRISPR/Cas9 edited SYP-BFP/PLP-GFP brain organoid as a potential tool to accelerate neurotoxicity assessment by combination with high content imaging.
control activity levels by 14 days. These data suggest that NIMP exposure can elicit day (57%) and at 4 days. Recovery to control activity was evident by 7 days. Brain signs. There was significant inhibition of serum ChE at 4 h (80%) with recovery at 1 isopropyl methylphosphonate) to investigate the sub-lethal effects of nerve agent thal OP exposures. In this study, we used the sarin surrogate NIMP (nitrophenyl inhibition of acetylcholinesterase (AChE) leading to accumula – tion of the neurotransmitter acetylcholine. This accumulation induces hyperactiv – ity in the peripheral and central cholinergic system with death resulting from the loss of respiratory function. Exposure can produce prolonged repetitive seizures and status epilepticus, a medical condition that causes high morbidity. While nerve agent exposure is frequently considered mainly a military threat, events in Japan in the 1990’s and in Syria in the 2010’s have clearly demonstrated that the civilian population can be targeted as well. A component of this population is thought to be the most susceptible is the pediatric population. However, the effects of nerve agents on juveniles have not been studied to a great extent. In the majority of studies involving exposure of preclinical models to OP nerve agents or surrogates, the OP exposure has been lethal dosages and were accompanied by co-administration of the therapeutics against OP-induced lethality (i.e., atropine and 2-PAM). However, less is known about the persistent effects of high sub-lethal OP exposures. In this study, we used the sarin surrogate NIMP (nitrophenyl isopropyl methylphosphonate) to investigate the sub-lethal effects of nerve agent exposure. Male and female 16-day old rat pups were exposed subcutaneously to either the vehicle multi-dose (170 ng/kg NIMP). Rats were sacrificed at 4 h and 1, 4, 7, and 14 days post-exposure and brain (cerebral cortex) AChE and serum ChE activities were determined. The NIMP treated rats exhibited episodic seizure-like signs. There was significant inhibition of serum ChE at 4 h (80%) with recovery at 1 day (57%) and at 4 days. Recovery to control activity was evident by 7 days. Brain AChE was also inhibited (54%) and remained inhibited (55%). Inhibition was still present at 4 days (26%) and at 7 days (13%) with recovery to control activity levels by 14 days. These data suggest that NIMP exposure can elicit episodic seizure-like signs at moderate levels of brain AChE inhibition.

Benzo[a]pyrene (BaP) is a widespread pollutant that exerts neurotoxic effects on early brain development. In our previous studies, we found that Cyp1a2(-/-) and Cyp1a1(-/-) mice were more susceptible to developmental BaP neurotoxicity using only wild-type mice. We treated pregnant dams with either 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle from gestational day 10 (GD 10) to weaning at postnatal day 25 (P25). We used three levels of treatment in addition to the control condition of no exercise. Dams exercised for two weeks prior to mating and through GD10. Pups exercised from P30 to P60, or both dams and their offspring exercised. All were given free access to running wheels for 1 hour/day, and activity was monitored using sniffing bags. After behavioral testing was completed ~P12, brains were harvested and high-performance liquid chromatography with electrochemical detection was used to quantify dopamine, serotonin and their metabolites in the hippocampus, striatum, prefrontal cortex and hypothalamus. We found a main effect of BaP treatment with significantly lower levels of serotonin and the metabolite S-HIAA in the hippocampus (P < 0.01) and significantly low levels of S-HIAA in the prefrontal cortex (P < 0.01), striatum (P < 0.05) and hypothalamus (P < 0.01). Exercise had the greatest impact on levels of dopamine and its metabolite DOPAC. In the hippocampus, dopamine was significantly lower in the offspring where only the dam exercised (P < 0.05). In the striatum, dopamine and DOPAC levels were highest in control mice and lowest where only the pups exercised (P < 0.05). In the hypothalamus, dopamine levels were highest in offspring where only the dams or only the pups exercised (P < 0.05) although offspring where both exercised were also higher than control values. Dopamine turnover appeared to differ though as DOPAC was highest in offspring where only the dam exercised whereas the lowest DOPAC levels were found in offspring where both dams and pup exercised. Together, these data suggest that BaP negatively impacts monoamines neurotransmitter levels, but the effects of exercise vary by level of treatment and brain region.

P5 3997 Inhibition and Recovery of Cholinesterase Activity in Juvenile Rat Brain and Serum following Acute Exposure to a Nerve Agent Surrogate


The most toxic chemical warfare agents are organophosphates (OP) that are commonly referred to as nerve agents or nerve gases. The compounds exert their toxicity through the inhibition of acetylcholinesterase (AChE) leading to accumulation of the neurotransmitter acetylcholine. This accumulation induces hyperactivity in the peripheral and central cholinergic system with death resulting from the loss of respiratory function. Exposure can produce prolonged repetitive seizures and status epilepticus, a medical condition that causes high morbidity. While nerve agent exposure is frequently considered mainly a military threat, events in Japan in the 1990’s and in Syria in the 2010’s have clearly demonstrated that the civilian population can be targeted as well. A component of this population is thought to be the most susceptible is the pediatric population. However, the effects of nerve agents on juveniles have not been studied to a great extent. In the majority of studies involving exposure of preclinical models to OP nerve agents or surrogates, the OP exposure has been lethal dosages and were accompanied by co-administration of the therapeutics against OP-induced lethality (i.e., atropine and 2-PAM). However, less is known about the persistent effects of high sub-lethal OP exposures. In this study, we used the sarin surrogate NIMP (nitrophenyl isopropyl methylphosphonate) to investigate the sub-lethal effects of nerve agent exposure. Male and female 16-day old rat pups were exposed subcutaneously to either the vehicle multi-dose (170 ng/kg NIMP). Rats were sacrificed at 4 h and 1, 4, 7, and 14 days post-exposure and brain (cerebral cortex) AChE and serum ChE activities were determined. The NIMP treated rats exhibited episodic seizure-like signs. There was significant inhibition of serum ChE at 4 h (80%) with recovery at 1 day (57%) and at 4 days. Recovery to control activity was evident by 7 days. Brain AChE was also inhibited (54%) and remained inhibited (55%). Inhibition was still present at 4 days (26%) and at 7 days (13%) with recovery to control activity levels by 14 days. These data suggest that NIMP exposure can elicit episodic seizure-like signs at moderate levels of brain AChE inhibition.

P5 3998 A Pilot Study to Understand the Effects of Exercise on Monoamine Neurotransmitters in Mice Exposed to Benzo[a]Pyrene during Gestation and Lactation

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Pregnant women are ubiquitously exposed to low concentrations of multiple endocrine disrupting chemicals (EDCs) known to disrupt estradiol function. During pregnancy, mothers undergo a surge in estradiol levels from 100 to 1000-fold greater than non-pregnant levels. This surge is critical for maternal and fetal development with disruptions in estradiol associated with increased cardiometabolic and neurobehavioral disease risk. Our previous research indicates that gestational exposure to a mixture of anti-estrogenic EDCs decreased the estradiol surge during late gestation in mice. Serum estradiol has been shown to alter neurotransmitter concentrations in the central nervous system. Understanding the effect of EDC-induced estradiol reductions on maternal neurochemistry during pregnancy is essential, because approximately 1 in 10 women experience postpartum depression, often with increased anxiety. Research typically ignores the influence of EDC exposure during pregnancy on maternal health, while prioritizing fetal development and offspring health. To assess the effect of EDCs on maternal health through modulation of critical neurotransmitters during pregnancy, pregnant dams were exposed to either a mixture of four EDCs (MIX: atrazine (10mg/kg), bispheanol-A (50µg/kg), perfluorooctanoic acid (0.1mg/kg), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (0.036µg/kg)) or vehicle (control) per day beginning on gestational day (GD) 0.5 until birth. Serum concentrations for each EDC and metabolites were quantified. We quantified neurotransmitter concentrations along mesocorticollimbic pathways in frontal cortex, hippocampus, and striatum on GD 13.5, 17.5, and at birth utilizing LC/MS carried out by a Dionex Ultimate 3000 UHPLC coupled to a Q Exactive Plus mass spectrometer. We measured norepinephrine (NE), dopamine (DA), 3,4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), tyrosine (Tyr), glutamate (Glut), GABA, glutamine (Gln), kynurenic acid (Kyn), serotonin (5-HT), 5-Hydroxytryptophan (5-HTP), 5-Hydroxyindoleacetic acid (5-HIAA), and tryptophan (Trp). Significant changes in amino acid and serotoninergic compounds occurred as a result of gestational day and birth alone. In all three brain regions, Kyn showed
significant reductions at birth in both MIX and control dams compared to GD 17.5. In contrast, Tyr was elevated at birth compared to GD 17.5 in both MIX and control dams. MIX exposure lowered SHT and NE before the surge in estradiol at GD 13.5 in fetal cortex. At the end of the estradiol surge during pregnancy at GD 17.5, MIX exposure altered serotonin metabolism lowering 5-HIAA levels in striatum. At birth, MIX exposure altered serotonin metabolism with lower 5-HIAA levels in all three regions. In fetal cortex, frontal cortex, and hippocampus, overall, more alterations occurred in serotonergic pathways, compared to dopaminergic pathways which showed minimal effects. MIX also altered Glu, Gln and GABA homeostasis. To evaluate the functional effects of these neurotransmitter changes, spontaneous locomotor and elevated plus maze (EPM) behavior was evaluated at weaning. MIX exposed female mice showed significantly increased stereotypic behavior along the edge of the locomotor chamber. MIX significantly increased time spent in the closed arms during the first five minutes of EPM testing. In conclusion, a mixture of anti-estrogens EDCs triggers neurotransmitter dysregulation in multiple brain regions, with serotonergic pathways most vulnerable. Future studies are needed to evaluate effects during lactation and to measure changes in maternal behavior. Pregnancy itself is an interesting critical window for long-term maternal health and more studies focused on postpartum depression are needed. Supported by P30 ES001247 and T32 ES070706.

4001 Behavior-Based Identification of Chemical Mechanisms in Larval Zebrafish

One in six children is diagnosed with a developmental disability including learning and intellectual disability. Chemical exposure during prenatal and early postnatal development is a known cause of neurodevelopmental disorders. However, due to extensive time, cost, and animal number requirements of developmental neurotoxicity (DNT) studies in rats, the potential hazard of most chemicals remains unknown. To fill this gap, we devised a battery of eleven automated behavior assays in larval zebrafish, a 3R-compliant model amenable to higher-throughput chemical screens. The battery captures various stereotypical visual and acoustic behaviors including habituation, a form of non-associative learning. We hypothesized that behavior-rich phenotyping of chemicals provides a comprehensive readout of potential adverse outcome pathways (AOPs) that dictate a broad range of events and interactions that orchestrate nervous system development and function. According to the AOP “Impairment of Learning and Memory”, we focused on the NMDA receptor (NMDAR) as a target of chemical exposure. In an initial step, the DNT-AOP Approach used a high-throughput (HAT) method to screen against seven drugs with distinct mechanisms including NMDA and GABA receptor (GABAR) antagonism. In line with effects reported in rats, exposure to the prototypical NMDAR antagonist MK-801 caused a deficit in habituation learning. Surprisingly, cluster analysis revealed high phenotypic similarity to the GABAR antagonist picrotoxin, pointing to a potential second mechanism of MK-801, GABAR antagonism. The battery was further evaluated against a set of ten US EPA ToxCast chemicals positive for MK-801 caused a deficit in habituation learning. Strikingly, in addition to an NMDAR-mediated learning deficit, clorophene-induced DNT. Strikingly, in addition to an NMDAR-mediated learning deficit, clorophene exposure caused ‘paradoxical excitation’, a phenomenon linked to GABAR agonism. Pharmacological intervention using GABAR antagonist picrotoxin blocked clorophene-induced sedation, substantiating its GABAergic interaction. In summary, these non-exhaustive examples highlight the capacity of this behavior-based NAM to illuminate AOPs, to reduce/replace existing in vivo models, and to accelerate DNT research.

4002 Measuring Neurotoxic Effects of Antiretrovirals (ARVs) Using a Human iPSC-Derived Neural Progenitor Cell (NPC) Screening Platform

HIV-infected (HIV+) individuals often develop HIV-Associated Neurocognitive Disorders (HAND), which present as a spectrum of cognitive impairments that can interfere with daily functioning and quality of life. Combination antiretroviral (ARV) therapy (cART) prevents HIV replication and has been shown to improve the likelihood of many HIV+ individuals; however, these treatments may also be neurotoxic and contribute to the development of HAND. Particularly concerning is HIV+ pregnant women taking cART to prevent perinatal HIV transmission. The ARVs dolutegravir, Tenofovir Disoproxil Fumarate, Tenofovir Alafenamide, or Bictegravir, were seeded in 384-well plates and exposed to ARVs (Emtricitabine, Etraviride, Dolutegravir, Tenofovir Disoproxil Fumarate, Tenofovir Alafenamide, or Bictegravir), either alone or in clinically relevant combinations. Following a 3-day treatment, we performed Click-IT Edu assay (Thermo Scientific) to measure DNA replication. Then, cells were fixed with paraformaldehyde and labeled with antibodies for self-renewal (SOX2) and epigenetic markers (anti-H3K27ac antibody). Nuclei were counterstained with Hoechst 33342. Image acquisitions were performed using Vala Sciences’ fully automated Structured Illumination Microscopy (SIM®) and resulting images were analyzed with Vala Sciences’ CyteSeer® automated image analysis software, which can identify cell structures and biomarkers at the single-cell level. Our results revealed that the HIV integrase inhibitor Etraviride reduced live cell count alone and in combination with the nucleoside analog reverse transcriptase inhibitors Tenofovir Disoproxil Fumarate and Emtricitabine. The second-generation integrase inhibitor Bictegravir also reduced live cell count alone and in combination with Tenofovir Alafenamide and Emtricitabine. The Microscopic imaging of Epigenetic Landscapes (MIEL) assay identified ARV-induced changes in the distribution of the H3K27ac epigenetic marker. Epigenetic changes were most pronounced in NPCs treated with Etraviride alone and with the combination therapies. Altogether, our results suggest that cART can affect neural progenitor cell function in vitro, which may translate to an impact on neurogenesis. We are currently developing tri-culture model systems from hiPSC-derived neurons, -astrocytes, and -microglia to test the effects of cART on differentiated cells. Preliminary data show a neurotoxic effect of Etraviride in this system. Although terminally differentiated, our cell culture system represents young neurons. Thus, these findings are indicative of toxicity to the developing fetus or to young children. We aim to use our NPC and the tri-culture model systems to identify cART regimens with reduced developmental neurotoxicity.

Characterization of Neurotoxicity Induced by Engine Oil-- Hydraulic Fluid--Derived Contamination in Aircraft Cabin Air
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Most environment control systems (ECS) in commercial airplanes use bleed air to pressurize, heat, and ventilate the cabin. Since bleed air is extracted by the turbine compressors, there is ongoing debate on whether contaminated bleed air originating from engine oil or hydraulic fluid leakage in the ECS can cause neurological health complaints. We therefore investigated the neurotoxic potency of engine oil- and hydraulic fluid-derived fumes in an in vitro model. First, we prepared fume extracts from four commonly used engine oils and two hydraulic fluids. To do so, oil-contaminated air was generated by a laboratory bleed air simulator that heats engine oil (50°C) and pressurizes (3 bar), and generated aerosols were collected on filters. Secondly, we exposed primary cortical cultures grown on microelectrode arrays (MEAs) to these fume extracts (1 - 100 µg/mL), and effects on spontaneous neuronal activity were recorded after acute (0.5-hour) and sub-chronic (24-hour and 48-hour) exposure. While at doses > 30 µg/mL all tested fume extracts decreased spontaneous neuronal activity without inducing cytotoxicity, differences in the potency and persistence of the effects were observed. Fume extracts originating from the two hydraulic oils were significantly more potent (3 - 5-fold) than those from engine oils, in particular during acute exposure. Acute exposure to 1-3 µg/mL hydraulic fluid-derived fume extracts reduced mean spike rate (MSR) significantly to < 75% of control resulting in half maximal effect concentrations (EC50) values of 2.3 and 5.8 µg/mL. However, after sub-chronic exposure, neuronal activity partly recovered in cells exposed to hydraulic fluid-derived fume extracts. Below 10 µg/mL, MSR was unaffected resulting in increased EC50 values of 15 - 17 µg/mL. In contrast, acute exposure to engine oils-derived fume extracts only caused a significant reduction of MSR at 30 - 100 µg/mL. Integrally, the reduction in neuronal activity was persistent and EC50 values remained in a comparable range for acute and sub-chronic exposure (EC50 acute: 39 - 121 µg/mL; EC50 sub-chronic: 37 - 98 µg/mL). Our data show that bleed air contaminants originating from engine oil or hydraulic fluids exhibit neurotoxic hazard in vitro, but there are clear differences in the potency, with contamination deriving from hydraulic fluid leakage being most potent. This work was funded by the European Union’s Horizon 2020 research and innovation programme under grant agreement No 814978 (TUBE), the European Commission-DG MOVE Service Contract MOVE/BE/SER/2016-363/SI2.748114, the European Commission-DG MOVE Service Contract MOVE/BE/SER/2016-363/SI2.748114, the National Institute for Public Health and the Environment (RIVM, The Netherlands), and the Faculty of Veterinary Medicine (Utrecht University, The Netherlands).
Hearing-related injuries are the most common military service-related disabilities. Excessive levels of noise exist on U.S. Navy aircraft carriers due to flight deck operations, putting personnel at risk of exposure to elevated noise levels during occupational flight deck activities. Even in off-duty areas, elevated noise levels and intermittent impulse noise have been measured, extending the duration of these exposures past the occupational timeframe. In addition to high noise levels, exposures to jet fuel on Navy carriers are also common and some jet fuel components have been identified as ototoxicants. The effects of the combinations of these exposures on hearing function have not been well characterized. The objectives of this study were to evaluate the impact of extended duration noise exposures and inhalation of jet fuel aerosols and vapor on hearing-related injury in a rodent model. Adult Long Evans rats (n=8-10 per exposure group) were exposed to 6 hours a day of steady noise, (~89 decibels (dB) 8-hour equivalent, meant to represent an occupational noise exposure), an extended duration of 120 dB impulse noise for 16 hours a day (8 impulses/hour), an extended duration 75 dB steady noise for 16 hours a day, 1000 mg/m³ of a JP-5 jet fuel aerosol/vapor mixture for 6 hours a day, or combinations of these four exposures. A control group was exposed to <60 dB background noise during the same time period. Eight kHz octave band noise was used for all noise exposures. Rats were exposed for five days a week with a two day total. Auditory function was assessed prior to and four weeks following the exposure. Peripheral auditory function was assessed using distortion product otoacoustic emission (DPOAE) testing, while the function of the peripheral and brainstem segments of the ascending auditory neural pathway were assessed by measuring auditory brainstem response (ABR). Cochleae from each rat were prepared and processed for histopathological and histochemistry of cochlear structures. Exposure to extended duration impulse noise significantly increased auditory thresholds in DPOAE by approximately 25 dB at 4 kHz and 8 kHz and an approximately 15 dB increase in click ABR thresholds. Consistent with these data, rats exposed to extended-duration impulse noise demonstrated a significant 10% reduction in cochlear hair cells. These changes in hearing thresholds were detected within four weeks following the final exposure, indicating that the changes in auditory function were likely permanent. JP-5 exposure did not significantly impact auditory thresholds alone, although decrements in neural auditory processing were present. Future work will examine the effects of JP-5 exposure on neural auditory processing as well as to identify safe extended-duration impulse noise exposure limits in the rodent model.

4004 Effects of Exposure to Impulse Noise and Jet Fuel Inhalation on Hearing-Related Injury in a Rodent Model

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The peripubertal period is associated with a heightened risk of eating disorder onset. Bisphenol-A is an endocrine disruptor that is known to dysregulate the normal functioning of hypothalamus, a key mediator of pubertal and feeding outcomes. To date, however, no study has examined how BPA exposure during adolescence affects puberty and feeding outcomes. Here, we tested this directly in female rats, and explored how these effects might be mediated by changes in the functioning of the hypothalamic orexin (hypocretin) neurone system. Female Long-Evans rats were exposed to BPA (0, 25, 250µg/kg/day) via their drinking water between post-natal days (PND) 29-56. Rats were monitored for vaginal opening (VO) and cystology throughout the BPA exposure period. Following BPA exposure, a subgroup of rats was tested for binge-like intake of palatable food using a paradigm where sweetened fat (10% sucrose and vegetable shortening) was made available intermittently (twice/week; 30 minutes) across four weeks. These rats were subsequently tested for anxiety-like behavior on the open field test. A separate group of rats were trained to lever press for sucrose pellets on a fixed ratio (FR) 1 schedule. On PND 97, brains were collected for immunohistochemistry and qPCR analyses. BPA (25 and 250µg/kg) exposure was associated with irregular cycling, earlier VO, and enhanced anxiety-like behavior. BPA (250µg/kg) was also associated with reduced binge-like eating and FR1 responding for sucrose. BPA dose-depending decreased orexin gene expression in hypothalamus, and reduced reactivity of orexin neurons to food-associated stimuli. Together, these data indicate that peripuberal BPA exposure is associated with advanced puberty onset and a restrictive feeding phenotype in early adulthood. BPA exposure was also associated with a general downregulation in the functioning of the orexin system, pointing to this neuropeptide as a potential mediator of these outcomes.

4006 Peripubertal Bisphenol A Exposure is Associated with Dysregulated Puberty and Feeding Outcomes: A Role for the Orexin (Hypocretin) System

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The hypothalamic orexin (hypocretin) system is involved in several physiological functions, including arousal, wakefulness, motivation, and sleep. Preclinical studies indicate that orexin-1 receptor (Ox1R) antagonists have promising therapeutics for the management of substance use disorder, as they reliably suppress drug seeking across all drug classes of abuse tested. Here, we tested the potential off-target effects of the Ox1R antagonist SB334867 (SB), which reliably blocks drug seeking at a dose of 10mg/kg (i.p.). We carried out a dose-response study focusing on cognitive outcomes following treatment with SB. Male and female Long-Evans rats (n=22) were trained on the rodent psychomotor vigilance task (PVLT), an adapted version of the T-maze, to measure sustained attention and fatigue in mice and rats. Briefly, rats were trained to make a rapid response on a lever in response following presentation of a light cue that varied in onset time (3-10 seconds after trial initiation); correct responses were rewarded with sucrose pellets. We first validated the task by testing the effect of three drugs with known attention-modulating properties (d-amphetamine, 0.3, 10, 30 mg/kg; guanfacine, 0.3, 10, 30 mg/kg) and atipamezole (0, 3, 10, 30 mg/kg). Next, all rats were tested following injections of SB (0, 3, 10, 30 mg/kg; i.p.). All drugs were tested in a within-subjects design and the order of all doses was fully counterbalanced. Consistent with their known attention-enhancing properties, d-amphetamine and guanfacine dose-dependently improved performance; d-amphetamine at the baseline dose significantly improved performance. SB dose-dependently reduced performance on the PVLT, however significant deficits were only observed at 30 mg/kg (reduced accuracy and increased reaction time). At doses that reliably reduce drug seeking, the Ox1R antagonist SB has no effect on a rat assay of cognitive fatigue and sustained attention. These data support the development of selective Ox1R antagonists for the treatment of addiction, as they would be predicted to have limited off-target effects on cognitive outcomes. Grant: MHJ is supported by a grant from the National Institute on Drug Abuse (R00 045765).

4007 Orexin-1 Receptor Antagonist SB-334867 Impairs Sustained Attention but Only at Suprapharmacologic Doses

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Genotoxic stress is a prominent feature in COVID-19 that may link environmental toxicant exposure and disease severity. A. Trupp1, X. Tiao1, H. Zhang1, M. Grames2, C. Hauer, K. Keys, E. Knott, and X. Lu1,1

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Novel Coronavirus (COVID-19) has killed more than 6 million people worldwide since its outbreak in late 2019 and continues to mutate, causing an increase in infection, hospitalization, and death across the globe. According to CDC, 1 out of 5 adults who are infected with COVID-19 develops longer-lasting chronic symptoms termed “long COVID.” Persistent symptoms of this so-called “long COVID” are

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characterized by fatigue, “brain fog,” difficulty breathing, heart palpitations, joint and
muscle pain, and most notably, death, among other symptoms. According to CDC,
more than 1 in 5 adult COVID survivors in the U.S. may develop post-acute sequalae
of SARS COV-2(PASC). Chronic neurocognitive and neurologic symptoms: “brain
fog,” mood changes, sleep problems, and anosmia/ ageusia are prominent in
PASC patients. Aging, underlying disease, and environmental exposure are the
main risk factors for the adverse outcomes of COVID-19. However, the common
pathogenic mechanism that exists across different demographic characteristics
is still unknown. Previous work in our lab supports the hypothesis that persistent
genotoxic stress associated with aging and environmental toxicant exposure are
pathogenic and may contribute to the susceptibility and severity of COVID-19.
To determine if genotoxic stress is indeed a prominent feature of COVID-19, we
examined six COVID-19 postmortem tissue samples and six control cases. DNA
and RNA samples were extracted from postmortem samples. We have identified
significant upregulation of genes associated with genotoxic stress in lung tissue,
including telomere attrition, inflammation (cytokines), DNA damage response
(DDR), and cell senescence biomarkers. Pathology study via immunohistochemical
(IHC) staining was performed to examine the DDR, cell senescence, and neuroinflammation with γH2AX, p21, and Iba1 antibodies, respectively. γH2AX, a phosphorylated histone protein that indicates DNA damage response, was increased in the
lung and brain of the deceased COVID-19 patients compared to that of the controls.
Cell senescence maker p21 also was increased in patients but did not reach statistical significance. Lastly, Iba staining in the patient brain revealed increased cell
density and microgliosis, suggesting neuroinflammation. These observations
revealed that genotoxic stress is a prominent feature in COVID-19 and could be a
mechanistic link between environmental toxicant exposure and COVID-19 susceptibility and severity. Our studies highlight the need for mechanistic and longitudinal studies in animal models to confirm a causal pathogenic role. Funding: NIEHS
R21ES031211and LSUHS COVID-19 awards to XHL. The study was approved by
LSUHS IRBY00002040.

4009

The Adverse Outcome Pathway Framework Applied to
Neurological Symptoms of COVID-19

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Several reports have shown that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has the potential to also be neurotropic. Many outcomes
observed in corona virus disease 2019 (COVID-19) are associated with the nervous
system. However, the mechanisms by which SARS-CoV-2 induces neurologic injury,
including neurological and/or psychological symptoms, remains unclear. During
the pandemic, the CIAO project “Modelling the Pathogenesis of COVID-19 using the
Adverse Outcome Pathway (AOP) Framework” was launched with a dedicated neuro
working group that aims at organizing the available knowledge on the neurobiological mechanisms underlying COVID-19 using the AOP framework. Biological key
events (KEs) were identified including binding to ACE2, blood-brain barrier (BBB)
disruption, hypoxia, neuroinflammation and oxidative stress. The group collected
scientific evidence to develop four AOPs leading to neurological adverse outcomes
(AO): anosmia, encephalitis, stroke, and seizure. The modularity of AOPs allows
construction of AOP networks offering a unique visualization of the core pathways
and shared mechanisms. Here, the construction of an AOP network for neurological
effects in COVID-19 highlights that long-term anosmia occurs following binding of
the spike protein to ACE2 located in endothelial, neuronal, and glial cells while short
term anosmia involves binding to ACE2​found in sustentacular cells of the olfactory
epithelium. In addition, neuroinflammation and BBB disruption are emerging as
common KEs to many individual AOPs, highlighting areas of overlap with toxicological mechanisms that could lead to increased disease severity when combined
with chemical stressors and other environmental exposures. Various challenges
with applying the AOP framework to such a complex disease were identified. For
example, the working group investigated the impact on the neurological AOPs of
COVID-19 by modulating and multiscale factors such as age, psychological stress,
nutrition, poverty, and food insecurity. It was then explored how these factors as
well as their impact on the disease could be encompassed and integrated into new
multiscale pathway perspectives. Future directions include developing additional
traditional AOPs and multiscale pathways and exploring the neurological impact of
long COVID. Organizing existing knowledge along an AOP framework can represent
a valuable tool to understand the mechanisms of a disease, to characterize the
potential for increased susceptibility due to concurrent toxicological effects, and
to identify data gaps and potentially contribute to treatment and prevention. This
AOP-aligned approach also facilitates synergy between experts from different
backgrounds while the fast-evolving and disruptive nature of COVID-19 emphasizes
the need for interdisciplinarity and cross-community research.

4010

Identifying Biomarkers for Acute Neurotoxicity in a PilocarpineInduced Rat Convulsion Model

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There remains a need for sensitive soluble biomarker panels to detect acute
neuronal toxicity which is a challenging issue in drug development. To identify
biomarkers for monitoring acute neuronal injury, we used a pilocarpine status
epilepticus model with characteristic gliosis due to recurring convulsions.
Female Sprague Dawley rats received a single intraperitoneal (IP) dose of Methyl
Scopolamine (MS, 1 mg/kg) or MS at 30 minutes prior to Pilocarpine (175 or 350
mg/kg). Identification of candidate neurotoxicity biomarkers were elucidated
from serum, CSF and brain tissue samples collected at 3 hours post dosing. At
175 mg/kg, clinical signs of pilocarpine toxicity included moderate tremors,
decreased activity, chewing, irregular breathing, lacrimation and hypersalivation.
At 350 mg/kg, clinical signs of pilocarpine toxicity included convulsions, severe
tremors, decreased activity, chewing, lacrimation and Straub tail. The neuronal
injury marker neurofilament light chain protein (NfL) was significantly increased in
CSF samples of 350 mg/kg group, with upward trends in serum, when compared
to controls, suggesting that CSF may be the most useful biological matrix when
monitoring for acute pilocarpine neurotoxicity in rats. Serumbased GRO/KC/CINC-1
and CSF-based IL-10 and MIP-2 levels were significantly increased amongst 27
cytokines/chemokines measured in the 350 mg/kg group. A Nanostring rat miRNA
(423) Panel was utilized to investigate miRNA expression level in the hippocampus.
Altered miRNAs included 31 and 41 in the 175 and 350 mg/kg groups, respectively,
when compared to concurrent controls. Among these miRNAs, 14 were downregulated and 27 were upregulated (uncorrected P<0.05) in 350 mg/kg group compared
to controls. The most upregulated miRNA “rno-miR-7b” with predicted target genes
of 401 (miRDB database) has been shown to affect necrotic cell death and cellular
response to chemical stress pathways as top adverse outcome pathways (AOPs).
The most downregulated miRNA “rno-miR-3590-3p,” has 316 predicted target
genes of (miRDB database) and has been shown to affect brain development and
translational initiation pathways as top AOPs. These findings provide evidence for
NfL, cytokine (IL-10 and MIP-2) chemokine (GRO/KC/CINC-1) and miRNA profile
changes as promising neurotoxicity biomarkers in a convulsion model of rats at a
unique timepoint of 3 hours post dose.

4011

iNOS Activity Is Required for Rapid Inhibition of P-Glycoprotein
at the Blood-Brain Barrier by Erastin

S. M. Brown, and R. E. Cannon. NIEHS, Research Triangle Park, NC.
Erastin is a small molecule known to selectively target tumor cells that express
small T oncoprotein and oncogenic RAS. It has demonstrated its utility in cancer
therapies due to its ability to induce ferroptosis, an iron dependent cell death
pathway. Cells treated in vitro with micromolar levels of erastin undergo ferroptosis
and exhibit depleted cellular antioxidants. The loss of antioxidants is attributed to
erastin’s ability to lower intracellular glutathione production by inhibiting the cystine/
glutamate antiporter, Xc-, triggering oxidative stress. Here, we used freshly isolated
brain capillaries from both male and female Sprague Dawley rats and measured the
effect of micro- and nano-molar levels of erastin on P-glycoprotein, an ABC (ATP
Binding Cassette) Transporter that resides within the blood-brain barrier (BBB).
The BBB resides in the vast array of microvessels of the brain. The microvessels
are formed of a tri-laminate design containing luminal facing endothelial cells with
outward layering of pericytes and astrocytes. P-glycoprotein is an efflux transporter
that is localized to the endothelial lumen of the barrier, preventing xenobiotics,
drugs, or harmful metabolites from entering the brain. P-glycoprotein is the most
studied of the ABC transporters because of its wide spectrum of substrates and
the role it plays in cancer drug resistance. To determine erastin’s rapid effect on
the BBB, we measured P-glycoprotein transport activity after exposing rat brain
capillaries in vitro to 1x10-12 up to 1x10-6 molar erastin for 1-4 hours. We found that
1 nM erastin rapidly inhibited P-glycoprotein transport activity in the brain capillaries of both male and female rats. To determine the kinetics of P-glycoprotein inhibition to 1 nM erastin, we measured transport activity hourly for 4 hours in rat brain
capillaries. P-glycoprotein transport was maximally inhibited at 3 hours in both
male and female rats. Additional experiments demonstrated the effects of erastin
on the P-glycoprotein transport are rapidly reversible and blocked by pretreatment
with either N-acetylcysteine or cysteine, as well as NOS inhibitors. A cystine uptake
assay was adapted to determine the effect of erastin at 1 nM on the Cystine/
Glutamate antiporter using a cystine analog, selenocystine. We extended our work
to humans by treating tumor cells expressing P-glycoprotein with 0.1µm erastin.
Human tumor cells treated with 0.1µm erastin exhibited increased cell killing by
adriamycin, a substrate for P-glycoprotein. This work is significant because it
suggests that an alternative low concentration and erastin dependent mechanism
exists that can improve drug delivery to the CNS. This research was supported by
the DTT research program at NIEHS.

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Xenobiotic induced neurotoxicity is an important liability of modern therapeutics and environmental pollutants. While there are several in vitro neurotoxicity screens to assess neuronal response, the cells are often exposed to concentrations that far exceed levels what may traverse the Blood-Brain Barrier (BBB). The goal of this project is to extend the current High Throughput Toxicokinetic (HTTK) prediction of steady-state systemic exposure (Css) of environmental chemicals to predicting doses of concern. To this end, we have implemented an assessment of their propensity of transporting across the BBB and into the brain. We have developed a novel, layered triculture in vitro BBB model that mimics the physiologically relevant in vivo barrier. We layer astrocytes, pericytes and brain microvessel endothelial cells (BMECs, HBEC-5i) in direct contact to establish cell-cell contact and signaling creating a BBB mimetic that restricts paracellular, passive transcellular, and transporter mediated transfer across our BBB mimetic layer. We used a Design of Experiment (DOE) approach to determine optimal assay conditions. The DOE maximized transendothelial electrical resistance (TER) and minimized Dextran (40kD) transport by adjusting seeding densities for the three cell types, the days in culture and the choice of extracellular matrix. We have used this model to measure bidirectional permeability of selected compounds, obtaining forward and reverse permeabilities (rates) and net partitioning coefficients (ratios). To date, we have measured more than 25 compounds including pollutants such as bisphenol-A and paracetamol, as well as a select number of CNS active drugs such as clozapine and fluoxetine. In our assay, the apparent permeability coefficients (Papp) in the apical (Ap) to basolateral (Bl) range from 0.05x10^-6 to 2x10^-6 cm/sec and Efflux Ratios (Bl→Ap/Bl) range from 4.0 to 0.02. In parallel with our in vivo model, we have developed a simple two compartment TK model and are extending the HTTK R library developed by the EPA as our models. The HTTK R model extension includes a CNS compartment with a BBB separating that compartment from the arterial blood flow. Using our measured forward (Ap→Bl) and reverse (Bl→Ap) Papp values, and the EPA PBTK reference data for compound parameters, we are developing a prediction of toxicant accumulation in the CNS. Using our computational model, we can set “Concentrations of Concern” in systemic exposure and blood levels relative to a range of Papp values. Then refine that prediction using compound specific HTTK parameters coupled with our measured BBB permeability. Our approach provides predictions of compounds that accumulate in the CNS as a function of environmental exposure and the resulting predicted blood levels, as well as the opposite case, where expected exposure levels would not be expected to lead to significant accumulations in CNS. We have further extended the HTTK R library by funding from the USA EPA in grant USEPA 840207. We also acknowledge Dr. Robert Stratford who helped initiate this project and provided critical early direction.

Inhibition of cystine uptake into cells indirectly reduces intracellular glutathione levels that disarms the cell of its natural defense mechanisms against oxidative stress and increases the likelihood of oxidative dependent cytotoxic and cytocidal events. Here we describe a modified fluorescent-based assay that measures maternal cysteine uptake through the Xc Cystine/Glutamate antiporter exchange system of rat brain astrocytes. To validate the assay, we used the ferroptotic inducer of a chemotherapeutic agent, erastin, and the Xc exchange inhibitor, sulfasalazine, as investigative tools affecting this antiporter’s activity. We used, sulfora-

Phthalates belong to a family of synthetic chemical compounds that are commonly used as plasticizers and found in construction materials, textiles, and medical products, among others. Industrialization and anthropogenic activities around rivers are the main sources of phthalates in freshwater ecosystems, which can result in exposure to aquatic species as well as human. Mono-2-ethylhexyl phthalate (MEHP) is a pro-mutagenic metabolite of the Di-2-ethylhexyl phthalate (DEHP). The aim of this research is to determine and characterize the toxicity of MEHP in brain cells of zebrafish and compare it with toxicity on human brain cells (HMC3). Cell death mechanism has also been assessed by measuring the activation of apoptosis and autophagy cell death processes. Our hypothesis proposes that phytochemicals and human cells exposed to MEHP reduce cell viability, increase reactive oxygen species (ROS) levels, and then refine that prediction using compound specific HTTK parameters coupled with our measured BBB permeability. Our approach provides predictions of compounds that accumulate in the CNS as a function of environmental exposure and the resulting predicted blood levels, as well as the opposite case, where expected exposure levels would not be expected to lead to significant accumulations in CNS. We have further extended the HTTK R library by funding from the USA EPA in grant USEPA 840207. We also acknowledge Dr. Robert Stratford who helped initiate this project and provided critical early direction.

Exposure to polychlorinated biphenyls (PCBs) is associated with development- al neurotoxicity and neurodegenerative disorders; however, the underlying mechanisms of pathogenesis are unknown. Because normal brain function is largely controlled by synaptic transmission, it is highly susceptible to xenobiotics that act as neurotransmitter antagonists or agonists. In the present study, we hypothesized that gestational exposure to PCBs could elicit microglial activation, at higher concentrations (compared to age- and sex-matched control mice exposed to clean air only) throughout the brain. WHO estimates that approximately 90% of people around the world are exposed to polluted air (through auto exhaust, factory emissions, etc.). With this in consideration, it is crucial to identify potential adverse effects of air pollution on fetal development. The findings of this study will improve our current understanding of the relationship between pre-birth UFP exposure and susceptibility to neurodegenerative diseases.
The IC$_{50}$ of oxazepam to impact spontaneously active neuronal cells was found to be 1.2 µM indicating greater potency. Contrasting these canonical benzodiazepines positive allosteric modulators of the γ-aminobutyric acid-A receptor (GABA$_A$), reduced neuronal activity, in line with the notion that benzodiazepines primarily act on neuronal networks of neurons. Each test compound concentration-dependently reduced neuroinflammatory levels, resulting in the functional implications of these findings in PCB-mediated neurotoxicity. This work was funded by ES005605, ES013661, ES029033.

**Selective Inhibition of Soluble TNF Attenuates Hippocampal Neuroinflammation and PSD-95 Expression to Improve Neurological Functions in a Rat Model of Gulf War Illness**


Systemic inflammation is a major contributor to poor brain pathology across many disease conditions. Specifically, the upregulation of the pro-inflammatory cytokine tumour necrosis factor α (TNFα) in the hippocampus activates its tumour necrosis factor receptor 1 (TNFR1), reducing AMPA receptor trafficking to impair LTP and associated behavioral outcomes. Studies using animal models of Gulf War Illness (GWI) have shown both a chronic upregulation of TNFα and impaired neurological function. Therefore, this study aimed to investigate whether selectively inhibiting only the soluble forms of TNF (sTNF) alters behaviour. It was hypothesized that reducing sTNF could reverse neuroinflammation to improve neuroplasticity and neurological function.

GWI was induced in rats by injection of disopropyl fluorophosphate (DFP) (or vehicle) for 5 consecutive days. Six months later, the rats were treated with XPro1595 (or vehicle) for 2 weeks to selectively inhibit sTNF, after which they were subjected to a battery of behavioural tests (cognition, anxiety-related, depressive-like, and neuropathic pain). MRI brain scans were performed, and the animals were euthanized for brain pathological analysis. The hippocampus of the GWI rats had significantly increased neuroinflammatory levels, resulting in edema and reduced AMPA receptor trafficking to the post-synaptic membrane that collectively contributed to impairments in memory, anxiety, depression, and neuropathic pain. However, treating the rats with XPro1595 in the chronic environment attenuated the neuroinflammatory response, reducing edema and improving AMPA receptor trafficking, allowing for improvements in all areas of neurological function. Overall findings suggest that selectively inhibiting sTNF using XPro1595 reduces neuroinflammation, synaptic plasticity, and overall function when administered in the chronic setting of a rat model of GWI. This data supports the use of XPro1595 in Veterans with GWI.

**Neurotoxic Effects of Unregulated "Designer" Benzodiazepines**


Once believed to be side effect-free wonder drugs for the treatment of anxiety, panic attacks, and sleep disorders, benzodiazepines have now been shown to elicit strong side effects, withdrawal effects, and general cognitive decline. Despite being illegal for recreational use, benzodiazepines are often consumed in substantial quantities for pleasure. Furthermore, there has been an explosion in the development of “designer” benzodiazepines, analogous compounds which mimic the effects of traditional benzodiazepines but have slightly different chemical compositions thus evading criminal classification. In the UK, while the number of deaths caused by legal high misuse is smaller (76) in comparison to other drugs such as cocaine (1,752) and heroin (7,748), the short-, mid-, and long-term impacts of these drugs remain unknown. Neurotoxic hazard characterization of these compounds is therefore of the utmost importance.

In this study, several benzodiazepine analogues were synthesized for toxicological studies, characterization, and the "designer" benzodiazepines; flunitrazolam, and fluetizolam. Effects of these compounds on neuronal activity were studied in spontaneously active rat primary cortical cultures grown on microelectrode arrays. Key functional readouts were obtained detailing spontaneous spiking activity and bursting behavior of individual neurons and networks of neurons. Each test compound concentration-dependently reduced neuronal activity, in line with the notion that benzodiazepines primarily act as positive allosteric modulators of the γ-aminobutyric acid-A receptor (GABA$_A$), an inhibitory neurotransmitter receptor. The canonical benzodiazepine, diazepam had an IC$_{50}$ of 3.8 µM when measuring the inhibitory capacity of neuronal cell spiking. The IC$_{50}$ of oxazepam to impact spontaneously active neuronal cells was found to be 1.2 µM indicating greater potency. Contrasting these canonical benzodiazepines with those of the designer benzodiazepines, it is clear to see the designer drugs were far more potent. Flunitrazolam had a much greater inhibitory effect at a lower dosage than diazepam, with an IC$_{50}$ of 0.03 µM. These findings are consistent with the idea that preferentially activates the α$_1$ subunit which manifests the same great inhibitory potency exceeding an IC$_{50}$ of 0.1 µM. The most potent canonical benzodiazepine, oxazepam, was observed to significantly inhibit neuron network bursting activity with an IC$_{50}$ and 2.5 µM when contrasted against flunitrazolam, the least potent "designer", the same effect was observed with the much lower dose of 0.04 µM. This study is the first to demonstrate the clear potent effects of several “designer” benzodiazepines on rat primary cortical neuronal cultures which are shown to be much stronger than those of canonical benzodiazepines. This is particularly concerning given those abusing the drug tend to ingest them in large doses as standard, it is clear to see that these “designer” benzodiazepines are not only poorly regulated but open to a much greater margin of error when dosing.

**In Vitro Neurotoxicity of Micro- and Nanoparticles Measured Using Microelectrode Array (MEA) Recordings in Rat Cortical Cultures**


Micro- and nanoparticles (MNPs) are small plastic particles in varying shapes, compositions and sizes. Levels of MNPs in the environment are increasing and humans are exposed to MNPs through dust, drinking water, and daily use products. However, not much is known about the potential human health risks of MNPs, including potential neurotoxic effects of MNPs. We demonstrated using a proof-of-principle in vivo study in mice, orally exposed to 1 or 10 µm polystyrene beads for one or ten days (4 mg/day), that polystyrene particles have the ability to reach the brain upon systemic uptake. We therefore subsequently investigated the ability of MNPs (different particle types and sizes, 10 µg/mL) to affect neuronal activity of rat primary cortical cultures using multi-well microelectrode array (MEA) recordings during acute (30 min) and chronic (21 days) exposure. The different sizes and types of plastic did not affect cell viability, but decreases in neuronal activity were seen for polyvinylchloride (PVC; size ranges <1 µm and 10-30 µm), polypropylene (PP; <1 µm and 5-10 µm) and polyamide 6.6 (PA6; 6-100 µm) during acute exposure. These decreases were not seen for other sizes of these plastic types, their leachates and other non-plastic nanoparticles (nano-talc and titanium dioxide). Remarkably, chronic exposure shows increases in neuronal activity after 3 days of exposure for PVC (<1 µm), PP (<1 µm), PA6 (1-1.5 µm) and PVC Leachate. PVC (90-150 µm) and PP (90-150 µm) show an increase in neuronal activity after 14 days and 10 days of exposure, respectively. All these effects persisted until 21 days of exposure. Leachate of PA6.6 also shows an increase in neuronal activity, but only after 21 days of exposure. Other sizes and other nanoparticles show no affect. Taken together, these results give a first, urgently needed insight into the human neurotoxic health hazards and risks of different types, sizes and shapes of MNPs. The MOMENTUM project (45800110) has been made possible by ZonMw Programma Microplastics and Health, and Health-Holland, Top Sector Life Sciences & Health.

**Critical Role of Mitochondrial Carrier Protein in PFOS-Led Toxicity**

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Per/polyfluoroalkyl substances (PFAS) are synthetic organo-fluorine chemicals used as flame-retardants, stain repellents, and coating additives in nonstick cookware and the food packaging industry. Designed to repel oil and water, PFAS are resistant to environmental and biological degradation rendering long (often environmental) and extracellular half-lives. While diverse adverse outcomes have been identified, a more recent body of literature suggests that the nervous system may be vulnerable to PFAS, where multiple PFAS cross the blood-brain barrier (BBB) and alter neurotransmission. Much work has focused on perfluorooctane sulfonate (PFOS), which is present in most human blood, crosses the human BBB, and has been found to alter neurophysiology in a number of experimental systems.

Our previous studies have shown the selective vulnerability of dopaminergic neurons in Caenorhabditis elegans (25 µM) along with loss of mitochondrial viability observed at far lower doses (~ 2 µM), while the mitochondrial DNA content remained unaltered. As a critical next step, we tested the hypothesis that PFOS neurotoxicity was mediated through interactions with specific mitochondrial targets. Here, we investigated the effect of PFOS on mitochondria by exposing them to different concentrations of PFOS (10 to 500 µM for 15 minutes). Acute PFOS treatment in isolated mitochondria showed inhibition of complex II (succinate dehydrogenase) and complex III (cytochrome c reductase). The inhibitory effect on complex III activity was relatively severe compared to other complexes. There is currently a poor understanding of how PFOS enters mitochondria. By utilizing inhibitors specific to individual mitochondrial carrier proteins, we identified a significant role of oxoglutarate carrier protein in the uptake of PFOS and a lesser role of dicarboxylate carrier protein. Notably, these carrier proteins are also responsible for the uptake of GSH in mitochondria. Previous studies have indicated that GSH is critical for PFOS-induced neurotoxicity since GSH administration ameliorated dopaminergic neurotoxicity. Furthermore, we have also identified that gamma-glutamylcysteine, an intermediate in GSH synthesis, is critical to ameliorating PFOS-induced neurotoxicity. Cytotoxic-produced, mitochondrial GSH (GSH$_m$), is a peroxyl radical scavenger. An IC$_{50}$ of 0.03 µM of this radical antioxidant for mitochondria. While possibly reduced GSH biosynthesis has been identified, an additional mechanism pertaining to the competitive effect on the uptake of GSH by mitochondria given that the same carrier proteins are utilized by both GSH and PFOS. In addition, there was no alteration in mRNA expression of genes involved in GSH biosynthesis, gcs-1.
Brain organoids, a human 3D brain model, are derived from induced pluripotent stem cells (iPSCs). They simulate some important functional aspects of the brain. This model contains different types of neurons, astrocytes, oligodendrocytes, and iPSC-derived microglia which can be added to the model. Brain organoids are electrophysiologically active. Since animal-based approaches for Developmental Neurotoxicity (DNT) have limitations, new models for neurotoxicology need to be developed. Our approach highlights the need to dose-reverse test in defined environment derived brain organoids. In this project, we have developed a brain organoid cryopreservation protocol, so it is possible to establish a bank of brain organoids and have them on demand for any application. A series of endpoints were measured to make sure that cryopreservation does not perturb main functionality of the brain organoids and they can be used for the downstream applications. To generate brain organoids, we differentiated human iPSs to neural progenitor cells (NPCs) and brought NPC cell suspension to form 3D brain organoids under constant gyration for up to 8 weeks. In order to test the best point for cryopreservation, we used four different ages of the brain organoids - 1, 2, 4, and 6 weeks of differentiation. We started with seven different freezing media (mFsc3T3, Crystor CS10, 3DGR0, NFC Freezing Medium, Cell-banker2, BFS/DMSO and TheraPEAK ProFreeze freezing Medium). Organoid viability and morphology were assessed after defrosting at 0 hours, 24 hours and 1 week later. Four (NPC Freezing Medium, Cell-banker2, BFS/DMSO and TheraPEAK ProFreeze freezing Medium) out of 7 media were chosen for cryopreservation due to low viability after defrosting, Neurite outgrowth, glia migration, resazurin reduction assays were performed to test functionality and viability of the recovering organoids. Immunohistochernistry was performed to assess the morphology of different cell types. As ultimate functional endpoint electrophysiological activity after freezing was assessed. One day after thawing, the brain organoids showedfringed borders and a lot of debris were observed in all the conditions. All conditions recovered in 2-4 weeks. The organoids frozen at 2 weeks showed the best homogenous shape and more transparent center after 4 weeks of recovery period in all three media. These organoids also showed higher viability compared to those frozen at weeks 4 and 6. The neurite outgrowth and glia migration were comparable among all the groups and even higher than the control group after recovery. The Mitochondrial membrane potential was not significant different in frozen organoids vs. the control group. Organoids frozen at 2 weeks in Crystor CS10 medium were plated on the microelectrode array system after a 4-week recovery period and showed spike and burst activities. The results showed that cryopreservation of rather immature brain organoids is more feasible for older cultures and it can serve as a time-saving opportunity to optimize toxicity studies on demand. Further optimization steps are necessary to cryopreserve more mature cultures, as this will allow more flexibility in experimental designs.

Toxicity is a leading reason why drugs are withdrawn from the market, with neurotoxicity responsible for 16%. Peripheral nerves are particularly susceptible to off-target effects resulting in permanent sensory-motor deficits, and chemotherapy-induced peripheral neuropathy (CIPN) occurs with a 68% incidence rate and 30% retaining effects after 6 months. CIPN can also affect clinical outcomes, with 91% of CIPN patients experiencing a 45% and 60% drop in drug effectiveness. Preclinical animal models are historically expensive and low-throughput and have largely failed to deliver results that translate to success in the human system. Peripheral nerves (PNs), in particular, lack predictive human-in-relevant in vitro drug screening models, with less than 7% of neurological drug candidates reaching the market. Microphysiological systems (MPS), including organ-on-chips, which utilize human induced pluripotent stem cell (iPSC)-derived cells to emulate specific organs, have emerged as promising screening platforms to bridge the gap between preclinical and clinical success. These systems are attracting attention from the pharmaceutical industry in the hope that they will curb attrition rates, lower costs, and reduce reliance on animal models. Engineering 3D tissues relevant to the nervous system, particularly PNs, is challenging because of the complex ultrastructure and necessity of functional outputs. AxxoSim has developed a high-throughput Compass® MPS platform, with human iPSC-derived sensory neurons and primary human Schwann cells for screening neurotoxic compounds using an embedded electrode array (EAA) to record action potentials (CAPs) from peripheral nerve cultures. The efficacy of this system was demonstrated by recording cultures exposed to Paclitaxel (PTX). The NerveSim® MPS is a custom tissue culture plate with integrated electrodes to allow in situ electrophysiological recordings. Each well contains a single cell- restrictive polymer mold and a cell-permissive inner gel that guides axonal growth across a series of 10 microelectrodes. Coculture spheroids were produced from human sensory neurons and Schwann cells in round-bottom multiwell plates then plated in one of the inner cell-permissive gels. Spheroids were cultured for 4 weeks in a series of culture media that promote neurite outgrowth through the gel, Schwann cell migration and alignment with axons, and axon guidance. The study showed that robust neurite outgrowth of ~500 μm was consistently observed, with Schwann cell migration and alignment along neuronal axons confirmed by S100 immunostaining. Using this system, multiple NerveSim® EAA cultures were stimulated in parallel at multiple distal sites with a stimulation current ramp while recording the CAPs at the cell body and axons. From these data, we collected the conduction velocity (CV), peak response amplitude (AMP), and threshold stimulus strength (TSS), which are all clinically relevant electrophysiological metrics in response to several known to cause peripheral neuropathy. Additionally, histopathology shows phenotypic responses of peripheral neuropathy including decreased fiber densities and increased degenerated fibers. The ability to clinically relevant data for drug efficacy as a modeling of CIPN towards screening of therapeutics for neuroprotection and neuroregeneration. Current efforts are focused first on increasing the number of myelinated nerve fibers in the NerveSim® sample, and second, on quantifying the effects of well-known demyelinating compounds, such as Cuprizone, to further establish the utility of NerveSim® as a drug screening platform.
Numerous toxicants, both endogenous (e.g., Glu) and exogenous (e.g., pesticides), compromise function of the nervous system and pose as risk factors for later diseases and/or mediators of damage. For example, when extracellular levels of the excitatory neurotransmitter Glu rise, aberrant synaptic signaling leads to excitotoxicity and oxidative stress, thought to contribute to neurodegeneration. Numerous pesticides, e.g., dieldrin, are known to induce oxidative stress and cause injury to neurons. In previous reports, limonoids such as fraxinellone showed significant neuroprotective effects against Glu excitotoxicity and oxidative stress. Given these findings, a library of novel fraxinellone analogues was synthesized with the goal of identifying a means of neuroprotection. In addition, the mechanism of action was explored. In vitro methods were used to assess the protective activity of the novel fraxinellone analogues, determine their mechanism of protection, and if these analogues protected against oxidative stress. Dieldrin and rotenone were selected as test agents. Cells were pre-treated with each compound at a range of 0.05 to 1.0 μM for 30 minutes before addition, we utilized activators (e.g., sulforophane) and an inhibitor (ML-385) to confirm Nrf2 involvement. In addition, reactive oxygen species was measured following Glu exposure with and without fraxinellone analogues. Several of the compounds protected against Glu excitotoxicity and toxicity from rotenone and dieldrin. When cells were pre-treated with the compounds that afforded protection before adding the insult, we saw enhancement of gene expression of antioxidant response element, a key mechanism of Nrf2 activation. Antioxidant gene expression was not seen for a compound that did not provide protection. Sulforaphane provided similar protection against Glu toxicity when co-administered with the Glu insult but at higher concentrations when compared to the active fraxinellone analogues. ML-385 antagonized beneficial activity of a fraxinellone analogue. A protective analogue significantly decreased levels of reactive oxygen species from Glu exposure. Our findings suggest the fraxinellone analogues protect against exogenous and endogenous insults through Nrf2 activation and induction of an antioxidant response.

4025 Novel Analogues of the Natural Product Fraxinellone Protect against Exogenous and Endogenous Toxicants

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4027 Nonclinical Safety Assessment of AMX0114: An Antisense Oligonucleotide in Development for the Treatment of Amyotrophic Lateral Sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative disease. Degeneration of motor neurons leads to loss of motor function, resulting in loss of speech, fine motor skills, and mobility and eventual respiratory failure. It is believed that axonal degeneration is a key contributor to the clinical presentation and pathogenesis of ALS, beginning in the early stages of disease. As a result, therapies which target axonal degeneration are of interest in the treatment of ALS. AMX0114 is an antisense oligonucleotide (ASO) targeting calf-2, a critical effector of axonal degeneration in ALS and other neurodegenerative diseases. AMX0114 is being developed with the goal of disrupting a key pathway leading to axonal degeneration, thereby affecting the disease course of ALS. ASOs represent an emerging therapeutic modality which mediates cellular processes at the RNA level. As a result, ASOs are a promising option for the treatment of ALS. However, previously approved systemic ASOs have exhibited a number of common adverse effects, including hepatotoxicity, renal toxicity, and hypersensitivity. Modern intrathelial ASOs with phosphorothioated chemistries and 2’O-methoxyethyl (MOE) modifications have generally not shown these toxicological signals. However, monitoring of these toxicities remains of interest given previous findings in earlier generation systemic antisense therapies. To support the initiation of a first-in-human (FIH) clinical trial, we have developed a nonclinical, IND-enabling safety program for AMX0114. This program includes studies to evaluate the potential systemic toxicity, genotoxicity, and any undesirable pharmacodynamic effects (safety pharmacology). We will review and discuss these studies and their preliminary results.

4028 Real-Time Intraparenchymal Dosing Using MRI-Guided Convection-Enhanced Delivery in Beagle Dogs

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Convection-enhanced delivery (CED) enables targeted delivery to parenchymal tissues and is used routinely in patients for neurological indications using a range of therapeutic modalities. Non-clinical development of cell and gene therapy products typically requires the same delivery methods to be used in toxicology studies. Standard procedures for CED include real-time MRI to target and confirm local delivery to brain tissues using a step-infusion paradigm and contrast agent (e.g., gadolinium). CED procedures are well established in non-human primates (NHPs) for dosing novel neurological therapeutics in multiple brain regions based on neuroanatomical atlases. With this foundation, a Beagle dog model was developed as a non-clinical alternative with dosing of up to six different parenchymal sites per animal. Dose volumes ranged from 30 to 100 μL per site with final infusion rates at 120 to 300 μL/hour. Brain infusions of gadolinium-containing solutions achieved an estimated <0.5 mm radial error from the planned target while real-time MRI scanning allowed for monitoring dose distribution. Similar to NHPs, Beagle dogs recovered well from surgeries, presenting with expected procedure-related clinical signs including mild tremors, swelling, and decreased activity which resolved within approximately 4 days. The present work highlights an additional preclinical model for brain CED that utilizes clinically relevant methods and devices and addresses the need for characterize alternatives to NHPs in toxicology.
adult neuronal and brain tissue preparations, such as spheroids or organoids, are under intense investigation for cell-based high-throughput screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as iPSCs, have allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of neuron electrophysiology to be used in screening applications in drug discovery and safety. The ability to develop and validate functional neuronal assays that quantifies the relationship between spiking activity of individual neurons and the network activity embedded in the local field potential (LFP) is crucial. Planar microarrays embedded in the substrate of each well of a culture plate interfaces with cultured cellular networks to continuously monitor broadband (1 - 5000 Hz) electrophysiological data from rodent cortical neuronal networks. The broadband voltage signal was then separately processed for action potential detection (200 - 5000 Hz) and low frequency oscillations (1 - 50 Hz). The power spectral density was computed from the low frequency signal sampled after network burst events, and then absolute power was computed in the delta (1 - 4 Hz), theta (4 - 8 Hz), alpha (8 - 14 Hz), beta (14-30 Hz), and gamma (30-50 Hz) bands. The spectral power within the LFP was correlated with the emergence of bursting activity within the rodent cortical neurons by 14 days in culture, and intra-burst oscillations in the alpha band were detected at 21 days in culture. Known reference compounds were then added to the cultures and the change in LFP features were quantified and compared with more traditional functional endpoints based on spiking activity. For example, Amoxapine reduced the burst duration and the burst frequency increased the network burst oscillations and decreased the intra-burst oscillations in the alpha band. These results support the continued development and use of in vitro neural assays for high throughput drug discovery and safety assessment.
into the ventricles were evaluated microscopically. Following coordinate verifica-
tion, another batch of animals were administered an intact reference agent (Cell Wash Solution) and monitored for up to 60 days. Clinical condition was monitored daily and body weights weekly. Following simultaneous bilateral ICV administration, a limited number of animals exhibited a few transient clinical signs immediately upon waking which were absent by the end of day. Body weights and body weight gains were unremarkable. In addition, a single transient bilateral ICV administra-
tion in mice induced a limited number of transient clinical signs and is considered an acceptable rodent model, potentially reducing the need for larger animals to accommodate a larger dose volume.

4034 Hippocampal Brain Slices as a Model for Traumatic Brain Injury and ischemia

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The culture system of brain slices has been established in many brain regions including cortex, striatum, hippocampus, cerebellum. The hippocampal slices in young animals are highly viable with high plasticity and can last for weeks without significant neurodegeneration. However, over weeks these underdeveloped cells may gradually lose their morphological properties and are more likely to differen-
tiate into different types of neural cells. Therefore, an acute slice system retaining original brain neuroanatomy and local network activity is needed for studying acute brain injury within hours, such as ischemia and traumatic brain injury (TBI). We developed an acute hippocampal slice system as a model for ischemic hypoxia and TBI using mature brain slices from adult rats in order to study how the brain responds to acute insults. In this model, hippocampal tissues were isolated and sliced rostrally-caudally along the hippocampus, where the cornu ammonis (CA1) and dentate gyrus (DG) areas of the hippocampus could be visualized and evaluated directly. During the preparation of this experimental model, the process of mechanical slicing created damages in neurons, astrocytes, glial cells, endothe-

telial cells and the axons of neurons resembling brain injury, while the core of brain slices could develop hypoxia due to little/no access to oxygen within the short term. The advantage of this model is that the traumatic tissue edge caused by slicing, and the hypoxic core at a depth of 100-200µm below the tissue surface, can both be viewed directly and monitored side-by-side simultaneously in one single brain slice. Here we used pimonidazole to evaluate hypoxia in hippocampal slices and hence provide an ex vivo model to mimic hypoxia in ischemia. With Cresyl violet staining, we were able to differentiate cell survival status and count cell viability using Image J software (p<0.001). There was a significant increased hypoxia over 6-hour period in the core of rat brain slices (p<0.05). We also observed signifi-
cant time-dependent dendritic injury over the 6-hour hypoxic period using microtu-

4035 Analysis of Historical Control Locomotor Activity Data from Vehicle Control Groups in Adult Rats

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The identification of possible locomotor effects of a compound, resulting in motor activity deficits (hyper- or hypo-activity), compared with concurrent controls is crucial to aid in the assessment of potential toxicity. Locomotor activity is required in regulatory laboratories and on compound screens to support preclinical development studies [Organisation for Economic Co-operation and Development (OECD) 407 and 408, respectively, and Office of Chemical Safety and Pollution (OCSPP) 870.3050 and 870.3100 guidelines, respectively] and can be added to non-clinical pharmaceutical studies as indicated by target or mechanism of action. Locomotor activity is often performed with an abbreviated set of sensorimotor and reactivity behavioral assessments or a full functional observational battery in adult rats in the last week of a 28 or 90-day repeat dosing study. This data may be used as an endpoint to establish absence of test compound-related locomotor effects, confirm test compound-related locomotor effects, or trigger additional neurotoxicity studies to evaluate potential test compound-related locomotor effects. While concurrent control data are the gold standard for determining test compound-related effects or lack of effects, historical control data are often necessary to support the final interpretation. Potential shifts in background activity may be uncovered and used to explain study specific data. The purpose of the current study was to establish a site-specific (Greenfield Indiana) historical control database that could be used to support compound-related locomotor activity based on strain of rat. We compiled locomotor activity data; basic movements, X

4036 Impact of Prenatal Exposures to Per- and Polyfluoroalkyl Substances (PFAS) on the Developing Neonatal Immune System

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Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic chemicals widely used in industrial processes and consumer goods, and they are ubiquitous in the environment and humans, including women of reproductive age. There is compelling evidence of PFAS-associated immunotoxicity vis-a-vis lowered antibody responses to vaccination; however, little attention has been given to how these toxicants might affect early life T cell development. Fetuses and infants are particu-
larly sensitive to environmental exposures due to their rapid growth and developing systems. During fetal development, each immune cell type develops at different gestational stages, thus perturbations during pregnancy or in the early postnatal period could affect multiple types of immune cells. To test the hypothesis that prenatal PFAS exposures impact the neonatal immune system, a cohort of 266 pregnant women in Rochester, NY, were enrolled between 2015 and 2019. Second-trimester maternal PFAS concentrations were determined by high-performance liquid chromatogra-
phy (HPLC) and tandem mass spectrometry (MS/MS). Infant immune population frequencies were measured at birth, 6, and 12 months of age using mass cytometry (n= 284 samples, 199 subjects). Results show the phenotypic and time-dependent complexity within the CD4+ and CD8+ T cell pool and the presence of 36 unique CD4+ and 14 unique CD8+ clusters of cells identified through high-dimensional analysis. We further show that higher PFAS concentrations are associated with lower IL-21 producing CD4+ T cells. IL-21 is a cytokine essential for the germinal center reaction and supports antibody maturation. Thus, our data suggest a possible link to suppressed antibody responses associated with PFAS. These findings provide new insight into the complexity of the immune response during infancy, and the impact of antenatal exposures on reshaping the fetal developmental program.

4037 Aryl Hydrocarbon Receptor Activation Alters Monocytic Cell Responses during Coronavirus and Influenza A Virus Infection

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Respiratory viruses pose a significant threat to global health, causing morbidity, mortality, and socioeconomic loss. One mystery associated with respiratory viral infections, such as with influenza A viruses (IAV) and coronaviruses (CoV), is the broad variability in disease severity that is observed, even during outbreaks of the same viral strain. Environmental exposures are a likely contributor to disease variability; however, the cellular mechanisms that explain connections between exposures and variable outcomes are poorly defined. One way through which environmental factors modify the immune response is via the aryl hydrocarbon receptor (AHR). The AHR is activated by a broad range of small molecules from the environment, including ligands that regulate inflammation and aid in tissue repair, however, they can also differentiate into interstitial macrophages and dendritic cells (moDCs). AHR activation significantly reduced lung moDCs and supported antibody maturation. Thus, our data suggest that suppressed antibody responses associated with PFAS. These findings provide new insight into the complexity of the immune response during infancy, and the impact of antenatal exposures on reshaping the fetal developmental program.

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be implicated in the pathogenesis of many diseases, including arthritis, diabetes, multiple sclerosis, and chronic lung diseases. Thus, AhR modulation of monocytes has the potential to affect the course of many diseases.

4038 Activation of the Aryl Hydrocarbon Receptor Alters Emergency Hematopoiesis during Respiratory Viral Infection

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Hematopoietic stem and progenitor cells (HSPCs) give rise to immune cells through a process called hematopoiesis, which is crucial for maintaining the appropriate number of blood and immune cells at steady state, and in response to stress. Stressors, such as infections, result in immune cell recruitment to the site of infection, triggering HSPCs in the bone marrow to produce more leukocytes, via a process known as emergency hematopoiesis. This ability to sense and quickly respond is essential for maintaining well controlled immune defenses; yet, how environmental signals influence emergency hematopoiesis during infection is not fully understood. The aryl hydrocarbon receptor (AhR) is an environment-sensing intracellular receptor that is expressed in all hematopoietic cells. Previous research has shown exposure to AhR ligands influences immune responses to a range of antigenic challenges, including respiratory pathogens. Interestingly, AhR modulation influences steady state hematopoiesis, suggesting that AhR could regulate emergency hematopoiesis. Yet, the impact of AhR activation on emergency hematopoiesis during infection is poorly understood. Thus, the goal of this study was to delineate how AhR modulation affects emergency hematopoiesis during respiratory viral infection. To determine the impact of AhR activation, mice were exposed to the prototype AhR agonist, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and infected with either a mouse coronavirus (CoV) or a human influenza A virus (IAV). We observed that during CoV infection, AhR activation altered the proportion of HSPCs, with a significant increase in the proportion of myeloid-biased progenitor cells and a decrease in the proportion of megakaryocyte-biased progenitor cells in the bone marrow. There were also fewer granulocyte/monocyte progenitor cells in the bone marrow, and reduced monocyte counts in the blood and lungs of TCDD-exposed mice during infection. Additionally, AhR activation reduced the number of megakaryocytes during infection. Overall, these data provide novel evidence that AhR activation influences emergency hematopoiesis during respiratory viral infections. These findings further support that AhR is a key mediator of hematopoiesis. Given the impact of respiratory viruses on human health, understanding how environmental signals, mediated through the AhR, influence immune responses to these common pathogens will advance our knowledge of factors that influence outcomes from infection.

4039 Ahr Controls Pulmonary Inflammation from Cannabis Smoke

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Δ-THC, a major cannabionid in Cannabis sativa. While THC may be anti-inflammatory, there is inconclusive literature on the effects of cannabis smoke on lung inflammation. This is relevant as inhaling cannabis smoke is the most common way that cannabis (marijuana) is consumed. Although THC may be anti-inflammatory, there is inconclusive literature on the effects of cannabis smoke on lung inflammation. This is relevant as inhaling cannabis smoke is the most common way that cannabis (marijuana) is consumed. This study was to assess the potential immunotoxicity of NaVO3 containing products and inhalation of fumes and dust. The objective of this study was to assess the potential immunotoxicity of NaVO3. Female B6C3F1/N mice were exposed to 0, 31.1, 62.5, 125, 250, and 500 ppm NaVO3 in drinking water for 28 days and evaluated for effects on immune cell populations, innate, cellular, and humoral-mediated immunity. There was a decreasing trend in body weight (BW) and BW gain in NaVO3-exposed mice, with a significant decrease in BW gain at >250 ppm, relative to control. Conversely, increasing trends in relative spleen, liver and kidney weights, and an increase (p<0.05) in the organ:BW ratio at >250 ppm NaVO3, were observed. At >125 ppm NaVO3, >500 ppm NaVO3, in kidney were observed. Erythrocyte counts in B6C3F1/N mice exposed to NaVO3 were significantly increased (p<0.05) compared to control, in concert with a decrease in erythrocyte size (MCV) and hemoglobin metrics, suggesting microcytic hypochromic anemia. NaVO3 exposure altered antibody production against sheep red blood cells (SRBC); antibody forming cells (AFC)105 spleen cells exhibited a decreasing trend, with NaVO3 impedes CD8+ T cell activation and effectors corepressor (Nrf2) and a decrease in erythrocyte size (MCV) and hemoglobin metrics, suggesting microcytic hypochromic anemia. NaVO3 exposure altered antibody production against sheep red blood cells (SRBC); antibody forming cells (AFC)105 spleen cells exhibited a decreasing trend, with NaVO3 impedes CD8+ T cell activation and effectors.

4040 Nr2f2-Dependent and Independent Effects of tBHQ on Dendritic Cell Function

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Dendritic cells (DCs) are professional antigen presenting cells that initiate both the innate and adaptive immune responses upon detecting antigen. Through antigen processing, DCs can activate naïve T cells by presenting antigenic peptides on MHC class II molecules. Previous research from our lab demonstrated that tert-butyldihydroquinone (tBHQ), a potent activator of nuclear factor erythroid 2-related factor (Nrf2) and a widely used food additive, blunts the expression of CD107a, fas ligand and CD44, which are markers of activation and effector function, on CD8+ T cells. This suggests that tBHQ impedes CD8+ T cell activation and effector function, but the mechanism for this is unclear. Since DCs bridge the innate and adaptive immune systems and play an important role in T cell activation, we hypothesized that exposure to tBHQ in dendritic cells inhibits expression of MHC class II and other co-stimulatory molecules involved in T cell activation and effector functions in a Nrf2-dependent manner. In our current in vitro study, isolated DCs and unfractio- nated splenocytes were collected from spleens of female wildtype or Nr2f2-/- C57BL/6 mice. The cells were activated by Influenza A Virus (IAV) in the presence or absence of tBHQ for 24 hours, after which the expression of markers for DC maturation, activation, and T cell priming was measured. tBHQ treatment led to a significant decrease in DC maturation marked by a decrease in MHCII expression. In addition, tBHQ significantly suppressed DC activation, as evidenced by a decrease in CD80 and CD86 expression, which occurred in a Nrf2-dependent manner. Likewise, tBHQ also reduced secretion of IL-6 and IL-12 by IAV-activated dendritic cells, whereas TNFα secretion was not affected. Overall, our data suggest that tBHQ inhibits the expression of MHC class II and other co-stimulatory molecules expressed by activated DCs with the decrease in CD80 and CD86 occurring in a Nrf2-dependent manner. Furthermore, the tBHQ-mediated impairment of dendritic cell activation and maturation may account in part for the suppression of T cell activation by tBHQ. This study was supported by NIH R01 E0324966 and R21 ES033830.

4041 Evaluation of Immunotoxicity of Sodium Metavanadate following Drinking Water Exposure in Female B6C3F1/N Mice in a 28-Day Study

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Sodium metavanadate (NaVO3) is a pentavalent vanadium compound used in the metal industry and in dietary supplements; human exposure occurs primarily through ingestion of contaminated drinking water, as well as through ingestion of NaVO3-containing products and inhalation of fumes and dust. The objective of this study was to assess the potential immunotoxicity of NaVO3. Female B6C3F1/N mice were exposed to 0, 31.1, 62.5, 125, 250, and 500 ppm NaVO3 in drinking water for 28 days and evaluated for effects on immune cell populations, innate, cellular, and humoral-mediated immunity. There was a decreasing trend in body weight (BW) and BW gain in NaVO3-exposed mice, with a significant (p<0.05) decrease in BW gain at >250 ppm, relative to control. Conversely, increasing trends in relative spleen, liver and kidney weights, and an increase (p<0.05) in the organ:BW ratio at >125 ppm NaVO3, were observed. At >125 ppm NaVO3, >500 ppm NaVO3, in kidney were observed. Erythrocyte counts in B6C3F1/N mice exposed to NaVO3 were significantly increased (p<0.05) compared to control, in concert with a decrease in erythrocyte size (MCV) and hemoglobin metrics, suggesting microcytic hypochromic anemia. NaVO3 exposure altered antibody production against sheep red blood cells (SRBC); antibody forming cells (AFC)105 spleen cells exhibited a decreasing trend, with NaVO3 impedes CD8+ T cell activation and effectors corepressor (Nrf2) and a decrease in erythrocyte size (MCV) and hemoglobin metrics, suggesting microcytic hypochromic anemia. NaVO3 exposure altered antibody production against sheep red blood cells (SRBC); antibody forming cells (AFC)105 spleen cells exhibited a decreasing trend, with NaVO3 impedes CD8+ T cell activation and effectors corepressor (Nrf2) and a decrease in erythrocyte size (MCV) and hemoglobin metrics, suggesting microcytic hypochromic anemia. NaVO3 exposure altered antibody production against sheep red blood cells (SRBC); antibody forming cells (AFC)105 spleen cells exhibited a decreasing trend, with NaVO3 impedes CD8+ T cell activation and effectors.
Dawley rats were orally exposed to the EDC mixture daily from gestational day 8 to 18. Pups were spontaneously delivered and kept with the dams until weaning. Dams were assessed 3 weeks after birth at the time of weaning. Male and female offspring were assessed at 10 weeks old. Pancreas and blood were collected from all samples. Extracellular vesicles were isolated from dam plasma and quantified by Nanoparticle Tracking Analysis on the Zetasizer. Plasma levels of EDCs were measured by LC-HRMS and cytokine levels in whole blood were measured by FACS. Further investigations with longer exposure periods could be necessary to determine the long-term effects of exposure. These effects are dependent on MP size, MP dose, and mouse sex. Further investigations with longer exposure periods could be necessary to determine the long-term effects of exposure. These effects are dependent on MP size, MP dose, and mouse sex. Additional studies are needed to determine if gestationally exposed offspring have persistent alterations to the pancreas. Further research will focus on the potential role of extracellular vesicles in mediating maternal and offspring metabolic dysfunction.

### 4043 Polypropylene Microplastics—Mediated Potential Immunotoxicity to Mice Subacutely Administered through Intragastric Intubation

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Microplastic is one of the major problems as it is ingesting into the ecosystem and affecting the food chain in an unexpected way. Humans are being exposed to microplastics through contaminated foods, inhalation or dermal penetration. These exposures may cause oxidative stress or immune toxicities including inflammation in target organs. Heterogenous effects of microplastics (MPs) exist, depending upon the route of exposure and size range. Polypropylene microplastics (PP-MPs) have a wide range of applications and have been merely studied for its toxicity. Two different sizes of PP-MPs (5 and 50 μm diameter) were used to evaluate their immunotoxicity in 4-week-old mice. PP-MPs were administered to male and female mice at 0 (vehicle control), 500, 1000, 2000 mg/kg/day for 4 weeks. No significant differences were observed between groups in the major splenic immune cell populations, including thymic CD4+, CD8+, CD4+CD8+ T lymphocytes, and splenic helper T cells, cytotoxic T cells, and B cells. The ratio of interferon-γ to interleukin (IL)-4 in culture supernatants from polyclonally activated splenic mononuclear cells ex vivo (48 h) was dose-dependently decreased in female mice that received small- and large-size PP-MPs. Level of IL-13, a representative cytokine involved with predominance of type-2 helper T cell response, was significantly upregulated in female mice that received small- and large-size PP-MPs. The serum IgG2a/IgG1 ratio was dose-dependently increased in exposed offspring at 10 weeks old, though to a lower concentration than in dams. Exposed males have decreased pancreatic islet area and inflammation (CD68 and S100A8) at 10 weeks old. The results of this research demonstrate a persistent effect of PP-MPs exposure on the immune system. Additionally, we recently presented that CD4+ T-cells from non-autoimmune female mice (C57/B16) were disproportionately affected by TCE exposure compared to male C57BL/6 mice. Naive CD4+ T-cells from females treated with TCE in vivo were more likely to differentiate towards IFN-γ secreting Th-1 cells compared to males. The IFN-γ secreting Th-1 cell subset is the cell that is involved in the progression of autoimmunity and accelerates the pathology in autoimmune-prone (MRL) mice. Our preliminary data shows that CD4+ T-cells play a role in the progression of autoimmune-prone (MRL) mice. Generally, males are less susceptible to development of autoimmune, but the underlying mechanisms remain unclear. Thus, we hypothesized that CD4+ T-cells from male C57BL/6 mice are less likely than females to skew towards an IFN-γ secreting Th-1 subset because of altered gene expression due to TCE exposure. TCE needs to be metabolized in vivo into trichloroacetaldehyde hydrate (TCAH) to produce effects and is used here, in vitro to investigate effects on CD4+ T-cell differentiation in male C57/B16 mice. We differentiated CD4+ T-cells into the Th-1 subset in the presence or absence of 0.5 mM TCAH. Then after 4 days the cells were collected and assessed for changes in gene expression using RNA sequencing (RNA-seq). Lastly, we investigated gene regulation in mRNA expression in the Th-1 subset when treated with TCAH. Pathway analysis revealed major interferon signaling genes like the interferon gamma receptor 1 and multiple interferon stimulated genes e.g., interferon-induced protein with tetracopeptide & interferon induced transmembrane protein were significantly downregulated in TCAH-treated Th-1 cells. Future experiments will compare both sexes and additional chronic time points to look for gene expression changes that may explain the sex bias in autoimmunity.

### 4044 TCDD Increased Populations of B Cells That Produce IL-10 In Vivo and in Experimental Autoimmune Encephalomyelitis

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Continued investigation into the mechanisms by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters immune function might delineate important mechanisms for any hydrocarbon receptor (AhR)-mediated toxicity, reveal important information for the development of less toxic AhR ligands as therapeutics, and, or increase our understanding of the role of AhR in immune homeostasis. We have used the experimental autoimmune encephalomyelitis (EAE) model to study multiple sclerosis and found, similar to what others have also shown, that TCDD suppressed EAE clinical symptoms and decreased effector T cell function, i.e., T, and B cell, cytokine production and increased regulatory T cells. The goal of this project was to determine if the mechanism by which TCDD suppressed effector T cell function was due in part to TCDD-mediated increases in regulatory B cells. Indeed, we previously showed that TCDD increased the percentage of CD19+CD24CD38+ cells producing IL-10. In EAE, TCDD did not affect the percentage of the CD24+CD38+ cell population in CD19, B220 or CD5 B cells in splenocytes (SPLC), lymph nodes (LN) or BM cells at end-stage disease, but TCDD increased the CD19+CD24+CD38+ percentage in the spinal cord (SC). Moreover, TCDD increased the percentage of CD19+CD24+CD38+ cells in EAE mice modestly reduced the ability of naive effector T cells to express interferon (IFN)-γ and tumor necrosis factor (TNF)-α and IL-10 staining in the spleens of EAE mice treated with TCDD showed a modest increase as compared to vehicle-treated EAE mice. Together these data show that TCDD can induce regulatory functions in B cells, although it was not obvious simply by examining the expression of regulatory markers but by assessing function by cytokine production or mixed lymphocyte responses. Supported by grant # 202003120002, Korea Ministry of Environment (MOE) and by MOE-Educational training program for the management of information on the hazards and risk of chemical substances.

### 4045 Th-1 Cells from Male Mice Downregulate IFN-γ Related Genes during Trichloroethylene Metabolite Exposure In Vivo

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Trichloroethylene (TCE) is an industrial chemical used for a variety of applications acting as a degreaser, solvent, or detergent. Exposure to this toxicant is usually associated with the progression of autoimmune and accelerates the pathology in autoimmune-prone (MRL) mice. Our preliminary data shows that CD4+ T-cells play a role in the progression of autoimmune lesions, males have more pathology in the male system, and exposed gestationally exposed have persistent alterations to the pancreas. Further research will focus on the potential role of extracellular vesicles in mediating maternal and offspring metabolic dysfunction.

### 4046 Delta-8-Tetrahydrocannabinol Attenuates Experimental Multiple Sclerosis through Modulation of Ileal Microbiota

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Delta-8-tetrahydrocannabinol (delta-8-THC) is a psychoactive substance found in the Cannabis sativa plant. However, delta-8-THC is less psychoactive than delta-9-THC. For this reason, delta-8-THC is becoming popular for recreational use and alleviating pain. Virtually nothing is known about the effect of delta-8-THC on the immune system. In the current study, therefore, we investigated the effect of delta-8-THC on Experimental Autoimmune Encephalomyelitis (EAE), a murine model for Multiple Sclerosis (MS), an autoimmune disease resulting in nerve damage, and loss of communication between the brain and the body. EAE was induced to female C67BL/6 mice by injecting myelin oligodendrocyte glycoprotein followed by intraperitoneal administration of delta-8-THC (10mg/kg body weight) or vehicle every day. The treatment with delta-8-THC led to amelioration of paralysis symptoms in EAE mice and reversed the weight loss when compared with vehicle-treated group. Delta-8 THC also suppressed neuroinflammation in EAE mice. Because gut microbiota play a crucial role in the regulation of EAE, we next examined gut microbiome dysbiosis in delta-8-THC-treated EAE mice. The comparison of ileal and fecal microbiota from our 16s RNA sequencing performed on V3-V4 hypervariable regions of bacterial DNA revealed that the stability of ileal microbiome was more crucial for EAE attenuation than fecal. The ileal microbiome disclosed significant decreases in the abundance of Bacteroidetes phylum which appeared to be higher in EAE diseased group. Treatment with delta-8-THC also caused homeostasis at class level in the ileum microbiota by significantly increasing the abundance of Bacilli and decreasing Bacteroidia and Clostridia. Additionally, the beneficial effects of delta-8-THC treatment was also associated with the stabilization of the abundance of Lachnospiraceae in ileum, the members of whose markers but by assessing function by cytokine production or mixed lymphocyte responses. Find up-to-date information at www.toxicology.org/2023 | #2023SOT | #ToxExpo | 330
together, the current study demonstrates for the first time that delta-8-THC attenuates neuroinflammation in EAE by stabilizing the ileal microbiome. This work was supported in parts by NIH grants R01ES020014, R01AT003961, P20GM103641, and R01AI123947, R01AI160896.

**4047** Dysregulation of Gut Microbiota-Derived Short Chain Fatty Acids in Trichloroethylene-Mediated Autoimmune Diseases

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Trichloroethylene (TCE), a ubiquitous environmental contaminant, is found in 34% of the U.S. drinking water supply that can potentially affect over 14 million Americans in 36 states. Exposed individuals can suffer from skin disorders, lupus-like disease (SLE) and acute hepatitis due to systemic inflammatory responses. Studies have shown that exposure to TCE via drinking water leads to hepatic inflammation, autoimmune hepatitis (AIH) and SLE-like disease in female MRL+/+ mice. Environmental factors, including diet and microbiota-derived metabolites, among which short chain fatty acids (SCFAs) are major players can influence AIH and SLE pathogenesis. Therefore, it is important to delineate the contribution of metabolic and signaling pathways as a result of microbiota dysbiosis in the induction of TCE-mediated ADs. This study was therefore, focused on determining the specific SCFAs and the involvement of fecal SCFAs-producing bacteria and SCFAs metabolism-related pathways in MRL+/+ mice after chronic TCE exposure (0.5 mg/ml, via drinking water for 52 weeks). Liquid chromatography-mass spectrometry (LC-MS) analysis of cecum SCFAs, fecal SCFAs-producing bacteria and SCFAs metabolism-related pathways were evaluated using whole gene sequencing (metagenomic) and RT-PCR approaches in the control and TCE-exposed mice. TCE exposure led to a significant shift in the microbiota composition and remarkable changes in the levels of microbiota-derived metabolites, which were associated with the generation of various autoantibodies. In particular, the levels of acetate, isobutyrate, 2-methyl-butyrate and isovalerate were decreased significantly in TCE-treated mice. Furthermore, TCE exposure led to reductions in fecal acetate producing bacteria (Bifidobacterium pseudolongum) and butyrate producing bacteria (Faecalibacterium prausnitzii), together with inhibition of amino acid metabolic pathways, including arginine, leucine, lysine, alanine and tryptophan. It is thus evident from our data that TCE exposure leads to dysregulation in SCFAs and SCFAs-producing bacterial composition and genes related to microbial metabolism pathways. Our findings fill critical knowledge gap towards better understanding of the contribution of TCE-mediated dysbiosis of gut microbiota and their metabolites/SCFAs in ADs.

**4048** Intestinal Immune Dysfunction in Zinc Deficiency Reduces the Production of Secretory IgA and Causes Bacterial Translocation to the Liver

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Nutritional zinc deficiency and its relationship to the gut-associated lymphoid tissues (GALT) immune system remains unclear. We evaluated the effects and underlying mechanisms of the intestinal GALT immune system on the sigla barrier caused by Zn deficiency. Five-week-old male rats were fed a standard diet (17 g/day), and two groups were fed a zinc-deficient diet (n = 7 each). Each group was also injected with saline or IL-4 (zinc deficient/standard). The sigla concentrations, MUC-2 concentration, Th2 (IL-4+/5)+ cells, IL-19/IL-3+ cells, and mRNA expressions of tight junction in the small intestine on zinc deficiency rats were significantly decreased than those other groups. On the other hand, LPS+ area/bacteria of small intestine on zinc deficiency rats were significantly increased. For the IL-4 administration for zinc deficiency, sigla concentrations in the small intestine were significantly decreased. After zinc supplementation for zinc deficiency, all the measurement indices were recovered. In conclusion, zinc deficiency can cause bacterial migration through the portal vein to the liver by lowering the GALT immune system (sigla) in the intestinal tract. The IL-4 administration or zinc supplementation to the zinc-deficient rats, although the beneficial effects on respectively GALT-immune system were different, suggests that bacterial translocation to the liver could be prevented.

**4049** Interactions between Aryl Hydrocarbon Receptor and the Microbiome Regulate Intestinal Macrophages in Non-obese Diabetic Mice

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Type 1 diabetes (T1D) is an autoimmune disorder which arises from a complex interaction between genetic and environmental factors. Multiple environmental signals predicted to impact T1D development occur at the intestinal surface, as a result of exposures through ingestion, dietary antigens, and microbial colonization. It is hypothesized that an inappropriate immune response to these exposures precipitates overt T1D, but a mechanism of action remains elusive. We propose a novel mechanism by which environmental surveillance interacts with the immune system: immunosuppressive lamina propria (LP) macrophage regulation via the aryl hydrocarbon receptor (AhR). To determine how AhR influences the intestinal immune response during T1D development, we generated NOD AhR knockout mice. Female AhR knockout mice had reduced insults at 12 weeks of age, and a decreased incidence and onset of hyperglycemia in comparison to their wild-type littermates. We discovered a population of resident-like macrophages (CD1Ib+CX3CR1+MHCIi+) in the small intestine LP that were negatively regulated by AhR (AhR-MFs). These cells have a very strong positive correlation to LP Tregs, and correlate with protection against insulitis. NOD AhR knockout mice were derived as germ free and the AhR-MF population was measured. In the absence of the microbiome both AhR knockout and wild-type mice had high levels of AhR-MFs, demonstrating that the microbiome is required for AhR-mediated regulation of LP macrophages. Current work is aimed at single cell sequencing of CD45+ LP cells from AhR knockout and wild-type mice to identify how AhR alters macrophage differentiation.

**4050** Extracellular Metallothionein Can Influence the Progression of Type 1 Diabetes in NOD Mice

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Metallothionein is a small molecular weight, cysteine-rich metal-binding stress response protein that is readily inducible in many tissues upon exposure to a variety of stressors, including divalent heavy metal cations, aromatic hydrocarbons, reactive oxygen species, bacterial infection, and irradiation. Metallothioneins (MTs) can function as a reservoir of essential metals such as zinc and copper and can protect cells from toxicants including heavy metals and free radicals. In addition to its basic housekeeping duties, MT has many immunomodulatory functions, and has been shown to stimulate lymphocyte proliferation and chemotaxis. Despite lacking a signal sequence, MT can be found in many extracellular spaces, and this pool of MT has been implicated in the progression of several inflammatory diseases such as inflammatory bowel disease and drug induced hepatotoxicity. The work described here relates to autoimmune Type 1 diabetes (T1D) and the role played by MT in disease onset and progression. T1D is characterized by immune cell infiltration into the islets of Langerhans, resulting in the destruction of insulin-producing beta cells and uncontrolled hyperglycemia. In the non-obese diabetic (NOD) mouse model, the onset of lymphocyte infiltration is thought to start several weeks in advance of glucose dysregulation and overt diabetes. Recruitment of these immune cells is mediated by several cytokines, including CXCL10, while other cytokines, including SDF-1a, can confer protective effects. A better understanding of the cumulative roles of these signaling molecules could lead to a treatment option that would block immune cell recruitment and subsequent destruction of beta cells. A review of global gene expression analysis in the pancreas of pre-diabetic (NOD) mice revealed increased expression of several isoforms of MT. Analysis of single cell RNA sequencing of human islets in prediabetic, autoantibody positive, patients has also showed increased expression of metallothionein in insulin-producing beta cells compared to normal controls and patients with established T1D. Beta cells can release MT into the extracellular environment and that pool of extracellular MT can synergistically affect chemotactic responses of Th1 cells to CXCL10, and interfere with chemotaxis of Th2 cells to SDF-1a. In vivo; these inflammatory effects can be blocked with a monoclonal anti-MT antibody, clone UC1-MT. When administered to NOD mice for a two-week period before the onset of diabetes, UC1-MT reduces inflammation and prevents the development of diabetes when compared to NOD mice treated with an isotype-matched control antibody. Manipulation of extracellular MT with UC1-MT may be an important approach in maintaining beta cell function and preventing the development of T1D in prediabetic patients. Furthermore, it suggests that environmental toxicants that upregulate extracellular MT levels can potentiate the progression of T1D in susceptible populations.
Atopic dermatitis (AD) is a multifactorial disease, and its symptoms have been reported to be affected by living environments, such as indoor temperature and humidity, smoking, and chemicals. In this study, we focused on indoor volatile organic compounds (VOCs). VOCs have been reported to be one of the exacerbating factors of allergies and are constantly emitted from interior materials and fixtures indoors. A high concentration of VOCs can affect healthy people with sick building syndrome; therefore, indoor VOC regulation regulations have been established. On the other hand, there are no regulations for VOCs, and therefore, there needs to be more research on the effects of VOCs below indoor guideline values on disease development. We hypothesized that if indoor VOCs affect disease development, they may also affect the treatment of skin diseases. Initially, mice were exposed, respectively, to an indoor air (control group) and a chemically filtered air (CF group), whose VOCs were removed by an activated carbon chemical filter, and their pathophysiology of atopic dermatitis was compared. A mouse model of atopic dermatitis was developed in female NC/Nga mice by weekly topically sensitized with Toluene-2,4-isocyanate (TDI), a hapten that induces Type 2 helper T cells. The VOCs in the chemically filtered air were analyzed by a GC-FID method, and were found to be removed by about 90% compared to the indoor air. In the control group, showed significant suppression of transepidermal water loss and skin thickness during the initial two weeks; however, they found to be comparable from two weeks to four weeks. Significant reductions of T and B cell counts in auricular lymph nodes and inflammatory responses in the pathological evaluation were found in the CF group compared to the control group. Co-stimulation with CD3/CD28 significantly suppressed IL-4, IL-6, and IL-17 produced by immune cells in the lymph nodes in the CF group. Furthermore, the CF group also significantly suppressed TNFα, IL-4, and IL-17 produced by ConA stimulation. Secondly, in order to investigate the indoor air quality in veterinary hospitals, air sampling was conducted in several hospitals, and their indoor and outdoor VOCs were analyzed by GC-FID and GC-MS. The results showed differences in the VOCs’ composition and concentration of the sampled indoor air that could be caused by the size of the hospitals and the differences in ventilation systems. The chemicals measured included alcohols, aromatic compounds, and solvents. The results of this study suggest that the control of gaseous chemicals, including indoor VOCs, is critically important for the indoor environment control of skin diseases since VOCs at concentrations similar to those in a typical hospital room and outdoor air can affect the treatment of skin diseases. Initially, mice were exposed, respectively, to an indoor air (control group) and a chemically filtered air (CF group), whose VOCs were removed by an activated carbon chemical filter, and their pathophysiology of atopic dermatitis was compared. A mouse model of atopic dermatitis was developed in female NC/Nga mice by weekly topically sensitized with Toluene-2,4-isocyanate (TDI), a hapten that induces Type 2 helper T cells. The VOCs in the chemically filtered air were analyzed by a GC-FID method, and were found to be removed by about 90% compared to the indoor air. In the control group, showed significant suppression of transepidermal water loss and skin thickness during the initial two weeks; however, they found to be comparable from two weeks to four weeks. Significant reductions of T and B cell counts in auricular lymph nodes and inflammatory responses in the pathological evaluation were found in the CF group compared to the control group. Co-stimulation with CD3/CD28 significantly suppressed IL-4, IL-6, and IL-17 produced by immune cells in the lymph nodes in the CF group. Furthermore, the CF group also significantly suppressed TNFα, IL-4, and IL-17 produced by ConA stimulation. Secondly, in order to investigate the indoor air quality in veterinary hospitals, air sampling was conducted in several hospitals, and their indoor and outdoor VOCs were analyzed by GC-FID and GC-MS. The results showed differences in the VOCs’ composition and concentration of the sampled indoor air that could be caused by the size of the hospitals and the differences in ventilation systems. The chemicals measured included alcohols, aromatic compounds, and solvents. The results of this study suggest that the control of gaseous chemicals, including indoor VOCs, is critically important for the indoor environment control of skin diseases since VOCs at concentrations similar to those in a typical hospital room and outdoor air can affect the treatment of skin diseases.

**4052 Removal of Volatile Organic Compound (VOC) by Chemical Filter Significantly Alleviates the Development of Mite Allergen–Induced Allergic Asthma in a Mouse Model**

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Gaseous chemicals in the atmosphere such as Volatile organic compounds (VOCs) and ozone gas have long been realized as the risk factor for respiratory diseases. Initially, mice were exposed, respectively, to an indoor air (control group) and a chemically filtered air (CF group), whose VOCs were removed by an activated carbon chemical filter, and their pathophysiology of atopic dermatitis was compared. A mouse model of atopic dermatitis was developed in female NC/Nga mice by weekly topically sensitized with Toluene-2,4-isocyanate (TDI), a hapten that induces Type 2 helper T cells. The VOCs in the chemically filtered air were analyzed by a GC-FID method, and were found to be removed by about 90% compared to the indoor air. In the control group, showed significant suppression of transepidermal water loss and skin thickness during the initial two weeks; however, they found to be comparable from two weeks to four weeks. Significant reductions of T and B cell counts in auricular lymph nodes and inflammatory responses in the pathological evaluation were found in the CF group compared to the control group. Co-stimulation with CD3/CD28 significantly suppressed IL-4, IL-6, and IL-17 produced by immune cells in the lymph nodes in the CF group. Furthermore, the CF group also significantly suppressed TNFα, IL-4, and IL-17 produced by ConA stimulation. Secondly, in order to investigate the indoor air quality in veterinary hospitals, air sampling was conducted in several hospitals, and their indoor and outdoor VOCs were analyzed by GC-FID and GC-MS. The results showed differences in the VOCs’ composition and concentration of the sampled indoor air that could be caused by the size of the hospitals and the differences in ventilation systems. The chemicals measured included alcohols, aromatic compounds, and solvents. The results of this study suggest that the control of gaseous chemicals, including indoor VOCs, is critically important for the indoor environment control of skin diseases since VOCs at concentrations similar to those in a typical hospital room and outdoor air can affect the treatment of skin diseases.

**4053 IL4Rα Signaling in Myeloid Cells Cause Pathological Outcomes Associated with Allergic Asthma in Mice**

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IL-4 and IL-13 play an essential role in the pathogenicity of allergic asthma via their common receptor, i.e., IL4Rα. However, the myeloid cell-specific role of IL4Rα-mediated signaling in allergic asthma has remained unclear. We hypothesized that myeloid cell-specific IL4Rα signaling is essential for granulocytic inflammation and associated pathological outcomes in allergic asthma. To test our hypothesis, we challenged myeloid cell-specific IL4Rα-deficient (LysMcre+/IL4Rαlox/lox) and myeloid cell-specific IL4Rα-sufficient (LysMcre+/IL4Rα+/-) mice with either mixed allergens (MA) or ovalbumin (OVA) and showed that in MA-challenged myeloid cell-specific IL4Rα-deficient mice that had exhausted immune cell recruitment, the MA-challenged myeloid cell-specific IL4Rα-deficient mice had significantly reduced immune cell recruitment into the lung airspaces. This reduction in immune cell recruitment in MA-challenged myeloid cell-specific IL4Rα-deficient mice was attributable to a significant decrease in the numbers of eosinophils and lymphocytes. Mice that had exhausted immune cell recruitment, the MA-challenged myeloid cell-specific IL4Rα-deficient mice had significantly reduced levels of primary eosinophil chemotractant, eotaxin-2, as well as Th2 cell chemotractants, particularly TARC (CCL17) and MDC (CCL22). The levels of IL-33 (a master regulator of Th2 inflammation) and Th2 cytokines, i.e., IL-4 and IL-13, were also significantly reduced in MA-challenged myeloid cell-specific IL4Rα-deficient mice as compared to MA-challenged myeloid cell-specific IL4Rα-sufficient mice. Additionally, while MA-challenged myeloid cell-specific IL4Rα-sufficient mice exhibited pronounced peribronchiorl and perivascular lung inflammation, myeloid cell-specific IL4Rα deletion significantly ameliorated the peribronchiorl and perivascular lung inflammation. MA-challenged myeloid cell-specific IL4Rα-deficient mice also exhibited reduced airway hyperresponsiveness, had significantly reduced serum IgE levels, and showed mitigation of sub-epithelial lung fibrosis compared to MA-challenged myeloid cell-specific IL4Rα-sufficient mice. Interestingly, mucous cell metaplasia (MCP), which arises from the functional differentiation of the lung epithelium, was unaltered in MA-challenged myeloid cell-specific IL4Rα-deficient mice when compared to MA-challenged myeloid cell-specific IL4Rα-sufficient mice, suggesting the dispensable role of myeloid cell-specific IL4Rα signaling in mixed allergen-induced MCM and mucins production. Taken together, our data show that in MA-induced allergic asthma model, myeloid cell-specific IL4Rα signaling is critically required for eosinophil and Th2 lymphocyte recruitment, production of the Th2 cytokines and chemokines, airway hyperresponsiveness, IgE antibody production, subepithelial fibrosis, and inflammatory lung pathology. Findings from this study could be applied towards developing cell-specific therapeutics against allergic airway and other eosinophilic disorders.

**4054 Sex-Based Differences in Inflammatory Responses to Silver Nanoparticles**

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Sex-based differences in the innate and adaptive immune systems are well recognized. There is an ongoing need to investigate the potential effects of nanoparticles on the immune system. Silver nanoparticles (AgNPs) are used in consumer, healthcare, and industrial products, such as food packaging, feminine hygiene products, cosmetics, implants, and bandages due to their anti-microbial properties. With the growing use of AgNPs, there is an increase in concerns between male and female. Therefore, the objective of this study aims to investigate the potential sex-based differences in immune system function due to AgNP exposure using human peripheral blood mononuclear cells and plasma from healthy donors. AgNPs (30 nm) were characterized for their hydrodynamic size by dynamic light scattering (DLS) and their primary size by transmission electron
microscopy (TEM). Inflammamase activation was determined by monitoring the IL-1β expression level after 6- and 24-hours of exposure to AgNPs. Leukocyte proliferation was measured by cell viability assay after an initial 24-hour exposure to AgNPs and 72-hours of phytohemagglutinin-M (PHA-M)-induced leukocyte prolif- eration. Complement activation was determined by measuring the C3-split product. Plasma coagulation was determined by measuring prothrombin time and activated partial thromboplastin time. Inflammamase activity was highest in females after exposure to AgNPs for both exposure times. The 6-hour exposure to the AgNPs caused significantly more inflammamase activity in females than males. The 24-hour exposure exhibited more inflammamase activity in females than males but was not statistically significant. Further analysis was conducted to compare the inflammamase activity within two age groups: younger and older. Inflammamase activity was higher in young females compared to young males; however, a differ- ence was not observed in the older age groups. Interestingly, young males showed more inflammamase activity than older males after a 6-hour exposure, but there was no observed difference after a 24-hour exposure to AgNPs. Young females only showed more inflammamase activity for the 24-hour exposure with similar activity for both age groups. Thus, AgNPs showed a sex-and-age-dependent response in the inflammamase activation. AgNPs suppressed the PHA-M-induced leukocyte proliferation with males demonstrating more tolerance than females to the presence of the AgNPs over the 72-hour period than the females. This indicated that the females exhibited more leukocyte cytocytotoxicity after prolonged AgNP exposure. AgNPs did not affect the complement activation or coagulation. The results highlight that there are distinct sex-based differences in inflammamase activity and leukocyte proliferation after exposure to AgNPs, which promotes the importance of developing a deeper understanding of the health risks associated with exposure to AgNPs for both women’s and men’s health.

4055 Investigating the Relationship between Chemosensory Neurons and Immune Cells in the Main Olfactory Epithelium (MOE)


The nasal epithelium acts as a first-line defense, continuously exposed to foreign material including microbes (e.g., viruses and bacteria), aeroallergens, chemicals, and pollutants. Part of the nasal epithelium, the main olfactory epithelium (MOE) is the principal site of olfaction (the sense of smell). Disruption of normal functions of this tissue can result in hyposmia, a reduction in the sense of smell or anosmia, total loss of smell, and significant loss of quality of life. Hyposmia and anosmia are still poorly understood, and it remains unclear whether inflammatory responses influence olfactory function, and also whether olfactory modulation influences resident immune cell populations. Given the capacity for bidirec- tional neuronal-immune communication in the MOE, especially relevant given the evidence of olfactory dysfunction associated with diseases such as allergic rhinitis and respiratory viral infections, we have initiated a study investigating this topic in mice. The MOE contains multiple cell types, including olfactory sensory neurons (OSNs), which detect odorous molecules, supporting epithelial cells, secretory cells, and immune cells. Many known odorants, such as peptides, proteins, etc., have potentially immunogenic properties, but little is known regarding the intercellular interactions between immunogen-sensing OSNs and resident immune cells. We hypothesize that OSNs influence the immune cell populations and functions in the presence of immunomodulatory odorants. Using flow cytometry and single-cell RNA sequencing, we are able to identify OSNs and multiple immune subsets in the MOE. Functionally, using transgenic mice expressing genetically encoded Ca2+ indicators, we are able to identify how immunogenic and non-immunogenic odorants impact neuron activity. This was done with mice expressing GCaMP6s, a Ca2+-responsive fluorescent protein in OSNs. We isolated the MOE en bloc and exposed the epithelium to immunogenic, non-immunogenic, and immunomodu- latory stimuli, and imaged GCaMP6s fluorescence using objective-coupled planar illumination (OCPI) microscopy to identify activated OSNs (those with increased fluorescent microscopy) from the OCPI approach suggest that OSNs are activated by common olfactory ligands, immunogenic ligands, and immunomodulatory ligands such as Delta-9-tetrahydrocannabinol (THC), which is well known for its recreational use but also has anti-inflammatory properties. This experimen- tal system establishes a platform to conduct detailed studies investigating how olfactory activity impacts nasal immune responses, filling gaps in our knowledge about the relationships between OSNs and immune cells in the MOE informing our understanding of olfaction and immunomodulation, and potentially informing our understanding of hyposmia and anosmia.

4056 Dose Response of the Food Additive tert-BHQ on OVA-Elicited Food Allergy in Mice

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There has been a steady rise in the prevalence of food allergy for the past couple of decades for reasons that are not completely understood. While previous studies from our lab demonstrated that tert-butylhydroquinone (tBHQ), a food additive present in many processed foods, promotes the development of allergy in mice, our current experiment focuses on the dose response of tBHQ in the mice. Mice were fed AIN-93G with 0.0014% tBHQ, 0.0007% tBHQ, and 0.00028% tBHQ or control diet. Mice were exposed to OVA once every week for 4 weeks during the sensitization phase. Sensitization to OVA was assessed by the rise in OVA-specific IgE. Upon oral challenge, mice were monitored for hypothermia shock response (HSR) and mast cell protease (mMCP)-1 response. Sensitization with OVA elicited a more robust OVA-specific IgG antibody response in mice on the tBHQ diet with high concentration. Likewise, in response to OVA challenge, a greater decrease in body temperature was observed in the mice on the high concentration of tBHQ diet post-challenge compared to control animals. In addition, because tBHQ is a robust activator of the transcription factor Nrf2 in immune cells, we aimed to determine the role of Nrf2 in these tBHQ-mediated effects. Therefore, we performed a PCR array on splenocytes from OVA-sensitized Nrf2 wild-type and Nrf2 knockout mice who received either vehicle or wild-type CD4 T cells or Nrf2-knockout CD4 T cells and were sensitized to OVA. We found that the Th2-related genes were downregulated, but Treg and Th1-related genes were upregulated in mice that received Nrf2-null CD4 T cells. Taken together, these data suggest that exposure to tBHQ through diet promotes OVA sensitiza- tion and exacerbates anaphylactic response to OVA challenge in a mouse model of food allergy. This effect is tBHQ dose-dependent and dependent on the Nrf2 transcription factor.

4057 Environmental Pollutant Trichloroethylene Enhances Responses to Egg Protein in a Human-Relevant Mouse Model of Food Allergy


Food allergy is a growing health problem with a prevalence of 8%-10% in children and 4% in adults. In the US, food allergies cause 50,000 visits to the emergency room per year with 150-200 deaths and an estimate of 500 million in costs. Common food allergies include proteins in egg, milk, wheat, soy, peanuts, tree nuts, shellfish or fish (i.e., the “red flag” foods). The cause of the increase in food allergy is unknown. One potential environmental risk factor includes exposure to environ- mental pollutants. While several environmental contaminants such as particulate matter and air pollution have been linked to allergic responses there is virtually no data on potential environmental contaminants that enhance risk for food allergy. The industrial solvent, trichloroethylene (TCE) is a halocarbon best known as a metal degreaser. It has polluted many of the water systems in the U.S., and human environmental exposure is not uncommon. Occupational TCE exposure promotes autoimmune and immune-mediated inflammatory disorders including skin rash with eosinophilia, exfoliative dermatitis, mucous membrane erosion, and epidermal necrosis. This immunological response is consistent with type I hypersensitivity reactions (i.e., allergy). Our goal is to investigate the effect of TCE in the development of food allergy in a human relevant epicutaneous mouse model of food allergy. We hypothesized that TCE exposure would enhance allergic responses in mice. We used three age groups with 12 wild-type mice per group, (3-8 weeks of age) were exposed to low-dose TCE (5 μg/ml or <1 mg/kg/day) or vehicle in the drinking water for a total of 9 weeks. Subsets of TCE and control mice were sensitized to egg protein (OVA; 100 μg) applied to depilated abdominal skin once a week for 6 weeks during the TCE exposure. After 6 weeks, mice were orally challenged with 50 mg OVA by gavage serum levels of OVA-specific IgG1 and IgE both pre- and post-challenge were assessed by ELISA. After oral challenge mice were assessed for inappropriate clinical responses (anaphylaxis). There was variability in the OVA-specific Ig response when comparing serum levels (ng/ml) obtained both pre- and post-challenge. However, the mean percentage of OVA-sensitized mice that developed OVA-specific antibodies was significantly higher in mice exposed to TCE compared to vehicle controls (66% vs. 44%, n=6 per group, respectively). After OVA challenge, mice were evaluated for clinical signs of anaphylaxis by measuring rectal temperature every 5 minutes for 60 min. Strikingly, TCE exposure significantly reduced rectal temperature and expressed a greater degree of TCE-sensitized vehicle controls (p<0.05). Mice not exposed to OVA (saline controls) did not exhibit OVA-specific IgG or experience a drop in body temperature as expected. Further study will address the impact of TCE as a potential environmental trigger of food allergy. Future studies will focus on the role of gut mucosal immunity in TCE-mediated responses to food proteins after exposure during sensitive time periods of immune development.

4058 Development of a Human In Vitro De-risking Approach to Improve the Relevance of Drug-induced Mast Cell Activation Evaluations for Nonclinical Drug Safety Assessment


Mice cells play critical roles in allergic response, anaphylaxis, and defense against pathogens and toxins. Mast cells secrete vasoactive and proinflamma- tory mediators including histamine, serotonin, and proteases stored in secretory granules to promote anti-pathogen immunity during infections. Uncontrolled mast cell degranulation (MCD) results in clinical signs of anaphylaxis consisting of flushing and/or more adverse events including changes in blood pressure, heart

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rate, respiratory rate, and in severe cases, mortality. Substructures within many peptide and small molecule drugs such as basic residues and aromatic or aliphatic hydrophobic residues have the potential to trigger activation of mast cells and subsequently pseudoanaphylaxis by inducing mast cell degranulation in humans and animals. We have previously established an ex vivo rat peritoneal mast cell degranulation assay as a tool to elucidate the role of mast cells in the mechanism of toxicity for peptide and small molecule drug candidates. Although the assay has demonstrated a strong in vitro-in vivo correlation and is highly predictive of recent toxicity, it was unclear whether these findings offer clinically relevant prediction of pseudoanaphylaxis risk in humans. In the present study, we report on the evaluation in vitro human MCD assays using either LAD2 human mast cells or CD34+ stem cell differentiated mast cells. Over 60 benchmark compounds with diverse chemical structures, including those reported to cause pseudoanaphylaxis risks in the clinic, were evaluated. Our results demonstrate a high correlation between the rat mast cells and LAD2 based human MCD assay. Additionally, several drugs known to cause pseudoanaphylaxis reactions in human can induce histamine release in both rat peritoneal and human LAD2 mast cells, suggesting correlation between in vitro and in vivo effects. In differentiated human mast cells, however, did not show consistent effects in histamine release with these compounds which likely attributed to differential expression of key mast cell activation receptors among different cell batches. Overall, our results showed a high correlation between rat peritoneal and human LAD2 based in vitro mast cell degranulation effects, suggesting the LAD2 assay may be predictive of human pseudoanaphylaxis risk. Compounds activating rat and human mast cells shared similar features in structures which suggest pseudoanaphylaxis risk by these structures are shared between human and rats. Our results also showed that LAD2 cells can be used to establish a relatively high throughput and low variability platform which will reduce animal usage for pseudoanaphylaxis derisking.

4059 Priming Treatment with T Cell-Redirecting Bispecific Antibody ERY974—Reduced Cytokine Release without Lost Cytotoxic Activity In Vitro by Changing Chromatin State


CD3-bispecific antibodies (CD3-BsAbs) are an emerging form of cancer immunotherapy, but their adverse effects in clinical contexts are difficult to manage. Cytokine release syndrome (CRS), a potentially life-threatening systemic inflammatory response associated with elevated levels of circulating cytokines, commonly occurs with this type of antibody. CRS frequently strikes after the first dose, and its severity may be increased subsequent to repeated treatment. The administration of CD3-BsAbs has been shown to reduce CRS, the mechanism behind this remains unclear. The purpose of this study is to elucidate the mechanism underlying reduced cytokine induction after repeated treatment with ERY974, a GPC3/CD3 BsAb. In this study, human peripheral blood mononuclear cells (PBMCs) and GPC3/CD3 BsAb treatment were tested for 72 hours, with PBMCs then cultured for one week before being treated again with ERY974 or negative control. We examined cytokine concentrations, mRNA expression of cytokines, CD3 expression, CD3-mediated signal transduction, T-cell activation markers, cytokotoxic activity, and T-cell chromatin state. The repeated ERY974 treatment showed lower cytokine levels (mRNA and protein) than the first treatment, and ATAC-seq analysis revealed that the priming treatment changed chromatin accessibility in T cells. The priming treatment decreased chromatin accessibility at the transcriptional regulation region of IL2 and decreased IL-2 expression. CD3 expression, CD3-mediated signal transduction, T-cell activation markers, and cytotoxic activity after repeated ERY974 treatment were similar to that after the first treatment. These results suggest that epigenetic changes to T cells after the priming treatment play an important role in the mitigation of cytokine release after the repeated ERY974 treatment.

4060 Developing In Vitro Models for Assessing Off-Target Effects of Antibody-Drug Conjugates


Antibody-Drug conjugates (ADCs) are an emerging modality that hold promise for improved therapeutic index through targeted drug delivery. ADCs with cytotoxic payloads targeting tumor antigens are of particular interest; however, adverse events still limit the full benefit of these molecules in the clinic. Studies have demonstrated that ADC payloads accumulate in cells capable of activating both ADCC and ADC, as neutrophils and megakaryocytes. Accordingly, non-target mediated uptake is implicated as an underlying off target ADC toxicity. To gain mechanistic insight and better characterize ADC safety, we established in vitro approaches to examine non-target mediated ADC toxicity in neutrophils and megakaryocytes. A series of ADCs, including HER2-targeted, HER2-HER2 DM1, and HER2-HER2 DM4, were used for proof-of-concept studies. Human CD34+ hematopoietic stem cells were differentiated into neutrophils or megakaryocytes and subsequently treated with ADCs at a range of concentrations. Cell viability was measured using ATP or flow cytometry to determine in vitro cytotoxicity IC50s. We found that megakaryocytes were more sensitive to Kadcyla-induced cytotoxicity as compared to Polivy, consistent with the incidence of clinical thrombocytopenia observed in patients treated with these ADCs. Our preliminary results suggest that in vitro assays can differentiate susceptibility to ADC induced cytotoxicity between cell types associated with clinical adverse findings. Future work, including the assessment of additional cell types/ADCs, mechanisms of cellular ADC internalization, and in vitro/in vivo correlataion will contribute to a better understanding ADC mediated toxicity. Overall, our results with neutrophils and megakaryocytes are consistent to the ADC mediated adverse effects observed in the clinic and can potentially be considered in projecting therapeutic index alongside in vitro/in vivo preclinical efficacy data.
cell infiltration in the tumor micro-environment were similar between IL-1α-MPs and rIL-1α. These results indicate that CPH:SA-based IL-1α-MPs generated a slow and sustained systemic release of IL-1α, resulting in reduced systemic inflammation and toxicity accompanied by an adequate anti-tumor response. Therefore, MPs based on CPH:SA formulations may be promising as delivery vehicles for IL-1α to achieve safe, effective, and durable antitumor responses for head and neck squamous cell carcinoma (HNSCC) patients.

**4063 Identification of Strategies to Block Nuclear Translocation of Interleukin-1a in Head and Neck Tumors**

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Interleukin 1a (IL-1α) is a proinflammatory cytokine that is believed to play a pro-tumorigenic role and is associated with tumor aggression and metastasis. Unfortunately, efforts to target IL-1α as a cancer therapy have been unsuccessful in clinical trials due to the strong immunological responses that generate cytokine releases against CAR-T treatment and the tumor, and downstream toxicity. In the present study, we evaluate various cryopreserved nonhuman (NHP) cell populations isolated from the whole blood of preclinical animal models. These data can be used to screen drug candidates and otherwise de-risk potential therapeutics prior to long and expensive in vivo safety studies. In the current study, we compare the performance of several commonly used CAR positive control compounds against samples isolated from nonhuman primates, an important pre-clinical safety species. These results show that sera were made across the two most commonly used CRA formats, whole blood and peripheral blood mononuclear cells (PBMCs) stimulated in the liquid phase. Whole blood or PBMCs from at least three animals were stimulated with either PMA and ionomycin, pokeweed mitogen, anti-CD3 (OKT3), phytohemagglutinin, or all five in combination for a nominal 24 hours. Plasma or cell culture supernatants from stimulated samples were assessed in the liquid phase. Whole blood or PBMCs from at least three animals were stimulated with either PMA and ionomycin, pokeweed mitogen, anti-CD3 (OKT3), phytohemagglutinin, or all five in combination for a nominal 24 hours. Plasma or cell culture supernatants from stimulated samples were assessed in the liquid phase.

**4064 Novel PBMC Humanized Mouse Model That Can Distinguish a Distinct CAR-T Response against Different Tumor Types**


Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a revolutionary treatment for certain hematological malignancies. While several CAR-T therapies showed an efficacious response in selected patients, this wider adoption has been challenged due to various side effects, such as cytokine release syndrome (CRS), and lack of efficacy. We have developed a novel peripheral blood mononuclear cell (PBMC)-humanized mouse platform to assess the efficacy and safety of CAR-T therapy. The model allows assessing CAR-T efficacy and expansion, CRS, and downstream toxicity altogether in the same mouse. Here, we established a tumor-bearing PBMC humanized mice using two different tumor cell lines, Raji B cell lymphoma and Nalm6 leukemia. We compared the effect of CAR-T therapy in different tumor types and tumor burden, focusing on CAR-T efficacy and expansion, CRS, and downstream toxicity. To establish a tumor-bearing PBMC humanized mouse model, we used Raji/IL-1α and Nalm6/IL-1α humanized mice. We injected heterogenous human T cells into NHPs and monitored the tumor burden and cytokine releases against CAR-T treatment and the tumor, and downstream toxicity. Here, we established a tumor-bearing PBMC humanized mice using two different tumor cell lines, Raji B cell lymphoma and Nalm6 leukemia. We compared the effect of CAR-T therapy in different tumor types and tumor burden, focusing on CAR-T efficacy and expansion, CRS, and downstream toxicity altogether in the same mouse. Here, we established a tumor-bearing PBMC humanized mouse model, using Raji/IL-1α and Nalm6/IL-1α humanized mice. We injected heterogenous human T cells into NHPs and monitored the tumor burden and cytokine releases against CAR-T treatment and the tumor, and downstream toxicity. Here, we established a tumor-bearing PBMC humanized mouse model, using Raji/IL-1α and Nalm6/IL-1α humanized mice. We injected heterogenous human T cells into NHPs and monitored the tumor burden and cytokine releases against CAR-T treatment and the tumor, and downstream toxicity.
quality attributes in flow cytometry analysis. Overall, our study identified multiple cryopreservation methods to enable retrospective immunophenotyping analysis of nonhuman primate peripheral blood samples (both thymus and cymolympho -macaques). Samples stored in FBS with 10% DMSO can be used for at least 6 months without negative impact in data quality. These methods can be benefi -cial for nonclinical studies where retrospective immunophenotyping is needed. Retrospective immunophenotyping could also facilitate sample shipment across sites and allow for the ability to analyze samples from different timepoints concur -rently, which could limit assay variability.

The role of AHR agonist TCDD in early human hematopoietic differentiation is not well understood. We developed and characterized an in vitro culture system to study the effect of AHR activation by 1 nM TCDD on hematopoietic differentiation of human cord blood CD34+ hematopoietic stem and progenitor cells (HSPCs) over a period of 28 days. Using single cell RNA-sequencing (scRNA-seq) and flow cytomet -ry, we identified that our model facilitates the formation of most major hematopoi -etic lineages except T cells. scRNA-seq analysis suggested that TCDD treatment led to a decrease in development of cells of lymphoid and megakaryocyte-erythroid lineages and an increase in pro-myelocytes and monocytes compared to vehicle (0.02% DMSO). Flow cytometric and immunofluorescence (CFDA/CFDA-e) in CD10+ lymphoid progenitors and CD19+ B-cells, and a significant increase in CD14+ (monocyte) and CD66b+ (granulocyte) progenitors with TCDD treatment at multiple time points. Differential gene expression analyses showed that genes critical for development of B-cells (EBF1, BCL1A, LEF1) were downregulated in the lymphoid cluster (p-value < 0.01) by TCDD treatment. BCL1A protein expressing CD10+ CD19+ lymphoid progenitors were also reduced with TCDD on days 14 and 21. scRNA-seq analysis demonstrated monocyties from TCDD group had significant suppression of genes associated with immune competence (IFTIM2, HLA-DR, TIBHS1, etc.). In late monocytes, genes mediating lipid transport (APOE, APOC1) and genes associ -ated with an M1 macrophage phenotype (ADAM12, LOC422521, etc.) were upregu -lated. Macrophages that developed in presence of TCDD showed a reduced gene signature for genes associated with M2 macrophages. Transcription factor activity inferred from gene expression revealed lower activity of IRF4 and IRF8, important modulators of macrophage polarization, in the TCDD group. Overall IRF6 protein expression was significantly suppressed in TCDD treated day 14 and 21 in T cell and CD34+ cells. In conclusion, using a novel in vitro model of hematopoiesis that can be useful in developmental immunotoxicity testing, this study demonstrates that TCDD treatment skews human hematopoietic differentiation towards monocytes and granulocytes. We also identify changes in expression of key mediators that may account for the development of an aberrant hematopoietic profile with TCDD.

Exposure to combustible products of cigarette smoke (CS) is associated with several toxic outcomes. Hence, non-combustible tobacco products, such as heated tobacco products (HTP), which mainly release nicotine and humecants, could reduce and neutralize the toxicities caused by CS, and be considered as a potential harm reduction tool for current smokers. CS can trigger a variety of diseases by activating or suppressing physiological functions, including the immune system. T CD4+ are adaptive immune cells with high phenotypic plasticity, which can acquire an effector or regulatory profile. CS is a well-known risk factor for autoimmune disease because it disrupts the effector/regulatory T CD4+ balance; however, the impact of HTP remains unclear. To address the effects triggered by CS or HTP on T CD4+ cells, we employed in vitro and in vivo exposures to evaluate their outcomes on T CD4+ phenotype, activation, and functions. Human T CD4+ were isolated from peripheral blood mononuclear cells collected from healthy donors using microbe -ads. For such, we employed single-cell RNA sequencing (scRNA-seq) combined with surface protein staining to classify CD4+ T cells from smokers (n = 14) and non-smokers (n = 12) into four major subsets: naïve (Tn), central memory (TCM), effector memory (TEM), and late effector-memory (TEMra). Single-cell differential gene expression analysis revealed that compared to non-smokers the Tn cells of smokers had elevated expression of MYC (FDR = 6.58 x 10^-10), which plays a role in T cell transition from quiescence to proliferation upon activation, as well as MYC target genes (AHI1, DTT, IMPDH2, RPL9, RPS3, SRSF3, and CCT). Smokers’ CD8 Tn cells also showed elevated levels of CD27 (costimulatory molecule for T cell activation; FDR = 1.98 x 10^-9) and DHFR (FDR = 4.86 x 10^-21). Repression of DHFR expression is critical for maintenance of Tn cell quiescence. Notably, we detected expression of DHFR in only 1.6% of non-smokers’ CD8 Tn cells, whereas 8.8% of smokers’ CD8 Tn cells expressed DHFR. Taken together, these transcriptional changes indicate that CD8 Tn cells of smokers may be primed to exit quiescence, which provides a potential explanation for how cigarette smoke may contribute to memory inflation. We are currently analyzing single-cell T cell receptor data from these donors to determine whether Tn cells with loss of quiescence are clonally expanded or derived from a diverse group of CD8 T cells. Since escape from quiescence requires changes, we plan to further analyze scRNAseq data to confirm that smokers’ CD8 Tn cells show metabolic transcriptional signatures consistent with quiescence exit.
exposures impaired T cell proliferation and led to a hypo-responsive phenotype. Altogether, our findings show that CS and HTP exposures affect CD4+ T biology in different ways favoring an enhanced or impaired T cell response, which can culminate in immune-mediated disorders.

**4074 Δ^2-Tetraydrocannabinol (THC) and JWH-015 Treatment Suppress CD8^+ T Cell Secretion of Interferon Gamma (IFNγ), Interleukin 2 (IL-2), and Tumor Necrosis Factor Alpha (TNFα)***

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Δ^2-tetraydrocannabinol (THC) is a cannabinoid identified in Cannabis sativa and has been well characterized to possess immunomodulatory properties in addition to its psychotropic activity. THC is known to bind cannabinoid receptor (CB) 1 and CB2, which are predominantly expressed in the central nervous system and the immune system respectively. There is currently a knowledge gap on how THC affects primary human CD8^+ T cells, which is critical because of the role these cells play in various inflammatory conditions, including HIV associated neurocognitive disorders and irritable bowel syndrome. The purpose of these studies is to characterize the effects of THC treatment on primary human CD8^+ T cell inflammatory cytokine responses. For these studies, cultured primary human pan CD8^+ T cells were utilized containing both naive and memory cells to assess the secretion of interferon gamma (IFNγ), interleukin 2 (IL-2), and tumor necrosis factor alpha (TNFα) in response to T cell receptor (TCR) engagement. To determine the kinetics of cytokine secretion, a 5 day time course was conducted. The cells were activated via the TCR by plate bound anti-CD3 and soluble anti-CD28 antibodies. It was determined that the 3 days post activation was the peak time of response to sample supernatants for cytokine secretion. The following experiments implemented a 30 minute treatment with increasing concentrations (0.1, 0.5, 1, 5, or 10μM) of either THC or the selective CB2 agonist JWH-015 prior to activation. After pretreatment cells were activated as previously described and supernatants were collected 3 days post activation. THC and JWH-015 suppressed secretion of IFNγ, IL-2, and TNFα at the 1, 5, and 10μM concentrations, with no effect on cell viability. Additionally, THC treatment was capable of suppressing the secretion of all 3 cytokines at concentrations below the 1μM treatment, while JWH-015 was not. These data suggest that THC is capable of suppressing the CD8^+ T cell contribution to inflammation, and that this inhibition is partially mediated by cannabinoid ligation of CB2. Supported by NIH R01 DA053047 and T32 ES007255.

**4073 Asbestos Exposure-Characteristic Alteration in Gene Expression in Human CD8^+ T Cell Line Clarified by Transcriptome Analysis***

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We have earlier reported the features about asbestos exposure-caused suppression in immune functions by cell culture experiments, some of which were also found in patients with malignant mesothelioma or people exposed to asbestos. However, it is unclear what is the key event in the functional suppression. Recently, it was found that human CD8^+ T cell line EBT-8 continuously exposed to chrysotile asbestos shows decreased in intracellular perfom and production of IFN-γ. Then, five types of sub-lines were made by long-lasting cultures with or without two kinds of chrysotile (CA, JAWE), crocidolite (CR) fibers and titanium dioxide particles (TiO₂), and all of the sub-lines of CA, JAWE and CR showed gradual decrease in IFN-γ mRNA level with the increasing days of culture, whereas those of TNF-α and other examined genes do not decrease. Therefore, these five sub-lines were analyzed for transcriptome by next-generation sequencing (NGS). NGS analysis informed 29,638 consensus transcripts to be compared about expression levels, where there are five transcripts with 4 times or more alteration in expression common to CA, JAWE and CR, while 78 transcripts showed double that amount. Five those transcripts included IFN-γ, two known and two unknown genes. The expression levels of those four genes showed significant and good correlation with IFN-γ expression (r>0.9). Those alterations in expression of the two known genes were confirmed by real time RT-PCR. In contrast, CR also showed unique types of alteration in gene expression, where some part of gene expressions in CR negatively correlated with those in CA. Those findings indicate that continuous exposure to asbestos causes gradual alteration in gene expression pattern, which is common to different types of asbestos. It is possible that accumulated asbestos fibers in draining lymph nodes might cause those alterations in gene expression, leading to suppressed immune functions in the pleural cavity upon inhalation exposure to asbestos related with asbestos-related malignant mesothelioma. In addition, it was found that crocidolite asbestos exposure causes a different type of alteration in gene expression compared with chrysotile in addition to common feature, suggesting different immunological effects between those asbestos fibers.

**4071 Heated Tobacco and Cigarette Smoke Exposures Impair Human Macrophages Functions***

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Xenobiotics released by cigarette smoke (CS) exert pro-inflammatory and immunotoxicological effects, which are associated with respiratory and cardiovascular diseases, cancer, and autoimmune diseases. Non-combustible tobacco products are arising as a potential harm reduction alternative for current smokers. Heat-not-burn tobacco and HTP devices heat tobacco but do not burn it. HNBT releases mainly nicotine, and humectants and lower emission of toxic xenobiotics than CS. While it is known tobacco products exert dual effects in regulating immunity, resulting in complex mechanisms of toxicity, the immunotoxicity caused by HNBT is not known. Hence, we evaluated the effects of HNBT, CS, or nicotine exposures on US37-differentiated macrophages activated or not by lipopolysaccharide of E. coli (LPS). U937 monocytic lineage was differentiated into macrophages by phorbol 12-myristate-13-acetate (100 nM) for 48 hours. Afterward, cells were exposed for 30 min (2s of smoke/vapor followed by 28s of airflow) using peristaltic pumps, followed by culturing in the absence or presence of LPS (1μg/mL). In vitro exposure was characterized by quantifying total polycyclic aromatic hydrocarbons (PAH) amounts in the culture medium by gas chromatography-mass spectrometry (GC/MS). Macrophage viability was evaluated 24h after exposure by annexin V and 7AAD labeling was assessed by flow cytometry. Macrophage activation was evaluated in different periods after exposure to verify: oxidative stress markers (1 hour, flow cytometry), zymosan phagocytosis (2 hours, flow cytometry), cytokines release (24h, ELISA) and polarization (48 hours, flow cytometry). Levels of PAHs were reduced in HNBT exposed culture medium; exposures to HNBT and CS did not affect the viability of the macrophages; both HNBT and CS exposures increased the production of total reactive oxygen species (ROS), however, the exposures impaired the ROS induced by LPS. The same effects aforementioned by HNBT were observed in macrophages cultured with growing concentrations of nicotine (0.01, 0.1, 1μg/mL). Furthermore, pre-incubation of macrophages with a δ7-nicotine-aceetylcholine (o-BTX) receptor antagonist abolished the effects promoted by HNBT and nicotine exposures, which was not observed for CS. Only CS exposure per se increased the IL-10 release, and both HNBT and CS exposures impaired the IL-18, TNFα, and IL-10 secretion induced by LPS. Moreover, HNBT exposure increased the expression of CD163 M2 macrophage and CS exposure enhanced CD80 M1 macrophage. Altogether, our data show CS or HNBT exposures modify macrophage functions and polarization. CS or HNBT drives macrophages into inflammatory and anti-inflammatory phenotypes, respectively, and the HNBT effects are mediated by nicotine receptor.

**4072 Role of Autophagy in E-cig Vapor Condensate-Induced Inflammation in A549 Cells***

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In recent years e-cigarettes have rapidly gained popularity among youth and non-smokers due to extensive marketing to quit smoking. However, recent investigations suggest that the molecular consequences of vaping are quite like that of conventional smoking. Autophagy is a self-degradative process that is important for balancing sources of energy at critical times in development and in response to nutrient stress. Autophagy also plays a housekeeping role in removing misfolded or aggregating proteins, clearing damaged organelles as a way to eliminate intracellular pathogens. Thus, autophagy is generally thought of as a survival mechanism, being aggregated proteins, clearing damaged organelles as well as eliminating intracellular pathogens. Therefore, autophagy is an important process for balancing sources of energy at critical times in development and in response to nutrient stress.

Inflammation in A549 Cells

3 days post activation. THC and JWH-015 suppressed secretion of IFNγ, IL-2, and TNFα at the 1, 5, and 10μM concentrations, with no effect on cell viability. 3 days post stimulation was the peak time of response to sample supernatants for cytokine secretion. The following experiments implemented a 30 minute treatment with increasing concentrations (0.1, 0.5, 1, 5, or 10μM) of either THC or the selective CB2 agonist JWH-015 prior to activation. After pretreatment cells were activated as previously described and supernatants were collected 3 days post activation. THC and JWH-015 suppressed secretion of IFNγ, IL-2, and TNFα at the 1, 5, and 10μM concentrations, with no effect on cell viability. Additionally, THC treatment was capable of suppressing the secretion of all 3 cytokines at concentrations below the 1μM treatment, while JWH-015 was not. These data suggest that THC is capable of suppressing the CD8^+ T cell contribution to inflammation, and that this inhibition is partially mediated by cannabinoid ligation of CB2. Supported by NIH R01 DA053047 and T32 ES007255.

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Cannabidiol (CBD), first discovered in 1940, is one of 113 cannabinoids found within the Cannabis sativa plant and is considered non-psychotropic phytocannabinoid due to its extremely low binding affinity to the cannabinoid receptor (CB1). In recent years there has been a dramatic increase in use of CBD as an additive to skin creams and to food and beverage products. CBD is known to have immune modulating effects. This research study was prompted by a case study that reported that Epidiolex, a epilepsy drug made from CBD, caused some patients to experience liver toxicity. Preliminary experiments in our laboratory were conducted using RNA-seq on a human hepatocyte cell line (HepAR2) that was treated with varying concentrations of CBD to determine what genes were differentially regulated in response to CBD treatment. One gene of interest in particular, BTN2A2, was increased with CBD. BTN2A2 belongs to the butyrophilin family of genes that resemble the B7 protein (CD28). BTN2A2 has been noted to inhibit the stimulation of T lymphocytes, along with inhibiting proliferation in both CD4+ and CD8+ T cells. The objective of this research project is to determine the protein expression of BTN2A2 on the different cell types found in human peripheral mononuclear cells (PBMC) and the effect CBD has on the expression of BTN2A2 in human PBMCs. Human PBMC were treated with CBD (1, 3, 10, and 32mM) and incubated for various times (6, 24, 48 and 72 hours). RNA was isolated and RT-qPCR was performed for the expression of BTN2A2 and showed a modest upregulation in BTN2A2 after exposure to CBD at 6 hours post addition. Since PBMC are heterogeneous, cell surface staining was used to characterize the expression of BTN2A2 on specific cell types within the PBMC population (monocytes, B cells, T cells, NK and plasmacytoid dendritic cells (pDC) along with intercellular antibody staining for BTN2A2 and analyzed by flow cytometry. Monocytes showed the highest percent (91.3 ± 2.4%) and the highest mean protein expression per cell (geometric mean fluorescence index (gMFI) = 155116) followed by pDC (23.8 ± 7.9%, gMFI = 20424), B cells (21.4 ± 3.7%, gMFI = 45077), NK cells (10.6 ± 3.5%, gMFI = 41849) and then T cells (1.5 ± 0.2%, gMFI = 16508) (N=12). These studies show that the cell types with the highest expression of BTN2A2 are antigen-presenting cells (i.e., monocytes, pDC and B cells). Understanding how CBD affects the expression of BTN2A2 in human PBMCs could be a potential mechanism, in part, by which CBD elicits its immune modulating effects. Supported in part by NIH R01 DA407180 and R01 DA053047.

Impact of Endocrine Disruptors on In Vitro T Cell Differentiation

Endocrine disruptors (ED) are a class of synthetic or naturally occurring substances able to interfere with the endocrine system, altering their system's secretion, synthesis, transport, action, metabolism, or elimination. Recently, an increased interest was posed on ED, due to their negative impact on human health is rising. The exposure to ED may lead to adverse outcomes, mainly involving reproductive and hormonal diseases, but also immune disorders. The interconnection between endocrine and immune system is well established, and it is known that hormones and substances perturbing the endocrine pathways can influence immune cell functions. Therefore, it is reasonable to hypothesize that ED can interfere with the immune system and modulate it. In this study we selected 6 different classes of ED: atrazine (herbicide), cypermethrin (insecticide), ethyl phthalate (plasticizer), ethyl estradiol (contraceptive drug), perfluorooctanesulfonic acid (persistent organic pollutant), and vinclozolin (fungicide). The aim of this study was to analyze if the selected ED showed the ability to interfere with the expression of BTN2A2 in human PBMC could be a potential mechanism, in part, by which CBD elicits its immune modulating effects. Supported in part by NIH R01 DA407180 and R01 DA053047.

Cannabidiol (CBD) and Δ9-Tetrahydrocannabinol (THC) Differentially Suppress Inflammation in Toll-Like Receptor (TLR) 4, 7, and 8 Stimulated Human Monocytes

Cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) are two of the most studied cannabinoids in recent years due to the legalization of cannabis in many of the United States. Both cannabinoids have been observed to have anti-inflammatory effects in humans. When activated with various pathogen- and damage-associated molecular patterns, human monocytes will form multicellular protein complexes called inflammasomes. A hallmark of inflammasome formation is the relocalization of the apoptosis-associated speck-like proteins containing a caspase recruitment domain (ASC) which recruits inactive pro-caspase 1. Oligomerization of pro-caspase 1 induces apoptosis and results in active caspase-1. Caspase-1 will then cleave pro-forms of pro-inflammatory cytokines including interleukin-1β (IL-1β) into its active form which is then secreted from the cell. Previous work in the Kaminski lab has shown that both CBD and THC suppress IL-1β secretion in toll-like receptor (TLR) stimulated human monocytes. To determine if CBD- and THC-mediated suppression of IL-1β secretion by human monocytes is through the inhibition of inflammasome formation, human peripheral blood mononuclear cells (PBMCs) were pre-treated with either CBD (10 µM) or THC (10 µM) before a 7-hour incubation with various TLR agonists, including lipopolysaccharide (LPS), R837, and ssRNA40 (TLR4, 7, and 8 agonists, respectively). After the 7-hour incubation, flow cytometry was used to determine the expression of BTN2A2 on the different cell types found in human peripheral mononuclear cells (PBMCs). Supernatants were quantified via ELISA for IL-1B. THC significantly suppressed inflammasome formation in TLR4 stimulated monocytes (26.9%), while having no effect on inflammasome formation in TLR7 and 8 stimulated monocytes. CBD significantly suppressed inflammasome formation in TLR7 stimulated monocytes (40.3%), while having a modest effect on inflammasome formation in TLR4 and 8 stimulated monocytes. These results suggest that CBD and THC are mediating their immunomodulatory effects through different mechanisms to suppress inflammasome formation. Supported by NIH R01 DA053047 and T32 ES007255.

Cannabidiol (CBD) and the Influence of Blood Mononuclear Cells (PBMC) and the Influence of Cannabidiol (CBD)

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In conclusion, this study evidenced the ability of the selected ED to modulate the immune system, in particular T cell differentiation, with different profiles.
Evidence from animal and human studies indicate that perfluoroalkyl substances (PFASs) cause adverse effects on the immune system. The CONTAM Panel of EFSA defined a health-based guidance value of a series of PFASs based on human data on PFAS-induced decreased antibody response for certain vaccines. So far, mechanisms underlying PFAS-induced decreased antibody responses are poorly understood. Such data are important to support causality between chemical exposure and adverse effects observed in human observational studies. Also, these data may help to select readouts in in vitro test systems to study potency differences between PFASs and/or to provide in vitro points of departure for next generation risk assessment. In a project funded by the European Food Safety Authority (EFSA), we assessed the effects of PFDA and PFOS on test systems that were selected to be of biological relevance for the T-cell dependent antibody response, including a dendritic cell model, a cell model for T cell differentiation and activation, a model on B cell differentiation and antibody production, and an in vitro model mimicking the primary antibody response. PFDA and PFOS caused immunosuppressive effects in all cell models, showing model- and chemical-related differences in potencies. To better understand molecular and cellular effects underlying this immunosuppression, we performed RNA seq studies, to understand the effects of PFDA and PFOS on dendritic cell maturation, and T cell differentiation and activation. Various cellular processes showed to be affected, of which some were shared by both PFASs. Altogether, this study provides in vitro mechanistic insight that increases our understanding of reported immunotoxic effects of PFASs in vivo. This information will allow the selection of test systems and readouts to study the in vitro immunotoxicity of other PFASs for which no data are available.

Arsenic is a naturally occurring contaminant that is commonly found in water supplies. Chronic exposure to arsenic has been associated with multisystem disease, including respiratory diseases such as viral infections. Arsenic is known to regulate innate cell activity and has been shown to alter NK cell function and activity. However, our knowledge on effects of arsenic on NK cell response to influenza remains limited. The following study aims to identify the effects of arsenic trioxide on NK cell activation and function against influenza A virus in human primary immune cells. Primary human peripheral blood mononuclear cells were treated with environmentally-relevant concentrations of arsenic trioxide prior to an influenza A virus challenge. Flow cytometric analysis of NK cells showed arsenic trioxide significantly decreased NK cell viability. Additionally, NK cell activation markers, CD69 and NKp46, were reduced with increasing concentrations of arsenic. Consistently, the production of NK cell effector mediators, including IFN-γ, granzyme B and perforin, were significantly impaired with treatment of arsenic. Overall, these data suggest that environmentally relevant concentrations of arsenic trioxide impaired NK cell responses to influenza A virus, which increases our understanding of reported immunotoxic effects of PFASs.

This study was supported by the NIH R01 ES024966 and R21 ES038380.

Acute respiratory distress syndrome (ARDS) is a major trigger of mortality associated with coronavirus-induced disease (COVID-19) due to development of life-threatening acute lung injury (ALI). Leukocyte infiltration, pro-inflammatory cytokine production, poor oxygenation, and respiratory failure are major clinical presentations of ALI. At present, there is no effective and specific treatment against such lethal symptoms in ARDS patients. Our lab already showed that 2, 3, 7, 8-Tetrachlorodibenz-p-dioxin (TCDD), one of aryl hydrocarbon receptor (AhR) ligands and a potent toxicant, attenuated several inflammatory processes in different murine preclinical models of inflammatory, autoimmune, and hypersensitivity diseases. Using Lipopolysaccharide-endotoxin (LPS)-induced murine preclinical model, our data showed that TCDD treatment effectively improves pulmonary functions. Furthermore, reduction of pulmonary leukocyte infiltration and circulating cytokine tissue necrosis factor alpha (TNFα) were major observations in ALI-female mice treated with TCDD. We aimed epigenetic regulation of TCDD on LPS-activated lung mononuclear immune cells (MNCs) in which specific pathways such as leukocytes trafficking and migration, and immune cells apoptosis and proliferation were altered. MicroRNAs (miRs) are 21-23 nucleotides, single-stranded, non-coding RNA molecules that epigenetically control gene expression. Microarray analysis revealed that 127 miRs were dysregulated (71 were up-regulated and 56 were down-regulated) in pulmonary MNCs upon TCDD treatment. Interestingly our analysis showed that miR-34a was up-regulated, while both miR-23a and miR-27a were downregulated in TCDD treated mice. Ingenuity pathway analysis (IPA) predicted that immunosuppressive genes were among the targets of these miRs. Pulmonary MNCs in vitro transfection studies confirmed that immunosuppressive genes such as Arginase1, TGF-β, and FOXP3 were direct targets of these miRs and their expression was dysregulated accordingly as well. Furthermore, apoptosis induction genes of pulmonary MNCs were validated as target genes of these dysregulated miRs.

Together, our study demonstrates that TCDD mitigates LPS-induced ARDS progression through epigenetic regulation of pulmonary MNCs via cytokine reduction. Thus, AhR could be a target of potential therapeutic strategies for more effective and specific treatment of ARDS. This work was supported in parts by NIH grants R01ES020144, P01AT003961, P20GM135641, and R01AI123947, R01AI160696.

Immune function can be impaired by environmental contaminants. One class of chemicals recently shown to interfere with the immune system is per- and polyfluoroalkyl substances (PFAS). PFASs are ubiquitous in both the environment and human tissues and have been shown to interact with the immune system, though disruptions to the innate immune system have also been identified. These studies indicate that PFAS exposure can influence the numbers of innate immune cells, cellular signaling, and functional endpoints. For example, we reported that certain PFAS can reduce the oxidative burst in vivo in larval zebrafish (Danio rerio), in vitro in a human neutrophil-like cell line, and ex vivo in primary human neutrophils. To complement these neutrophil studies, we are evaluating how macrophages are affected by a 2-day (in vitro) and 4-day (in vivo) exposure to ten PFASs: perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), Nafion Byproduct 2, perfluoro-2-methoxyacetic acid (PFMOAA), and hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). In single-cell PFAS cytokicity studies with macrophage-like THP-1 cells, exposure to 320 μM PFOA, PFNA, PFOS, and Nafion Byproduct 2 significantly reduced viability. We observed no changes in cell viability at or below exposures to 80 μM PFAS. We are currently investigating how phagocytosis is affected during PFAS exposures using both zebrafish larvae and THP-1 cells: macrophage populations derived from zebrafish and THP-1 cells will be challenged with fluorescent heat-killed E. coli. Phagocytic index and number will be measured with flow cytometry. Thus far, we have observed that PFOS, but not PFOA, increases the average extent of phagocytosis. Based on these functional assays, 2-3 PFASs will be selected for further studies to elucidate currently unknown molecular mechanisms of PFAS immunotoxicity.

Understanding how PFAS affect innate immunity will help us better understand how these chemicals can alter an organism’s ability to recognize and destroy pathogens in its environment as well as infected or transformed cells.

Alcohol is an underestimated toxicant, and binge drinking is remarkably prevalent in the US. Acute alcohol intoxication can cause impaired brain function, dilation of blood vessels, increased risk of certain cancers, stroke, and liver diseases like cirrhosis, and suppression of inflammatory responses. Macrophages are scavenger cells and a fundamental part of innate and adaptive immune responses, and they are important in wound repair and tissue remodeling. The functions of macrophages include engulfing and destroying pathogens, processing and presenting antigens, initiation of inflammation, secreting cytokines and other inflammatory mediators, and participating in the maintenance and repair of tissues. There is clear evidence that consuming excessive amounts of alcohol impairs macrophage function. However, the mechanism is not fully understood. We hypothesize that there is no tolerance of macrophage polarization, a process of differentiation to a specific cell type. To assess the effects of the toxicity of alcohol on macrophage polarization, the mouse macrophage cell line RAW 264.7 cells were employed as an in vitro model which were extensively used for the study of macrophages responses and their products. Three sets of experiments have been conducted. Set One, a

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Organophosphate esters (OPEs) are an emerging class of toxicants comprising a diverse group of chemicals used as flame retardants. Some OPEs are immunotoxic. In macrophages, exposure to individual OPEs has been found to induce significant functional alterations. However, research thus far has focused on single OPEs. In the environment, OPEs exist as mixtures in which 1) certain OPEs have an increased relative abundance; and 2) some OPEs have opposing activities. Previous research from our lab focused on an environmentally relevant mixture of OPEs modelling Canadian household dust. We found that macrophages exposed to the Canadian household OPE mixture develop a dose-dependent increase in lipid accumulation as assessed by Nile red staining. Based on these data, we hypothesize that the OPE mixture disrupts lipid homeostasis with resultant deficits in macrophage function. In the present study, THP-1 human monocytes were exposed to phorbol 12-myristate 13-acetate to induce differentiation before treatment with diluted concentrations of the Canadian household OPE mixture (1x10^-6, 5x10^-4, 2x10^-4) for 48 hours. Utilizing confocal imaging of the PLIN2 lipid droplet marker, we found that exposure to OPEs increases the number, but not the volume, of lipid droplets. This is consistent with previous observations. We further show that cholesterol is significantly increased following a 48-hour exposure to OPE mixtures (5x10^-4 and 2x10^-4) using an AMPLEX red assay for the measurement of total and free cholesterol. Thus, cholesterol accumulation correlates with increased lipid droplets. The accumulation of lipid in macrophages is known to generate functional deficits such as reduced effectorcytosis and migration. We assessed effectorcytosis, the recognition and degradation of apoptotic cells, by fluorescently labelling apoptotic T-cells and measuring their engulfment by THP-1 macrophages exposed to the OPE mixture for 48 hours. Preliminary data suggest that macrophages pre-treated with the OPE mixture have reduced migratory capacity at a dilution of 2x10^-4, the same dilution found to suppress effectorcytosis. Together, these data suggest a link between OPE exposure, cholesterol accumulation, and functional deficits in macrophages. Funded by CIHR.
4088 The Cross-Talk Between Complement Components and Neuro-Immu-Metabolic Pathways in in Vitro Alzheimer’s Disease Model
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Hyperglycemia and oligomeric amyloid-β (OAB) accumulation induced insulin resistance (IR) is one of the prominent causes of oxidative stress leading to the neuronal death in diabetic patients experiencing Alzheimer’s Disease. The cross talk between complement factors and neuro-immuno-metabolic pathways becomes increasingly important in reprogramming of neuronal cells. To model the neuronal damage under conditions mimicking the Alzheimer’s disease, SH-SYSY cells were exposed to OAB at high glucose and insulin concentrations in different periods of time. The effect of de nova synthesis of C1q, CsA and CD59 on neuron viability were evaluated. The alteration in the IR51, GLUT3 and complement factors were determined by ELISA, while NOx concentrations were measured spectro photometrically. In the neurons exposed to high glucose-insulin, the decrease in GLUT3, NOx and IRS1 levels were the result of neuronal IR, in which the increases in oxidative stress and glutamate toxicity were accompanied (p<0.05). Whereas, high glucose-insulin-OAB group could not meet the IR criteria. Both CD59 and CsA showed parallel alterations with C1q in all experimental conditions, in which the presence of glucose was mandatory. The resistance of neurons to complement damage was increased via the modulation of C59 and C1q, while IR decreased the C1q and Cd59 levels. This study was supported by TUBITAK, 214S112.

4089 Effect of Sex Hormones on Extracellular Vesicle Function
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The American Burn Association estimates nearly ~500,000 individuals suffer from burn injuries in America each year that require medical burn care; resulting in ~3500 deaths per year. Mortality is due to bacterial infection as a consequence of severe cytokine dysfunction and impaired wound healing. Previous research conducted in our group has shown females have worse outcomes than males following burn injury, but the reasoning is unknown. We hypothesize that concentrations of Estrogen can create a pro-inflammatory effect in epithelial cells, which is controlled in a negative feedback loop. To test this hypothesis, we utilized an in vitro cell model using human Airway Epithelial Cells (hAECS). We stimulated hAECS with 250 nM of estradiol for 24 hours and isolated extracellular vesicles (EVs) from the supernatant. miRNA was isolated from the EVs using Qiagen miNeasy Mini Kit and differential expression of miRNA was assessed using NanoString technolog (nCounter Human v3 miRNA panel). Following estradiol stimulation several miRNAs were significantly induced by estradiol-stimulation compared to control, including miR-378a, miR-384a and miR-100-5p (p-value < 0.05). Ingenuity Pathway Analysis (IPA) was performed to assess miRNA-miRNA interactions. IPA resulted in several miRNA-miRNA targets that play role in biological pathways of immunity or pathogen response, for example miR-100-5p has known targets to several miRNAs including MTOR, RPTOR and TLR2. These data suggest following exposure to estradiol, there are differential expression of miRNA within EVs, which may affect miRNA regulation by altering the immune pathways leading to the described sex differences in burn injury.

4090 The Proximity of High-Traffic Roads Affect the Immunotoxicity of Pollen
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Pollen and air pollution particles are both significant respiratory health stressors worldwide. Studies have shown that the properties of pollen can be influenced by interactions with air pollutants, thereby altering their allergenicity and even the severity of pollen-induced symptoms. This study aimed to examine whether birch pollen harvested from trees grown in locations with different levels of air pollutants differ in their immunomodulatory properties. We collected pollen samples from six different locations, including downtown, sites along major roads, and parks distant from major roads in Kuopio, Finland. Pollen samples were analyzed for elemental composition with a scanning electron microscope. Cell model of the first line of defense i.e., culture of human alveolar epithelium (AS49) and macrophages (THP-1) was exposed to three doses of pollen extracts (concentrations: 0.625 mg/ml, 2.5 mg/ml, and 10 mg/ml) for 24 h. LPS and commercial reference pollen were used as controls. Toxicological endpoints (viability, metabolic activity, oxidative stress), cell cycle phases, and several cytokines (eotaxin, GM-CSF, IFN-γ, IL-10, IL-1β, IL-6, MCP-1, MDC, MIP-1β and TNFα) were analyzed. Pollen samples collected near high-traffic roads contained more metallic elements. When assessing the toxicological properties of pollen, no differences in cell viability and only a few differences in metabolic activity were seen, whereas oxidative stress responses differed between pollen samples collected from different locations. Pollen harvested from trees growing along the freeway showed the highest oxidative potential. Pollen collected downtown induced the highest cytokine release (i.e., TNF-α, GM-CSF, and MIP-1β), while pollen collected near minor roads and parks induced the lowest secretions. Our study shows that the growing location affects the immunotoxicological properties of pollen.

4091 A New Model to Detect and Analyze the Human T Lymphocyte Response to Chemical Sensitizers
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Allergic contact dermatitis, caused by contact sensitizers, is a delayed type IV hypersensitivity reaction that is T-cell dependent. The study aims to set up a highly sensitive model to detect human T lymphocytes responding to chemical sensitizers and to semi-quantify their frequency. We have developed an in vitro human monocye-derived dendritic cells (mo-DC) / T lymphocyte co-culture assay, allowing us to evaluate the activation of T lymphocytes and their subpopulations (CD4+ or CD8+) using human peripheral blood mononuclear cells (PBMCs) from healthy donors. Purified T cells were seeded in multiple wells and stimulated weekly by autologous mo-DCs previously loaded overnight with the chemical sensitizers in media supplemented with 10% human AB serum. Chemical-specific T lymphocytes were detected after three rounds of stimulation using an IFN-γ Elispot assay. The analysis was considered valid if the number of spots counted was greater than 30 in the loaded wells and if the spot count was at least two-fold higher in the presence of the chemical sensitizers compared to unloaded DCs. A second IFN-γ Elispot was performed on positive cell lines using antibodies directed to MHC class I or II molecules. We were able to detect dinitrobenzene sulfonic acid (DNBS), cinnamaldehyde (Cia), isoeugenol (IscE), nickel sulfate (NiSO4), cobalt chloride (CoCl2), and methylisothiazolinone (MIT) responding T lymphocytes in 4/24, 2/3, 3/2, 2/3 and 2/2 of tested donors respectively. We calculated a mean frequency of 1.06 for DNBS, 0.97 for Cia, 0.30 for IscE, 2.00 for NiSO4, 0.58 for CoCl2, and 1.05 for MIT per million circulating T lymphocytes using a Poisson distribution law. We tested twenty-one T-cell lines specific to DNBS and seventeen specific cell lines to Cia in a secondary Elispot assay. The results confirmed the positivity of these T-cell lines and showed that the response was primarily dependent on MHC class I molecules for DNBS, indicating the implication of CD8+ T-cells. However, CD4+ T-cell lines mostly responded to Cia. Our results contribute to a better understanding of the mechanism of immunization to chemical sensitizers and propose a T-cell assay to identify T lymphocytes responding to chemical sensitizers.

4092 A Role for Microplastic Fibers and Azobenzene Disperse Dyes in Allergic Airway Disease

Microplastic fibers (MPFs) of various sizes have been detected in human lung tissue and may harbor chemical additives, such as azobenzene disperse dyes (ADDs), which are of high toxicological concern as they have been shown to have carcinogenic and mutagenic properties. The effects of both MPFs and ADDs on the pulmonary system remain underexplored despite epidemiological reports finding occupational asthma to be associated with microplastic and ADD exposure. To examine the reactivity of ADDs to airway epithelial cells (SAECs) and human immature monocyte derived dendritic cells (iMDDCs), we exposed to three ADDs that constitute an abundant black dye used in textiles: disperse blue 373 (DB373), disperse violet 93 (DV93), and disperse orange 61 (DO61). Cells were exposed to a range of ADD concentrations (0.01µg/mL to 4µg/mL) and cell viability was quantified after 48hrs or 24hrs using a CellTiter-Glo assay. To identify elevated mRNA expression of genes associated with an asthmatic response in vitro, RT-qPCR was performed on SAECs and iMDDCs exposed to 1ug/mL of DB373 and 1ug/mL of DV93 for 24 hours. Results showed that DV93 lead to the greatest decrease in cell viability for both cell types. For SAECs, DV93 and DB373 resulted in an upregulation of lung injury and asthmatic genes (TGF-β1, IL-8, TSLP, etc.) compared to control cells. For iMDDCs, DV93 resulted in an upregulation of Nrf2-regulated genes (HMox1 and Srxn1), highlighting an important molecular initiating event involved with sensitization. Next, intracellular reactive oxygen species (ROS) production was determined in iMDDCs following exposure to a range of ADD concentrations for 6 hours. Results showed that DV93 resulted in the greatest decrease in cell viability for both cell types. For iMDDCs, DV93 and DB373 resulted in an upregulation of lung injury and asthmatic genes (TGF-β1, IL-8, TSLP, etc.) compared to control cells.

For iMDDCs, DV93 resulted in an upregulation of Nrf2-regulated genes (HMox1 and Srxn1), highlighting an important molecular initiating event involved with sensitization. Next, intracellular reactive oxygen species (ROS) production was determined in iMDDCs following exposure to a range of ADD concentrations for 6 hours. Results showed that DV93 resulted in the greatest decrease in cell viability for both cell types. For iMDDCs, DV93 and DB373 resulted in an upregulation of lung injury and asthmatic genes (TGF-β1, IL-8, TSLP, etc.) compared to control cells.
Maine, Augusta, ME.

Ca ++ was inhibited by 30 min CPC pre-exposure. Plasma membrane potential (PMP) and efflux. CPC inhibits Syk phosphorylation following 30 min pre-treatment detected documented VLS. These results provide evidence that IL-2-mediated permeability changes at concentration ranges relevant to clinical exposure not associated with adverse findings which confirm the specificity of the transwell assay. Taken together, these results support an in vitro HUVEC-based transwell assay which can identify risks associated with drug-induced vascular permeability changes. Incubation of HUVEC cells with supernatants collected from pre-treated PBMCs can detect effects of drugs that induce secondary immune-mediated vascular permeability changes. This in vitro approach can potentially be used to investigate clinically relevant vascular leakage associated with cytokine drug candidates.

Cetylpyridinium chloride (CPC), the positively-charged broad-spectrum antimicrobial, has been used widely in cosmetic products and agricultural processes at concentrations up to 3 mM, thus exposing much of the U.S. populace to significant levels of CPC. However, minimal information exists on the eukaryotic toxicology of CPC; hence, there is an urgent need for information. Mast cells, ubiquitous throughout the human body, particularly at interfaces, are implicated in many diseases and key players in normal immune and nervous system functioning. We have demonstrated that CPC potently (low-µM) inhibits antigen (Ag)-stimulated function of RBL-2H3 mast cells, including degranulation. We have interrogated the molecular mechanisms underlying CPC’s inhibition of degranulation. Following 30 min pre-treatment, CPC drastically inhibits Ag-stimulated store-operated Ca ++ entry (SOCE) into the cytosol, a core mediator of the degranulation pathway. Inhibited SOCE may be caused by CPC’s inhibition of Ca ++ influx from the endoplasmic reticulum (ER) into the cytosol, a trigger of SOCE. In turn, Ag-stimulated mitochondrial Ca ++ uptake from the ER is reduced by CPC. Inhibited ER Ca ++ influx can be caused by CPC’s interference with Ag-stimulated tyrosine phosphorylation and phosphatidylinositol 4,5 bisphosphate, which together provide the trigger signal for ER Ca ++ influx. CPC inhibits Syk phosphorylation following 30 min pre-treatment detected via ELISA and western blot. Using in-Cell Western, global tyrosine phosphorylation was inhibited by 30 min CPC pre-exposure. Plasma membrane potential (PMP) and cytosolic pH, contributors to SOCE, are not affected by CPC. Dampered cytosolic Ca ++ leads to CPC inhibition of microtubule polymerization, which is necessary for degranulation. This work outlines biochemical mechanisms underlying the effects of CPC on immune signaling and allows the prediction of CPC effects on disparate cell types that share similar signaling elements.

Vascular Leakage Syndrome (VLS) is characterized by an increase in capillary permeability accompanied by a progressive extravasation of fluids and proteins which often result in edema and organ failure. Several cytokine and cytokine muten therapy candidates, including tumor necrosis factor α (TNFα) and interleukin-2 (IL-2), have been reported to induce VLS in humans via primary or secondary immune-mediated effects on the endothelium. Several endothelial cell-derived transwell assays have been historically been developed to inform on the potential for drug-induced vascular permeability changes. These systems showed some promise in detecting agents which directly impact endothelial functions; however, they were known to be less sensitive to cytokine-induced endothelial damage through secondary immune activation mechanisms. In the present study, various in vitro models were utilized to establish a platform that can detect both primary and secondary effects of cytokine-mediated vascular permeability changes. Human umbilical vein endothelial cells (HUVECs) were cultured in a hanging intent to allow for the formation of a monolayer with tight junctions. Inserts were either treated with cytokines directly, co-cultured with peripheral blood mononuclear cells (PBMCs) in transwells or incubated with supernatants collected from PBMCs pre-treated with different cytokines. Results showed that incubation of cytokines known to directly interact with endothelial cells (i.e., TNFα, or IL-1β) induced a dose-dependent increase in permeability after a 24-hour incubation, demonstrated by an increase in fluorescently labeled dextran across the cell monolayer. In contrast, increases to HUVEC monolayer permeability were not observed following incubation with IL-2 alone for up to 72 hours. However, incubation with supernatants collected from IL-2-treated human PBMCs resulted in increased permeability within 24 hours. Concentrations of IL-2 used to activate PBMCs were in the range of Cmax observed in Aldesleukin (recombinant human IL-2) patients with documented VLS. These results provide evidence that IL-2-mediated permeability changes are mainly attributed to a secondary effect via treatment-related release of cytokines or potentially other soluble factors from activated PBMCs. Increased permeability was not observed in HUVEC/PBM coculture up to 72 hours due to a suppression of IL-2-mediated lymphocyte activation by hydrocortisone in the HUVEC culture media, which further indicated the importance of lymphocyte activation in vascular permeability changes. The cytokine not known to be associated with VLS in the clinic, including IL-4, did not induce permeability changes at concentration ranges relevant to clinical exposure not associated with adverse findings which confirmed the specificity of the transwell assay. Taken together, these results support an in vitro HUVEC-based transwell assay which can identify risks associated with drug-induced vascular permeability changes. In particular, incubation of HUVEC cells with supernatants collected from pre-treated PBMCs can detect effects of drugs that induce secondary immune-mediated vascular permeability changes. This in vitro approach can potentially be used to investigate clinically relevant vascular leakage associated with cytokine drug candidates.

The activation of aryl hydrocarbon receptor (AhR) in the gastrointestinal tract plays a role in maintaining intestinal homeostasis. Tryptophan metabolites are so far the most studied endogenous ligands of AhR. There are three tryptophan metabolic pathways in the gut including the kynurenine and serotonin pathway in the intestinal cells, and the indole pathway in the gut microbiota. This study aimed to identify the involvement of intestinal tryptophan metabolism in the pathogenesis of inflammatory bowel disease (IBD) and investigate their role in intestinal AhR activation. In this study, clinical information, dietary intake data and fecal samples were collected from patients [10] undergoing colonoscopy in a patient population with ulcerative colitis (UC) patients in active status (n=20) and remission (n=20). Cohn’s disease (CD) patients in active status (n=20) and remission (n=20). Fecal water (FW) was extracted and added to human colonic epithelial cells (Caco-2), to measure their AhR activation levels by using ethoxyresorufin-O-deethylase (EROD) assay. Tryptophan metabolites were measured by liquid chromatography-quadrupole-time-of-flight-tandem mass spectrometry. As a result, the FW derived from IBD patients induced significantly lower AhR activation in Caco-2 cells, when compared to FW from HCs. A negative correlation was observed between AhR activation by FW and intestinal inflammation, as indicated by increase in ileal Caco-2 intestinal permeability (Coefficient=-0.655, P<0.0135). There is no difference in either dietary tryptophan intake or fecal tryptophan concentration between groups. However, tryptophan metabolite indole was significantly increased in FW from IBD patients, especially patients with active inflammation. Fecal indole-3-acetic acid (IAA) and serotonin levels were significantly decreased in IBD patients, with more reduction in CD patients. In addition, the activation of kynurenine pathway (elevated kynurenine/tryptophan) is positively correlated to intestinal inflammation, but negatively correlated to AhR activation ability of FW. On the contrary, the enhanced serotonin pathway and IAA production by gut microbiota (increased serotonin/tryptophan and IAA/tryptophan) were negatively associated with AhR activation ability of FW. The dominant AhR agonist in the FW still needs to be further identified.

Vascular Leakage Syndrome (VLS) is characterized by an increase in capillary permeability accompanied by a progressive extravasation of fluids and proteins which often result in edema and organ failure. Several cytokine and cytokine muten therapy candidates, including tumor necrosis factor α (TNFα) and interleukin-2 (IL-2), have been reported to induce VLS in humans via primary or secondary immune-mediated effects on the endothelium. Several endothelial cell-based transwell assays have been historically been developed to inform on the potential for drug-induced vascular permeability changes. These systems showed some promise in detecting agents which directly impact endothelial functions; however, they were known to be less sensitive to cytokine-induced endothelial damage through secondary immune activation mechanisms. In the present study, various in vitro models were utilized to establish a platform that can detect both primary and secondary effects of cytokine-mediated vascular permeability changes. Human umbilical vein endothelial cells (HUVECs) were cultured in a hanging intent to allow for the formation of a monolayer with tight junctions. Inserts were either treated with cytokines directly, co-cultured with peripheral blood mononuclear cells (PBMCs) in transwells or incubated with supernatants collected from PBMCs pre-treated with different cytokines. Results showed that incubation of cytokines known to directly interact with endothelial cells (i.e., TNFα, or IL-1β) induced a dose-dependent increase in permeability after a 24-hour incubation, demonstrated by an increase in fluorescently labeled dextran across the cell monolayer. In contrast, increases to HUVEC monolayer permeability were not observed following incubation with IL-2 alone for up to 72 hours. However, incubation with supernatants collected from IL-2-treated human PBMCs resulted in increased permeability within 24 hours. Concentrations of IL-2 used to activate PBMCs were in the range of Cmax observed in Aldesleukin (recombinant human IL-2) patients with documented VLS. These results provide evidence that IL-2-mediated permeability changes are mainly attributed to a secondary effect via treatment-related release of cytokines or potentially other soluble factors from activated PBMCs. Increased permeability was not observed in HUVEC/PBM coculture up to 72 hours due to a suppression of IL-2-mediated lymphocyte activation by hydrocortisone in the HUVEC culture media, which further indicated the importance of lymphocyte activation in vascular permeability changes. The cytokine not known to be associated with VLS in the clinic, including IL-4, did not induce permeability changes at concentration ranges relevant to clinical exposure not associated with adverse findings which confirmed the specificity of the transwell assay. Taken together, these results support an in vitro HUVEC-based transwell assay which can identify risks associated with drug-induced vascular permeability changes. Incubation of HUVEC cells with supernatants collected from pre-treated PBMCs can detect effects of drugs that induce secondary immune-mediated vascular permeability changes. This in vitro approach can potentially be used to investigate clinically relevant vascular leakage associated with cytokine drug candidates.
Aryl hydrocarbon receptor (AhR) plays an important role both as an environmental sensor and a regulator of the immune response. In the current study, we investigated the role of AhR in colitis, a chronic inflammatory disease of the gastrointestinal tract. Colitis is caused by the multifaceted interplay between microbial dysbiosis, dysregulation of host immune response, environmental factors, and host genetic profile. To that end, we investigated the effect of natural AhR ligands such as indole-3-carbinol (I3C) on 2,4,6-trinitrobenzenesulfonic acid (TNBS) or dextran sulfate sodium (DSS)-induced colitis. We found that I3C attenuated colitis which was associated with decreased inflammation in the colon. Microarray analysis of intestinal epithelial cells from TNBS-induced colitis mice treated with I3C revealed a significant increase in AhR signaling pathways and induction of antimicrobial peptides (AMPs) such as cryptdin, Reg3b, and mucins, including muc2 and muc3, when compared to vehicle-treated mice with colitis. Our analysis also found that LPS/IL-1 pathway was upregulated in TNBS-induced colitis mice suggesting that TNBS enhances LPS-producing bacteria and inflammation. However, I3C decreased the LPS/IL-1 pathway by modulating the microbiome in the ileum. We validated the data by studying the colonic expression of AMPs and mucins. Our analysis found that I3C significantly increased the mRNA expression of AhR, antimicrobial peptides such as β-defensins, cryptdin, Reg3b, and mucins such as Muc2, when compared to vehicle-treated mice with colitis. Similarly, the mRNA levels of AhR, AMPs, and mucins were significantly increased in DSS+I3C-treated MC-38 cells (murine colon carcinoma cells) when compared to DSS-treated MC38 cells. Together, the current study suggests that I3C attenuates colitis primarily through AhR-mediated induction of AMPs and protective mucins, resulting in the enrichment of beneficial gut microbiota. This work was supported in part by NIH grants R01ES030144, P01AT003961, P20GM103641, and P01AI123947, R01AI160896.

4097 Indole-3-Carbinol Prevents Colitis through Induction of Antimicrobial Peptides and Mucins in an AhR-Dependent Manner

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C57BL/6j female mice were challenged with HDM, and PBS was used as a control. Significant increase in asthma scores after HDM exposure was observed in female mice compared to males. HDM-exposed female mice had increased expression of pro-inflammatory mediators including TNF-α, IL-6, and IL-13 when compared to male mice. Our results indicate that sex determines the response of the immune system to HDM, and this sex difference may be attributed to hormonal effects. Further, the expression of miR-135a-5p was significantly higher in female mice compared to males. We hypothesize that hormonal effects or chromosomal mechanisms responsible for the higher susceptibility of females to asthma. This work was supported by NIH grants R01ES030144, P01AT003961, P20GM103641, and R01AI123947, R01AI160896.
The role of lysosomal cholesterol in the re-emergence of coal workers pneumoconiosis reported in certain U.S. Appalachian states was investigated by employing an experimental rat model of lung toxicity. A computer-controlled, automated aerosol generation system was custom-built and employed to generate aerosols containing crystalline silica (Min-U-Sil 5) or coal dust (Keystone Mineral Black 325BA). Determination of the particle size distribution in the aerosol samples generated, using a micro-orifice uniform deposit impactor (MOUDI), showed a mass median aerodynamic diameter of 1.6 μm (geometric standard deviation (g.s.d.) 1.6) and 1.36 μm (g.s.d. 2.3), respectively, for the crystalline silica and coal dust particles. Male Fisher 344 rats (n=4/group) weighing approximately 200 g were used in the whole-body inhalation exposure lung toxicity study. The four exposure groups were: 1. Filtered-Air (6 hours/day, 5 days/week during week 1 followed by 6 hours/day, 4 days/week for weeks 2, 3, and 6 hours/day, 3 days during week 4), 2. Min-U-Sil 5 (15 mg/m³, 6 hours/day, 5 days during week 1 followed by filtered air for 6 hours/day, 4 days/week during weeks 2-5), 3. Coal dust (filtered air for 6 hours/day, 5 days during week one followed by coal dust, 10 mg/m³, 6 hours/day, 4 days/week during weeks 2-5), and 4. Min-U-Sil 5 + coal dust (Min-U-Sil 15 mg/m³, 6 hours/day, 5 days during week 1 followed by coal dust, 10 mg/m³, 6 hours/day, 4 days/week during weeks 2-5). At the end of the fifth week, since the initiation of the first exposure, the rats were euthanized, and bronchoalveolar lavage (BAL) was performed to determine the induction of lung injury. Exposure of rats to Min-U-Sil 5 or coal dust alone did not change lactate dehydrogenase (LDH) activity in bronchoalveolar lavage (BAL) fluid at the post-exposure time interval when the analysis was performed. On the other hand, combined exposure of the rats to Min-U-Sil 5 and coal dust, at the same post-exposure time interval, resulted in a 1.3-fold increase in LDH activity suggesting a modest induction of lung toxicity in the rats, compared to the individual agents. Similarly, the number of PMNs detected in the Min-U-Sil 5 alone, coal dust alone, or Min-U-Sil 5 + coal dust exposed rats were 1.41, 1.13, and 4.24-fold higher than air controls. Interestingly, in rats co-exposed to combined exposure, a similar trend in the generation of oxidants by the lung phagocytes was detected in the rats exposed to the test agents alone or in combination. Collectively, these results indicate that the combined exposure to Min-U-Sil 5 (crystalline silica) and coal dust results in lung toxicity induced by the agents separately, under the conditions employed in the current study, did not result in significant lung toxicity. This data is consistent with the theory of potential involvement of crystalline silica in the re-emergence of coal workers pneumoconiosis reported in the US.
differentiation. To examine if ACAT-1 inhibition limits macrophage activation, K-604 was co-administered with M-CSF in wild-type derived cells. Cells were then treated with LPS on d7 and harvested after 24h. Nitrite, Nos2 expression, and iNOS protein density were determined through nitrite colorimetric measurement, RT-qPCR, and western blot, respectively. Seahorse™ Glycolysis and Mito Stress Tests determined glycolytic function and oxygen consumption rate. Nitrite (μM) and cAMP (μM) were measured utilizing the CEA assay and Western Blot (WB) respectively. DEP+LPS elicited IL-1β and TNF-α secretion. Expression of these inflammatory markers showed a dramatic increase (NLRP3: 5.0 ± 0.6 (mean ± SEM) fold vs. filtered air (FA) + saline control; IL-1β: 12.9 ± 2.8; caspase-4: 2.0 ± 0.6; IL-6: 29.8 ± 5.7; MCP-1: 4.1 ± 7.6; and TNF-α: 4.6 ± 0.7) compared to the saline control group. Mice that inhaled DEP and underwent LPS challenge showed increases in IL-1β up to 18.9 ± 10.2 fold; IL-6 (142.2 ± 56.0 fold), and MCP-1 (154.4 ± 48.6 fold) expression compared to those that inhaled FA similarly underwent LPS challenge. Immunoblots showed significantly elevated expression (3.0 ± 1.0 fold) and cleavage (3.7 ± 0.6 fold) of pro-IL-1β in mice that underwent DEP inhalation and subsequent LPS challenge. The amount of secreted IL-1β in BAL fluid increased with DEP inhalation (FA+LPS vs. DEP+LPS; 25.1 ± 10.9 vs. 64.8 ± 10.6 pg/mL). Cleavage of caspase-1 (2.3 ± 0.7 fold) and expression of NLRP3 (0.4 ± 0.2 fold) demonstrated an increased trend. Expression of GSDMD, another important component of the inflammasome pathway, was also increased (2.9 ± 0.7 fold). Elevated IL-1β production and secretion, combined with increases in GSDMD, NLRP3, and caspase-1, are consistent with enhanced inflammasome activation. In conclusion, our data show that repeated DEP inhalation stimulates the inflammatory response to subsequent LPS-mediated acute lung injury through activation of the inflammasome pathway. The finding that responses were still elevated 27 days after exposure suggests that a population of memory immune cells is responsible for persistent inflammasome activation. These results provide new insight into mechanisms by which chronic inflammation stimulates disease progression.

Acute inflammatory exacerbations (AE) represent precipitous deteriorations of a number of chronic lung conditions, including pulmonary fibrosis (PF), COPD, and asthma. AEAs are marked by diffuse and persistent polyclonal alveolitis that profoundly accelerate lung function decline and mortality. Excess monocyte mobilization during AE, and their persistence in the lung are linked to poor disease outcome. Guided by clinical evidence, we have developed an inducible model of pulmonary fibrosis leveraging the PF-linked missense isoleucine to threonine mutation at position 73 [I73T] in the alveolar type-2 cell-restricted Surfactant Protein-C [SP-C] gene [SFTPC]. We have previously shown that genetic and pharmacological ablation of infiltrating monocytes results in reduced disease progression and lung histopathology, improved disease control and survival, and overall inflammation following mutant SP-C induced lung stress. Notably, deletion of CCR2 monocytes yielded significant reduction in TGFB1 signaling, as shown by reduced Smad2/3 phosphorylation. Using single cell sequencing we annotated and phenotypically characterized resident and recruited monocytes/macrophages populations intervening during the peak of AIE. PF. We found that inflammatory exacerbations are accompanied by influx of activated monocytes (tgα/tgα + cc2 + cx3cr1+) producing a pro-inflammatory transcriptional signature (ccl and cxcl, and galectin signaling). Comparatively, use of CellChat Explorer database identified an activated mature macrophage subset (cd68 + tgα + tgα + arg1+) coordinating innate immune tfgb1, fn1, fibronectin, and profibrotic signal- ing 14 days after SP-C mutant induction. These results provide a clear picture of the fibrogenic role of specific activated monocyte/macrophage subsets responding to alveolar epithelial stress. Identification of phenotypically analogous populations in the context of chemical induced injury and fibrosis has the potential to establish convergent inflammatory mechanisms of fibrotic injury.

Asthma is a chronic inflammatory disease of the airways. Despite treatment, many patients experience suboptimal symptom control, in part, due to variability in genes that dictate drug disposition and response. 170 single nucleotide polymorphisms (SNPs) in genes associated with asthma, and of unknown significance, were evaluated for an effect on symptom control in children with asthma. The rs11572080 SNP in the CYP2C8 gene was associated with improved asthma control score. Guided by clinical evidence, we have developed an inducible model of asthma exacerbation using a combination of Toll-like receptor (TLR) ligands and a histone deacetylase inhibitor (HDACi). The model is corroborated using a murine asthma model. In this model, we found that inflammatory cytokines including IL-6, MCP-1 and TNFα were also minimal, indicating an absence of active inflammation with either DEP or filtered air. In individuals that were administered LPS, expression of these inflammatory markers showed a dramatic increase (NLRP3: 5.0 ± 0.6 (mean ± SEM) fold vs. filtered air (FA) + saline control; IL-1β: 12.9 ± 2.8; caspase-4: 2.0 ± 0.6; IL-6: 29.8 ± 5.7; MCP-1: 4.1 ± 7.6; and TNF-α: 4.6 ± 0.7) compared to the saline control group. Mice that inhaled DEP and underwent LPS challenge showed increases in IL-1β up to 18.9 ± 10.2 fold; IL-6 (142.2 ± 56.0 fold), and MCP-1 (154.4 ± 48.6 fold) expression compared to those that inhaled FA similarly underwent LPS challenge. Immunoblots showed significantly elevated expression (3.0 ± 1.0 fold) and cleavage (3.7 ± 0.6 fold) of pro-IL-1β in mice that underwent DEP inhalation and subsequent LPS challenge. The amount of secreted IL-1β in BAL fluid increased with DEP inhalation (FA+LPS vs. DEP+LPS; 25.1 ± 10.9 vs. 64.8 ± 10.6 pg/mL). Cleavage of caspase-1 (2.3 ± 0.7 fold) and expression of NLRP3 (0.4 ± 0.2 fold) demonstrated an increased trend. Expression of GSDMD, another important component of the inflammasome pathway, was also increased (2.9 ± 0.7 fold). Elevated IL-1β production and secretion, combined with increases in GSDMD, NLRP3, and caspase-1, are consistent with enhanced inflammasome activation. In conclusion, our data show that repeated DEP inhalation stimulates the inflammatory response to subsequent LPS-mediated acute lung injury through activation of the inflammasome pathway. The finding that responses were still elevated 27 days after exposure suggests that a population of memory immune cells is responsible for persistent inflammasome activation. These results provide new insight into mechanisms by which chronic inflammation stimulates disease progression.
Inflammatory Signaling

Cell Lines to Unravel Mechanisms of Toxicity with a Focus on Inflammation

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Supported by the Netherlands Organization for Scientific Research. There is no objection to its presentation and/or publication. The opinions expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Department of Defense. This Joint Undertaking receives support from the innovation program and EFPIA.

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longitudinal separations were evident in LD10 and LD50 cohorts, respectively, but not in HSD. This observation corroborated with the fact that the fentanyl exposure is both time and dose dependent. Acute assaults of high doses of fentanyl differentially impacted the cortex gene profile. A 2-way ANOVA was computed to curate those gene profiles that were significantly altered by the co-factors, namely dose and time. Applying the cut-off at p<0.05 and fold change >1.5, we curated 48, 313 and 530 genes that were differentially expressed (DE) due the effects of dose, time, and their cumulative effects (dose x time). Supervised analysis mined those gene sub-families that demonstrated shifts in expression values in sync with increasing dose or time delay. Functional analysis of DE gene profile identified networks linked to reward circuitry, inflammation and neuronal functions. A time- and dose-sensitive dynamics of gene functions threw light on fentanyl driven biomechanisms. Validation work and additional omics assay are underway to present a confirmatory, holistic picture. Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

4113 Use of Transcriptomics in Early Stage Agrochemical Research to Identify the Model Candidates

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A candidate chemical for crop protection development needs proposed uses that are both efficacious and safe to humans and the environment. Therefore, a suitable lead candidate must have an acceptable predicted chronic toxicity profile. To predict this, we performed subacute dietary toxicity studies in male and female rats (4/sex/group) and transcriptomics with benchmark dose modeling (BMD) to provide information on the biological mode of action (MoA) and predict the chronic no-observed-effect-level (NOEL). Three structurally similar gamma-aminobutyric acid (GABA)-gated chloride channel antagonists (GABA-C) were evaluated following a 14-day exposure to inform target organ toxicity and aid dose level selection for subsequent studies. Based on the acidental endpoints in the subacute toxicity studies and the MoA for GABA-C chemicals, we selected paraffin-embedded formalin-fixed tissue sections from the tests in males and the adrenal glands, heart, liver, spleen, thymus, and thyroid in both sexes for transcriptomics analysis using the TempO-Seq© (Templated Oligo-Sequencing) Whole Transcriptome Assay (BioClavis). Differentially expressed genes and pathways (DEGs/DEPs) were identified. Gene set over-representation was used to assess the biological context of apical changes, and BMD analyses were conducted to identify a molecular point of departure to identify key tissues to forecast chronic toxicity and provide a benchmark for dose level selection. All three compounds were well tolerated when administered up to ~40 mg/kg body weight/day in the diet. Two compounds (C1 and C2) were welltolerated up to the highest dose tested (~300 mg/kg body weight/day) and elicited no clinical signs, but one compound (C3) resulted in severe toxicity at dietary levels >100 mg/kg body weight/day and these animals were euthanized prior to the end of the study. Among surviving animals, each compound resulted in increased serum cholesterol and increased adrenal gland weights in both sexes. For C1 (lead compound), there were no additional effects besides increased cholesterol levels at dose levels or greater than 115/10 mg/kg/day (males/females) and increased adrenal weights at or greater than 12/30 mg/kg/day (males/females). C1 did not elicit a robust transcriptional response and BMD analyses identified few dose-responsive pathways across any evaluated tissue, including those limited by small sample size. In contrast, C2 and C3 had more apical findings, including more severe effects in the adrenal glands (C2, C3) and effects in the spleen (C3), and BMD analyses identified significant dose-responsive genes and pathways in these tissues for both compounds. In addition, C2 and C3 had subsets of regulated genes that are not diagnostic of the same toxicity. Most of the identified gene activation or repression were linked to regulatory pathways or disease pathways. BMD analysis identified the heart as a sensitive tissue for C2. We conclude that short-term transcriptomics studies combined with phenotypic data can be used to inform earlier decisions in new chemical development and predict registration study outcomes and project risks.

4114 The Utility of Toxicogenomics to Assess Biological Relevance of a Putative Proliferative Response

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Toxicogenomics can be used to support assessment of in vitro experiments, including those limited by small sample size. The potential biological relevance of a small increase in replicative DNA synthesis (RDS) observed in hepatocytes from only one of 4 donors in a donor NLW, at one exposure concentration of a new pesticide, TYMIRIUM® technology (cyclobut trifluoromethyl) was explored using RNA-seq. Samples from three donors were sequenced and a combination of differential expression (DEG), congruence, gene ontology (GO) and functional enrichment analyses were performed. Following this, a targeted analysis of genes and pathways associated with cell-cycle/proliferation was conducted. DEG and pathway analysis found that genes in the cytochrome P450 2B and 3A families were upregulated across all donors, congruent with previous studies. A small number of genes associated with some pro-proliferation signaling pathways were differentially expressed in more than one donor; however, these were not uniformly altered, suggesting that enrichment of these pathways was probably not indicative of a proliferative response. No other DEGs, Reactome pathways, GO terms associated with proliferation or cell-cycle hierarchies were observed in NLW that were unique to the test item. Furthermore, common marker genes associated with cell-cycle and proliferation such as MKI67, were not differentially expressed in any donor. Gene set enrichment analysis of proliferation-related Hallmark signatures found an overall inconsistent response after test item exposure across all three donors. Some of these gene sets were more positively enriched in NLW samples compared to the other two donors. In particular, signatures associated with MYC target signaling showed modest positive enrichment in NLW in both test item and phenobarbital treated samples. However, the upstream regulator MYC was not differentially expressed, again indicating that enrichment was likely not indicative of a proliferative response. Although there was more positive enrichment of some proliferation-related Hallmark gene sets in donor NLW, there was little evidence for overexpression of DEGs, nor enrichment of Reactome pathways/GO-terms associated with cell-cycle and proliferation. Overall, there was a lack of consistent response of proliferation/cell-cycle genes in any donor. Consequently, the small apparent increase in RDS in donor NLW, was not consistent with the underlying biochemical changes and therefore was not considered biologically relevant. This demonstrates how toxicogenomics can be used as a robust tool to investigate unusual in vitro findings.
Compounds extracted from urine samples were analyzed with a high-resolution NMR as well as their controls. Subjects with e-cig were recruited for urine collection. The unbiased mapping study data. More details will be presented. The gene lists generated by the alteration patterns of histone marks would further reveal the upstream signaling pathways activated/repressed by the repeated treatment. Hypomethylated areas have been modified. And ChIP-Seq against H3K4me3, H3K27me3, H3K27Ac, and H3K9me3 are performed to interpret the BR and TR responses of thalidomide and methyl cellulose, in addition to CC4, Valproic acid, Clofibrate, and Corn oil. To narrow down the enhancer-promoter regions of interest, hypomethylated areas identified by WGBA was used efficiently. A 14-day repeated dosing of thalidomide induced increase in BR for about 400 genes including Cp2bp10, Cyp2a5 and Cds2. Many, if not all, are accompanied by increase in the signal of H3K4me3 and or H3K27Ac, i.e. activation marks. Our comprehensive data would explain the effects of the repeated BR treatment on the expression of Histone marks. The gene lists and the expression patterns were further used to narrow down the thalidomide related pathways that were repressed in the urine of e-cig users compared to cigarette smokers. These data suggest that e-cig use may metabolomics demonstrated differences in the chemical landscape in the urine of e-cig users. To address this gap, we applied liquid chromatography mass spectrometry (LC-MS) to profile the urine of e-cig users. The overall findings suggest that e-cig use may provide a drug testing strategy that accounts for the inherent complexity of tumors. Furthermore, as cytotoxic perturbations in metabolism is often observed prior to cell death, metabolic profiling could become a key issue in drug development. Therefore, we established 3D models of a breast cancer cell line (MDA-MB-231), lung carcinoma cell line (A549), and two thymic carcinoma cell lines (TYB2 and 1889c) using 3D molds for 1889 and MDA-MB-231 cell lines and simple rotation for A549 and TYB2 cell lines. Characterization of the 3D models were performed using live imaging with a variety of dyes that detect viability and hypoxic conditions, as well as structural analyses. Subsequently, the effect of colchicine, doxorubicin, paclitaxel and bortezomib were compared between the 2D and 3D models using toxicity assays - CellTiter-Glo® and MTT. For the first time, we could produce reproducible spheroids using different media recipes to decrease/increase differentiation. Metabolic/Molecular profiling of the 2D and 3D models were performed using Nuclear Magnetic Resonance (NMR) that is suitable for measuring ultra-small volume samples, as 14 as real time PCR. 3D models showed higher IC values compared to 2D models in all cell lines, indicating that cells in a 3D configuration were more resistant to the tested drugs than cells grown in a monolayer. Finally, we could improve our established technique for noninvasive toxicity testing employing NMR. The present findings using 3D models of a number of different cancer cell lines revealed a higher resistance to drug-induced toxicity as compared to 2D models. Moreover, our newly designed NMR technique gives access to the investigation of small tissue-like models, normally unaccessible using standard analytical techniques. Analytical techniques known so far are both destructive and deliver merely a "snap-shot" of the measured time point. For this reason, specimens have to be dissected and imposed to multiple measurements to obtain 3D information. Our technique based on NMR spectroscopy does not face the aforementioned limitations.

The survival of a tumor depends on its ability to accommodate changes of pH, reactive oxygen species (ROS), nutrient supplies and hypoxia. 2D cell culture fails to reproduce the 3D interactions observed in tumors, including the effect of cell-cell interaction and nutrient gradients. This may be a reason for the failure of newly developed anti-cancer drugs to succeed in vivo after showing promising results in 2D cell culture. In recent years, improved protocols for the production of 3D cell culture models have been established, allowing for a reliable production of 3D spheroids from different cell types. The measurement of metabolic responses in the optimized 3D tumor models instead of 2D cell culture would provide a drug testing strategy that accounts for the inherent complexity of tumors. Furthermore, as cytotoxic perturbations in metabolism is often observed prior to cell death, metabolic profiling could become a key issue in drug development. Therefore, we established 3D models of a breast cancer cell line (MDA-MB-231), lung carcinoma cell line (A549), and two thymic carcinoma cell lines (TYB2 and 1889c) using 3D molds for 1889 and MDA-MB-231 cell lines and simple rotation for A549 and TYB2 cell lines. Characterization of the 3D models were performed using live imaging with a variety of dyes that detect viability and hypoxic conditions, as well as structural analyses. Subsequently, the effect of colchicine, doxorubicin, paclitaxel and bortezomib were compared between the 2D and 3D models using toxicity assays - CellTiter-Glo® and MTT. For the first time, we could produce reproducible spheroids using different media recipes to decrease/increase differentiation. Metabolic/Molecular profiling of the 2D and 3D models were performed using Nuclear Magnetic Resonance (NMR) that is suitable for measuring ultra-small volume samples, as 14 as real time PCR. 3D models showed higher IC values compared to 2D models in all cell lines, indicating that cells in a 3D configuration were more resistant to the tested drugs than cells grown in a monolayer. Finally, we could improve our established technique for noninvasive toxicity testing employing NMR. The present findings using 3D models of a number of different cancer cell lines revealed a higher resistance to drug-induced toxicity as compared to 2D models. Moreover, our newly designed NMR technique gives access to the investigation of small tissue-like models, normally unaccessible using standard analytical techniques. Analytical techniques known so far are both destructive and deliver merely a "snap-shot" of the measured time point. For this reason, specimens have to be dissected and imposed to multiple measurements to obtain 3D information. Our technique based on NMR spectroscopy does not face the aforementioned limitations.

The passing of the Agriculture Improvement Act of 2018, along with the perception that cannabidiol (CBD) could have beneficial effects, has led to a dramatic increase in the number of CBD- and hemp-containing consumer products in the US market; however, large data gaps remain regarding their safety. Previous studies reported effects in several organ systems upon oral exposure to CBD, including in the liver and male reproductive system. We have evaluated the effects of a developmental oral exposure to CBD in Sprague-Dawley rats. Pregnant dams were exposed orally to 0, 15, 30, 100, or 250 mg CBD/kg bw/day by gavage from gestational day 6 until the start of parturition and their pups were dosed daily by gavage from the day after birth (postnatal day [PND] 1) until PND 21. Animals were sacrificed on PND 21 or at the end of a recovery period on PND 180. No differences were observed on body or organ weight at either timepoint, except for a significant trend of decreasing testicular weight with increasing CBD dose at PND 21. A dose-dependent increase in the number of mitotic figures was observed in the hepatocytes of the liver of males and females at PND 21, but not at PND 180. No other CBD-related histopathological changes were observed. Sperm parameters, evaluated at PND 180, were not affected by the CBD exposure. The expression of genes encoding enzymes of the testosterone biosynthesis pathway was decreased at the highest dose of CBD at PND 21. These data are expected to help the FDA in its assessments of the potential adverse outcomes of CBD use during pregnancy and early life.
The incidence of hepatocellular carcinoma (HCC) is increasing in the Western world over the past decade. Within the United States, rise of HCC cases has been mainly observed in the South-West part of the country including states of Texas, New Mexico and Arizona. In New Mexico, elevated numbers of HCC cases are seen among Hispanic and Native American populations of the state. Historically, these racial populations are prone to key risk factors of HCC such as diabetes and obesity. However, those risk factors alone are not sufficient to explain the rapid increase in HCC incidence observed in the past decade. We hypothesize additional environmental risk factors (e.g., heavy metal exposures) play a key role in the South-West United States including New Mexico. Cadmium (Cd) is a major heavy metal pollutant of global implications. Cd exposures are reported to be associated with chronic metabolic alterations driving rises in cases of HCC such as obesity, diabetes and non-alcoholic fatty liver disease (NAFLD). Our lab is focused on understanding the impact of chronic, low-dose exposures of Cd (Clec) as a key driver of metabolic alterations, such as insulin signaling dysfunction, leading up to diabetes, NAFLD and HCC. In the current abstract, we use a combined systems biology-experimental approach to understand dysfunction of a key insulin signaling master regulator, Akt, due to chronic Cd exposures. A well-characterized HCC cell line of liver function, HepG2, was grown for 24-weeks under conditions of normoglycemia (5.6 mM) and hyperglycemia (15 mM) at two Cd dosing levels (0 nM, and 1000 nM). All of the exposure conditions were designed to mimic physiologically relevant dosing levels observed in humans. HepG2 cells obtained using the CLEC exposure protocol were subsequently assessed for the insulin responsivity and Akt signaling with the following protocol - Each test condition was exposed to 100 nM insulin and total protein was collected at 0 sec, 20 sec, 40 sec, 1 min, 5 mins and 30 minutes intervals. The collected protein was then assessed for the phospho-Akt (S473)/total AKT, phospho-mTOR/total mTOR, phospho-hsp-70/total hsp-70 and total IRS-1 signaling activity to assess the dynamics of insulin responsivity in each CLEC exposure condition. The ratio data obtained from the time series activity of Akt activation is then incorporated into an ordinary differential equation (ODE) model comprised of four equations describing a simplified Akt-mTOR-IRS signaling loop with negative and positive feedback. This model is based on powerful high-resolution metabolomics (HRM) and environmental chemical (HRE) analysis approaches. We used archived samples available in the Child Health and Development Studies (CHDS) in California, US, one of the largest prospective pregnancy cohorts, to analyze for HRE and positive feedback model. We identified several key risk factors for breast cancer on a population level, predicting an individual’s risk of breast cancer is not yet possible. In this study, we used highly informative, existing pregnancy cohort biospecimens to predict individual breast cancer risk by identifying key factors for breast cancer on a population level, predicting an individual’s risk of breast cancer is not yet possible. In this study, we used highly informative, existing pregnancy cohort biospecimens to predict individual breast cancer risk by identifying key factors for breast cancer on a population level, predicting an individual’s risk of breast cancer is not yet possible. 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During the Vietnam War era, the Diamond Alkali pesticide manufacturing plant dumped thousands of gallons of Agent Orange and Dioxin into the Passaic River. Fish and crabs inhabiting the Passaic River/Newark Bay area, now a superfund site in NJ, are contaminated with toxic agents including heavy metals and polycyclic and polychlorinated aromatic hydrocarbons (PAHs and PCBs.) Consumption of fish in this region is associated with the highest cancer risks and noncancer health hazards compared to other exposure pathways (ingestion, dermal contact of surface water). Despite published warnings for susceptible populations to avoid consumption of contaminated fish, this practice continues in high numbers, particularly amongst anglers and their families in low-income communities. We hypothesized that consumption of this contaminated (aka “dirty”) fish produces alterations in the GI tract microbiome. The GI tract microbiome encompasses metabolic, immune, and endothelial communication and function in the host. Alteration, or dysbiosis, of this community can have negative implications in disease development and overall homeostasis. While microbiota disruption is common in humans, characterization of the response to environmental stressors like dioxin, PCBs, and heavy metals remains a gap in research. To test our hypothesis, C57BL/6 mice were fed a 7-day baseline diet followed by 10 days of diets supplemented with 2.5% or 7.5% “dirty” or “clean” eel. 16s ribosomal RNA was collected from feces and sequenced at ~10,000 reads per sample using Illumina MiSeq. Principal Component Analysis (PCA) was used to examine the effect of diet and sex on GI tract microbial diversity in the mice. On day 1 of the dirty eel supplement, the community structure began to diversify, and by day 9, a significant difference in microbiota makeup was seen in male mice fed the 7.5% dirty eel diet. Taxonomy analysis indicates that beneficial microbes involved in lipid metabolism and gut barrier integrity are reduced at higher doses of “dirty” feed. This data demonstrates that consumption of contaminated aquatic species alters the microbiome in the mammalian gut, with a sex-dependent effect. This suggests that everyday environmental exposures for Newark residents can result in significant changes to one of the key communities of organisms that are responsible for the upkeep of overall health.

Protoporphyrinogen oxidase (PPO) catalyzes the oxidation of protoporphyrin IX to protoporphyrin IX, which is the final common enzyme in both the heme and chlorophyll biosynthetic pathways. PPO inhibitors (PPOs) are used as agrochemicals for weed control. Regenerative anemia is a common treatment related effect in repeat dose toxicity studies of PPOs and is characterized by decreased red blood cells (erythrocytes) and an increase in immature erythrocytes in the blood. Design of new PPOs would benefit from having a quantitative biologically-based computational model of PPO-induced anemia. To inform this modeling, the temporal progression of putative key events in the adverse outcome pathway (AOP) of PPO inhibition was evaluated. Female Han Wistar rats, 5/group, were treated with an exemplar PPOi by daily oral gavage of 0, 1, 3, or 100 mg/kg bwt/d, for up to 14 days with interim sampling and sacrifices on day 1, 6, 7 and 8. Liver, bone marrow, spleen, heart, and kidney were examined histologically and using whole transcriptome TempSeq-mRNA sequencing. Clinical pathology was performed for blood hematology, plasma erythropoietin (EPO), and bone marrow smears. Blood plasma and liver extracts were subjected to metabolomics analysis. PPOi treatment caused dose-dependent increases in porphyrins in blood and liver from 4 hours. Simultaneously bone marrow smears showed dyserythropoiesis. Subsequently blood reticulocyte counts substantially decreased. The decreased reticulocyte counts progressed to decreases in red blood cell mass with increased plasma erythropoietin and hepatocyte EMH in the liver and spleen. Consistent with these pathological changes, differentially expressed genes in liver, bone marrow and spleen were associated with hypoxia, heme metabolism and inflammatory pathways. After 14 days, the reticulocyte counts partially recovered, remaining minimally lower than controls. However, marked decreases in multiple red blood cell parameters and indices remained as did increased plasma EPO and EMH in liver and spleen. In conclusion, we identified the temporal progression of key events in the AOP from PPO inhibition to anemia. Early biochemical changes in the porphyrin pathway trigger a rapid primary alteration in the bone marrow erythropoietic stem cells, which then secondarily cause anemia due to decreased erythropoiesis. This anemia triggers the well-established EPO-driven compensatory response that attempts to restore red blood cell mass. Therefore, the parameterization of a quantitative computational model of this AOP requires an understanding of the cell autonomous role of heme in controlling erythropoietic stem cell differentiation, and later key events can be parameterized using the existing models of anemia induced EPO feedback control.

Non-alcoholic steatohepatitis (NASH) is a chronic liver disease (CLD) that alters the pharmacokinetics of numerous drugs which may lead to drug reactions. Genetic polymorphisms in OCT1, MATE1, MATE2, and MRP4 have also been shown to alter drug pharmacokinetics. Therefore, the purpose of this study was to establish a genetic screening for single nucleotide polymorphisms (SNPs) and genetic factors altering drug elimination in NASH patients in order to determine the role of NASH in altered disposition. DNA was isolated from patient blood using a QIAamp DNA Blood Midi/Maxi kit, concentrations determined using a NanoDrop 2000 Spectrophotometer. Samples were diluted to 10 ng/µl using Ultrapure DNase, then genotyped in an Illumina HumanOmniOne array. A total of 541 common and 1,660 rare SNPs were genotyped.

Protoporphyrinogen oxidase inhibiting herbicides (PPOi) are used to control weeds in a variety of crops. These herbicides inhibit heme biosynthesis and photosynthesis, leading to disruption of porphyrin production which can cause the breakdown of cell membranes. The first PPOi was introduced in the 1970’s and are now widely registered globally. In mammals, the inhibition of PPO increases porphyrin levels, blocks the production of hemoglobin and erythrocytes, resulting in anemia. However, there are significant species differences in the levels of porphyrin accumulation resulting from exposure to PPOis, and subsequent toxicity correlating with porphyrin accumulation. In this work, we utilize an in silico approach to rationalise these species differences in porphyrin accumulation and understand species relevance to human health risk assessment. We have developed a novel mechanistic in silico model for PPO related toxicity, which consists of a systems-based description of the haem biosynthetic pathway coupled to a pharmacokinetic (PK) model for systemic exposure of the pesticide active ingredient (AI). The final model takes as inputs PK measurements along with simple in vitro potency data of the AI against the PPO target. The model is then capable of predicting blood porphyrin accumulation levels, a key indicator of downstream toxicity, following PPO inhibition. The model has been validated against data on well-profiled exemplar compounds. With appropriate parameter adjustments, the model allows for interspecies extrapolation and thus an evaluation of potential multispecies risks associated with exposure to PPOis. The model also provides a multiscale platform which can be extended to cover the role of haem in controlling erythropoiesis and the systemic homeostatic system for anemia via erythropoietin (EPO) to get to a quantitative understanding of the entire Adverse Outcome Pathway (AOP) of PPO inhibition. This supports the transition from descriptive knowledge to a quantitative assessment of PPOi safety evaluation.
Rinrskor: Kineticokinetic Data Support High-Dose Selection for the Rat Carcinogenicity Study

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Rinrskor (XDE-848 benzyl ester) is a crop protection herbicde for which a 2-year rat carcinogenicity distict study was conducted at dose levels set at 10, 50, and 300 mg/kg bw/day. These were based on the apical and kinetic (TK) results of the 90-day toxicity study in which rats received 100, 300, and 1000 mg/kg bw/day. Evaluation of TK data from the 90-day toxicity study and the 14C-ADME (absorption distribution metabolism and excretion) study are compared to justify dose selection. A 300 mg/kg bw/day dose was selected for the 2-year study. Results from the 14C-ADME study indicated that XDE-848 is predominantly converted to the primary acid metabolite XDE-848. For both sexes in the 90-day toxicity study, daily systemic blood exposure (as area-under-the-curve, AUC) was evaluated to provide additional data for departure from linearity. The ADME study consisted of time-course 14C plasma collections for determination of peak plasma concentration (Cmax) and AUC, and metabolic identification and percentage of drug recovery in urine and feces. Total 14C excretion in urine decreased from 41.3-22.2% to 5.97-8.62% as the dose was increased from 10 to 300 to 3000 mg/kg bw/day. XDE-848 acid was the major metabolite in urine, and it declined 4.46-6.1-fold at the high vs low dose. The corresponding fecal excretion of total 14C increased from 50.7-51.2% to 73.4-80.5% with increasing dose. The major metabolite in the feces was the parent XDE-848 benzyl ester, and it increased 2.2-2.7-fold at the high vs low dose. The shift from excretion of XDE-848 acid to increased fecal excretion of parent XDE-848 benzyl ester with increasing dose fully contributes to the loss of dose proportionality for XDE-848 acid in blood and urine observed with increasing dose in the 90-day study. The saturation of absorption can be attributed to saturation of pre-systemic esterase metabolism and increased excretion in feces as in-unmetabolized drug. Therefore, increasing doses beyond 300 mg/kg bw/day would result in greater fecal excretion due to saturation of pre-systemic metabolism and diminished systemic blood concentrations. TK data from the 2-year chronic study indicated sub-linear departure from proportionality at the 300 mg/kg bw/day dose. The TK results from the 90-day, 14C-ADME, and 2-year studies did indicate that the 300 mg/kg bw/day was the highest dose selected for the 2-year chronic study provided sufficient systemic exposure to adequately assess chronic toxicity and carcinogenicity potential.

Spinosad: Pharmacokinetics and Metabolism in the Rat, Dog, and Human

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Spinosad, a natural product insecticide, is comprised of two major components, Spinosyn A and D. The pharmacokinetic and metabolic fate of these two compounds are reviewed to understand the identity and quantity of systemic exposure to parent Spinosyns and metabolites across dose levels, life-stages, and animal species. Overall, Spinosyns A and D are well absorbed via the oral route in rat and dog (66-80%). Metabolism involves glutathione (GSH) conjugation of the parent compound and formation of demethylated metabolites. Systemic exposure in rat blood and tissues is primarily to parent Spinosyns and demethylated metabolites. Non-dose proportional (supralinear) pharmacokinetics were observed in dog and non-pregnant rat mam. The nonlinear systemic exposure is likely due to perturbation of GSH homeostasis in the liver. The non-dose proportional systemic exposure to Spinosyns in the blood and uterus of the pregnant rat occurred at dose levels consistent with difficulties in parturition (dystocia) and was primarily comprised of Spinosyns A and B. In vitro metabolism of Spinosyns A and D was greater in liver microsomes and hepatocytes from rabbits or human donors vs. mouse, rat or dog. Metabolite profiles in vitro are comparable to in vivo results (primarily CYP-based dealkylations, GSH conjugations). Dermal bioavailability of Spinosyns A and D in human has been previously shown to be non-detectable following topical application of a Spinosad pectudication formulation. This was performed to elucidate the predictive potential of next generation risk assessment. The pharmacokinetic profile of Amiodarone is abnormal for a drug. It highly accumulates in tissues and has a long half-life in the body. The mechanism behind this pharmacokinetic profile is poorly understood and so, previous pharmacokinetic models for amiodarone were mostly empirical. Using physiologically based pharmacokinetic (PBPK) modeling to elucidate these mechanisms is challenging because, amongst others, reported physicochemical parameters for amiodarone vary greatly and quality of physicochemical properties are available only in silico parameterization data. A PBPK model for intravenously and orally administered amiodarone in rats was first developed. This PBPK model written in R was parameterized solely using in vitro and in silico-derived parameters. The uncertainty of these parameters was accounted for by setting probability distributions for the different parameters based on their variability found in literature. After a successful PBPK model was first developed, this model was used to generate different sets of input parameters. The model simulations for acute and repeated exposure in rats were evaluated against experimental pharmacokinetic profiles reported in literature. While most tissue concentrations were predicted within 3-fold of experimental data, brain and fat levels were overpredicted poorly. This allowed for the identification of the in vitro-in vivo extrapolation methods used. The variability in reported log D 7.4 had negligible impact on concentration-time profiles in tissues and blood. On the other hand, different intrinsic hepatic clearance values cause large differences on the predicted concentration-time profiles of amiodarone in tissues and blood. In addition to the variability in reported in vivo intrinsic clearance, different normalization and the scaling methods used resulted in vivo hepatic clearance values spanning three orders of magnitude. The PBPK model was subsequently adapted to predict tissue concentrations in a human population using the physiological parameters from the PopGen database. The human PBPK simulations were compared to concentrations in repeated exposure scenarios highlights the importance of including uncertainty propagation in PBPK modeling. Finally, the human PBPK model was used to perform QIVIVE using cell-associated maximum concentrations that caused lipid metabolism disruption in brain and hepatic in vitro models. This was performed to simulate dosing regimens predicted to cause steatosis or neurotoxicity. By incorporating parameter uncertainty as well as biological variability into amiodarone PBPK models, this study allowed the identification of which parameters PBPK models can rely on QSPR and which ones high-quality experimental data (e.g. intrinsic clearance) is needed. This information on PBPK parameterization can help next generation risk assessment of data-poor highly lipophilic chemicals.
constituents and their amounts in mg per gram of botanical. Ninety-five (95) constituents were identified. The recommended dose of each botanical was obtained from commercial products and expressed as a range of milligrams, e.g., Ashwagandha's recommended dose varied from 400 to 1,000 mg. The minimum, maximum, and average recommended dose of each botanical was multiplied by the amount of constituent per gram of botanical to yield minimum, maximum, and average dose, respectively. These doses were classed into four groups: high, low, and average dose for each constituent. The dose range was 4.6 mg for raubasine in yohimbe to 132 mg for (-)-epigallocatechin-3-O-gallate in green tea. ADMF, e.g., pkα, solubility, permeability, logD, of each constituent were predicted using in silico models in ADMET Predictor®. The average dose, predicted intrinsic solubility, and human jejunal permeability were used to classify each constituent according to the Biopharmaceutical Classification System (BCS). Most of the constituents (60) were in class I (high solubility/high permeability). Twelve compounds were in class II (low solubility/high permeability). Twenty-one compounds were in class III (high solubility/low permeability) and 2 compounds were in class IV (low solubility/low permeability). Twenty compounds in classes III and IV are glycosides and are expected to have low fraction absorbed. The sugar groups on the aglycones can be cleaved by enzymes in the gastrointestinal tract and the aglycones will have higher permeability and lower solubility which will move them into classes I or II. Classes based on the Extended Clearance Classification System were also predicted. Most of the compounds (~59%) are in classes 1A and 2 that are assigned clearance via metabolism. Thirty-eight percent are in classes 3A and 4 (renal clearance). No compounds were in class 1B where hepatic uptake is the limiting factor for clearance. Pharmacokinetic simulations were performed in ADMET Predictor using a 70 kg subject and the human liver microsomal clearance model. Using the average dose, the fraction absorbed varied from 4.6 (withanoside IX/ashwagandha) to 100% with an average of 82%. Predicted oral bioavailability was calculated as 0.2 (asha with the maximum fraction absorbed of 63.9%). The highest Cmax was 8.7 µM for aristolochic acid I in Aristolochia. The highest AUC was 54.4 µM*h for (-)-epigallocatechin-3-gallate in green tea. When the maximum dose was used as input, (-)-epigallocatechin-3-gallate in green tea had the highest Cmax (9.8 µM) and AUC (90.5 µM*h). These estimated internal concentrations for constituents help assess safety of botanicals at the recommended dose in commercial products.

Toxicokinetics of 14C-Labeled 2,2',5,5'-Tetrachlorobiphenyl in Rat Tissues following Intratracheal Administration by V. Adamu,a,b P. Adamakova-Dodd,a X. Jing,b D. K. Lee,b and P. S. Thorneb, aUniversity of Iowa, Iowa City, IA, and bBayero University Kano, Kano, Nigeria.

Despite significant evidence supporting adverse outcomes associated with inhaled lower-chlorinated polychlorobiphenyls (PCBs), assessment of their health risks has been hampered by the lack of toxicokinetic data on various PCB congeners. Tetra-chlorinated PCBs dominate the congener profile of indoor air in schools, with PCB 52 being found at the highest concentration. Therefore, we selected PCB 52 for this study because tissue levels correlated with the available toxicokinetic data and because models for risk assessment because of its high prevalence in both environmental and human samples. Male Sprague-Dawley rats (262 ± 16 g) were intratracheally dosed with 160 µg/kg or 16 µg/kg body weight for the high and low dose groups. Radioactivity was measured using a scintillation counter for 36 different tissue types and digestive matters collected at 10, 100, 200, 360, 720, and 1440 min post-exposure. Exhaled air, urine, and feces were also sampled and analyzed at designated intervals. The total radioactivity concentration-time course data were analyzed by non-compartmental analysis using Phoenix WinNonlin® 8.3 to derive toxicokinetic parameters. Metabolites were fractionated using a GC MS validated liquid-liquid serial extraction method. Pulmonary uptake was near complete (>99%) and was independent of the dose. The recovery rates of the administered [14C]-PCB 52 ranged from 79.3 to 98.9 % for both high and low doses. Absorption was rapid, with serum concentrations increasing from 4 to 7 folds in 30-60 min. The absorbed [14C]-PCB 52 was distributed throughout the tissues, gastrointestinal digesta and digestive matters, and exhaled air within minutes. The disposition initially showed a marked affinity for highly perfused tissues and subsequently for fat-containing tissues. The maximum tissue concentration in serum, liver, and heart were achieved at 12 minutes after lung dosing. Muscle and liver were the primary early deposition sites due to the large tissue volume and high perfusion, respectively. Redistribution processes resulted in high radioactivity in adipose and liver tissues at later time points because of their high lipid content. More than half of the administered [14C]-PCB 52 remained in adipose and skin tissues, and 33.1% in gut content 24 h post-exposure. Our findings demonstrate metabolism and biphasic elimination of PCB 52 from blood and highly perfused tissues, including the lung, liver, trachea, heart, esophagus, and kidneys. More than two thirds of the total blood PCB was metabolized within 24 h. High and low dose groups of [14C]-PCB 52 resulted in high and low tissue concentrations, respectively, and dose-normalized blood concentration time-course dispositions demonstrated a linear toxicokinetic profile. The metabolism of [13C]-PCB 52 was extensive, with more than half of the parent [13C]-PCB 52 converted to phase I and II metabolites. The major metabolites were hydroxylated and human metabolites demonstrated a range of in vitro in vivo correlations. Total elimination was 32.7% by 24 h after exposure and it was primarily via feces and, to a smaller extent, urine, with a serum terminal elimination half-life of 37 h. The absorption and distribution of PCB 52 following intratracheal exposure was rapid and extensive. Biomonitoring for PCB 52 in the blood reflects exposure within one to two days. PCB 52 undergoes extensive metabolism, with phase II metabolites as the significant component. Our results provide a foundation for toxicokinetic modeling to inform risk assessment.


Microcystins (MC) represent a family of cyclic peptides with approximately 250 congeners, many of which are demonstrated to be toxic to humans. The toxicological profile of MC is characterized by active cellular uptake via organic anion transporting polypeptides (OATPs) and the subsequent irreversible inhibition of primarily ser/thr phosphatases (PPP) amongst a number of other cellular proteins. Despite that a comparison between rodents and humans is hampered by the absence of MC receptor surrogates for humans with regard to the i) type of OATP expressed in the various tissues, ii) the affinity and iii) capacity of the expressed OATPs for specific MC congener transport. The current risk assessment is still based on i) a single MC congener and ii) 90-day toxicity study in mice. The fact that humans demonstrate major differences in OATP expression and thus susceptibility to MC only compounds the fact that current risk assessment premises could severely underestimate the potential toxicities of MC due to their congener-specific kinetics. With the use of 21 structurally different MC congeners, covering the known spectrum of MC hydrophobicity, a range of molecular weights, and included common as well as unusual modifications of the consensus structure, we could demonstrate MC congener-dependent transport in OATP1B1 and OATP1B3 expressing HEK293 cells. Using in silico calculated LogP values, we show a strong correlation between the hydrophobicity of the MC molecule in OATP1B1 and OATP1B3 mediated transport into the cell. For both OATP1B1 and OATP1B3 expressing HEK293, having comparable PPP expression levels, we demonstrated that with increasing hydrophobicity of the congener, cell toxicity increased. Based on the observations that both transporters clearly preferred hydrophobic MC, the significant differences between OATP1B1 and OATP1B3 were investigated. MC congeners with a highly hydrophilic arginine residue at position (2), e.g. MC-RR, or a hydrophilic tyrosine at position (2), e.g. MG-YR, appeared to provide for the differences observed in the HEK293 cells.

To test the hypothesis that position (2) is critical for uptake into cells via OATP1B1, MC pairs with a highly hydrophobic amino acid and a highly hydrophilic amino acid residue were synthesized: MC-RR and MC-RF; MC-YR and MC-RY; MC-LR and MC-KL. Accordingly, these MC pairs with transposed positions (2) and (4) were tested and allowing the direct comparison of MC pairs with identical overall hydrophobicity of the MC molecule yet allowing to determine the role of the positions. While reduced transport via OATP1B1 was observed only with a phenylalanine at position (2), hydrophobicity at position (2) was critical for transport in OATP1B3 transfected cells. By using MC pairs, e.g. MC-YR and MC-RY, we demonstrated that hydrophilic residues at position (2) could significantly increase MC concentrations required to reduce the cell viability to 50%.

Improved Pharmacokinetic Profile of a Poorly Water-Soluble Compound through the Development of a Sustained Release Formulation for Intravitreal Administration in Animals by A. Salvatore1, P. Piccinni2, D. Codoni3, S. Bertani2, and E. Del Vesco, 2Evotec, Abingdon, United Kingdom; and 1Evotec, Verona, Italy. Sponsor: S. Bertani, Safety Pharmacology Society.

Intravitreal drug administrations have become an efficient approach to deliver drugs to the retina and/or choroid with the great advantage of the spatial and temporal control on the release of the payload, avoiding the undesirable side effects of the systemic administrations. Several animal models, such as pigs, mice and rabbits, are commonly used to conduct intravitreal pharmacokinetic studies. Despite some anatomical and functional differences, the pharmacokinetic parameters (clearence, volume of distribution, half-life) of the human and animal eye have good correlation and comparable absolute values. The present work focused on the development of a sustained release formulation of a neutral poorly water-soluble API at 10 mg/mL concentration for intravitreal administration in animal studies. A number of compounds for the treatment of an ocular disease were developed and topical solutions of these APIs (API active pharmaceutical ingredient) were tested in an animal model. Suboptimal bioavailability was observed and deemed due to low API permeability through the corneal epithelium and/or rapid drainage through the nasolacrimal ducts. To enhance the bioavailability of these drug candidates further development work was required to design and manufacture sustained release formulations. The poor solubility of the compound in water was used to develop aqueous suspensions and obtain a depot to be administered and achieve sustained release. To develop suitable aqueous microsuspension for intravitreal administration, the particle size distribution of the API is a key quality attribute. Measurements of the particles size distribution (PSD) of the API showed that the compound had a mean size of 40.82 µm and this was considered unacceptable for an intravitreal suspension (target PSD ~10 µm). Hence, an attempt was made to reduce the API particle size using a wet milling approach. The particle size of the API was reduced.
to 8.29 µm following optimization of the milling time and an aqueous suspension suitable for intravital injection, with a mean API concentration of 0.125 mg/ml, successfully prepared by means of the two vehicles detailed thereafter: vehicle A contained sodium carboxymethyl cellulose, tween 80, mono sodium phosphate monohydrate, disodium phosphate dihydrate and NaCl while vehicle B contained sodium hyaluronate, tween 80, mono sodium phosphate monohydrate, disodium phosphate dihydrate and NaCl (0.9%). The subcutaneous release of CBD from the nStrada device on day 0 (D0) and blood was obtained several times a week over 35 days. Following the maximum concentration (Cmax) at D1 of 74.3±26.9 ng/ml and 26.3±16.5 ng/ml in MCT and SES, respectively. The subcutaneous release of CBD from the nStrada implants exhibited sustained in vivo release kinetics by D14 until termination of the study on D35. The average steady-state levels were 0.8 ng/ml for CBD delivered in MCT and 0.4 ng/ml for CBD delivered in SES. At necropsy on D35, serum alanine aminotransferase (ALT) was determined, and liver histopathology was evaluated, and neither revealed liver toxicity. Skin histopathology surrounding the implanted devices revealed changes consistent with fibrosis as expected. In vitro release data confirm consistent delivery of CBD into media containing phosphate buffered saline (PBS) with 5% Labrasol. Overall, these results provide a pre-clinical evaluation of CBD PK following implantation of a constant release device over 35 days and confirm that there is no evidence of liver toxicity at these CBD plasma levels.
The Relationship between the Physicochemical Properties of Industrial Chemicals and Their Percutaneous Absorption

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Industrial chemicals can enter the body via dermal contact and cause a variety of damages including cancers, and understanding their percutaneous absorption, therefore, is important for the risk assessment and exposure prevention of the chemicals. Some factors, such as molecular size, vapor pressure and hydrophobicity/hydrophilicity, are known to influence the degree of dermal absorption. For chemicals with molecular weight below 500, low solubility and the octanol:water partition coefficient (log Kow) is mostly important to the dermal absorption. However, the quantitative relationship between the two parameters is not well known. We measured the degree of dermal absorption of chemicals with a wide range of log Kow values and a quadratic curve was produced. When evaluated with this curve, not only the absorption, but also the transapncy as well as the accumulative property of chemicals could be rated. 3D cultured human skin was used to evaluate the dermal absorption. A piece of the skin was put into a Franz diffusion cell, and 14-labelled chemical in PBS was applied onto it. The radioactivity in the skin, the receiving phase as well as the upper PBS solution, was measured at multi points after the addition. Tr50, the time (h) half of the added amounts that was translated from the upper PBS solution to the lower receiving phase, was calculated. A total of nearly 40 chemicals was evaluated. It was found that the amounts of chemical that remained in the upper PBS solution, accumulated within the skin and passed into the lower receiving phase, were different for chemicals with different log Kow values. With the log Kow being the horizontal and the Tr50 the vertical axis, a quadratic curve could be drawn with the R2 being 0.9118. When referring to the absorption fastness (Tr50), the log Kow could be divided into three portions. Chemicals with log Kow below 0.5 could be hardly absorbed into the skin, those with log Kow between 0.5-3 are highly skin absorbable and easily transparent, and those with log Kow beyond 3.5 could easily be the skin layer, the more the skin is much slow, suggesting high accumulation. With this simple model, a chemical could be estimated how easy and how fast it could be absorbed via skin, and also whether it is accumulative within the skin. Taking the information of the skin absorption as well as toxicity into consideration, measures of skin exposure prevention could be implemented for industrial chemicals, while caution in the sampling time of blood/urine for biomonitoring could be given for those with accumulative property.

Improving Computational Derivation of PFAS Toxicokinetic Half-Lives


Per- and polyfluoroalkyl substances (PFAS) are a diverse class of long-lasting, man-made chemicals that have been used for a variety of industrial and consumer purposes. Long-term exposure to PFAS has been linked to a range of adverse health effects, including immunosuppression and liver damage. PFAS have been found in the environment and in drinking water across the US, and in the blood stream of humans and other animals globally. The toxicokinetic (TK) half-life - the amount of time required for 50% of the chemical analyte to be eliminated from the body - is a critical metric for characterizing potential adverse health effects. We present a chemoinformatics-ready process for computationally deriving half-lives of PFAS utilizing the existing EPA/ORD developed invivoPKfit R package. invivoPKfit is available as an add-on package on the GitHub repository of ExpCast-invivoPKfit. Concentration versus time (CvT) experimental data for various PFAS were extracted and harmonized from a variety of literature, including both peer-reviewed studies and grey literature. When applied to the PFAS CvT dataset, invivoPKfit systematically and consistently estimates TK parameters for each combination of chemical and species in the dataset. invivoPKfit provides the initial estimates of TK parameters, such as volume of distribution (Vd) and elimination rate (Keq), by using non-compartmental heuristics. The package then calculates and optimizes the log-likelihood of the model by varying the TK parameters. invivoPKfit repeats the process for every unique combination of chemical and species in the dataset. Akaike Information Criterion (AIC), used to estimate prediction error, is calculated for both 1-compartment and 2-compartment models as well as a null (no-time response) model. The model with the lowest AIC is chosen as the best model. The estimated Keq values from the chosen model allow for the direct calculation of chemical half-lives. Derived TK parameters are then made publicly available via the USEPA/CompTox Dermal Toxicology (CompTox) Chemicals Dashboard. Toxicokinetic half-lives of PFAS provide critical information to stakeholders and decision makers as they seek to understand and mitigate the public health impacts from this widespread and persistent class of chemicals. This abstract does not necessarily reflect the US EPA policy.

Application of a High-Throughput Physiology-Based Pharmacokinetic/Toxicokinetic (httk) Dermal Route Using Human In Vivo Caffeine Exposure Data

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Dermal absorption represents an important route of exposure to chemicals from multiple sources: environmental, pharmaceutical, occupational, and consumer products. A high throughput approach is well suited for estimation of potential health risk from dermal exposure. Our approach consists of developing a generic and open-source high throughput toxicokinetic (httk) model that can be used with multiple chemicals. The httk estimates can then help inform data gaps for chemicals of concern. A dermal route has been added to httk and used to predict systemic dose following dermal absorption. Dermal permeability (Kp) is a key chemical-specific parameter needed to quantify dermal absorption. Using caffeine as an example, we used two different methods to calculate dermal permeability, both based on quantitative structure activity relationships (QSAR). The Potts-Guy QSAR equation is the default approach used by the US EPA to calculate dermal absorption permeability. The UK Surrey model makes a novel combination of a QSAR approach with a diffusion-based "brick and mortar" model representative of the stratum corneum (SC). Realistic membrane thickness and composition are used to represent the SC and sequential layers underneath the SC, assumed to be used in httk, and we are exploring the inclusion of an evaporation calculation to the model. Our work is also considering the volatility of different chemicals to be used in httk and are exploring the inclusion of an evaporation calculation to be added to our model. Preliminary inclusion of simultaneous absorption and evaporation from the SC is being explored as an additional factor necessary to explain our simulation results. In summary, a hybrid approach combining QSAR and mechanistic concepts have been applied using caffeine as an example chemical. Continued model refinement of the httk model is expected to provide additional insights into mechanisms needed to improve our dermal representation for httk application. This abstract does not reflect US EPA policy.

Development and Analysis of High-Throughput Physiology-Based Pharmacokinetic/Toxicokinetic (PBPK/TK) Dermal Exposure Model

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Dermal absorption of chemicals represents a important route of exposure in pharmaceutical, occupational, and environmental settings. There are thousands of chemicals in use with little or no toxicity or toxicokinetic data and it is not feasible to collect in vivo data for all these chemicals. One alternative is to estimate human toxicokinetics using high-throughput methods. The aim of this study was to develop a generalized physiology-based pharmacokinetic/toxicokinetic (PBPK/TK) dermal exposure model for the R package httk. For dermal exposures this model can be used in a high-throughput manner to estimate human blood and tissue concentrations and estimate risk for many chemicals. The structure of the dermal PBPK/TK model was based on Campbell, Clewell, Gentry, Anderson, and Clewell, "Computational Toxicology, 929, 439 (2012). Chemical-specific metabolism and protein binding data were obtained from the literature as collected by R package PKInfo. For chemicals that do not have metabolism data available, we used the US EPA Computational Toxicology (CompTox) Chemicals Dashboard. The model was constructed to allow comparison of dermal, oral, and intravenous exposures. Over 26 exposure scenarios across 14 chemicals were modeled and compared to published concentration-time in vivo data from the EPA Cvt database [Sayre, Wambaugh, and Gruke, Scientific Data, 7, 122 (2020)]. Of these 14 chemicals, four are pharmaceuticals, 10 are lipophilic log of the octanol to water partition coefficient, logP > 2), three have low water solubility (< 10 mg/L), four have high water solubility (> 1000 mg/L), and eight are considered volatile. The Root Mean Squared Error (RMSE) between log-transformed simulated and observed concentrations was calculated to determine how
Malignant melanoma is one of the most aggressive types of human cancer with a high fatality rate due to its resistance to current therapies. On the other hand, the extensive deregulation of normal epigenetic marks is associated with melanoma onset and progression. Given the well-established association between epigenetic modifications and alterations in gene expression, together with the reversibility of such modifications, it becomes apparent that they have attracted the scientific interest as potential therapeutic targets. Recently, our group has shown that isothiocyanates (ITCs; bioactive compounds in cruciferous vegetables) possess significant anti-melanoma activity in addition to acting as epigenetic regulators by modulating the expression of various acetylases/deacetylases, methyltransferases/demethylases and their associated H3 and H4 histone marks. Finally, Tazemetostat (TAZ) is a third-generation epigenetic drug very recently utilized, in the clinical setting, for its capacity to act as an inhibitor of the enhancer of zeste homolog 2 (EZH2) histone lysine methyltransferase which constitutes the enzymatic catalytic subunit of the polycomb repressive complex 2 (PRC2). To this end, EZH2 has been shown to act as a chromatin-modifying enzyme in maintaining transcriptional repression thereby contributing to silencing of tumor suppressor genes consequently promoting uncontrolled cell proliferation and cancer progression. The aim of the present study was to delineate the underlined mechanism of therapeutic effectiveness of TAZ either alone or in combination with various ITCs [sulforaphane (SFN), iberin (IBN), phenethyl (PEITC) and benzyl (BITC)] in a number of human malignant melanoma and non-tumorigenic immortalized keratinocyte (control) cells. Our results revealed, for the first time, that exposure to TAZ significantly reduced cell viability, in a dose- and time-dependent manner (via activation of apoptotic pathways in these cells while 20% DNA methylation was unaffected. In addition, combinatorial treatments resulted in further reduction of cell viability an effect that was accompanied by higher apoptotic rates as well as increased expression levels of critical genes involved in intrinsic (BAD, APAF1), extrinsic (TNF, TNFRSF1A, TRADD, TRAF5) and other (CASP2) apoptotic pathways as well. Moreover, treatment with TAZ reduced expression levels of EZH2, embryonic ectoderm development (EED) and suppressor of zeste 12 (SUZ12), all of which constitute PRC2’s major subunits, an effect accompanied by decreased expression levels of trimethylation of histone H3 on lysine-27 (H3K27me3; an epigenetic regulation and is associated with various diseases. DNA hypomethylation on the promoter region contributes to transcriptional silencing, and the global DNA hypomethylation in the body region is associated with genomic instability. Since Pb accumulates in erythrocytes, the hematopoietic system is one of the targets of Pb poisoning. It inhibits the biosynthesis of heme by inhibiting a protein called δ-aminolevulinic acid dehydrase (ALAD). Thus, ALAD activity is considered a biomarker indicator for the early Pb effect. LINE-1 is an interspersed repeated DNA that is used as a surrogate marker for estimating genomic DNA methylation levels and is used as a proxy marker in a wide range of diseases, including cancer. It has been examined whether Pb affects epigenetic alterations in patients and adults based on global DNA methylation, CpG site-specific DNA methylation, and differentially methylated regions. However, no study has examined the effects of Pb exposure on epigenetic alterations in healthy children exposed to Pb. We, therefore, examined global DNA methylation using LINE-1 and CpG-specific DNA methylation using ALAD gene in children exposed to environmental Pb in Kabwe, Zambia, one of “The 10 World’s Most Polluted Places” due to its Pb-Zn mine, which operated in Kabwe for almost a century (1902-1994). Blood samples were collected from children aged 2 to 10 years old in Pb-polluted and Pb-unpolluted areas with permission from the Ministry of Health, Zambia. The methylation status of ALAD and LINE-1 was investigated using MSP and pyrosequencing techniques, respectively. The ALAD profile methylation in Pb-polluted area (mean BLL = 24.0 µg/dL) showed significantly high methylation rate (84.3% vs 42.1%) compared to the unpolluted area (mean BLL = 7.9 µg/dL). Moreover, the methylated ALAD gene showed an increased risk of Pb poisoning (adjusted OR = 7.84, p<0.001). Regarding LINE-1, global DNA methylation was found to be inversely associated with BLLs (β = -0.046). The highest quartile of having the highest DNA methylation rate. Lower levels of Pb, the lower the methylation of LINE-1. Thus, Pb-induced health problems could arise from hypomethylation of CpG site-specific DNA at the promoter region, such as ALAD, as well as hypomethylation of global DNA due to high BLLs.

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The development of cancer in humans is caused by irreversible modifications of the genome through genetic or epigenetic alterations resulting in the acquisition of multiple heritable abnormal cellular pathways. DNA methylation is the major and most-studied epigenetic mechanism that may cause alterations in the expression of genetic information without changing in the primary DNA sequence. In the mammary glands, a target organ for lorcaserin carciogenicity in rats, by reduced representation bisulfite sequencing. Lorcaserin exposure resulted in dose-dependent DNA alterations in the mammary glands, as evidenced by the presence of 1591 and 1961 significantly differentially methylated CpG sites (a Benjamin-Hochberg adjusted p < 0.05 and 20% DNA methylation difference) in the 30 and 100 mg/kg bw/day treatment groups, as compared to the control group. Pathway enrichment analysis of these differentially methylated CpG sites demonstrated their strong representation in genes associated with cell morphology, cellular function and maintenance, cellular response to stress or damage, and cell differentiation, among 437 hypermethylated CpG sites, 437 CpG sites were present in both treatment groups, with 401 being changed in the same direction. A detailed analysis of differentially methylated CpG sites demonstrated that while the number of hypomethylated CpG sites did not differ between treatment groups, the number of hypermethylated CpG sites was 1.3 times greater in rats treated with 100 mg/kg bw/day of lorcaserin compared to rats treated with 30 mg/kg bw/day. In summary, we have demonstrated that lorcaserin induced extensive DNA methylation changes in mammary glands at early pre-neoplastic stages of lorcaserin-induced rat carcinogenesis. These findings suggest the importance of epigenetic alterations in the carcinogenicity of lorcaserin.

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Lead (Pb) exposure among children is a global concern, especially in developing countries. Its exposure can change transcriptional and post-transcriptional epigenetic regulation and is associated with various diseases. DNA hypomethylation on the promoter region contributes to transcriptional silencing, and the global DNA hypomethylation in the body region is associated with genomic instability. Since Pb accumulates in erythrocytes, the hematopoietic system is one of the targets of Pb poisoning. It inhibits the biosynthesis of heme by inhibiting a protein called δ-aminolevulinic acid dehydrase (ALAD). Thus, ALAD activity is considered a biomarker indicator for the early Pb effect. LINE-1 is an interspersed repeated DNA that is used as a surrogate marker for estimating genomic DNA methylation levels and is used as a proxy marker in a wide range of diseases, including cancer. It has been examined whether Pb affects epigenetic alterations in patients and adults based on global DNA methylation, CpG site-specific DNA methylation, and differentially methylated regions. However, no study has examined the effects of Pb exposure on epigenetic alterations in healthy children exposed to Pb. We, therefore, examined global DNA methylation using LINE-1 and CpG-specific DNA methylation using ALAD gene in children exposed to environmental Pb in Kabwe, Zambia, one of “The 10 World’s Most Polluted Places” due to its Pb-Zn mine, which operated in Kabwe for almost a century (1902-1994). Blood samples were collected from children aged 2 to 10 years old in Pb-polluted and Pb-unpolluted areas with permission from the Ministry of Health, Zambia. The methylation status of ALAD and LINE-1 was investigated using MSP and pyrosequencing techniques, respectively. The ALAD profile methylation in Pb-polluted area (mean BLL = 24.0 µg/dL) showed significantly high methylation rate (84.3% vs 42.1%) compared to the unpolluted area (mean BLL = 7.9 µg/dL). Moreover, the methylated ALAD gene showed an increased risk of Pb poisoning (adjusted OR = 7.84, p<0.001). Regarding LINE-1, global DNA methylation was found to be inversely associated with BLLs (β = -0.046). The highest quartile of having the highest DNA methylation rate. Lower levels of Pb, the lower the methylation of LINE-1. Thus, Pb-induced health problems could arise from hypomethylation of CpG site-specific DNA at the promoter region, such as ALAD, as well as hypomethylation of global DNA due to high BLLs.


Pervasive toxicants, such as the metal lead and the plasticizer DEHP, are well studied in toxicology. While the primary health effects of these compounds have been delineated, the mechanisms underlying exposure-induced effects on neurodevelopment, growth, metabolism, and other disease risks are poorly understood. Molecular mechanisms underlying these impacts may include changes in the epigenome, including that of DNA hydroxymethylation, which plays a key role in neurodevelopment and the maintenance of health. To investigate the relationship between developmental exposure to Pb and DEHP and DNA hydroxymethylation, at collaborative, NIEHS-sponsored consortium, TaRGET II, initiated longitudinal mouse studies of developmental exposure to human-relevant lead and DEHP. Briefly, perinatal exposures to 32 ppm lead-acetate in drinking water (equivalent to 16-60 µg/dL in human drinking water) or 25 mg DEHP/kg of food (approximately 144 ng/mL in mouse blood) were administered to nulliparous adult female mice. Exposure began 2 weeks before breeding, and continued through-out pregnancy and weaning, until offspring were 21 days old. At 5 months of age, blood and cortex tissue were collected from perinatally exposed mice. For a total of 25 male mice and 17 female mice (n=5-7 per tissue and exposure, 1 male and 1 female per group).
1 female per litter), DNA was extracted and hydroxymethylation was measured using hydroxymethylated DNA immunoprecipitation sequencing (HiMeDIP-seq). Differential peak analysis was conducted in R using Bioconductor packages DiffBind and DESeq2, comparing across exposure types, tissue types, and animal sex. The R Bioconductor package annotat was used to annotate differentially-hydroxymethylated regions (DHMRs). Lead exposed females had no statistically significant difference in DNA hydroxymethylation or cortex hydroxymethylation compared to controls. Lead exposed males, however, had 385 DHMRs (all increased, FDR<0.15) in cortex, but no DHMR was identified in blood. DEHP exposed females had two regions with lower hydroxymethylation in blood (FDR<0.15) and no statistical differences in regional cortex hydroxymethylation. For DEHP males, 10 DHMRs in blood (six increased and four decreased, FDR<0.15) and 246 DHMRs (242 increased and 4 decreased, FDR<0.15) in cortex were identified. There was overlap in regions or genes comparing exposures across sexes and comparing tissues across females. There were four genes in both DEHP and lead exposed male cortex that overlapped, including two genes important for brain function: calmodulin-lysine N-methyltransferase (Ckmnt) and acid-sensing ion channel 2 (Asc2); a metabolism gene N-acetylglycolaldehyde dehydrogenase 2 (Gald2) and an IncRNA gene, coiled-coil domain containing 192 (Ccdc192). Overall, perinatal exposure to human-relevant levels of two common toxicants showed specific differences in DNA hydroxymethylation based on sex, exposure type, and tissue, but male cortex had the highest number of differences by exposure. Because hydroxymethylation occurs at the highest levels in the brain and is essential to normal brain development and function, these differences could have impacts on brain health throughout life. Future assessments will combine multiple epigenetic measures from the same animals to assess how sets of epigenetic marks are collectively impacted by perinatal exposure to lead and DEHP.

4148 Activation of Progesterone Receptor by DEHP and Endogenous Progesterone Differentially Impacts Cell Growth
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Many everyday consumer products such as food containers, personal-care products, and children’s toys contain phthalates, a class of chemicals that are used to make plastics more durable and soluble. One of the most toxic phthalates is Di(2-ethylhexyl)phthalate (DEHP). Its major metabolite is Mono-2-ethylhexyl phthalate (MEHP). DEHP has been hypothesized to bind to progesterone receptor (PR) via QSAR modeling. Additionally, exposure to DEHP has been linked to increases in the risk of breast cancer. We hypothesize that DEHP exposure can increase PR expression, which has been shown to alter the histone landscape and lead to an increase in breast cancer risk. We aimed to make a comparison of the disease outcomes. To test whether DEHP and its major metabolite, activate PR, we exposed T47D cells to progesterone (P4), DEHP, and MEHP (1.5-15,000mM). Cell growth and protein levels were assessed by running crystal violet, cell count, and protein expression analysis. Overall, perinatal exposure to human-relevant levels of two common toxicants showed specific differences in DNA hydroxymethylation based on sex, exposure type, and tissue, but male cortex had the highest number of differences by exposure. Because hydroxymethylation occurs at the highest levels in the brain and is essential to normal brain development and function, these differences could have impacts on brain health throughout life. Future assessments will combine multiple epigenetic measures from the same animals to assess how sets of epigenetic marks are collectively impacted by perinatal exposure to lead and DEHP.

4149 Discovering the Putative Epigenetic Adverse Outcome Pathway for Reproductive Toxicity of Chemical Additives Using Caenorhabditis elegans

Adverse Outcome Pathway (AOP) was introduced as a framework to assemble, integrate, evaluate, and visualize toxicological data and knowledge relevant for adverse effects by a stressor, linking the molecular initiating event (MIE), a cascade of key downstream events (KE), and an adverse outcome (AO) into a pathway network. Although histone modifications have been proposed as the toxic mechanism of various environmental chemicals due to its role in transcription regulation, relatively little is known about the hazard information and AOPs for chemicals inducing these epigenetic changes. Thus, this study aims to investigate the alteration of repressive histone marks by environmental chemical exposure and apply to an AOP leading to reproductive toxicity. To this end, we conducted germline de-silencing screening on 18 environmental chemicals, human exposure-based chemicals with known histone modifications (HMTs) activity, measurements and BMD responses to these chemicals. Through histone modification screening, histone methylated protein expression and gene expression analysis, and reproductive toxicity evaluation in C. elegans. In addition, benchmark doses (BMDs) for experimental values with multiple concentrations were calculated to quantify the POD of each endpoint and compare the sensitivities to exposure. Among the 18 chemicals, based on the exposure information (EPA CompTox dashboard and CTD database), di(2-ethylhexyl) phthalate (DEHP), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), bisphenol A (BPA), and triclosan were selected as chemicals with a high probability of exposure to reproductive aged females. The selected five chemicals were chemical additives in plastics, and triclosan and TBBPA showed the higher reproductive toxicity and increased HMTs activity in concentration-dependent manner. Protein expression analysis and screening of germine de-silencing demonstrated that HMTs activity by these chemicals is suggested as a MIE of triclosan and TBBPA exposure to cause following KEs, H3K9 and H3K27 methylation. We also identified genes that are likely to be regulated by histone methylation through the comparative analysis of BMD for HMT activity and gene expression. Lastly, reproducibility of two additives was alleviated by H3K27-specific HMT inhibitor exposure, suggesting that repressive histone modification play a key role in adverse outcome. To further propose epigenetically regulated transcriptional responses as KEs in epigenetic AOP of which MIE is “HMT activation”, gene expression (AO-associated genes) analysis using HMT inhibitors should be conducted. Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, Ministry of Science and ICT) (NRF-2020R1A2C300683).

4150 Diethylstilbestrol Induces Multigenerational Alterations in the Expression of microRNA in the Thymus Indicative of Long-Term Effects on Immune Functions and Disease Susceptibility
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Diethylstilbestrol (DES) is a well characterized endocrine disruptor and has been shown to mediate translational toxic effects in humans and animals. Studies have shown that prenatal exposure to DES causes thymic atrophy in the fetus, alters thymic T cell differentiation, and causes changes in immune functions, thus potentially increasing the risk of autoimmune and immune diseases. In the current study, we investigated the effect of DES on microRNA (miR) expression profile in parent (F0), F1, and F2 generations by examining miRs in the thymocytes. To that end, pregnant mice (GD 14) were injected with DES (10 µg/kg body weight). The thymus of mothers (F0), F1, and F2 generations of mice were harvested and high throughput miR array was performed. Data obtained from this study showed that more than 100 miRs were common to and dysregulated greater than 1.5-fold in thymocytes of all three generations of mice post-DES exposure, when compared to controls. miR array data demonstrated changes in miR profile affecting expression of a large number of genes, known to regulate thymic atrophy, cancer, immune suppression, apoptosis, autophagy, signaling, and other physiological pathways. Also, miR array data showed dysregulation of miRs by DES that may affect various pathways involved in diseases including cancer, autoimmune diseases, and immune dysfunction. Our study suggests that prenatal DES exposure triggers long-term and multigenerational effects by altering the miR profile that regulates genes involved in the development of disorders. Our findings support the concept of fetal basis of adult disease following exposure to DES. This work was supported in part by NIH grants R01ES030144, P01AT003961, P20GM103641, and R01AI123947, R01AI160896.

4151 Characterization of Extracellular MicroRNA Profiles in HepaRG to Identify Biomarkers of Chemical-Mediated Toxicity and Mechanism-of-Action

Previous in vivo and in vitro chemical exposure studies have indicated that dose responsive microRNAs (miRNAs) are linked to the known mechanisms of action (MoA) of chemical perturbations. The plausibility of using extracellular miRNA profiles as non-destructive biomarkers of chemical exposure is currently being investigated. We had previously identified the presence of 18 extracellular miRNAs of human hepatic HepaRG cells by small RNA sequencing, and a panel of 65 were chosen for direct measurement in media by Abcam Fireplex assay based on highest level of presence. Cells were treated with a set of 40 chemicals representing 14 MoAs. After nine days in culture, cells were dosed over a 4- or 8-point range with half-log spacing. MicroRNA and lactate dehydrogenase (LDH) release into the media were measured after 24h of exposure. MicroRNA profiling identified three basic patterns: 1) response of most measured miRNAs, 2) a partial response of miRNAs (either higher or lower compared to vehicle control), and 3) no or little change. A response of many measured miRNAs usually correlated with cellular toxicity as indicated by significant LDH release. In contrast, the Cyp5a4 inhibitors amiodarone, ketoconazole and itraconazole demonstrated a reduction of release into the media compared to control for most of the measured miRNAs. Many chemicals/doses not resulting in overt toxicity demonstrated a partial pattern of released miRNAs into the media, some of which were consistent within chemical MoA. Benchmark dose (BMD) analysis indicated the potential dose-responsive miRNA biomarkers common within a MoA group. Unsupervised hierarchical clustering of both miRNA measurements and BMD responses revealed some clustering of chemicals by MoA for several groups, including HMGCR inhibitors (statins), HDAC inhibitors, PPAR-gamma activators, unfolded protein response and heat shock response. BMD estimates identified examples of altered extracellular miRNA release that were more sensitive than measurements of toxicity. This included 19 miRNAs that demonstrated BMDs one-half below the BMD for LDH release when HepaRG were exposed to statins. Future analyses will examine paired intracellular miRNA and miRNA profiles to further link findings with known mechanistic pathways of toxicity.
In summary, extracellular miRNAs may be useful as accessible early indicators of toxicant exposure. Further work to define functional mechanisms could be invaluable for screening of chemicals and mixtures of unknown effect by a simple and non-destructive sampling of the media. This abstract does not necessarily reflect EPA policy.

4152 Impact of Lead Exposure on Epigenetic Regulation during SH-SYSY Neural Differentiation: Focus on piRNA and Transposable Element DNA Methylation

Early neurotoxic exposures can have long-lasting implications for cognition and health, due in part to effects on epigenetic mechanisms governing neurodevelopment. In addition to DNA methylation and histone modifications, small, non-coding RNA serve as an epigenetic link between environmental exposures and adverse health outcomes. One such class, PIWI-interacting RNA (piRNA), associate with PIWIL proteins to regulate transposable elements (TEs), and this complex interacts with DNA methylation machinery as a part of this function. While piRNAs were long thought to be exclusively expressed in the germline, work from our group and others has overturned this conclusion with somatic piRNA expression in both mice and humans. In early developmental human tissues (gestational days 90-105), PIWI mRNA expression in the brain was comparable (PIWILT and 2) or exceeding (PIWI3 (182%) and 4 (294%)) that of germline tissues. Our group has previously demonstrated that developmental lead (Pb) exposure, from conception to weaning, disrupts TEs within the mouse brain, with 3.86% (16pm Pb dose), 2.83% (32pm Pb dose), and 1.77% (32pm Pb dose) less DNA methylation at intracisternal a particle (IAPs) 110, 236, and 506, respectively. Given the presence of the piRNA system as well as Pb-associated hypomethylation at TEs in the brain, we hypothesize that disruption of the piRNA system plays a mechanistic role in this relationship. We are testing this hypothesis in the SH-SYSY neuronal differentiation cell model, wherein we found PIWILT expression increased 89% on Day 6 with 10µM Pb exposure, as well as 131%, 240%, and 170% by Day 12 with 0.16µM, 1.26µM, and 10µM Pb exposure, respectively, relative to control. Correlated with these results, we saw significant changes in molecular and morphological markers of SH-SYSY differentiation with Pb exposure. Upon the completion of differentiation (Day 18), we observed increased cellular proliferation at the 0.16µM and 1.26µM doses, with 333% (435% increase in cell number, and 125%-181% increase in neuron branching and synapse generation, relative to control. Quantitative immunofluorescence revealed an almost doubling of the expression of β-tubulin II, a 33% increase in GAP43, and a 19-25% increase in MAP2 expression across doses, relative to control. We are currently exploring how the profile of expressed piRNA transcripts as well as LINE-1 DNA methylation changes under these same conditions. In conclusion, we have found Pb exposure significantly alters neural differentiation and is associated with changes in the expression of PIWIL, machined in vivo. Coupled with evidence of Pb-induced hypomethylation at TEs in the developing brain in vivo, further investigation into this relationship is key to understanding the mechanisms of early epigenetic programming and those of Pb-induced epigenetic disruption during neurodevelopment.

4153 Role of IncRNA AW11210 in Staphylococcal Enterotoxin B-Induced Inflammation
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Staphylococcal enterotoxin B (SEB) is a bacterial superantigen that induces massive expression of pro-inflammatory cytokines leading to toxic shock syndrome. In this study, we investigated the role of a long noncoding RNA, AW11210 in SEB-induced inflammatory responses in vivo and in vitro. In this study, we generated AW11210 knockout (KO) mice. After those mice were challenged with SEB, cells in popliteal lymph nodes were isolated to determine gene expression. We found that lymphocytes in KO mice had significantly decreased expression of pro-inflammatory cytokines IL-6, IL-12 and IL-17. On the other hand, the expression of anti-inflammatory cytokine IL-10 was increased. RNA-seq revealed that about 250 genes were down regulated while about 200 genes were upregulated in KO mice compared to wildtype mice. Pathway analysis indicated that many dysregulated genes were involved in LPS/ IL-1-mediated inhibition of RXR function and FcyRIIB signaling in B lymphocyte pathways. These results indicate that IncRNA AW11210 is a key regulatory factor in inflammatory responses. Overall, this study has provided novel insights into the epigenetic regulation of inflammatory response in a mouse model. Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788, R01AI123947 and R01AI160896 to PSN and MN.

4154 Early-Life Exposure to Inorganic Arsenic Primises the Offspring to Increased Airway Hyperresponsiveness: Insights from Integrative Analysis of Epigenome
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Over 200 million individuals worldwide are exposed to inorganic arsenic (iAs) in drinking water and/or food at levels above the WHO provisional guideline value of 10 parts per billion (ppb). Furthermore, it raises public concern about the inhalation of iAs in tobacco smoke and electronic cigarettes. Prenatal exposure to iAs may increase the risk of adverse health effects during early childhood as well as later in life, perhaps through epigenetic modifications. In humans, maternal iAs exposure has been shown to be associated with child lung inflammation and airway allergy, in part through disrupting immune responses and hindering lung function. However, less is known about how early-life exposure to iAs promotes inflammation and respiratory syndromes. In the present study, we aim to explore the unrecognized association between maternal iAs exposure and child lung inflammation and airway allergy, in part through the alteration of epigenome during critical windows. Female virgin C57Bl1/6J mice were exposed to 0 or 10 ppb iAs in drinking water two weeks before conception to the end of lactation for a total of eight weeks. Blood sample from five-month-old progenies was collected for transcriptomic and epigenomic analysis. Ingenuity pathway analysis revealed that the differentially expressed genes were highly enriched in inflammatory response and organismal development. In particular, the top networks linking TGFβ1 were validated through qPCR in blood and lung tissues, suggesting their role in regulation of airway inflammation and reactivity. Of note, there was a correlation with increased offsprings off airway hyperresponsiveness in response to second insult of house dust mite challenges. While F1 progenies did not receive iAs after weaning, these findings suggest that maternal iAs exposure may prime the offspring to inflammatory responses, through the modification of transcriptome and epigenome, causing increased risk of allergic airway diseases later in life.

4155 Epigenetic Mechanisms for the Antidepressant Actions of Ketamine in an Organophosphate-Based Rat Model for Gulf War Illness
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Gulf War Illness (GWI) is a chronic multisymptomatic disorder that afflicts approximately a third of veterans deployed during the First Gulf War. Amongst the various complaints, mood disorders, anxiety, depression, and memory difficulties are commonly reported by GWI veterans. It is believed that sustained exposure to the organophosphate (OP) category of compounds, including pesticides and military nerve agents, home to nerve agents, underlies GWI development. Using repeated, low-dose exposures to an OP and a nerve agent surrogate (diisopropyl fluorophosphate (DFP)), we previously identified signs of chronic depression in rats. We also reported reduced brain-derived neurotrophic factor (BDNF) and epigenetic histone dysregulations in this GWI model. Additionally, ketamine (KET) boosted BDNF levels and produced an antidepressant effect in GWI rats. In this study, we investigated whether DFP-induced BDNF reductions could allow for the persistence of GWI symptoms. We also examined whether KET was producing long-lasting antidepressant effects via an epigenetic pathway. Male Sprague-Dawley rats (3-m age) were injected with DFP (0.5 mg/kg, s.c., 1x daily for 5 days). After about 6-m, rats received either a single dose of KET (10 µg/kg, i.p.) or saline and, 24 hours later, rats were euthanized, their hippocampi was collected and processed for confocal imaging and western blot quantifications. In agreement with our published data, GWI rats displayed a significant decrease in BDNF levels. At the same time, KET produced a long-lasting antidepressant effect and was associated with significantly increasing BDNF levels in GWI rats. Epigenetic screening indicated that GWI rats expressed increased histone deacetylase (HDAC) activity, a significant increase in HDAC1 and HDAC5, and higher levels of HDAC3 proteins. In the KET-treated GWI samples, significant decreases in the expression of all HDAC enzymes (HDAC1, 3, and 5) were noted (n=5 rats/group, one-way ANOVA, p<0.05). Altered in spine morphology and density profoundly affect neuronal plasticity and disease outcomes, including depression. To directly evaluate whether DFP-induced changes to proteins involved in synaptic plasticity were producing dendritic remodeling, we assessed hippocampal spine density using Sil staining. Confocal imaging revealed a slight decrease in dendritic spine density in GWI rats, while KET treatment produced a small increase in spine density over saline-treated GWI rats. Interestingly, we noted dendritic remodeling in GWI rats as evidenced by reductions in plastic, thin spines (T-type) and an increase in immature spines (S-type). Treatment with KET boosted T-type spine numbers and decreased S-type spines in GWI rats. Spine maturation requires support from brain trofic factors such as BDNF. We hypothesize that KET, by blocking HDACs, overcomes the DFP-induced transcriptional block on Bdnf. The present study demonstrates that the favorable endpoints of KET collaborates frustrated pathological synaptic plasticity, and produces a long-lasting antidepressant effect in GWI rats.
Prenatal exposure to metal and metalloid contaminants can influence fetal programming via DNA methylation and have been linked to adverse birth and later life consequences. Epigenetic clocks utilize machine learning to estimate biological aging of a given tissue based upon DNA methylation of aging related CpG loci and have been considered potential health biomarkers. While previous studies have demonstrated that several maternal- and paternal-specific external cues like socioeconomic stress are associated with aberrant epigenetic gestational aging (eGA) in the placenta, a critical fetal organ during pregnancy, less is known about the relationship between exposures to heavy metals and eGA. In the present study, placental DNA methylation data from the Extremely Low Gestational Age Newborn (ELGAN) study (N=405) was associated with maternal and paternal eGA (0.68 weeks, 95% CI: 0.13, 0.01), while SB was associated with a modest acceleration of 0.01 weeks (95% CI: 0, 0.01). Further analyses on the DNA methylation of RPC CpGs revealed five RPC CpGs associated with prenatal Mn in males, which was annotated to genes including CYP24A1, DNMT3A, and CRTC1. Both DNMT3A and CRTC1 regulate transcription and have been associated with potential DNA methylation in placenta. Additionally, CYPA2 aids in the metabolism and bioavailability of vitamin D, which are vital for pregnancy and fetal growth. Overall, our findings suggest that prenatal Mn, Sb, and Cd are associated with placental eGA and differential RPC CpG methylation in a sex-dependent manner in ELGANs that could contribute to the toxicity of these contaminants. Further work is needed to validate these results as well as explore other mechanisms of the RPC surrounding exposures to other chemicals and mixtures.
However, all three XRES in the CYP1A promoter-enhancer were conserved, indicating that the observed loss of CYP1A inducibility was not due to sequence changes in AHR motifs. In embryos, we confirmed prior observations of blunted CYP1A induction by PAH in adapted fish. However, unlike prior reports, we did not observe similar responses at CYP1B and CYP1C, which suggests that this memory is specific to CYP1A and is not generalizable to other AHR targets. In addition, we observed enrichment of bivalent histone modifications at loci adjacent to the CYP1A promoter in PAH-adapted embryos. These data represent one of the first reports of environmental responsiveness of bivalent chromatin. Our findings represent an important advance in understanding the formation and persistence of a transcriptional memory at CYP1A in response to chronic PAH exposure. Since CYP1A induction is protective against PAH toxicity in multiple studies, human populations that develop similar memory in response to high dose PAH are likely to be at an increased risk of toxic outcomes, including cancer.

4160 DNA Methylation of Candidate Imprint Control Regions Associated with Alzheimer’s Disease in African Americans and European Americans
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Alzheimer’s disease (AD) is the sixth leading cause of death in the United States. AD exhibits ethnic disparities whereby the prevalence is 2-fold higher in African Americans (AA) compared to European Americans (EA). It is hypothesized that aberrant epigenetic memory caused by early life exposure to adverse environmental agents may contribute to AD risk later in life, although to date a comprehensive assessment of the imprinting state of the genome in AD-affected populations has not been reported. To address this hypothesis, we investigated cytosine methylation at genomic sequences that control imprinting in DNA derived from post-mortem tissues of AD cases and non-AD controls, using an expanded set of putative imprint control regions (ICRs) previously reported by our group, in which we identified 1465 previously unreported loci. When we performed whole genome bisulfite sequencing of DNA derived from the temporal cortex or cerebellum of nine AD cases (5 AA and 4 EA) and eight controls (4 AA and 4 EA), we identified 120 candidate ICRs that are differentially methylated in AD cases compared to controls. Of these, 13 were within known ICRs, whereas 107 overlapped with the expanded set of 1465 candidate ICRs. When we stratified the patient/control bisulfite sequencing dataset by race, we found that 58 of the 120 differentially methylated regions were unique to AA and 22 were unique to EA, with only one of the 120 differentially methylated regions common to both racial categories (chr17:5771278-5771364). These results suggest that AA harbor significantly more AD-related differentially methylated loci than their EA counterparts, whereas the near absent overlap of affected regions seen in the two populations may reflect differences in environmental exposures culminating in unique signatures of aberrant methylation. Next, we performed a limited clinical study using blood samples from six individuals, three AD patients (2 AA, 1 EA) and three controls (all AA), were subjected to targeted methyl sequencing and confirmed that the locus on chromosome 17 is hypermethylated in all three AD cases compared to the controls. Our results support the hypothesis that errors in imprinting, possibly due to adverse exposures during early development, contribute to later onset of Alzheimer’s disease, and that the increased prevalence of AD in African Americans may be the result of higher exposures to adverse environmental agents and their influence on DNA methylation. Furthermore, the concordance between post-mortem brain tissue and blood samples derived from extant patients at the chromosome 17 locus is consistent with an imprinting phenomenon and a promising avenue of research with the goal of identifying biomarkers of AD risk in a readily accessible tissue.

4161 mTOR-Mediated Blood-Tests Barrier Permeability Regulates Epigenetic Aging of Sperm
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The sperm epigenome is affected by a variety of environmental xenobiotics and other factors, such as age, paternal diet, and health. A range of adverse health outcomes in offspring, including impaired metabolic, reproductive, and neurode-velopmentally attributed abnormalities, such as total absence (azoospermia), low count (oligozoospermia), abnormal morphology (teratozoospermia), and/or abnormal motility (asthenozoozoepsemia). According to the genetic central dogma, diseases such as azoospermia are caused by abnormal gene expression due to behavioral choices leading to alteration in human gene expression profiles. We

Animal testing for evaluating potential reproductive toxicity from environmental chemicals remains a foundational piece in the regulation of drugs and chemicals used in commodity and consumer products. Testing under the current guidelines requires a large number of animals, ranging from 560 to 6000 animals per chemical or drug. Currently, in vitro models for testicular development and spermatogenesis are actively being developed. Advances in biomedical engineering provide a possible solution to the challenges associated with the sustained culture of adult-derived cells. Building on our previous primary rodent testicular model, we developed testicular organoids from the canine. In this study, we collected Beagle dog testicles from animal spay-and-neuter clinics, and three steps of enzymatic digestion were used to isolate the testicular cells. Testicular cells were cultivated in the gelled agarose micro-mold and equilibrated with an extracellular matrix (ECM) containing optimized growth factors. The organoids were examined daily and fixed after culturing 1-14 days for morphological characterization. High-content immunofluorescence assay was applied to quantitatively characterize these temporal changes of the testicular population in the culture. Spermatogonia, Leydig, and Sertoli cells were labeled with germ cell nuclear antigen 1 (GCNA1), hydroxysteroid dehydrogenase type 3 (HSD3D), and SOX9, respectively. By self-organization, we observed the formation of testicular organoids and various types of spermatocytes, spermatids, and spermatogonia. Furthermore, testicular toxicant cadmium and BPA treatment significantly disrupted organoid structure and decreased testicular cell-specific marker expression, including depletion of Leydig and Sertoli cells. Establishing the canine testicular organoid model with active spermatogenesis will provide a valuable in vitro model for examining effects on the male reproductive system. Supported by NIEHS R44 ES027374 and R43 ES031890.

4163 Transcription Analysis of Male Infertility for Predicting Genomics Changes Caused by Behavioral Factors
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Male infertility encompasses any health issue that impedes the likelihood of conception and can be caused by abnormal sperm function or obstructions that prevent ejaculation. Multiple factors, including illness, injury, chronic morbidity, and lifestyle choices, contribute to its onset and progression. Male infertility is attributed to abnormally low count, poor motility, abnormal morphology, altered genetic expression, and sexual behaviors. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming. Testing under the current guidelines requires a large number of animals and is costly and time-consuming.
try to investigate the connection of differentially expressed genes (DEGs) from azoospermia patients and the normal population to the daily behaviors in azoospermia patients biologically by means of transcriptome analysis. Differential gene expression (DEGs) analysis was performed by using the high-throughput molecular profiles of existing male infertility patients and case control groups in public databases Gene Expression Omnibus (GEO) in order to identify DEGs related to male infertility. Results were used to weight gene correlation network analysis (WGCNA) to get the co-expression DEGs between male infertility and risky behaviors. Then GO/KEGG enrichment analysis and gene set enrichment analysis (GSEA) was performed to determine the biological processes influenced by hub genes. Hub genes were obtained by establishing protein-protein network (PPI network), and was ultimately used for analyzing how behavioral factors contribute to the onset and progression of male infertility. We selected 3 GEO Series (GSE) related to male infertility, including GSE145467, GSE45585, GSE9210. According to the results of differential gene expression analysis in GSE145467, here are 5250 DEGs obtained, including 2113 upregulated genes and 3097 downregulated genes. After the WGCNA of GSE45585, we obtained 12 modules. The most relative module was used for detecting hub genes, with a functional network was constructed. According to GSEA in GSE145467, here are 5 top pathways obtained: (a) synthesis of PC; (b) sphingolipids; (c) endocrine therapy resistance; (d) Lissencephaly 1 (LIS1) pathway; (e) activation of the N-methyl-D-aspartate (NMDA) receptors and postsynaptic events. According to GO/KEGG enrichment analysis in GSE9210, we formed visualized results between the biological process and gene expression in male infertility. Through a series of transcriptome analysis of the exposed to endocrine disrupting chemicals (EDCs) is known to disrupt male reproductive functions, while fetal exposure to acetaminophen (AC) and non-steroidal anti-inflammatory drugs (NSAIDs) is associated with increased male reproductive disorders in humans. Infants can be exposed to the phytosterogen genistein (GEN) from soy formula, or to the plasticizer di(2-ethylhexyl) phthalate (DEHP) leaching from consumer product or medical devices. Meanwhile, fevers in infants may be treated with AC or NSAID ibuprofen (IB). Considering the essential role of Sertoli cells in germ cell development, disruptive effects of EDCs and analgesic/anti-pyretic drugs on Sertoli cells could jeopardize spermatogenesis and contribute to male infertility. In vitro and in vivo Sertoli cells exposed to AC, IB, GEN, and MEHP alone and as mixtures at 10, 50, and 100 μM were performed to assess gene and protein expression. The gene expression of the immature Sertoli cell marker Anti-mullerian hormone (AMH) was unregulated in a dose-dependent manner by exposure to AC+GEN mixtures in TM4 cells, whereas the expression of AMH decreased in a dose-dependent manner after exposure of AC+GEN mixtures in rat Sertoli cells. In vitro analysis of immortalized TM4 Sertoli cells and rat primary Sertoli cells after 24-hour exposure to AC, GEN, and AC+GEN mixtures. Similarly, TM4 cells exhibited decreased Sox9 protein expression after exposure to GEN and AC+GEN mixture at 50 μM by immunofluorescent (IF) staining. In previous experiments, we found that both AC and GEN alone or in combination decrease the production of prostaglandins PGD2 and PGE2 in a similar manner. Thus, we examined the expression of eicosanoid pathway related genes. Both Cox-1 and Cox-2 genes and proteins were downregulated by GEN alone and AC+GEN mixtures in TM4 cells, while Cox-2 expression was downregulated in rat Sertoli cells. In contrast, the gene expression of the PGD2 synthase Ptgsd was increased by 50 μM treatments in TM4 cells, but only by the mixture in rat Sertoli cells. Whole transcriptome RNA-seq and KEGG analysis of TM4 cells and rat Sertoli cells revealed more genes altered in TM4 cells than in primary Sertoli cells. Several functional pathways were altered in both cell types by 50 μM of either single compounds or their mixture. This included FoxO, TNF, CAMP, Hedgehog, Pisk-Akt, cell cycle and cellular senescence pathways. Interestingly, Sertoli cell senescence was strongly reduced in TM4 cells treated with GEN and AC+GEN, but not AC. These results suggest that exposures to AC and GEN, alone and mixed, significantly dysregulate immature Sertoli cell function They further highlight signaling mechanisms that could aid in elucidating novel EDC and drug targets that could impact male gonad development and contribute to the origins of male infertility.

Cannabidiol, commonly known as CBD, is one of the major cannabinoids found in the plant Cannabis sativa L. (hemp). The U.S. Food and Drug Administration approved Epidiolex (a CBD-containing prescription drug) in 2018 to treat seizures associated with two rare and severe forms of epilepsy (Dravet and Lennox-Gastaut syndromes) in patients one year of age and older. CBD has been reported previously to induce male reproductive toxicity in animal models. However, it is not clear whether CBD has similar effect on human reproductive health. In our previous study, we found that CBD-induced cytotoxicity phenotypes were similar in mouse and human Sertoli cells where CBD inhibited cell proliferation and DNA synthesis. In this study, we revealed the molecular mechanisms underlying CBD-induced cytotoxicity in primary human Sertoli cells by bioinformatic approaches combined with RNA sequencing analysis. Through the analysis of the dose dependent response of genes, DNA replication, cell cycle, and DNA repair were found to be the most significantly impacted pathways in human Sertoli cells by CBD. Additionally, we also observed dose-dependent changes in genes related to senescence associated secretory phenotype (SASP) and p53 pathway, a crucial upstream pathway playing in cellular senescence. As shown by the steady halt of proliferation and the activation of senescence associated-galactosidase (SA-β-gal), two hallmarks of senescence, long-term treatment with 10 μM CBD promoted cellular senescence in human Sertoli cells. Furthermore, we discovered a concurrent increase of p16, a crucial marker of cellular senescence, on both mRNA and protein levels.

A new approach for teratogenesis evaluation via semen exposed to thalidomide (THA) in rabbits has been developed. A new test system will be useful to determine whether, and for how long, male contraceptives should be used when making medica- tion, THA and its Shydroxylated metabolite (major metabolite pathway in humans) and S-hydroxylated metabolite (major metabolite pathway in rodents) concentra- tions in rabbit semen were analyzed using liquid chromatography-mass spectrom- etry, after providing oral THA teratogenic doses (250 and 500 mg/kg) for 14 days that were as same or slightly lower than those in plasma. Rabbit and human semen samples did not differ in pH. Based on the toxicokinetic information, an intravaginal THA dose (0.4 mg/kg) was administered to female rabbits daily during gestational days (GDS) 1-13. On GDS 28, caesarean sections were performed. No intravaginal THA administration effects on maternal clinical signs, fetal morphologic changes, viability, or body weight were observed. Liquid chromatography-mass spectrometry analysis showed no detectable THA or hydroxylated metabolites in dams or fetal plasma on GD28. On GD13, just shortly after the last THA administra- tion, the THA and metabolite concentrations in the intrauterine contents, such as the placenta, yolk sac membrane, and embryo, were lower than those in the dams’ plasma. Implantation position did not affect concentrations. In dams, the S-hydroxylated metabolite concentrations were higher than the Shydroxylated metabolite concentrations. Based on these results, the study concluded that semen-mediated thalidomide teratogenic effect are absent in rabbits.

Cannabidiol-Induced Transcriptomic Changes and Cellular Senescence in Human Sertoli Cells

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Possible Teratogenic Effects via Male Semen Exposed to Thalidomide in Rabbids

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Curcumin Confers Cytoprotective and Steroidogenic-Boosting Effects to In Vitro Leydig Cells

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Testosterone is the principal male sex hormone essential for male fertility and overall wellbeing. Approximately 95% of the total testosterone in the male body is produced by the specialized testicular cells, Leydig cells, in a process called testicular steroidogenesis. During aging, serum testosterone levels progressively decline in men, with concomitant diminished muscle growth, reduced energy level, and decreased sexual and cognitive functions. Studies have demonstrated that, although decline in the H-P-T endocrine axis plays some role, the root cause of aging-associated androgen deficiency, or the so called “andropause” phenomenon, is due to decreased Leydig cell numbers and their reduced steroidogenic capacity. The present study investigates whether herbal medicine can protect Leydig cells from exposure to environmental contaminants and may reinvigorate their steroidogenic capacity. Two herbal medicinal chemicals, luteolin and curcumin, were selected for the initial analysis on three mouse Leydig cell lines MLT-1C, MA-10, and I-10. Leydig cells were incubated with each chemical at 0, 3, 10, and 30 μM for 2, 4, and 6 days, with medium/chemical change every 2 days. At each time point, viable cells were assessed using ORFLO Moti Z automated cell counter and collected for subsequent total RNA extraction and real-time quantitative PCR.
analyses of expression of key steroidogenic genes (STAR, Cyp11a1, Hsd3b6, and Hsd17b3). Curcumin showed a concentration-dependent growth stimulating effect in all three cell lines, with MLTC-1 being the most responsive one (after 2, 4, and 6 days of treatment, 1.29-fold, 1.26-fold, and 1.17-fold at 3 uM; 1.25-fold, 0.97-fold, and 1.28-fold at 10 uM). Luteinide did not increase cell proliferation in MLTC-1 cells. We previously showed that herbicide atrazine adversely affected the viability of Leydig cells cultured in suspension. With curcumin as a positive hit, we proceeded with curcumin and atrazine co-treatment on MLTC-1 cells. Cells were incubated with atrazine at 30 and 100 uM, with and without curcumin (10 uM) for 3 and 6 days, with medium/chemical change every 2 days. The presence of curcumin modestly improved the viability of Leydig cells exposed to atrazine. With regard to the impact on Leydig cell’s steroidogenic capacity, curcumin treatment stimulated the expression of all four steroidogenic genes tested, particularly at 10 uM after 6-day treatment (STAR 1.47-fold, Cyp11a1 4.24-fold, Hsd3b6 1.98-fold, and Cyp17b3 2.08-fold). The co-treatment of curcumin with atrazine further increased the expression of Hsd17b3 gene (4.2-fold, atrazine 100 uM + curcumin 10 uM, after 6 days). In summary, herbal medicine curcumin exhibits a protective effect in cultured rodent Leydig cells, either alone or in combination with an established environmental pollutant atrazine. In addition, curcumin increased the expression of a panel of key steroidogenic genes in Leydig cells. These findings suggest that curcumin may have a potential to reinvigorate Leydig cell’s steroidogenic capacity and thus enhance testicular andrographin production.

**4168 Preconception Exposure to Ethylene Glycol Monomethyl Ether Alters Abundance of Developmentally Important miRNAs in Rat Sperm**

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Paternal preconception exposure to toxicants has been linked to adverse health outcomes. These effects can occur through epigenetic mechanisms. Spermatozoa carry thousands of RNAs, including both mRNA and small non-coding RNAs, such as miRNA, and there is evidence that sperm miRNAs are beneficial for embryonic development in the mouse. Disregulation of germ cell transcription, cytoplasmic exclusion, or other spermatogenesis differentiation processes could alter sperm RNA populations, resulting in dysfunctional sperm. Ethylene glycol monomethyl ether (EGME) is a well-characterized testicular toxicant that selectively kills primary spermatocytes. It was previously reported that at low doses that do not significantly increase testicular histopathology, measured as retained spermatid heads, EGME increases miRNA as a proportion of all small RNAs in rat sperm. Therefore, we hypothesized that EGME would alter the abundance of sperm miRNAs that have roles in regulation of developmental gene expression. RNA-seq data from a prior publication were analyzed to test this hypothesis. Adult male Fisher rats were exposed to 0.75 mg EGME/kg/d for 5 days, and epididymal sperm were isolated 5 weeks later, after primary spermatocytes have completed spermatogenesis. Sperm small RNA libraries were sequenced, and reads were aligned to the rat genome using sRNAbench. Differential expression analysis using DESeq2 identified 12 miRNAs that were significantly differentially regulated at all three doses and had a monotonic dose-response. After identifying high-confidence targets of those miRNAs, we performed an overrepresentation analysis that identified the Gene Ontology term “regulation of developmental growth,” “cell fate commitment,” and “mutations of a branching structure” as significantly overrepresented terms. We conclude that EGME alters the abundance of miRNAs in rat sperm in ways that are likely to be detrimental to early embryo development.

**4169 β-Aminoisobutyric Acid Ameliorated Melphalan- and Diabetes-Induced Male Germ Cell Toxicity: Mechanistic Studies in Experimental Rat Model**


Melphalan has been widely used for the treatment of several types of cancers despite its gonadotoxic effects. Due to its ability to cause mutations in the spermatogonial stem cells and spermatids, melphalan can exert negative impact on male reproductive health. β-aminoisobutyric acid (BAIBA), a myokine released by skeletal muscles, has the potential effects against several pathological conditions; however, its exact role in chemotherapy-induced germ cell toxicity is still unexplored. The present study aimed to determine the dose-dependent (25, 50 and 100 mg/kg) effects of BAIBA on melphalan-induced (1.5 mg/kg) germ cell toxicity in Sprague-Dawley (SD) rats. Further, an attempt has been made to investigate the molecular mechanisms involved in BAIBA mediated protection against the development of melphalan-induced germ cell toxicity in rats. The evaluation parameters included quantification of oxidative stress biomarkers, sperm count, sperm motility and head morphology, sperm and testicular DNA damage, sperm mitochondrial membrane potential, ultrastructural, histological and protein expression studies in testes. Melphalan treatment significantly altered all the above-mentioned parameters and the high dose of BAIBA restored melphalan-induced toxicity in a significant manner by exerting antioxidant, anti-inflammatory and anti-apoptotic effects. However, the medium dose of BAIBA decreased the toxicity of melphalan and the low dose of BAIBA failed to counteract the marked reduction induced by male germ cell toxicity. In the present study, antioxidant, anti-inflammatory and anti-apoptotic role of BAIBA in melphalan-induced gonadal damage in experimental rat model is one of the novel findings. Diabetes mellitus (DM) has been recognised as the ‘epidemic of the century’ due to its global prevalence. As per the International Diabetes Federation report of 2019, approximately 463 million adults were suffering from diabetes in 2019 and this number was projected to rise to 700 million by 2045. Male germ cell damage is one of the major complications of DM as evident by several pre-clinical and clinical studies. BAIBA has been reported to exert beneficial effects in diabetic nephropathy and cardiomyopathy; however, its exact role in diabetes-induced germ cell damage is still elusive. In the present study, an attempt was made to elucidate the molecular mechanisms of BAIBA mediated germ cell protection in diabetic rats. Adult male SD rats were subjected to either no treatment (control) or BAIBA (100 mg/kg; BAIBA control) or STZ (50 mg/kg; diabetic control) or low (25 mg/kg), medium (50 mg/kg) and high (100 mg/kg) dose of BAIBA in diabetic conditions. Significant alterations in sperm related parameters, oxidative stress, apoptotic and inflammatory biomarkers, histological, testicular and spermatogenic parameters, histostological changes, DNA damage and changes in immunoperoxidase of proteins in testes were observed in diabetic rats. High doses of BAIBA significantly ameliorated diabetes-induced testicular complications by alleviating oxidative stress, apoptosis and inflammation via activation of AMPK/SIRT1 signaling pathway. Medium doses of BAIBA partially restored the above-mentioned parameters whereas low dose of BAIBA was found to be insignificant in countering the toxicity. It is interesting to note that BAIBA protects male germ cell damage in diabetic rats by regulating the AMPK/SIRT1 signaling pathway. Above investigation demonstrated that BAIBA is effective in reducing melphalan- and diabetes-induced toxicity in the germ cell of rats. However, further dose-dependent and threshold-based analyses of different routes of exposure may elucidate its exact intervention, involvement of other molecular targets and usefulness for clinical efficacy.

**4170 Characterization of an In Vitro Model of Neonatal Testis**


A current limitation in predictive toxicology is inadequate availability of in vitro and microfluidic systems for male reproductive systems, which are particularly susceptible during post-natal development. Successful in vitro models of male reproductive toxicity need to recapitulate representative cell types and functional activities present in neonatal testis. To address this need, we built on previous organotypic testis culture work to develop a novel 3-D primary neonatal testicular cell culture system (TCS) using primary postnatal day five rat cells to model testicular toxicity during neonatal development. This model incorporates 3D co-culture of testis cells in an innovative microculture system developed at the University of Washington. The microculture plate contains an array of 20 experimental wells, each with a central culture area (16uL volume) and a concentric culture area (54uL volume), the well can be combined for a total culture volume of 90uL. The TCS is seeded with primary cells in media with 4.4 mg/mL Matrigel, protein, sufficient for formation of 3D structure. Using this microculture system the TCS reduces the number of animals required for evaluation of toxicants relative to in vivo studies, while still maintaining in vivo-like histology and physiological relevance of the TCS, we conducted a baseline characterization of testosterone production and gene expression across eight days in vitro (DIV). After a 48-hour acclimation, culture supernatant samples were collected on DIV 2, 4, 6, and 8 for testosterone analysis and RNA samples were collected on DIV 4, 6, and 8. Testosterone concentrations were determined through an ELISA assay (Neogen 402510). RNA samples were collected and submitted to Novogene Corporation for bulk RNA sequencing. Testosterone concentrations decreased over time in the baseline study (p<0.001). Transcriptomic data showed consistent expression of testis marker genes over time: Amh and Sox9 (Sertoli Cells), Cyp11a1 and Star (Leydig cells), Dazl (Germ cell marker) and Hsd17b3 (macrophages). We assessed the utility and physiological relevance of the TCS through quantification of testosterone production after addition of luteinizing hormone (LH) and follicle stimulating hormone (FSH) to the culture media. Hormones were added at a low dose (5mIU/mL FSH and 50mIU/mL LH) and high dose (20mIU/mL FSH and 500mIU/mL LH). The low-dose exposure was started at both experimental day 2 and experimental day 6 to test the change in response across time in culture. Both treatments were found to be significantly increased relative to the control (high p-value <0.001, low p-value <0.01) and experimental day was not found to significantly interact with treatment. The mean testosterone concentration was 23.0 ng/mL [95% CI: 15.3-30.7 ng/mL] in the high-dose group and 8.2 ng/mL [95% CI: 2.4-13.9 ng/mL] in the low-dose group. The TCS recapitulates physiologically relevant testosterone production in response to hormone exposure and maintains major testis cell types, requirements for a successful in vitro testis toxicological model. The TCS addresses a critical limitation in predicting reproductive toxicity during the post-natal period and will be further characterized to benchmark the temporal relevance compared to in vivo life course as well as recapitulation of functional dynamics occurring during development. (1) Open Microfluidic Coculture Reveals Paracrine Signaling from Human Kidney Epithelial Cells Promotes Kidney Specificity of Endothelial Cells. (Zhang, Tianz, et al., 2020).
A drastic decline in sperm parameters has been observed in the last five decades. The reasons for the decline are not well understood, although many recent findings suggest the role of environmental pollutants as a possible culprit. We hypothesize that at least some male reproductive toxicities may be mediated by the blood-testis barrier (BTB) dysregulation via the mechanistic target of the rapamycin (mTOR) pathway. mTOR is a molecular hub downstream of many molecular signaling cascades often triggered by environmental exposures, including oxidative stress, hypoxia, unfolded protein response, endoplasmic reticulum stress, and others. In Sertoli cells, the balance of the two mTOR complexes (mTORC1 and mTORC2) determines the BTB permeability, where mTOR complex 1 (mTORC1) promotes BTB restructuring and mTORC2 promotes BTB integrity. The BTB is one of the tightest tissue barriers in the mammalian organism which regulates the entry of diverse chemicals inside the lumen of the seminiferous tubule - the compartment in which several critical steps of spermatogenesis occur, including both meiotic divisions, epigenetic reprogramming, and spermiogenesis. Thus, in the current study, we test our hypothesis by the analysis of male reproductive outcomes in transgenic mouse models with an altered balance of mTOR complexes. Sertoli cell-specific inactivation of mTORC1 or mTORC2 was achieved by knockout of their corresponding components, Raptor and Rictor, using Cre-Lox approach. An in vivo biotin tracer assay was used to observe the integrity of the BTB in wild-type (WT) and knockout (KO) animals. Animals were euthanized at several time points and their testes were weighed. Testes weight and their relative weight were recorded and analyzed using eosin-hematoxylin staining. Sperm smears were used for sperm counts and sperm morphology analysis. Mitochondria DNA copy number was assessed using qRT-PCR. DNA extracted from epididymal spermatozoa of 8- and 22-week-old mice was used to prepare libraries for reduced representation bisulfite sequencing using Nugen technology. Sequencing data were analyzed using Bismark, Bowtie, MethylKit, and Metascape. A significant reduction of testes weight was observed in both knockouts as compared to respective wild-type animals at different time points. The biotin tracer assay confirmed that BTB integrity was compromised only in animals with suppressed mTORC2. The most drastic changes in male reproductive outcome were observed in these animals at all time points, confirming that increased BTB permeability has deleterious effects on spermatogenesis. Specifically, mice with suppressed mTORC2 had disorganized testes morphology, decreased sperm counts, increased morphologically abnormal sperm, and increased mitochondrial DNA copy numbers. In both KO groups, an age-related increase in the percentage of promoters with altered methylation was also observed as compared to respective wild-types. According to the enrichment analysis, the top affected pathways were relevant to embryo development suggesting intergenerational effects of mTOR signaling disruption in Sertoli cells.

**4171**

**mTOR-Dependent Dissociation of the Blood-Testis Barrier: A Potential Novel Mechanism of Male Reproductive Toxicity**

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Exposure to highly hazardous pesticides (HHPs) is a global public health problem. Some organophosphate pesticides (OP) are among HHPs, representing >50% of total use worldwide. Chlorpyrifos (CPF), one of the most widely used OP, is classified as moderately dangerous by the World Health Organization. CPF has been linked to reproductive effects, such as reduced testicular weight and sperm count, which involves matrix metalloproteinases (MMP-2 and -9) and their tissue inhibitors (TIMP-1 and -2). Apart from regulating trophoblast migration, both PI3K and AKT play a role in maintaining glucose homeostasis throughout development, affecting insulin-dependent heart and skeletal muscle growth. Both MMPs and TIMPs also participate in pathogenesis of neurodegenerative, cardiovascular, inflammatory diseases, and cancer. Human extravillous trophoblast HTRB/SVneo cells, seeded at 1x10^5 cells/well in 6-well plates, were treated 24-h later with media containing subtoxic concentrations of either: 1) 50:50 propylene glycol + vegetable glycerin (PG/ VG) (0.5%); 2) nicotine alone (0.1 mg/ml); 3) PG/ VG 1%+ nicotine 0.05 mg/ml; 4) PG/ VG + 24 mg/ml nicotine (0.5%); 5) PG/ VG + 24 mg/ml nicotine + 5 mg/ml menthol (0.5%); 6) aerosol condensate prepared from heated group 4 or 5 constituents (0.1 mg/ml). Data on control (0.1 mg/ml) media control condensate was processed using a Single Port E-cig Aerosol Generator. RT-qPCR, with appropriate gene primers, were subsequently performed. Results demonstrated that PG/VG plus nicotine, as well as their combination with menthol, strongly increased the expression of PI3K, MMP-2 and MMP-9 compared to control. Alternatively, heated aerosol condensates downregulated MMP-2. These findings support the hypothesis that aerosol constituents and heated aerosols alter gene expression in extravillous trophoblasts critical for the establishment of utero-placental exchange and proper placentalation. The ubiquity of the genes affected also suggest the possibility of effects on other organ systems. Supported by NYU GSoM Div. of Environ. Med.

**4173**

**In Vitro Exposure to E-cigarette (E-cig) Constituents and Aerosol Condensate Alters Gene Expression in Cultured Trophoblast Cells**

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Although it has been less than 20 years since their marketing in the US, electronic cigarettes (e-cigs) have gained an immense popularity both in the US and around the world. As they were once marketed as a healthier alternative to traditional cigarettes, their use is widespread among all groups of nicotine users; the 2016 National Youth Tobacco Survey reveals that almost 5% of middle school students and 20% of high school students used e-cigs within the previous 30 days. It is estimated that between 16 and 19% of women of childbearing age consume tobacco products, including 10% of pregnant women in the last trimester. Thus, it is a critical issue to determine the actual level of e-cig safety during pregnancy. We previously showed that e-cig constituents and their heated aerosol condensates alter trophoblast migration. Thus, studies were undertaken to investigate gene expression changes and possible pathways by which migration could have been affected. One of the most ubiquitously affected pathways is the PI3K/akt pathway, which involves matrix metalloproteinases (MMP-2 and -9) and their tissue inhibitors (TIMP-1 and -2). Apart from regulating trophoblast migration, both PI3K and AKT play a role in maintaining glucose homeostasis throughout development, affecting insulin-dependent heart and skeletal muscle growth. Both MMPs and TIMPs also participate in pathogenesis of neurodegenerative, cardiovascular, inflammatory diseases, and cancer. Human extravillous trophoblast HTRB/SVneo cells, seeded at 1x10^5 cells/well in 6-well plates, were treated 24-h later with media containing subtoxic concentrations of either: 1) 50:50 propylene glycol + vegetable glycerin (PG/VG) (0.5%); 2) nicotine alone (0.1 mg/ml); 3) PG/ VG 1%+ nicotine 0.05 mg/ml; 4) PG/ VG + 24 mg/ml nicotine (0.5%); 5) PG/ VG + 24 mg/ml nicotine + 5 mg/ml menthol (0.5%); 6) aerosol condensate prepared from heated group 4 or 5 constituents (0.1 mg/ml). Data on control (0.1 mg/ml) media control condensate was processed using a Single Port E-cig Aerosol Generator. RT-qPCR, with appropriate gene primers, were subsequently performed. Results demonstrated that PG/VG plus nicotine, as well as their combination with menthol, strongly increased the expression of PI3K, MMP-2 and MMP-9 compared to control. Alternatively, heated aerosol condensates downregulated MMP-2. These findings support the hypothesis that aerosol constituents and heated aerosols alter gene expression in extravillous trophoblasts critical for the establishment of utero-placental exchange and proper placentalation. The ubiquity of the genes affected also suggest the possibility of effects on other organ systems. Supported by NYU GSoM Div. of Environ. Med.

**4174**

**Extracellular Vesicle-Based Delivery of Anti-Inflammatory Cytokine IL-10 Effects on Pregnancy Immune Mediators HLA-G and PGRMC2**

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Preterm birth (PTB; < 37 weeks of gestation) is associated with a fetal-maternal inflammatory response and premature disruption of immune homeostasis that maintains pregnancy. Exposure to environmental toxins during pregnancy causes immune intolerance at the fetomaternal interface (FMI), specifically in the human fetal membranes (FM) surrounding the intrauterine cavity. Current therapeutics target the maternal inflammatory response to delay myometrial contractions and cervical ripening, but these treatments had limited success in reducing PTB rates. Interleukin 10 (IL-10), an anti-inflammatory cytokine, can regulate immune homeostasis and delay inflammation-induced spontaneous PTB; however, its short half-life diminishes IL-10’s therapeutic utility to reduce FMI inflammation. Packaging IL-10 into extracellular vesicles (EVs [eIL10]) has overcome this limitation, reduced the fetal inflammatory response, and delayed PTB in animal models. To further improve the tropism of eIL10 and targeted delivery to fetal tissues, we considered the major histocompatibility complex HLA-G, which is localized to the FM and placenta. EVs modified to specifically target HLA-G should show increased delivery to the target tissue and decreased off-target effects. Here, we assessed whether EVs engineered to contain an anti-HLA-G antibody could show specificity for HLA-G positive cells. To rule out any immune-toxic effects of IL-10 at the target sites (e.g., suppression of innate anti-inflammatory systems) due to feedback of immune pathways, we tested the effect of recombinant IL-10 exposure on the expression of HLA-G, which plays a role in FMI immune privilege, as well as progestosterone receptor PGRMC2, which functionally facilitates pregnancy maintenance hormone progesterone function. HEK293-derived EVs were linked to HLA-G antibodies to target immortalized chorion trophoblast cells from human fetal membranes (HMF-CMC) which express HLA-G. A. co-culture of HEK (HLA-G negative cells) and HMF-CCT (HLA-G positive cells) were treated with anti-HLA-G tagged EVs for four hours, and cell specific targeting was measured using immunocytochemistry. HLA-G and
PGRMC2 were measured in HFM-CTC, because these cells constitutively express both proteins. HLA-C and PGRMC2 were measured after 24 h of IL-10 exposure by western blot. We report successful targeted delivery of EVs to fetal cells that contribute to inflammation. Naïve EVs did not specifically target HLA-G expressing cells, and knockout of HLA-G in HFM-CTCs abolished the targeting effect, confirming HLA-G expression mediated targeting. Exposure to recombinant IL-10 did not change HFM-CTC expression of HLA-G or PGRMC2, suggesting lack of immune suppression. The constitutive expression of HLA-G after exposure to IL-10 also confirmed that this molecule can be used as a target and will not diminish after an initial dose of IL-10. We conclude: (1) HLA-G on fetal cells is a potential target molecule for specific delivery to FMI for efficient treatment of fetal inflammatory response associated PTB and (2) IL-10 inhibits fetal inflammatory signals, delays PTB, and does not cause deleterious immunosuppressive effects by downregulating other anti-inflammatory systems during pregnancy.

4175 Preconception Exposure to the Organophosphate Larvicide Temephos May Impair Intrauterine Growth of Offspring in Mice


Temephos (Tem) is an organophosphate larvicide used by public health authorities to control mosquito transmission of dengue, chikungunya and Zika. Tem usage in health campaigns is recommended by the World Health Organization (WHO) due to its classification as low toxicity in mammals. Nevertheless, female mice treated with Tem showed decreased litter size, reduced body weight gain, and oxidaive stress markers. Preconception exposures were performed for 90 minutes per exposure with a 30 W atomizer, and after the fifth estrus. At the end of treatment, females were randomly mated with unexposed males, and they were euthanized at day 17 of pregnancy. Litters and placentas were collected and weighed. Additionally, maternal PG/VG inhalation exposures were counted and intrauterine growth restriction (IUGR) was determined. Our data showed that pre-conception exposure to Tem did not alter the litter size compared to control. However, it significantly decreased litter and placental weight compared to control. Interestingly, pre-conception exposure to 150 µg/kg/day of Tem significantly increased IUGR of the offspring compared to those from controls. Further, pre-conception exposure to 150 µg/kg/day of Tem significantly increased embryo resorptions in litters compared to controls. Our data suggest that preconception exposure to Tem may be deleterious to offspring development. However, more experiments are warranted to elucidate the underlying mechanisms of toxicity.

4176 Inhalation Exposure to Propylene Glycerol and Vegetable Glycerin Results in Elevated Free Radical Production in Dams and Offspring

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Women continue to use electronic cigarettes (e-cigs) during pregnancy. We investigated the effects of propylene glycerol and vegetable glycerin (PG/VG), which are the main liquid components in e-cigs, inhalation exposures on maternal and F1 health outcomes. We hypothesized that PG/VG exposures would increase hydrogen peroxide ($H_2O_2$) production and xanthine oxidase (XO) in the exposed dams and the F1 generation. Our previous research shows that PG/VG inhalation exposure decreased pup mass and litter sizes and increased maternal oxidative stress markers in a dose-dependent manner (100 mg/kg/day did not increase XO activity), which has been observed at doses ≥50 mg/kg/day. Fetal weights were significantly reduced at 100 mg/kg/day, which correlated with ossification delays and were not significantly affected by maternal body weight losses, however, no treatment-related fetal malformations (teratogenic effects) were observed. There were no direct effects on embryo-fetal toxicity at any of the doses tested. Therefore, the NOAEL for embryo/fetal viability, growth, and development for Valbenzine was established at 50 mg/kg/day. Overall, these findings indicate that valbenzine does not significantly affect rat or rabbit embryo-fetal development even at dose levels producing maternal toxicity.

4177 Embryo Fetal Toxicity Studies in Rats and Rabbits Following Exposure to Valbenzine, a VMAT-2 Inhibitor in the Treatment of Tardive Dyskinesia


Valbenzine, a VMAT-2 inhibitor approved in the U.S. for the treatment of tardive dyskinesia was evaluated for its potential to cause maternal, embryo/fetal toxicity and teratogenicity in pregnant rats and rabbits via oral gavage during the period of organogenesis. Valbenzine administered daily by oral gavage to Sprague Dawley rats from GD 6 through 17 dosed up to 15 mg/kg/day was generally well-tolerated at all doses tested. Clinical signs of decreased activity and squinted eyes at 15 mg/kg/day were noted and were expected pharmacological effects of the compound due to its VMAT-2 activity. Statistically significant decreases in body weight parameters and food consumption were noted at ≥5 mg/kg/day. There were no effects on developmental toxicity parameters at oral dose levels tested (1, 5, 15 mg/kg/day). Based on the lack of adverse prenatal effects, the NOAEL for embryo/fetal viability, growth, and development was established at 15 mg/kg/day, the highest dose tested. In a separate study, New Zealand white rabbits were dosed at 20, 50, or 100 mg/kg/day of oral gavage from GD 7-20. Signs of maternal toxicity were observed at doses ≥50 mg/kg/day. Fetal weights were statistically significantly reduced at 100 mg/kg/day, which correlated with ossification delays and were not significantly affected by maternal body weight losses, however, no treatment-related fetal malformations (teratogenic effects) were observed. There were no direct effects on embryo-fetal toxicity at any of the doses tested. Therefore, the NOAEL for embryo/fetal viability, growth, and development for Valbenzine was established at 50 mg/kg/day. Overall, these findings indicate that valbenzine does not significantly affect rat or rabbit embryo-fetal development even at dose levels producing maternal toxicity.

4178 Endothelial Cell AHR Signaling and Dioxin-Induced Placental Adaptations

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Placenta involves the coordinated differentiation of trophoblast stem cells into various lineages with specialized functions such as invasive trophoblast cells. Invasive trophoblast cells migrate into the uterine compartment during pregnancy and remodel uterine spiral arteries. These pregnancy-dependent vascular restructuring facilitates nutrient flow to the placenta. Failures in trophoblast cell invasion are associated with pathological conditions such as preeclampsia, intrauterine growth restriction, and preterm birth. Gestational environmental exposures can alter molecular and cellular pathways controlling development of the organism. Cells respond to environmental challenges through alterations in gene regulation and expression that may affect developmental processes. Maternal exposures to environmental pollutants can modulate placental adaptations. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that modulates gene expression facilitating adaptations to environmental exposures. AHR ligands present in the environment can be referred to as endocrine disruptors because they affect homeostatic mechanisms controlling the physiology of the organism. The mouse is not a suitable model for investigation of the effects of environmental pollutants on intratherine trophoblast cell invasion. Unlike mouse, rat placenta shows deep trophoblast cell invasion into the uterus, similar to human placenta. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a dioxin and potent activator of AHR signaling, during sensitive and critical windows of gestation induces placental adaptations. This effect appears to be mediated by endothelial cell AHR signaling. To investigate the role of endothelial cell AHR signaling in placenta site responses to environmental exposures we generated an AHR conditional rat model. To this end, we used CRISPR/Cas9 genome editing to generate an endothelial cell specific AHR conditional rat model. In placentation site responses to environmental exposures we generated an AHR conditional rat model. This work was supported by National Institutes of Health Grant #R01 GM104942-05, NIH KO1 OH23220 (ECB), NIH KO1 ES051022 (TRN).
The Effects of Imidacloprid and Its Bioactive Metabolite Desnito-Imidacloprid on Ovarian Antral Follicles

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Imidacloprid (IMI) is a neonicotinoid pesticide used in large scale agricultural systems, home gardens, and veterinary pharmaceuticals. IMI is a small molecule that is more water soluble than other insecticides, increasing the likelihood of large-scale environmental accumulation and chronic exposure of non-target species. IMI is readily absorbed in the gastrointestinal tract of mammals where it travels to the liver and undergoes phase I biotransformation into a variety of intermediate metabolites such as desnito-imidacloprid (DNI), a bioactive metabolite shown to be significantly more toxic to mammals than IMI. Studies have shown that IMI reaches the ovaries immediately after oral exposure and that IMI exposure reduces ovarian weight and causes morphological abnormalities in ovarian follicles compared to control. However, little is known about the mechanisms by which IMI and DNI affect ovarian toxicity. Thus, we tested the hypothesis that IMI and DNI cause ovotoxicity through different mechanisms of action in vitro. Antral follicles were dissected from ovaries of CD-1 mice (31-41 days old) and cultured in media containing vehicle control or 0.2µg/ml - 200µg/ml of IMI or DNI for 48 hours and 96 hours. Follicle morphology was examined and follicle size was measured every 24 hours. At the end of the culture periods, follicles were collected for gene expression analysis of steroidogenic regulators (Star, Cyp11a1, Cyp17a1, Hsd17b1, Cyp19a1), estrogen receptors (Erα and Erβ2), the androgen hydrocarbon receptor (Ahr) and its targets (Cyp1a1 and Cyp1b1), and apoptotic factors (Bax and Bcl2). Culture media were also collected for estradiol, progesterone, and testosterone quantification via enzyme linked immunosorbent assays. After 48 hours, neither IMI nor DNI affected follicle growth compared to control. Interestingly, IMI (3.0µg/ml) and DNI (200µg/ml) significantly decreased follicular estradiol secretion compared to control. DNI (0.2-200µg/ml) exposure for 48 hours caused significantly more antral follicles to rupture compared to control follicles and IMI-treated follicles. After 48 hours, IMI, but not DNI, affected expression of steroidogenic regulators compared to control. IMI (0.2 and 200 µg/ml), but not DNI, affected expression of Cyp1a1, Cyp1b1, Bax, and Bcl2 compared to control. After 96 hours IMI did not affect follicle growth, whereas DNI (20 and 200µg/ml) inhibited follicle growth compared to control. DNI (200µg/ml) significantly decreased estradiol secretion compared to control and IMI (200µg/ml) significantly increased progesterone secretion compared to control. DNI (0.2-200µg/ml) exposure for 96 hours caused significantly more antral follicles to rupture compared to control follicles and IMI-treated follicles. After 96 hours, both IMI (0.2-200µg/ml) and DNI (0.2-200µg/ml) dysregulated expression of steroidogenic regulators in antral follicles compared to control. Neither IMI nor DNI dysregulated Erα, Erβ2, Ahr, or Cyp1a1 compared to control. However, DNI (200µg/ml) increased expression of Cyp1b1 and Bax and it decreased expression of Bcl2. These data indicate that IMI does not affect follicle growth and increases follicular estradiol transiently, whereas DNI disrupts follicle growth and decreases follicular estradiol long-term possibly through induction of Cyp1b1 expression. Supported by NIH F30 033914 and RO1 E0328661.
An Ex Vivo Mini-ovary Preserves Molecular Signatures of Gonadotropin-Dependent Folliculogenesis and Enables the Identification of Ovarian Toxicants

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As the female gonad, the ovary consists of various stages of follicles as its functional unit, which critically regulate women’s reproductive cycles and fertility. Both environmental estrogenic disrupting chemicals and clinical drugs have been shown to exhibit ovarian disrupting effects. However, it is challenging to identify ovarian toxicants and explore mechanisms involved due to the low throughput of whole animal model and limited biomasses of premenopausal human ovaries. We previously established a 3D hydrogel encapsulated in vitro follicle growth (eIVFG) system which faithfully recapitulates all key ovarian functions in vivo, including follicle-dependent follicular maturation, hormone secretion, and ovolation. Here, we further determined whether the molecular signatures of these follicular events are well preserved in follicles derived from eIVFG, thus enabling a truly scalable and highly controllable system to identify ovarian toxicants and mechanisms involved. Multi-layered secondary follicles were isolated from 16-day-old CD-1 female mice and cultured with eIVFG for 8 days. Follicles were collected on day 0, 4, and 8 for single-follicle RNA-sequencing (RNA-seq) analysis. Principal component analysis (PCA) separated follicles into distinct clusters by their cultured days, indicating the dynamic transcriptome changes during the gonadotropin-dependent folliculogenesis. Using all differentially expressed genes (DEGs), we performed multi-gene pathway and enrichment analyses at different days of eIVFG. Eight temporal gene expression clusters were identified, which can be further categorized into two groups: continuously increasing or decreasing genes, suggesting the crucial roles of either induction or suppression of these genes in follicle stimulating hormone (FSH)-induced follicle maturation. We next examined the expression of well-established follicle maturation marker genes, such as Fshr, Pappa, Cyp19a1, and Lhcgr. All these genes were remarkably induced during eIVFG and their expression patterns were consistent to previous results from in vivo models, indicating that eIVFG conserves the molecular signatures of gonadotropin-dependent folliculogenesis. So far, we have used eIVFG to examine the ovaries toxicity of 40 chemicals, including (HAB) toxins, 6 per- and polyfluoroalkyl substances (PFAS), 10 bisphenol analogues, 7 flame retardants, 8 pre-clinical compounds, and 3 micro & nano-plastics. For example, our results showed that microcystin-LR (MC-LR), one of the most common HAB toxin, acted as a selective inhibitor of protein phosphatase 1 (PP1) inhibitor to interfere with FSH-induced follicle maturation, which caused a secondary defect on ovulation. In addition, a commonly used long-chain PFAS, perfluorononaic acid (PFNA), compromised follicle growth, ovulation, hormone secretion of estradiol and testosterone, and expression of ovarian steroidogenic genes of Star, Hsd17b1, and Hsd3b1. Together, our study demonstrates that eIVFG preserves morphological, hormonal, and molecular signatures of gonadotropin-dependent folliculogenesis, representing a powerful model to identify ovarian toxicants and mechanisms involved.

Short-Term Exposure of Propylparaben Alters Expression Levels of Steroidogenic and Apoptotic Factors in Ovaries and Oviducts of Mice

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Parabens are prevalently found in cosmetics, foods, and other daily-use products. Limited studies indicated that paraben could impact female reproductive function. Yet, the effects of a short-term exposure of propylparaben on the ovary and oviduct are unknown. The ovaries and oviducts are central to mammalian female reproduction. The ovary is the site of oocyte production and the site of estrogen hormone biosynthesis. The ovary is the site of fertilization and actively transports the preimplantation embryo to the uterus. The current study tested the hypothesis that acute propylparaben exposure would alter steroidogenesis and increase apoptosis in the ovaries and oviducts of mice. To that end, adult CD-1 mice (n=6 per treatment group) were orally dosed with corn oil/vehicle or environmentally relevant doses of propylparaben (CAS No.94-13-3; 20 µg/kg/d, 200 µg/kg/d, and 20 mg/kg/d). After ten days of daily dosing, ovaries, oviducts, and sera were collected at diestrus and subjected to real-time qPCR and ELISA (estradiol and progesterone). Comparison between propylparaben treatment groups and controls, resulted in significant differences (p < 0.05). Specifically, in the oviducts, propylparaben exposure increased levels of Bcl2 (mean ± SD, 200 µg/kg/d 2.67 ± 0.64, 20 mg/kg/d 2.26 ± 0.73, control 1.17 ± 0.73, n=5-6) and Bax (20 µg/kg/d 2.20 ± 0.54, 200 µg/kg/d 2.42 ± 0.78, 20 mg/kg/d 2.27 ± 0.20, and control 1.19 ± 0.60 n=6), and decreased levels of Bcl2 (20 µg/kg/d 0.81 ± 0.15, 20 mg/kg/d 0.81 ± 0.12, and control 1.00 ± 0.13 n=6). Further, in the ovaries, propylparaben exposure decreased levels of Bax (20 mg/kg/d 0.65 ± 0.26, control 1.06 ± 0.38, n=5-6), and increased levels of Esr1 (20 µg/kg/d 0.81 ± 0.15, 20 mg/kg/d 0.81 ± 0.12, and control 1.00 ± 0.13 n=6). In addition, the ovaries progesterone/estradiol levels decreased levels of Bax (20 mg/kg/d 0.65 ± 0.26, control 1.06 ± 0.38, n=5-6), Esr1 (20 µg/kg/d 0.51 ± 0.19, control 1.07 ± 0.46, n=5-6), Star (200 µg/kg/d 0.47 ± 0.29, control 1.39 ± 0.95, n=5-6), 3βHsd (200 µg/kg/d 0.49 ± 0.14, control 1.11 ± 0.46, n=5-6), and Hsd17b1 (20 µg/kg/d 0.92 ± 0.44, 200 µg/kg/d 0.61 ± 0.11, control 1.01 ± 0.17, n=5-6). There were no statistically significant differences in estradiol and progesterone levels between propylparaben and control groups. Overall, these results suggest that a short-term exposure of propylparaben impacts gene expression of the ovary and oviduct and can potentially affect mouse reproductive health.

Spinosad: Mode-of-Action and Human Relevance Assessment of Dystocia in Rats

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Spinosad technical, a natural product insecticide derived from fermentation, associated with treatment-related adverse pregnancy outcomes which manifested as dystocia in the rat. A robust mechanistic study program was initiated to determine the MOA for dystocia in rats and relevance of this hazard to humans using the (WHO)/International Programme on Chemical Safety (IPCS) framework. A number of dose-related key events have been identified that characterise the rat MOA for Spinosad-induced dystocia. Dystocia was characterised by prolonged parturition which was associated with peri-partum maternal death and other peri-partum effects. Using in vivo and ex vivo contractility experiments, it was concluded that parturition became protracted due to inhibition of uterine muscle contraction, arising due to a pharmacological/receptor-mediated inhibition of action potential generation in uterine smooth muscle cells (myometrial cells). By using competition binding experiments with receptor ligands, it is hypothesized that the Spinosad receptor mediating uterine effects may be Translocator Protein (TSPO).

Investigation of the Pharmacodynamic Component of a Rat Mode of Action for Dystocia in the Rat

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Spinosad is a natural product insecticide derived from Saccharopolyspora spinosa fermentation. Spinosad is a mixture of structurally similar fermentation products named Spinosyns, that includes Spinosyn A and Spinosyn D. In a dietary rat teratology study, Spinosad was demonstrated to cause dystocia via uterine adverse effect of dystocia at the LOAEL levels showed that uterine concentrations in humans are expected to be approximately one order of magnitude lower compared to rats. The rat Spinosad adverse effect of dystocia will not be triggered in humans as tissue concentrations remain below the effect threshold for the dynamic (receptor-mediated) molecular initiating effect. Since, the rat mode of action is not plausible in humans, Spinosad does not pose a reproductive hazard to humans.

Investigation of the Pharmacodynamic Component of a Rat Mode of Action for Dystocia in the Rat

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Spinosad is a natural product insecticide derived from Saccharopolyspora spinosa fermentation. Spinosad is a mixture of structurally similar fermentation products named Spinosyns, that includes Spinosyn A and Spinosyn D. In a dietary rat teratology study, Spinosad was demonstrated to cause dystocia via uterine action potential generation and muscle contraction in rat uterus ex vivo. Spinosad exposure of 100 mg/kg/day inhibited rat uterine contractility in vivo during parturition. In ex vivo experiments with rat uterus, Spinosad A inhibited myometrial cell action potential generation and uterine contractility within minutes of exposure and an IC50 of approximately 3 µM in Translocator Protein (TSPO) ligand competitive displacement experiments in uterine membrane homogenates suggested that the Spinosad receptor mediating rat uterine action potential generation and contraction inhibition may be TSPO. Weight of Evidence was conducted, and several alternative modes of action were excluded such as endocrine effects.
cell factor 1, which modulates the transcriptional activity of FOXO3 and histone H4 acetylation, was increased 0.86-fold. 100% of females expressed the TNFα/7/2 complex subunit 18, which promotes HR, was increased by 0.95-fold; nucleolin which induces chromatin decondensation, was increased by 1.0-fold, while FACT complex subunit, a histone chaperone was increased by 2.7-fold (P < 0.05). Together the alterations in the abundance of these proteins suggest that ATM plays a role in the coordination of the ovarian DDR response during DMBA-induced oxtotoxicity. Supported by 1R01ES030341 from NIEHS.

4188 Ovarian Peroxidase Proplier—Activated Receptors Are Altered by Zearalenone Exposure and Heat Stress in Female Pigs


The earth’s temperature has risen by 0.08 °C each decade since 1880, with predicted average global temperature increasing by 0.36 to 0.70 °C by 2035. Heat stress (HS) is an increased core body temperature due to external thermal conditions coupled with basal heat production; phenotypically manifesting as hyperinsulinaemia, intestinal hyperpermeability, and inflammation. Negative reproductive metrics result from HS including anovulation, early pregnancy loss, and seasonal infertility. Zearalenone (ZEN) is a non-steroidal mycotoxin, and both ZEN and its metabolites can bind to estrogen receptors to mimic 17β-estradiol activity. In females, ZEN exposure causes negative reproductive outcomes, including abortion, vulvovaginitis, pseudopregnancy, stillbirth, and endocrine disruption. Peroxidase prolier-activated receptors (PPARs) are steroid hormone superfamily receptors that can be activated by a variety of natural and synthetic ligands. Exposure of female pigs to ZEN and/or HS was hypothesized to alter ovarian PPAR protein abundance differentially in thermal neutral (TN) and HS females. Crossbred prepubertal pigs were divided into six treatment groups: TN vehicle control (TC; n = 6), TN ZEN (40 µg/kg BW; TZ; n = 6), pair-fed vehicle control (PC; n = 6), pair-fed ZEN (40 µg/kg BW; PZ; n = 6), HS vehicle control (HC; n = 7) and HS ZEN (40 µg/kg BW; HZ; n = 7). The pair-feeding experimental design eliminated any confounding effects of HS-induced hypophagia. The abundance of ovarian PPARα, PPARβ/δ, and PPARγ were quantified via western blotting. In the absence of ZEN, HS decreased PPARα (P = 0.001), increased PPARβ/δ (P = 0.006) and did not alter PPARγ (P = 0.11). In TN females, ovarian PPARα (P = 0.86) and PPARγ (P = 0.21) were unaffected; however, PPARβ/δ was increased (P = 0.01) by ZEN exposure. Within HS females, ovarian PPARα (P = 0.004), PPARβ/δ (P = 0.0001), and PPARγ (P = 0.0007) were increased by ZEN exposure. In PF females, ovarian PPARα (P = 0.003) was increased, PPARβ/δ was reduced, while PPARγ was decreased (P = 0.02) by ZEN exposure. These findings suggest that ovarian PPARs are responsive to both ZEN exposure and HS, a molecular scenario that potentially influences fertility. This work was supported by the Iowa Pork Producers Association.

4189 In Vivo Evaluation of Local Tolerance of Vaginal Formulations and Medical Devices with a 3D Human Epithelial Model

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The goal of the study is to validate the utility of the 3D human in vitro vaginal tissue model as an alternate for the rabbit vaginal irritation (RVI) test requested for the regulatory evaluation of drugs and devices in contact with the vaginal mucosa. A double-blind study was conducted in vivo and in vitro with N=14 encoded test articles (TAs) including materials intended to be in contact with vaginal tissues in the form of preservatives, contraceptives, solvents, viscosity enhancers, antiseptics, and cleansing agents (surfactants). The TAs were topically applied in vivo and in vitro at 2% dose with 5 repeat exposures over 6 days. Dose volumes were propor-
tionally increased according to the form of the TA. The abundance of TA proteins was measured by Western blot analysis using antibodies specific for the TA. Confounding effects of HS induced hypophagia. The abundance of ovarian PPARα, PPARβ/δ, and PPARγ was quantified via western blotting. In the absence of ZEN, HS decreased PPARα (P = 0.001), increased PPARβ/δ (P = 0.006) and did not alter PPARγ (P = 0.11). In TN females, ovarian PPARα (P = 0.86) and PPARγ (P = 0.21) were unaffected; however, PPARβ/δ was increased (P = 0.01) by ZEN exposure. Within HS females, ovarian PPARα (P = 0.004), PPARβ/δ (P = 0.0001), and PPARγ (P = 0.0007) were increased by ZEN exposure. In PF females, ovarian PPARα (P = 0.003) was increased, PPARβ/δ was reduced, while PPARγ was decreased (P = 0.02) by ZEN exposure. These findings suggest that ovarian PPARs are responsive to both ZEN exposure and HS, a molecular scenario that potentially influences fertility. This work was supported by the Iowa Pork Producers Association.

4187 Manipulation of ATM Alters the Ovarian Proteome Response in Cultured Mouse Ovaries during DMBA Exposure

J. K. Rishi, and A. F. Keating, Iowa State University, Ames, IA.

Dimethylbenzenanthracene (DMBA) is a chemical carcinogen that causes ovarian DNA adduct formation. As a polycyclic aromatic hydrocarbon, DMBA is released during the combustion of organic material, including wildfires, car exhaust, and cigarette smoke. Genotoxic damage initiates the ovarian DNA damage repair (DDR) response, coordinated by Ataxia Telangiectasia (ATM). Inhibition of ATM previously prevented toxicant-induced follicle loss thus, this study investigated the hypothesis that DMBA exposure may alter ovarian DNA and protein abundance and ovarian DNA damage repair. Post-natal day four C57BL/6 female mice were treated with DMBA (10 nM). The total ovarian proteome was analyzed via liquid chromatography-tandem mass spectrometry. Comparison of protein abundance across treatments identified that DMBA altered 167 proteins (P < 0.05) compared to a lean female, potentially affecting female reproductive and general health. Supported by 1R01ES030341 and 1R01ES030341-S2 from NIEHS.

4186 Altered Hepatic mRNA and Protein Abundance in Lean and Obese Female Mice during Exposure to Dimethylbenzenanthracene

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Reduced female fertility can be caused by lifestyle and environmental factors. Obesity, which is associated with reduced oocyte quality, conception, and implantation, affects over 40% of US reproductively aged women. Environmental toxicant exposure can cause infertility through a variety of mechanisms and proper hepatic chemical biotransformation is necessary to prevent systemic toxicity. The polycyclic aromatic hydrocarbon, 7,12-dimethylbenzenanthracene (DMBA), is generated during combustion of organic material present in tobacco products, smoked meats, and diesel exhaust fumes. Obese female mice have increased DMBA-induced ovarian DNA damage and a reduced ovarian protective response compared to lean females. Whether differential hepatic function underlies these observations remains unclear, thus this study investigated the hypothesis that DMBA exposure may alter hepatic mRNA and protein abundance of genes associated with fatty liver disease in lean and obese females. Six-week-old female C57BL/6 mice (n = 40) were randomly assigned a Chow (n = 20) or high fat, high sucrose (HFHS; 45% kcal from fat and 20% kcal from sucrose; n = 20) diet ad libitum. When the HFHS group had ~30% increased (P < 0.05) body mass compared to lean mice, all received an intraperitoneal injection with corn oil (vehicle control; CT) or DMBA (1 mg/kg) for 7 d. Thus, there were four treatment groups: lean-CT, lean-DMBA, HFHS-CT, and HFHS-DMBA (n = 10 each). Euthanasia occurred at day two of diestrus at which time liver weight was increased (P < 0.05) by ~20% in HFHS-DMBA relative to lean-DMBA mice. Hepatic RNA was isolated and converted to complimentary DNA for qRT-PCR analysis using a Qiagen fatty liver PCR array. The HFHS group had decreased (P < 0.05) abundance of hepatic Nfkb1 (1.5-fold) and Star3 (1.4-fold) mRNA. In liver mice, DMBA decreased (P < 0.05) Cyp2e1 mRNA abundance by 2.9-fold. Liver protein was isolated and western blotting determined that in HFHS mice, DMBA exposure decreased (P < 0.05) NFKB1, STAT3, and CYP2E1 protein level but these effects were absent in lean mice. These findings support the hypothesis that the liver of an obese female may have an altered hepatic response to toxicant exposure compared to a lean female, potentially affecting female reproductive and general health.

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(9.5%) but the TEER values for 3 of 4 donors were non-irritating (>50%). Miconazole
nitrated treated in MT values > 50% (4/5) and 4 donors had 50% > 57.5%) but had TEER > 50% for all donors. Of the remaining test articles, 45 were
found to be non-irritants in both MT and TEER assays in all four donors. In conclu-
sion, the MT and TEER assays from the four donors were highly reproducible and a
decrease in MT and TEER appears to be useful endpoints for preclinical toxicity
screening of early and mature cardiovascular products. The use of this in vitro system
to assess the safety of topically applied vaginal care products, drugs, vaginally
inserted medical devices will be cost effective and could replace the use of animals
for experimentation.

4190 Using Rabbit IPS-C Derived Cardiomyocytes to Investigate Drug-
Induced Fetal Heart Malformations


Teratogenic events such as fetal heart malformations are serious and tragic
drug-induced toxicities. Preclinical studies conducted during drug safety evalua-
tion are intended to identify teratogenic risks using rodent and nonrodent models,
largely based on the unfortunate experience with thalidomide which is teratogenic
in humans and in rabbits but is refractory in rodents. To study the teratogenic
properties of thalidomide in rabbit, a novel method was designed to develop cardio-
myocytes (CMs) from rabbit induced pluripotent stem cells (riPSCs). A panel of
RT-PCR of pluripotency, endoderm, ectoderm, mesoderm, smooth muscle, and
cardiac markers were studied across a time course of directed differentiation. As
early as differentiation day (DD) 3 of the differentiation, riPSCs begin to express
early CM markers (e.g. NKX2.5 and cKMT) and mesodermal markers (e.g. DKK1
and Fox1). By DD 21, these riPSC-derived cells expressed mRNA and protein of
mature CM genes including TNN2, MYL2, HCN4, and KCNJ2. Spontaneous
beating was observed by phase contrast microscopy and electrophysiology as early
as DD 8, and live cell calcium indicator probes demonstrated pulsatile calcium flux
that correlated with cell contraction. Thalidomide (30 μM) treatment to riPSCs
CMs down-regulated mRNA and protein expression of early and mature cardiac
markers, suggesting suppressed differentiation. In addition, thalidomide treatment
abrogated the onset of spontaneously beating cells. Taken together, these data
indicate that riPSCs-CMs can recapitulate thalidomide-induced cardiac teratogen-
cyte observed in rabbits. In moving forward, this model can be deployed to investi-
gate the underlying mechanisms of rabbit teratogenicity and better understand
species-specific differences in drug-induced fetal heart malformations.

4191 COVID-19 Effects on Pregnancy, Prenatal, and
Postnatal Development

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over
98 million people in the U.S. alone with over 225,600 reported infections in pregnant
women (2020). Control and mitigation strategies are largely based on the unfortu-
nate experience with thalidomide which is teratogenic in humans and in rabbits but
is refractory in rodents. To study the teratogenic properties of thalidomide in rabbit,
a novel method was designed to develop cardio-

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The first hours and days of life are critical for newborns as they need to adapt to
the extra-uterine environment. Additionally, they are vulnerable to a wide range of
respiratory disorders. Juvenile animal models have made, and continue to make,
important contributions to neonatal medicine, mainly serving as models for lung
development. The advantages of the Inhalation administration route being that it
is less invasive, provides rapid onset of action, minimizes systemic side effects
and, offers direct access to the pulmonary system. Drug administration in neonates
represents specific and unique challenges. Drug administration procedures were
reevaluated in which 2 species (rat and dog) routinely used in preclinical juvenile
safety assessment studies via the inhaled route of administration with the objective
to refine and develop safe and proper procedures in younger animals. Sprague
Dawley rats were successfully dosed on a nose-only exposure system as young as
4 days of age (postnatal day [PND]) Daily dosing with milled lactose (Lactohale
control particles) was conducted for 7 consecutive days with a particle used
throughout, namely a 1 1/8 inches inside diameter polymericube. Animals
were conditioned on PND3 by separating them from their respective dam for a
target period of 60 minutes. Dams were removed and placed in a different bin with
same housing conditions as their litter. Duration of dosing was up to 120 minutes
after the procedure was completed, remaining pups were cleaned for any excess
material around the nostril area and returned to their respective dam and
 Cage. A few rat pups were cold to touch at the end of dosing however the animals
 gained back body temperature as soon they were returned to their dams. Beagle
dogs as of 5 days of age (postnatal day [PND]5) were habituated to a mask for 6
days. The animals were conditioned as early as PND5 to assure acceptance and
familiarization. Starting with 5 minutes per occasion for the first two days and
increasing the acclimation time for 30 minutes, having their respective dam
present through all steps (in the home cage and within the inhalation laboratory).
Puppies were held by technicians and covered with blankets to avoid any signs of
hyperthermia. The complete litter was present in the inhalation laboratory at the
same time to avoid any signs of distress. Polyurethane sheets (dental dam®) were
used to reduce the diameter of inhalation mask opening, creating a better fit
and ease around the muzzle. Cage side observations were performed on each puppy
after mask exposure. Inhalation behavior observations were recorded if abnormal-
ities were detected. Positive reinforcement of dams and providing a calm environ-
ment were shown to reduce several stress factors. Technicians were ever present,
desensitizing of dams with positive reinforcement before parturition to allow the
staff to start handling the puppies at an early age. Technicians showing a calm
and confident manner, using soft tones and quiet voice are essential for the success
of these type of studies. In conclusion, the use of the inhaled route in Sprague
Dawley and Beagle dogs for ages as young as PND4 and PND5, respectively, was shown to
be well tolerated, demonstrating that the inhalation route is an appropriate model for
investigating pulmonary development in juvenile animals.
Toxicity testing of agrochemicals requires a comprehensive battery of animal studies in multiple species covering various life stages and exposure durations. Data from these studies are used to perform human health risk assessments. Among the list of studies, the two-generation reproduction toxicity study (2-gen) is the most animal-intensive study which typically uses about 3000 animals. As we work towards the future goal of an animal-free testing paradigm, a framework was proposed in which this particular study could be waived while retaining the ability to perform robust and protective risk assessments for agrochemicals. A meta-analysis was performed utilizing 2-gen data from the European Food Safety Authority (EFSA) conclusions for 108 agrochemicals. The available rat subchronic (13-week) NOAELs and the 2-gen parental, reproductive, and offspring NOAELs were tabulated for each chemical. The NOAELs and developmental toxicity studies and the 2-gen. Weight of evidence from existing short-term systemic, 1-gen, and developmental toxicity studies can potentially be predictive of 2-gen outcome. In terms of if a second generation is needed, the extended 1-generation reproductive toxicity study (1-gen) screening (1-gen), developmental toxicity studies and the 2-gen. Additional analyses were conducted to compare the data gaps between 1-gen and 2-gen reproductive screening (1-gen), developmental toxicity studies and the 2-gen. Weight of evidence from existing short-term systemic, 1-gen, and developmental toxicity studies can potentially be predictive of 2-gen outcome. In terms of if a second generation is needed, the extended 1-generation reproductive toxicity study guideline has provided trigger criteria. Thus, data from existing repeated dose 1-gen, and developmental toxicity studies can be reviewed against those criteria for waiver justification. Herein, a framework for waiving the 2-gen is proposed. First, hazard identification can be informed by existing data. Second, from quantitative perspective, EF of 10 from rat subchronic NOAEL can serve as parental NOAEL being protective of overall 2-gen toxicity effects. Finally, if the chemical belongs to certain class of chemistry, read-across can be applied to increase confidence. Overall, the proposed framework provides both hazard and point of departure for assessing reproductive toxicity without conducting the 2-gen, thus providing a scientific basis for a 3Rs-based risk assessment approach for agrochemicals.

**Toxicity Testing of Agrochemicals**

Toxicity testing of agrochemicals for reproductive toxicity is required to advance predictive toxicology and chemical assessment. High-throughput (HTP) assays have been used as platforms for rapid assessment of reproductive toxicants; however, additional genotoxicity assays and method validation would further advance knowledge regarding fertility and reproductive health. We assessed yeast (S. cerevisiae) and roundworm (C. elegans) HTP assays related to germline function and other reproductive health endpoints by scoring toxicity of 127 environmental chemicals and modeling each dataset using a streamlined, semi-automated benchmark dose software (BMDS) approach. We then extracted and modeled mammalian in vivo data from the Toxicological Reference Database (ToxRefDB, Version 2.0). We potency-ranked the data set from each evidence stream by BMD and evaluated rank order correlation between datasets using Pearson and Spearman correlation coefficients. Finally, we constructed a prediction model using machine learning to incorporate model descriptors (including data on QSAR, chemical properties, etc.) and tested model performance in predicting toxicology outcomes with reasonable accuracy (Training R²: 0.97, testing R²: 0.61). Our findings indicate the HTP assays have great potential for making accurate predictions of human reproductive toxicity of environmental chemicals.

**Teratogenicity Assessment of Antimalarials Early in Drug Development Using the ReproTracker Assay**

Malaria is a widespread disease affecting millions of people, especially pregnant women, annually. Over the past decades, several novel antimalarials have been developed. Unfortunately, many of these have shown to be developmental toxicants (teratogenic). This highlights the need for improved development and development of novel antimalarial medicines with a low probability of developmental toxicity, which can be used during pregnancy, to reduce maternal and newborn mortality rates. Currently, assessment of potential developmental toxicant relicts on the human fetus is not incorporated into traditional embryo-fetal developmental (EFD) studies in mammals with new approach methodologies (NAMs), such as human stem cell-based assays (ReproTracker®), to assess the developmental toxicity of antimalarial drugs. ReproTracker is a state-of-the-art human induced pluripotent stem cell (hiPSC)-based biomarker assay that can identify the teratogenic potential of new drugs with high accuracy (R²=0.86), sensitivity (86%), and specificity (86%) and provide evidence as to the likely outcome of in vivo studies. Here, we tested 9 marketed (E.g., Artemesunate and Artether) and 5 MMV antimalarials in the ReproTracker assay and compared the results with the mammalian EFD outcomes in rat and rabbit. Compounds induced developmental toxicity in either one of the animal species were considered teratogenic. ReproTracker correctly identified the potential for developmental toxicity of 8 out of 9 marketed compounds and 3 out of 5 MMV antimalarials. In conclusion, this comparative study has demonstrated that ReproTracker, an animal-free screening platform, can be used for de-risking chemicals (antimalarials) early in drug development. This study also led us to conclude that we should consider integrated models for the early assessment of developmental toxicity.

**Rapid Screening of Environmental Chemicals for Human Reproductive Toxicity**

Rapid screening of environmental chemicals for reproductive toxicity is required to advance predictive toxicology and chemical assessment. High-throughput (HTP) assays have been used as platforms for rapid assessment of reproductive toxicants; however, additional genotoxicity assays and method validation would further advance knowledge regarding fertility and reproductive health. We assessed yeast (S. cerevisiae) and roundworm (C. elegans) HTP assays related to germline function and other reproductive health endpoints by scoring toxicity of 127 environmental chemicals and modeling each dataset using a streamlined, semi-automated benchmark dose software (BMDS) approach. We then extracted and modeled mammalian in vivo data from the Toxicological Reference Database (ToxRefDB, Version 2.0). We potency-ranked the data set from each evidence stream by BMD and evaluated rank order correlation between datasets using Pearson and Spearman correlation coefficients. Finally, we constructed a prediction model using machine learning to incorporate model descriptors (including data on QSAR, chemical properties, etc.) and tested model performance in predicting toxicology outcomes with reasonable accuracy (Training R²: 0.97, testing R²: 0.61). Our findings indicate the HTP assays have great potential for making accurate predictions of human reproductive toxicity of environmental chemicals.
Cell-based in vitro cytotoxicity assays are a valuable tool for screening compounds for toxicity evaluation. Many in vitro cytotoxicity assays rely on dyes, or labels, to measure cell death at a single timepoint after a predetermined exposure time. Here, we characterized a real-time, label-free cytotoxicity assay for high-throughput screening and dose response analysis. First, cancer cells (AS49, SKOV3) were seeded in 96-well microplates with embedded electrodes on the substrate. Electrical impedance measurements from the electrodes in each well detected the attachment and proliferation of the cancer cells. Cytotoxic compounds were added 24 hours after the cancer cells and cytolysis was monitored for an additional 72 hours. Cytotoxicity was measured by comparing treated wells to no treatment control wells, and the kinetics of cell death were determined from the time required for the compounds to kill half of the cancer cells (kill time 50, KT50). AS49 cells were cultured and treated with mitoxantrone at three concentrations to create “Max”, “Mid”, and “Min” treatment groups. The Z-prime was computed from the “Max” and “Min” treatment groups, corresponding to the lowest and highest concentrations of mitoxantrone, respectively, for each of three independent assays. The Z’ was 0.81 +/- 0.03 across the three plates for an HTS assay in duplicate, reflecting high sensitivity and repeatability across plates. Furthermore, assay performance was characterized spatially across the plate, with no evidence of drift or edge effects. Finally, the cytotoxicity assay was used to measure the EC50 of six compounds, including mitoxantrone, with an 8-point concentration response analysis. The Hill equation was fit to the cytotoxicity assay data at 48 hours after introducing the compounds to calculate the EC50 (mitoxantrone - 16 nM, paclitaxel - 2 nM, doxorubicin - 73 nM). Future work will further evaluate the value of impedance-based assays for revealing mechanistic information in toxicology.

Investigation of the Cytotoxic Effects of Artesunate in Combination with Cisplatin on Proliferation and Morphology of Androgen-Sensitive (LNCaP), Androgen-Insensitive (PC-3) Human Prostate Cancer Cell Lines and Normal Epithelial Prostate Cell Line (RWPE-1)


This study investigates the cytotoxic effect and morphological changes that occur when a combination of Artesunate and Cisplatin is administered to human prostate cancer cell lines (LNCaP and PC-3) and a normal epithelial prostate cell line (RWPE-1). In vitro anti-proliferative activity of 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyterrazolium bromide (MTT) assay was used to assess the cytotoxic effects of each drug, and the combination in ratios 1:1, 1:2, and 2:1 of Artesunate and Cisplatin respectively. The drugs were administered to LNCaP, PC-3, and RWPE-1 for 72-120 hour. Images of samples with and without the drugs were acquired using the TC20 automated cell counter. The study demonstrates that Artesunate and Cisplatin at 0.1 µM - 1 nM concentration range, leads to cell death and decrease cell viability. The combination of Artesunate with Cisplatin significantly decreases cell viability by more than 30%, and the most cytotoxic effect was seen on PC-3 cell line at 1:2 ratio. Increased cell viability was observed on RWPE-1 cell line for each drug and the combinations after 72-hr. This indicates that both drugs have little or no cytotoxic effect on the normal epithelial prostate cell line (RWPE-1). Changes were observed in the cells morphology with and without drugs using the TC20 automated cell counter. It was concluded that the combination of Artesunate with Cisplatin suppressed prostate cancer proliferation based on the dose and exposure time.
Enhancers are critical gene expression control regions that are marked by open chromatin signals and transcription factor binding sites. Previous reports indicate that low-dose hexavalent chromium (Cr(VI)) disrupts chromatin accessibility around transcription factor binding sites and alters CTCF binding dynamics to bring about gene transcription misregulation. These two aspects comprise a compelling argument to investigate the impact of Cr(VI) on enhancer function, given that enhancers are extensively bound by transcription factors. Using FAIRE-seq, ChIP-seq and STARR-seq, we show that Cr(VI) preferentially increases accessibility at Cr-specific subset of enhancers. Although Cr(VI) has a minimal impact on direct enhancer output as measured by luciferase assay, there are attendant epigenetic changes that include a global increase in H3K27 acetylation enrichment. Mechanistically, Cr(VI) alters promoter binding, including at Gardner’s enhancers that neighbor genes that are important for the phenotype. Overall, low-dose hexavalent chromium impacts gene transcription by altering transcription factor function on upstream gene regulatory regions.

4206 Arsenic-Exposed Humanized Mice Exhibit Sex Divergence in Molecular Profiles Relating to Insulin Resistance
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Arsenic is a pervasive environmental toxin that is ranked as the number one priority for investigation by the Agency for Toxic Substance and Disease Registry. Chronic exposure to arsenic has been associated with type 2 diabetes (T2D). However, the underlying mechanisms remain largely unknown. We and others have demonstrated that arsenic treatment of INS-1 832/13 pancreatic beta-cells impairs glucose-stimulated insulin secretion (GSIS), a hallmark of T2D, and that arsenic alters the microRNA (miRNA) profile in beta-cells more dramatically than other metals. miRNAs have a well-established regulatory role in both normal beta-cell function and T2D pathogenesis. In addition, other studies have highlighted the association of iAs exposure with insulin resistance in peripheral tissues and impairment of glucose homeostasis. However, an important caveat to the previous in vivo mouse studies is that the inherent differences in iAs metabolism between species. iAs is metabolized by the enzyme arsacid methyltransferase (ASM1). Utilizing our novel humanized line, wherein 129S6/SvEvTac mice express the human variant of ASM1, we designed a study in which iAs metabolism and circulating arsenic distribution more closely resembles what is seen in humans. While sexual divergence of metabolic phenotypes is appreciated in different contexts, it is not known whether this is the case with iAs exposure. We hypothesized that male humanized mice would exhibit impaired metabolic phenotypes and related molecular profiles in the liver and adipose, relative to their female humanized counterparts. To test this hypothesis, ~5 month old wild-type and humanized mice of both sexes were treated with 400ppb iAs for 4 weeks and then sacrificed for tissue collection. Our study highlights a sex divergence in metabolic phenotypes in iAs-exposed humanized (but not WT) mice. We propose a general model in which humanized male mice exposed to iAs have elevated miR-34a and reduced Klf11, indicators of insulin resistance. Also, in humanized female mice, genes that promote insulin sensitivity and glucose tolerance in both the liver and adipose are elevated compared to humanized male mice. These findings suggest that humanized females are protected from metabolic dysfunction relative to humanized males in the context of iAs exposure. Further studies should be aimed at determining the underlying mechanism for this sex-divergent effect.

4207 Optimizing Circadian Transcriptional Machinery
Output to Augment Cellular Resiliency in Operationally Extreme Environments
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The USAF is continuously looking for ways to improve Airmen health and performance, as well as prevent any adverse health effects due to operationally relevant stressors. The body's circadian rhythm is a sophisticated cycle that regulates many functions of human physiology. During military deployed operations, there is an inherent conflict between the internal body clock and operational stress environment. Disruptions of the circadian rhythm have been shown to have many adverse health effects like sleep disorders, susceptibility to infections, depression and metabolic syndromes. Health effects like these could be accentuated by the vulnerable condition of Airmen in response to external stressors imposed by unique austere environments. Over the past few years, there have been numerous studies showing that small molecules that target the circadian clock can have beneficial effects in various tissues. For example, circadian clock enhancing compounds including...
cryptochrome inhibitor KS15 and SR2878 have been shown to reduce DNA damage caused by chemotherapy drugs. Therefore, it is hypothesized that these existing small molecules that optimize the transcriptional output of the internal circadian clock cycle in cultured human keratinocytes can enhance the resilience of these cells when exposed to extreme stress environments. The main objective is to explore the possibility of restoring, maintaining or enhancing cellular response to extreme modulating the transcriptional output of the circadian machinery under conditions of exposure to extreme heat or extreme cold. CRISPR/Cas9 gene editing was employed to knock out BMAL1 (brain and muscle Arnt-like protein-1) in the HaCat keratinocytes. BMAL1 is one of the core clock genes and is crucial for a healthy circadian rhythm. Introducing clock modulating compounds to BMAL1 knockout cells will determine if protective effects provided by the clock machinery are acting on through the circadian feedback loop. In these experiments, BMAL1 knockout HaCat cells were exposed to clock modulating compounds under Air Force relevant stressors (extreme heat and extreme cold) and compared to wild type HaCat cells. Clock modulating compounds include cryptochrome inhibitor KS15 and SR2878 (Merck KGaA, Darmstadt, Germany). Here, we first confirmed the expression of functional merb enzyme activity in transgenic flies showing demethylation of MeHg in vivo. We next assessed the ability of merb to alter MeHg toxicity in development. With all combinations of merb-induced demethylation in vivo; ubiquitously (via an actin promoter) or in a tissue-specific manner (gut, muscle or neurons), we observe a rescue of MeHg-induced eclosion failure at the pupal to adult transition. In MeHg-fed larvae with ubiquitous or targeted (gut and muscle) merb expression, we see a significant decrease in MeHg body burden at the pupal stage relative to control flies. We also observe a significant increase in the MeHg elimination rate with merb demethylation induced in adult flies (control, 1.7 ± 0.2; merb flies, 1.9 ± 0.2). We also show that liver injections of Merb, a liver-specific merb transgene, increase the fraction of cells progressing from G1 to S phase after a shift to serum starved conditions in the Huh7 cells. In summary, our results show that clock modulating compounds may protect cells from extreme stress under different conditions. Distributions statement: A. Approved for public release. APRIL 2022-0008 Cleared on 2 November 2022.

4208 An NRF2-Bound Antioxidant Response Element Regulates the HIF1α Axis

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The transcription factor (TF) NRF2 regulates expression of many genes with antioxidant functions, and thus is a key player in the response to oxidative stress. Once activated, NRF2 binds to a regulatory DNA element known as the antioxidant response element (ARE). The downstream result of NRF2 activation in times of oxidative stress therefore is cytoprotective. However, its activity can have negative consequences, as NRF2 hyperactivation has been demonstrated to provide cancer cells with an advantage. Therefore, if we are to understand how NRF2 activity crosses the threshold from beneficial to detrimental we need to characterize NRF2 target genes that extend beyond just the canonical targets. Herein, we build on this work to validate the causal relationship between this ARE and HIF1α in the context of the genomic locus. We used CRISPR/Cas9 genome editing to generate HIF1A-AREMUT MDA-MB-231 human breast cancer cells. First, we used chromatin immunoprecipitation (ChIP) to measure direct binding at NRF2 to the ARE in breast cancer cells. We found that NRF2 occupancy at the ARE upstream of HIF1α was significantly decreased in the AREMUT cells compared to the WT (4.37 ± 0.13 & 5.57 ± 0.55, respectively; P<0.01), which contributed to a ~2-fold decrease in HIF1α protein. Thus, mutation of the ARE upstream of HIF1α results in altered cell cycle and glucose uptake phenotypes. First, for a CDKN1A-associated phenotype, we compared the percent of cells in the G1, S, and G2 phases of the cell cycle by using propidium iodide staining and cell cycle flow cytometry analysis. We found a significant increase in the fraction of cells progressing from G1 to S phase after a shift to serum starved conditions in the HIF1A-AREMUT cells compared to WT cells. Second, we compared alterations in the CDKN1A transcript (P<0.05, G2/M phase compared to G1/S phase). Third, for a CDKN1A-associated phenotype, we compared the percent age of cells in the G1, S, and G2 phases of the cell cycle by using propidium iodide staining and cell cycle flow cytometry analysis. We found a significant increase in the fraction of cells progressing from G1 to S phase after a shift to serum starved conditions in the HIF1A-AREMUT cells compared to WT cells. In summary, HIF1A is a noncanonical NRF2 target gene, and elimination of the NRF2 input at the HIF1α locus disrupts key parts of the HIF1α network. This ARE is highly conserved, and likely to be functionally relevant to cancer cell biology.

4209 Targeted Intracellular Demethylation of Methylmercury Enhances Elimination Kinetics and Reduces Developmental Toxicity in Transgenic Drosophila

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Methylmercury (MeHg) persists today as a priority public health concern. Mechanisms influencing MeHg metabolism, kinetics, and toxicity outcomes are therefore essential knowledge for informing exposure risks. Existing studies point to different toxic potencies of MeHg and inorganic mercury (Hg²⁺), highlighting the role for biotransformation (demethylation) in regulating MeHg toxicokinetics/dynamics. Whereas microbial MeHg demethylation in the gut is seen to influence elimination kinetics, the potential for systemic demethylation in tissues and target organs to influence MeHg toxicity remains uncertain. To investigate the consequences of MeHg demethylation in situ in various tissues in vivo we ectopically expressed the merB organomercurial lyase enzyme of the bacterial mer operon in Drosophila. We have previously established that merB is capable of demethylating MeHg, generating a reactive intermediate (Hg²⁺) for delivery to the cytosol. Here, we first confirmed the expression of functional merb enzyme activity in transgenic flies showing demethylation of MeHg in vivo. We next assessed the ability of merB to alter MeHg toxicity in development. With all combinations of merB-induced demethylation in vivo; ubiquitously (via an actin promoter) or in a tissue-specific manner (gut, muscle or neurons), we observe a rescue of MeHg-induced eclosion failure at the pupal to adult transition. In MeHg-fed larvae with ubiquitous or targeted (gut and muscle) merB expression, we see a significant decrease in MeHg body burden at the pupal stage relative to control flies. We also observe a significant increase in the MeHg elimination rate with merB demethylation induced in adult flies (control, 1.7 ± 0.2; merB flies, 1.9 ± 0.2). Furthermore, HIF1α FoxO pathway activity was significantly increased in merB flies (control, 2.6 ± 0.4; merB flies, 4.0 ± 0.6). We also show that liver injections of Merb, a liver-specific merb transgene, increase the fraction of cells progressing from G1 to S phase after a shift to serum starved conditions in the Huh7 cells. In summary, our results show that clock modulating compounds may protect cells from extreme stress under different conditions. Distributions statement: A. Approved for public release. APRIL 2022-0008 Cleared on 2 November 2022.
Sex-Specific Transgenerational Effects of Preconception Exposure to Arsenite in C57BL/6 Mice


Chronic exposure to inorganic arsenic (iAs) has been linked to diabetic phenotypes in humans and mice, but the role of iAs exposure prior to conception and its transgenerational effects are understudied. Our previous study showed that preconception exposure of C57BL/6J male mice to 200 ppb iAs changed gene expression profiles in paternal sperm and resulted in insulin resistance in male offspring. The present study used a transgenerational design to investigate transgenerational effects of iAs exposure on gene expression profiles in biological generations more closely resembling human conditions. Oocytes were exposed to 2 ppm iAs (arsenite) in drinking water for 10 weeks before mating. Indicators of diabetic phenotypes were measured in the first (G1) and the second generation (G2) of their offspring. RNA-sequencing was used to characterize transcriptomes in germ cells from all three generations, in livers of G1 and G2 generations, and in white adipose tissue of G2 generation. Pathway and functional protein networks associated with expression of significantly altered genes were identified using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING). We found that only one gene was differentially expressed in G0 sperm after iAs exposure, as compared to 2569 genes in G0 oocytes. Antigen processing and MHC class II protein complex were the most enriched protein networks in G0 oocytes. Parental exposure to iAs resulted in differential expression of 18 genes in oocytes from G1 mice, while no gene transcript was altered in G1 sperm. In comparison, 232 and 220 genes were differentially expressed in livers of G1 males and females from parents exposed to iAs, respectively. Multiple pathways related to long-chain fatty acid metabolism were enriched in livers of G1 females. In G2 sperm and oocytes, 42 and 334 genes were differentially expressed as a result of iAs exposure of G0 grandparents, respectively. Protein function networks associated with mitochondrial respiratory chain function, specifically complex I and IV, were the most enriched in G2 oocyte. In livers of G2 males, 3903 gene transcripts were altered, yet no gene transcript was significantly altered in female livers. In comparison, 940 and 2793 genes were differentially expressed in the white adipose tissue of G2 males and females, respectively. Insulin signaling pathway was among the enriched pathways in livers of both G2 males and females. A protein network associated with Dysregulated miRNA targeting in insulin (Insulin pathway was significantly upregulated) was significantly altered in female livers. Notably, exposure of G0 generation to iAs resulted in increased adiposity and insulin resistance in G2 males, but not G2 females. This data suggests that sex-specific transcriptoral effects in germ cells and/or tissues of G0 and G1 generations may underlie the diabetic phenotype of G2 males.

Zinc Supplementation Alters Tissue Distribution of Arsenic in Mus musculus with Corresponding Changes in Metal Transporters

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Arsenic (As) exposure is a global concern because it is found at elevated levels in contaminated soil and water supplies. The As from these sources can find its way into the human body through ingestion of foods grown in contaminated soils, dust inhalation, and consumption of tainted water. Prolonged exposure to elevated levels of As has been linked to a variety of diseases including cardiovascular disease, skin disorders, immune disorders, neurotoxicity and cancer. A variety of studies have been used to combat As exposure however zinc (Zn) supplementation remains a possible therapeutic option that requires further study. Cell-based studies have shown that Zn supplementation can be used to counteract some of the molecular impacts of As exposure. To investigate the distribution of As and Zn in different tissues and the alterations in tissue distribution when given combination treatment, mice were exposed to 5 ppm sodium arsenite, 10 ppm zinc chloride, or a combination of the two in drinking water for a total of six weeks. Plasma, liver, spleen, and kidney tissues were harvested to assess tissue distribution of As and Zn concentrations and qRT-PCR was performed to analyze changes in known As and Zn transporters. As treatment alone significantly increases the amount of As in all tissues while Zn supplementation alone did not alter the amount of Zn in any of the tissues. However, Zn supplementation decreased the levels of As in plasma, liver, spleen, and kidney. Highlighting possible causes of these results, in the liver the decreased accumulation of As in the presence of supplemental Zn corresponds with an increase in Mrp1 (a known As(GS)III exporter) expression. This increase is only seen in the presence of both metals. As treatment did not cause liver damage as measured by serum Alt-1. In the spleen, supplemental Zn increases expression of Glut1, which when combined with As exposure may assist in the reduction of arsenicals. In the kidney, As and Zn treatments individually decrease the expression of Apo9 (transporter that causes the efflux of methylated arsenic metabolites) and the result is additive in the combination treatment. The enhanced reduction of Apo9 may contribute to the reduced tissue arsenic observed in combination treatment. Of note, As treatment alone decreases the amount of Zn in the kidney and when supplemental Zn is given, the Zn concentration in the tissue returns to normal. This phenomenon corresponds to an increase in expression to Zip10 (a Zn importer) and Mrp2 (an arsenic exporter). Results from this study demonstrate that Zn supplementation can lower As accumulation in a variety of tissues, which may in turn lessen the negative impacts of arsenic exposure.

Toxicity of Metal Compounds in Human Precision-Cut Lung Slices Ex Vivo

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Metals are constant present in our daily life, for example as components of stainless steel, semiconductors or in medical devices. However, many metals reveal inflammatory and even carcinogenic potentials following inhalation. Hereby, the toxicity often depends on the respective metal species, decisive factors are oxidation state and solubility - in case of metal particles - their size (nano-/micro-scaled) as well as structure (granular or fibrous). Due to the ubiquitous distribution of metals and the associated need to limit exposure and set exposure limits for workplace and environment, determining the health hazards and elucidating the toxicological mechanisms of action of the various forms of metals are extremely relevant. The aim of this project was to compare and validate in vitro and ex vivo models for the toxicological evaluation and grouping of different metal-based compounds, which differ in their solubility and bioavailability. In this study, the toxicological effect by poorly soluble or biopersistent metal particles (nanoparticles) and fibers (nanowire), or soluble metal ions was investigated. To provide some insight into the underlying processes, human precision-cut lung slices (PCLS) as a valuable ex vivo model, were treated over 24 hours with copper (Cu) and silver (Ag) in three different forms (nanoparticles, nanowire and as a salt of nitrate or sulfate). Cytotoxic effects of the tested compounds were evaluated in a dose-dependent manner in PCLS obtained from three biological donors by LDH and WST-1 assays. Moreover, proinflammatory effect was evaluated by measuring IL-18 via ELISA. Ag nanoparticles were well tolerated in distal human lung tissue ex vivo in contrast to other Ag and Cu forms. This lack of toxic effect by Ag nanowires in human PCLS was observed at all tested concentrations up to the highest concentration of 500 mg/mL. However, the other Ag forms (Ag nanoparticles and Ag nitrate) and all Cu forms (Cu nanoparticles and Cu sulfate) showed potent toxic effects in human lung tissue (decreased metabolic activity up to 65% (Ag nanoparticles), up to 90% (Ag nitrate) and up to 50-60% (Cu nanoparticles, nanowire, sulfate)). To evaluate, whether toxic effects of the tested metals correlated with IL-18 secretion, IL-18 was measured extrinsically and intrinsically in human PCLS. Only a slight induction of IL-18, but no toxic effect by IL-18 release of 1B was observed within the acute time period of 24h for both Ag nanowire and Cu nanowire treated tissue. The soluble metal salt (Cu sulfate, Ag nitrate) showed intrinsic induction and also an extrinsic release of IL-1B at treated concentrations of 5 mg/mL and above. However, Cu and Ag nanoparticles showed a concentration-dependent increase of intrinsic IL-1B (Cu nanoparticles) and IL-18 (Ag nanoparticles) at up to 38 pg/mL for Ag nanoparticles in the highest applied concentration, compared with untreated control with 1.62 pg/mL. In conclusion, Ag nanoparticles showed the least toxicological and proinflammatory effect in human PCLS ex vivo. This initial toxicity evaluation in human PCLS shows that ex vivo metal testing in tissue culture is a promising approach for toxicity profiling. Furthermore, PCLS are a valuable model for elucidating toxicological mechanisms and provide a good pretest platform for reducing animal groups in future in vivo studies. Acknowledgement: The project is funded by the German Federal Ministry of Education and research BMBF (NanoCare 4.0, MetalSafety).

Evaluation of Mercury Contamination in Chickens (Gallus gallus) and Soils in an Artisanal Gold Mining Area in Colombia

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Mercury (Hg) is one of the heavy metals with greatest impacts on ecosystems. Its use in artisanal gold mining is linked to environmental contamination and health risks to humans. Studies have indicated that communities surrounding gold mining areas are exposed to Hg through consumption of contaminated fish, although miners also eat meat from farm animals raised near gold mining areas. The objective of this study was to evaluate total Hg (T-Hg) concentrations in feathers of chickens (Gallus gallus), as well as in soils, from a gold mining area (San Martin de Loba-SML), comparing them to those registered in a reference site (Arjona) in Bolivar, Colombia. A total of 40 chickens were sampled in SML and 31 in Arjona, all being housed with 30 and 21 soil samples in each location, respectively. Total Hg analysis was performed using a direct Hg analyzer. The average T-Hg concentrations in chest feathers, rachises, and bars from wing feathers were 2.37±0.42, 0.72±0.32, 2.97±1.26 µg/g in SML and 0.41±0.05, 0.23±0.04, 0.76±0.07 µg/g in Arjona, respectively. In soils, T-Hg levels were 45.5±12.4 µg/g in SML and 0.04±0.00 µg/g in Arjona.
μg/g in Arjona. Biological and soils samples from SML examined for Hg depicted significant average and range in SML concentrations in the reference area were catalogued as not polluted. In conclusion, Hg released by contamination and its negative impact on animal and human health. "Unicargena Support to Research Groups and Doctoral Programs, 2021-2022; Grant 155/2019, MinCiencias (Young Talent, 826/2020, and Doctoral formation, 647/2014)."

**4215 Assessing the Relationship between Toxic Metal Environmental Justice Indices and Preterm Birth in North Carolina**


Prenatal metal exposure has been associated with an increased risk of preterm birth (PTB). In NC, metal exposure may occur via unregulated, contaminated private wells and may be exacerbated by racial and economic disparities. Low-income and minority communities that may have greater exposure to metal well water contamination can be identified via Toxic Metal Environmental Justice Indices (TM-EJIs). TM-EJIs, calculated at a census tract level, are the product of three variables: the number of well water tests exceeding Environmental Protection Agency (EPA) standards for a specific metal, the difference between the state’s and census tract’s average of low-income and minority population, and the population density. TM-EJIs have been determined for four metals in NC (inorganic arsenic (As), cadmium (Cd), lead (Pb), and manganese (Mn)) and can be positive, negative, or equal to zero. Positive TM-EJIs identify geographic locations of concern where larger proportions of low-income and minority communities are exposed to private wells contaminated with As, Cd, Pb, or Mn. Negative TM-EJIs still identify locations where metal contamination occurs in private wells, but not necessarily among EJ communities. TM-EJIs are equal to zero when no well water tests exceed the national standards for the metal of interest. The aim of this study was to assess the relationship between the TM-EJIs and PTB prevalence at a census tract level in NC. Census tract PTB prevalence was calculated from 1.3 million birth certificates inclusive of all singleton non-anomalous live births in NC from 2003-2015. TM-EJI data, also at a census tract level (n=2038) for each metal, were transformed into indicator variables, to allow for the assessment of changes in PTB prevalence as a result of TM-EJIs going from zero to positive values, and from zero to negative values. Further, the models were used to analyze the relationship between the As TM-EJI and PTB, Cd TM-EJI and PTB, Pb TM-EJI and PTB, and lastly Mn TM-EJI and PTB. In total, data from n=1,935 census tracts were included in the regression models for the four TM-EJIs and PTB. Models were adjusted for the census tract percentage of mothers with less than high school education and the percentage of mothers younger than age 40 at delivery. The Pb and Mn TM-EJIs were related to the prevalence of PTB. Census tracts with positive Pb TM-EJIs had PTB prevalence on average 0.23% higher than census tracts with Pb TM-EJIs equal to zero (β: 0.23, 95% CI: -0.0084, 0.048, p-value: 0.059). Similarly, census tracts with positive Mn TM-EJIs had PTB prevalence on average 0.31% higher than census tracts with Mn TM-EJIs equal to zero (β: 0.31, 95% CI: -0.052, 0.053, p-value: 0.0077). Moreover, census tracts with negative Mn TM-EJIs had PTB prevalence on average 0.34% lower than census tracts with Mn TM-EJIs equal to zero (β: -0.34, 95% CI -0.54, -0.14, p-value: 0.00097). The results from the present study highlight that PTB prevalence is higher in census tracts with elevated Pb and Mn TM-EJIs. These findings highlight the impact of joint exposure of chemical and non-chemical stressors on PTB risk. The data highlight the importance of public health action and prioritization in communities that are impacted by racial and/or economic disparities.

**4218 Metalloneutrins Protects against Hg-Induced Nephrotoxicity in Models of Reduced Renal Mass**

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Metalloneutins are low-molecular-weight proteins that are rich in sulfhydryl groups. Consequently, they bind readily to metal ions such as mercury (Hg). Hg is an important environmental toxicant to which humans are exposed regularly. Individuals with renal insufficiencies, such as those with chronic kidney disease (CKD), may be more susceptible to the effects of Hg, and the progression of the relationship between the concentrations of eleven metals in umbilical cord and IQ (ages 10 and 15). Generalized linear models were used to examine the association of single and multiple metals and IQ. Quantile g-based computation was used to examine the mixtures-based effects in relation to IQ. Statistical significance was defined as p < 0.05. When data from male and female ELGANs were combined, when compared to the reference area were catalogued as not polluted. In conclusion, Hg released by artisanal gold mining is being bioaccumulated by birds utilized in the human diet, representing a health risk to the consumers. It is highly recommended that in gold mining areas, proper protective measures be adopted. As such, local regulations should be permanent in communities surrounding mining areas to reduce Hg contamination and its negative impact on animal and human health. "Unicargena Support to Research Groups and Doctoral Programs, 2021-2022; Grant 155/2019, MinCiencias (Young Talent, 826/2020, and Doctoral formation, 647/2014)."

**4217 Evidence for Zinc Toxicity in Insulin-Producing Beta Cells as a Contributor to Type 2 Diabetes Mellitus in Carriers of the Risk Allele rs13266634 of the Beta Cell Zinc Transporter ZnT8**

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Failure of insulin producing β-cells in the setting of insulin resistance is the underlying cause for type 2 diabetes mellitus (T2DM). This however only occurs in a subset of individuals susceptible to β-cell dysfunction and T2DM. Emerging experimental and epidemiologic evidence points to excess zinc (Zn) as a contributor to β-cell dysfunction and as a potential contributor to β cell failure in the setting of T2DM. This includes an increased risk for T2DM in carriers of a single nucleotide polymorphism (SNP; rs13266634) in the SLC30A8 gene which encodes the β cell transporter ZnT8. The frequency of this risk allele is 70-81% in most populations. ZnT8 is mainly expressed in β-cells, where it has been shown by others and us to contribute to achieving a high concentration of Zn in secretory insulin vesicles. Insulin producing β-cells contain an exceptionally high concentration of Zn while at the same time exhibit a high rate of Zn turnover owing to the co-secretion of high quantities of Zn with insulin. Most of cellular Zn is bound to buffering proteins such as metallothionein to mitigate the toxic effect of free Zn. Given the high Zn turnover in β-cells, it is likely that β-cells may be at increased risk of excess free Zn under certain conditions. Prior evidence by us and others, showing that the T2DM risk variant of rs13266634 to be associated with a significantly higher Zn concentration in native human pancreatic islets provides additional support for the possibility of β-cell Zn excess cell failure leading to T2DM. To investigate this, we conducted an analysis of urinary concentrations of trace metals and their association with 2-hour glucose values in a large international cohort of mothers and their offspring across 10 international field centers -the HAPO Follow Up Study (HAPO-FUS). Multiple linear regression analysis in mothers and children was performed to adjust for T2DM risk factors. The 2-hour glucose values during an oral glucose tolerance test (OGTT) were chosen as an outcome variable due to their strong predictive value for further development of T2DM. Metals included in the regression analysis were Zn, Se, Cd, and As. Our analysis showed a strong regional variation in urine metal profiles between international centers, with highest levels of Zn in SML (374±13 µg/g in Arjona). Biological and soils samples from SML examined for Hg depicted significant average and range in SML concentrations in the reference area were catalogued as not polluted. In conclusion, Hg released by artisanal gold mining is being bioaccumulated by birds utilized in the human diet, representing a health risk to the consumers. It is highly recommended that in gold mining areas, proper protective measures be adopted. As such, local regulations should be permanent in communities surrounding mining areas to reduce Hg contamination and its negative impact on animal and human health. "Unicargena Support to Research Groups and Doctoral Programs, 2021-2022; Grant 155/2019, MinCiencias (Young Talent, 826/2020, and Doctoral formation, 647/2014)."

**4216 Sex-Specific Associations between Umbilical Cord Essential and Toxic Metals and Neurocognitive Outcomes in Extremely Low Gestational Age Newborns (ELGANs)**

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The association between the exposure to metals and neurocognitive development among extremely premature children born prior to 28 weeks gestational age is understudied. This analysis aimed to determine if prenatal exposure to metals is negatively associated with intelligence quotient (IQ) in Extremely Low Gestational Age Newborns (ELGANs). A total of 7,976 subjects were included in the investigation of the relationship between the concentrations of eleven metals in umbilical cord and IQ (ages 10 and 15). Generalized linear models were used to examine an association of single and multiple metals and IQ. Quantile g-based computation was used to examine the mixtures-based effects in relation to IQ. Statistical significance was defined as p < 0.05. When data from male and female ELGANs were combined, when compared to the reference area were catalogued as not polluted. In conclusion, Hg released by artisanal gold mining is being bioaccumulated by birds utilized in the human diet, representing a health risk to the consumers. It is highly recommended that in gold mining areas, proper protective measures be adopted. As such, local regulations should be permanent in communities surrounding mining areas to reduce Hg contamination and its negative impact on animal and human health. "Unicargena Support to Research Groups and Doctoral Programs, 2021-2022; Grant 155/2019, MinCiencias (Young Talent, 826/2020, and Doctoral formation, 647/2014)."
Hg-induced nephrotoxicity. Enhanced expression of MT may slow the progression of nephrotoxicity, suggesting that increased expression of MT protects against renal injury in corresponding animals injected with zinc. Interestingly, renal injury in corresponding animals injected with zinc demonstrated less nephrotoxicity than controls. Histological analysis of kidney sections showed significant renal injury in animals injected with saline and exposed to 1.25 µmol HgCl₂. Interestingly, renal injury in corresponding animals injected with zinc demonstrated less nephrotoxicity than controls. Expression of mRNA encoding superoxide dismutase and glutamate cysteine ligase was also measured in the kidney using qPCR to show that MT reduced oxidative stress in these organs. Knockdown of Nrf2 or Keap1 in hepatic cells by targeting sepiapterin reductase (SPR), a NADPH-dependent enzyme that catalyzes the reduction of sepiapterin to dihydrobiopterin (BH2). Dihydrofolate reductase (DHFR), a key enzyme in one carbon metabolism, further metabolizes BH2 to tetrahydrobiopterin (BH4), a cofactor for enzymes important in aromatic amino acid hydroxylases and the nitric oxide synthases. In the present studies, we show that cadmium reduced the conversion of sepiapterin to BH2 and BH4 in a time- and concentration-dependent (3-10 µM) manner. Using recombinant human enzyme and lysates from healthy, term human placentas, cadmium was also found to inhibit SPR (IC₅₀ = 24.2 µM for the recombinant enzyme and IC₅₀ = 380 µM for placenta). In placental lysates, dithiothreitol (2 mM) reversed SPR inhibition by cadmium indicating that this heavy metal reversibly binds to thiol groups in SPR. Similarly, cadmium inhibited SPR in human placental BeWo chorionic carcinoma trophoblasts. Moreover, treatment of BeWo cells with BH2 readily formed BH4 in a time- and concentration-dependent manner. Cadmium inhibited BH2-mediated formation of BH4 (IC₅₀ = 1.6 µM); these data indicate that cadmium reduces DHFR activity. DHFR was found to be 2-3 fold more sensitive to disruption by cadmium than SPR. The Keap1-Nrf2 pathway is important in cellular defense against oxidative and/or electrophilic stresses. Knockdown of Nr2f or Keap1 in BeWo cells did not alter the inhibitory effects of cadmium on SPR or DHFR. Taken together, these data indicate that cadmium inhibits DHFR biosynthesis in placental trophoblasts by inhibiting SPR and DHFR in a process that is not mediated by oxidative or electrophilic stress. Inhibition of enzymes requiring BH4 in the placenta may be an important mechanism by which cadmium induces developmental toxicity. Supported by NIH grants AR050573, ES029275, and ES005022.

Lead is a ubiquitous environmental contaminant that is frequently detectable in various products as an unavoidable impurity, potentially resulting in consumer exposure. It is a neurotoxicant and also a reproductive and developmental toxicant. The Proposition 65 listing of lead as a reproductive and developmental toxicant is based on the formal requirement: when substances are identified as reproductive and/or developmental toxicants by Federal regulations, OSHA regulations in the case of lead, they are automatically added to Proposition 65. Lead’s MADL was established by OEHHA in 1987 and is not a health effect-derived value, but rather is based on blood lead levels measured among workers exposed to lead. Various bodies have also established risk values for lead. These values include LOAELs of 0.16 and 1.3 µg/kg/day for children and adults, respectively, identified by JECFA in 2011, a TDI of 3.6 µg/kg/day calculated in 2001 by RIVM, a limit of 10 µg/L in treated water (equivalent to 30.4 µg/day) established in 2021 by the U.S. EPA and in 2017 by the WHO, and an allowable level in bottled water of 5 µg/L (15.2 µg/day) and an interarm reference level (IRL) for women of childbearing age of 12.5 µg/day identified in 2020 by U.S. FDA scientists. Variability among toxicity criteria poses challenges for both raw material suppliers and product manufacturers needing to demonstrate safety as well as global regulatory compliance. We critically evaluated the basis for each available risk value to determine which is most appropriate for this purpose, the U.S. FDA’s IRL of 12.5 µg/day, estimated exposures for some product types are above OEHHA’s MADL. Such exceedances would be considered a regulatory compliance failure, even though they may not necessarily indicate a significantly increased risk of adverse health effects. In other words, OEHHA’s MADL may be health protective even though it is not a health-based value. In contrast, other criteria, such as the U.S. EPA’s 10 µg/L (30.4 µg/day) notification level for water established under the Lead and Copper Rule, may not be adequately protective. This work highlights the importance of product use-specific approaches to exposure assessment and consideration of various established risk-based values to identify the most health protective and relevant value for use in risk assessment.

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Total Mercury in Indigenous Communities of the Bolivian Amazon

The Amazon is a unique place on the planet because of its irreplaceable biodiversity and contribution to the regulation of global climate. Unfortunately, it is being destroyed by deforestation and impacts derived from gold mining, one of them, mercury (Hg) pollution, affecting the health of indigenous communities. This study aimed to quantify the concentration of total mercury (T-Hg) in human hair and fish in indigenous communities living in Bolivia. Analyses of T-Hg in biological samples were carried out using cold vapor atomic absorption spectroscopy. Hair samples were collected from indigenous communities living on the basins of the Beni (n=435), Madre de Dios (n=350), and Mamore (n=53) rivers, with total-Hg values in hair ranging from 1.2 to 27.1, 0.1 to 114.4 and 0.1 to 6.2 µg/g, respectively. Among all participants, 89.4% registered levels exceeding 1 µg/g. Mercury is a lethal and persistent contaminant that does not decompose readily in the environment. Mercury, as a pollutant, is distributed globally because of its volatile nature and its ability to convert between chemical forms. Humans are exposed to various forms of mercury by ingestion, inhalation, andermal absorption. Inorganic forms of mercury accumulate readily in the kidney and can cause significant cellular injury. The purpose of this study was to test the hypothesis that mercury has detrimental effects on cellular components and processes such as protein folding, calcium homeostasis, mitochondrial activity, and junctional complex function. We hypothesized that cadmium disrupts biopterin metabolism in neuronal-derived human enzyme and lysates from healthy, term human placentas, cadmium was further metabolizes. In placental lysates, dithiothreitol (2 mM) reversed SPR inhibition by cadmium indicating that this heavy metal reversibly binds to thiol groups in SPR. Similarly, cadmium inhibited SPR in human placental BeWo chorionic carcinoma trophoblasts. Moreover, treatment of BeWo cells with BH2 readily formed BH4 in a time- and concentration-dependent manner. Cadmium inhibited BH2-mediated formation of BH4 (IC₅₀ = 1.6 µM); these data indicate that cadmium reduces DHFR activity. DHFR was found to be 2-3 fold more sensitive to disruption by cadmium than SPR. The Keap1-Nrf2 pathway is important in cellular defense against oxidative and/or electrophilic stresses. Knockdown of Nr2f or Keap1 in BeWo cells did not alter the inhibitory effects of cadmium on SPR or DHFR. Taken together, these data indicate that cadmium inhibits DHFR biosynthesis in placental trophoblasts by inhibiting SPR and DHFR in a process that is not mediated by oxidative or electrophilic stress. Inhibition of enzymes requiring BH4 in the placenta may be an important mechanism by which cadmium induces developmental toxicity. Supported by NIH grants AR050573, ES029275, and ES005022.
the polyadenylated H3.1 mRNA in the human bronchial epithelial BEAS2B cells ing factors. To characterize the factors that regulate inhibition of H3.3 assembly Nucleosome assembly is regulated by histone chaperones and chromatin remodelers, thereby disrupting the balance between H3.1 and H3.3. However, it is unclear how tail. Polyadenylation of H3.1 mRNA increased its stability and H3.1 protein level, how changes in bone adipocytes following tungsten exposure contribute to this process.

Funding P20GM130422.

process.

Sdf1 different. Non-tumor bearing mice, tungsten increased expression of marrow niche was increased in the non-tumor bearing, tungsten-exposed mice. In 8 weeks. We found that the overall burden of Plin1+ adipocytes within the bone, female mice exposed to either tap water or tungsten in the drinking water (15 ppm, exposed to tungsten during intraoperative radiotherapy, our lab is currently investigating the effects of tungsten exposure on breast cancer progression and metastasis. Tungsten is known to accumulate within the bone, creating a site for long-term exposure and toxicity. Breast cancer is also known to metastasize to the bone. We have previously shown, using the 4T1 orthotopic breast cancer model, that oral tungsten exposure enhances metastasis, osteolysis and myeloid-derived suppressor cells in the bone niche. These findings suggest that tungsten deposition in the bone creates a favorable microenvironment to promote metastasis. Bone marrow adipocytes (BMA) play an important role in breast cancer metastasis to the bone through the secretion of adipokines that drive tumor cells homing, colonization, and growth by changing the microenvironment. In order to investigate the role of BMA in tungsten-enhanced breast cancer metastasis in the bone, we quantified the number of Plin1+ (Perilipin-1) adipocytes and evaluated the gene expression of adipokines in both non-tumor and 4T1 tumor-bearing BALB/c female mice exposed to either tap water or tungsten in the drinking water (15 ppm, 8 weeks). We found that the overall burden of Plin1+ adipocytes within the bone marrow niche was increased in the non-tumor bearing, tungsten-exposed mice. In the 4T1 tumor-bearing mice, tungsten exposure also slightly increased the number of Plin1+ adipocytes, however the overall number of adipocytes were significantly decreased. Tungsten exposure also shifted the adipokine profiles in the bone marrow niche, in both non-tumor and 4T1 tumor-bearing mice. Interestingly, the adipokines that were elevated following tungsten exposure in each group were different. Non-tumor bearing mice, tungsten increased expression of Adipok, Cxcl10, Fabp4, Il-6, and Sdf1 while 4T1 tumor-bearing mice, tungsten increased expression of Cxcl2, Il-1β, and Tnfa and decreased expression of Adipoq. These results suggest that increased adipokines in the bone marrow niche is beneficial for breast cancer metastasis to the bone. Future work will focus on investigating how changes in bone adipocytes following tungsten exposure contribute to this process. Funding P20GM130422.

Mechanisms for Displacement of Histone Variant H3.3 by Arsenic-Induced Polyadenylation of Canonical Histone H3.1 mRNA

Arsenic, as a ubiquitous metalloid, poses a global public health threat to more than 200 million people in the world. Exposure to arsenic is associated with an increased risk of cancers. Epigenetic mechanisms play an important role in arsenic-mediated carcinogenesis. Epigenetic changes include DNA methylation, histone modifications, expression of non-coding RNAs, and incorporation of histone variants into nucleosomes. Replication-dependent canonical histones, such as H3.1, can be replaced by replication-independent histone variants, such as H3.3, within the nucleosome to alter chromatin structure and influence gene expression. Our previous studies demonstrated that arsenic exposure inhibits deposition of H3.3 at certain regulatory elements in the genome, leading to polyadenylation of H3.1 mRNA. mRNAs for canonical histone genes do not end with a poly(A) tail. Polyadenylation of H3.1 mRNA increased its stability and H3.1 protein level, thereby disrupting the balance between H3.1 and H3.3. However, it is unclear how arsenic-induced polyadenylation of H3.1 mRNA compromises H3.3 assembly. Nucleosome asymmetric modification by histone chaperones and chromatin remodeling factors. To characterize the factors that regulate inhibition of H3.3 assembly following arsenic-induced polyadenylation of H3.1 mRNA, we either overexpressed the polyadenylated H3.1 mRNA in the human bronchial epithelial BEAS2B cells that stably express FLAG-tagged H3.3, or treated the cells with 1 μM of arsenic for 48 hours. H3.3 complexes were then isolated by FLAG affinity gel purification followed by mass spectrometry to identify the H3.3 binding partners and compare the binding affinity changes with both H3.1 mRNA polyadenylation and arsenic exposure. Based on the mass spectrometry results, the interaction between H3.3 and several proteins, including a histone chaperon protein, was decreased by both overexpression of polyadenylated H3.1 mRNA and arsenic treatment, indicating that this histone chaperone might be involved in the H3.3 displacement mediated by arsenic-induced polyadenylation of H3.1 mRNA. Decrease of association between the histone chaperone and H3.3 was further verified by immunoprecipitation-western blot analysis. These results suggest that the changes of association between histone chaperones and H3.3 caused by polyadenylation of H3.1 mRNA may play an important role in arsenic carcinogenesis and the underline mechanisms need to be further explored.

Particulate Arsenic Trioxide Increases Greater DNA Damage and Reactive Oxygen Species than Soluble Arsenite in Lung Epithelial Cells

Environmental arsenic exposure is associated with lung cancer. Arsenic is the first substance known to cause lung cancer by two distinct routes, ingestion and inhalation, in the form of soluble arsenite and particulate arsenic trioxide, respectively. In comparison to significant progresses in research on mechanisms for lung carcinogenesis of arsenic ingestion, inhalation arsenic exposure route in particular form and its lung carcinogenic mechanisms are relatively under-investigated. Fundamentally, it remains unclear whether particulate arsenic exposure is in a...
disolved form and whether particulate exposure yields higher damage. Utilizing dynamic laser scattering, particulate arsenic trioxide exposure in cellular system was shown to be in particulate form instead of dissolved form. Using immunofluorescence, particulate arsenic trioxide was demonstrated to generate dramatically higher oxidative DNA damage and strand break, as well as significantly higher superoxide, in lung epithelial cells such as BEAS-2B, HSAEC1-KT, and SAE, comparing to soluble arsenite exposure at same concentration. This study demonstrated that particulate arsenic trioxide exposure yields higher damage in lung epithelial cells and indicated that the inhalation route of particulate arsenic exposure plays an important role in lung carcinogenesis.

4227 Relative Oral Bioavailability of Manganese in Electric Arc Furnace Steel Slag Is Influenced by High Iron Content and Low Bioaccessibility

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Electric Arc Furnace (EAF) slag is a rock-like aggregate produced in association with carbon steel; it is used for several construction applications, including land and unpaved road cover. The potential human health risks due with EAF slag have recently been of interest because EAF slag is enriched with manganese (Mn) (~3%) and several other metals, including iron (Fe) (~18%). However, because these metals are bound in highly alkaline mineral matrices, in vitro bioavailability (IVBA) of Mn (27%) and Fe (10%) is limited. As an essential nutrient, Mn absorption and excretion is controlled by homeostasis, and absorption of Mn is also affected by an excess or deficiency of other divalent metals, including Fe. To assess Mn absorption from incidental EAF slag ingestion, as compared to Mn in diet, in an in vivo relative bioavailability (RBA) study using F344 rats was conducted. Mn in diet was used because the EPA oral reference dose for Mn is based on the upper bound of Mn in the human diet. At 9 weeks old, rats were divided into six dose groups (n=6 for dose groups 1 through 3; n=8 for dose groups 4 through 6) and administered Mn in their diet for 14 days. Dose groups 1 through 3 received 10 ppm (control), 250 ppm, and 500 ppm Mn in enriched chow, respectively. Dose groups 4 through 6 received the control diet (10 ppm Mn in enriched chow) in addition to 1000 mg Mn/kg in 3.5-g 6-g, and 8-g EAF slag droghballs, respectively. Total Mn doses ranged from 0.24 to 21 mgMn/kg-day in dose groups 1 through 3 and 17 to 40 mgMn/kg-day in dose groups 4 through 6. Mn and Fe concentrations in liver, and Mn in lung and striatum, the target tissue of the brain, were quantified using EPA Method 3051 and, following elimination of outliers, Mn levels in each tissue were fit by dose to linear, exponential, and polynomial models to evaluate their relative dose-response (RDC) relationships. Across the models evaluated for each tissue type (liver, lung, and striatum), the linear model had the best fit to the data and was therefore chosen to derive tissue-specific D-TC slope coefficients and ultimately, RBA values. The D-TC relationship was the most highly significant for the liver Mn content, while both the enriched chow and EAF slag had a positive slope (P < 0.05). The Mn in EAF slag had a D-TC relationship. The linear Mn content D-TC relationship showed a positive slope for the enriched chow administration group (P value < 0.05) but a slightly negative and statistically insignificant (P > 0.05) slope for the EAF slag doughball group, indicating that there may be a decrease in systemic Mn absorption with increasing Mn dose associated with Mn in enriched chow. The RBA for Mn in EAF slag based on lung data was 19%. In comparison to the liver and lung tissues, the striatum showed little relationship (P > 0.05) between Mn tissue concentration and D-TCs remained relatively constant from administration of the enriched chow and EAF slag, and an RBA could not be calculated. Importantly, Fe concentrations in the liver of the chow-dosed groups were significantly decreased with dose, indicating that Mn inhibited Fe absorption. However, increased Fe was observed in the livers of EAF slag dose groups, likely because slag contains 6 times higher levels of Fe than Mn, and the high iron content of EAF slag may have inhibited systemic Mn absorption. Mn homeostasis is maintained in the liver, and the positive absorption curve for enriched chow and EAF slag was due to relative differences in IVBA between Mn in enriched chow and EAF slag (41%). Lung and liver tissue support that systemic absorption of bioaccessible Mn is limited by competition with Fe, and striatum tissue data support a finding that, even at very high doses, homeostasis was maintained in the target tissue. Overall, these data indicate that incidental ingestion of Mn in EAF slag does not pose a human health hazard.

4228 The Leaging Ligand Structure of Platinum Complexes Impacts the Survival of Human Embryonic Kidney and Melanoma Cells

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Since the discovery of the cytotoxic properties of platinum compounds, three divalent platinum-based chemotherapeutics have been approved by the FDA for the treatment of a wide array of cancer diseases. Platinum-based drugs are so effective that approximately half of all patients who receive chemotherapeutic treatment are given one of these compounds. Both the FDA-approved and novel platinum(II)- based chemotherapeutics contain a central platinum(II) atom, which is coordinated to leaving and non-leaving ligands. It is well-documented in the scientific literature that cisplatin-like complexes effectively enter the cell, bind to a guanine residue, and induce p53-mediated cell death; however, the impact of leaving ligand structure on cell survival is yet to be elucidated. Thus it is vital to examine leaving ligand effects because non-leaving ligands remain attached to the central platinum(II) atom upon entering the cell, while leaving ligands are displaced from the platinum(II) atom before or concurrently with the remaining portion of the molecule. The presence of leaving ligands can significantly impact the toxicity of leaving ligands, dichloro(ethylenediamine) platinum(II) (Pt(en)Cl2) has two chlorine atoms that can be displaced from the molecular structure. In contrast, 1,1-cyclobutane dicarboxylato(ethylam- inediamine)platinum(II) (Pt(en)CBDDCA) has one cyclobutanedicarboxylato group whose two bonds coordinated to the platinum atom are displaced simultaneously. To test the hypothesis that differences in leaving ligand structure of platinum(II) complexes interacts with cellular viability, a calculated IC50 of 36.67 µM for Pt(en)Cl2 and an IC50 of 55.86 µM for Pt(en) CBDDCA was developed using human embryonic kidney (HEK293) or melanoma (SKMEL5) cells. HEK293 or SKMEL5 cells were plated, then exposed to increasing concentrations of the platinum compounds for 24 hours. The data indicates a significant impact of leaving ligand structure on cellular viability with a calculated IC50 of 12.5 µM for Pt(en)Cl2 and an IC50 of 108.52 µM for Pt(en)CBDDCA. Atomic absorption assays were completed for each compound, cell line combination after 24 hours of exposure to determine if leaving ligand structure impacted the intracellular accumulation of platinum.

4229 Mercury Assessment in Invasive Lionfish Pterois spp (Oken, 1817) from a Marine Protected Area in the Colombian Caribbean

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Lionfish (Pterois spp.) is a recognized invasive species, reported since 2009 in the Corales del Rosario and San Bernardo Natural National Park in the Colombian Caribbean. Some strategies have been proposed to control its abundance and dispersion, limiting the ecological damage it provokes, including its capture and consumption. The marine park is influenced by the vicinity of Cartagena Bay, an ecosystem impacted by mercury (Hg) pollution from an extinct chlor-alkali plant, and also by sediments loaded with mercury from the Dique Channel, an extension of Magdalena River, a receiver of residues from gold mining activities. The aim of this work was to evaluate total Hg (T-Hg) levels in lionfish to verify its suitability for human consumption. Total Hg in muscle tissue was measured using a direct mercury analyzer (DMA80). Submarine fishing in the park allowed the capture of 58 specimens of lionfish, with sizes ranging from 17.4 to 44.4 cm (mean = 28.0±0.63 cm). Mercury concentrations in fish muscle ranged from 0.01 to 0.38 mg/kg, fw, with an average of 0.11±0.01 mg/kg. Mercury levels were not correlated with fish length (ρ=0.04; p=0.72). In short, Hg concentrations in lionfish complied with legislation for fish consumption, but a precautionary approach and a permanent monitoring strategy are strongly advised.

4230 Impact of miR-186 Overexpression on Arsenite Exposure–Induced Gene Expression and Transformation

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miR-186 is overexpressed in human squamous cell carcinoma induced by chronic arsenic exposure relative to premalignant hyperkeratosis. The role that miR-186 overexpression plays in carcinogenesis was investigated by transfecting human keratinocytes (HaCaT cells) with a miR-186 expression plasmid and an empty vector plasmid. Clones were isolated and the 3 expressing highest levels of miR-186 from miR-186 transfected and lowest miR-186 from empty vector transfected cells were propagated in media containing 0 or 100 nM NaAsO2. RNA was isolated from cells after 9 and 29 weeks incubation. Differential gene expression was determined by RNA-seq of polyA mRNA, and differential miRNA expression determined by small RNA-seq. Differential alternative splicing was evaluated using the rMATs framework. Cellular transformation was evaluated by anchorage independent colony formation. Chronic arsenic exposure induced gene and miRNA expression, and alternative splicing changes in both empty vector and miR-186 overexpression transfected clones. Arsenic induced expression of a gene encoding for a phosphatase. miR-186 overexpression dramatically changed the differential gene expression patterns induced by arsenite exposure. Cells overexpressing miR-186 and exposed to 100 nM NaAsO2 for 29 weeks formed twice as many colonies as all other groups. The data indicates that miR-186 overexpression induces gene expression changes that accelerate cellular transformation.
**4231 Zinc Supplementation Prevents Mitotic Accumulation in Human Keratinocyte Cell Lines upon Environmentally Relevant Arsenic Exposure**

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Chronic exposure to inorganic arsenic (iAs) is a global health issue leading to multi-organ diseases, often characterized by disrupted cell cycle progression. Exposure to supratherapeutic concentrations of iAs causes cellular accumulation in G2 or M phase of the cell cycle in multiple cell lines by inducing cyclin B1 expression. It is not clear if iAs exposure at doses corresponding to serum levels of chronically exposed populations (~100 nm) has any effect on cell cycle distribution. Environmentally relevant iAs exposure will lead to mitotic accumulation of human keratinocytes by stabilizing ubiquitination substrates of RING finger E3 ubiquitin ligase ANAPC11. For dose-response experiments, human keratinocyte cell lines (HaCaT and Ker-CT) were exposed to iAs (0-5 µM, 24 h). For zinc supplementation experiments, HaCaT and Ker-CT cell lines were simultaneously treated with 0, 0.1 µM Cd (0.1 µM; 24 h) and zinc (0 or 1 µM, 24 h). RNA and whole cell lysates were isolated for each experiment. Cell cycle distribution was measured using flow cytometry. mRNA and protein levels of ANAPC11 substrates cyclin B1 and securin were measured by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and immunoblotting respectively. Data were analyzed by One-Way ANOVA with Dunnett’s post-hoc test (dose-response) or Two-Way ANOVA with Tukey’s post-hoc test (zinc supplementation).

For all tests, p<0.05 was considered significant. iAs exposure starting at 0.1 µM led to mitotic accumulation of cells in both cell lines, along with the stabilization of ANAPC11 ubiquitination targets cyclin B1 and securin, without affecting their steady state mRNA levels. Moreover, zinc supplementation successfully prevented iAs-induced mitotic accumulation and the stabilization of cyclin B1 and securin without affecting their mRNA levels. Environmentally relevant iAs exposure leads to mitotic accumulation possibly by displacing zinc from the RING finger of cell cycle regulating E3 ubiquitin ligase ANAPC11. iAs-induced cell cycle disruption could underpin the molecular pathogenesis of multiple diseases associated with chronic iAs exposure such as diabetes and neurodegeneration. These results suggest that zinc dysregulation effects of iAs exposure and could be an inexpensive way to combat effects of chronic iAs exposure. Supported by NIH grants P01ES030283, R01ES027778 and P20GM135004.

**4232 Cadmium Causes Apoptosis in MIN6 Cells in an In Vitro Model of Pancreatic Beta Cells**

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Type 2 diabetes mellitus (T2DM) is a growing worldwide epidemic. Decreased insulin secretion in the face of insulin resistance is the hallmark of T2DM. Epidemiological and experimental studies show that exposure to the environmental contaminant cadmium (Cd) is associated with pre-T2DM, T2DM and altered blood glucose levels. Cadmium (Cd) is found in a variety of foods and drinking water. As reported by the World Health Organization (WHO), groundwater contamination by arsenic has affected at least 140 million people in 50 countries around the world, with most patients suffering from chronic arsenicosis, most remarkably skin inflammation, anemia, and cancer. Arsenic is one of the common symptoms of arsenic poisoning. Erythropoietin (EPO) is a hormone required for hematopoiesis, and suppression of EPO production have not been clarified. Autophagy is also one of the cytoprotective effects and has been reported to be affected by arsenic. In this study, we evaluated the effects of cadmium on MIN6 cells and the effects of low-concentration long-term arsenic (pentavalent inorganic arsenic) treatment on EPO production and autophagy induction in EPO-producing HepG2 cells. The amount of erythropoietin mRNA in harvested cells was measured using real-time RT-PCR. Autophagy was induced by arsenic treatment and EPO production was measured by ELISA. The results showed that functional pathways associated with inflammation and energy metabolism are activated in mouse lung by Vat levels occurring with environmental exposures. Thus, environmental vanadium exposure could be a previously unidentified causal factor in idiopathic pulmonary fibrosis.

**4233 Evidence for Environmental Vanadium Impact on Glutathionylation and Multimics in Lung Fibrosis**

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Pulmonary fibrosis is a devastating lung disease which can occur without known cause and be fatal. Recent research by others shows that protein glutathionylation is an early and possibly pivotal step in pulmonary fibrosis. Vanadium is an important commercial metal with widespread environmental exposures and is known to induce oxidative stress. Importantly, global warming has potential to increase exposure due to increased commercial use of vanadium in battery-operated automobiles and tools and increased reliance upon vanadium catalysts for processing efficiency. To determine whether vanadium causes protein glutathionylation and the occurrence of transcriptional disturbance in fibrosis. Human lung fibroblast (HFL) were exposed to vanadium for 24 h and tested for protein glutathionylation and associated morphologic and metabolic responses. Female C57BL/6 mice were exposed to vanadium in drinking water for 7 months and lungs were isolated for metabolomics analyses and RNA-Seq analyses. Integrative omics was performed with a data-dependent bioinformatics software, xMIAx. Analyses of HFL showed dose-response effects of V on cell proliferation (10 to 20%), protein glutathionylation (>2-fold), cell senescence (>10-fold), metabolic responses in inflammatory lipids and increase in hydroxyproline (10 to 20%), which is characteristic of collagen turnover. Analyses of mice showed increased collagen deposition in lungs, effects on energetics and inflammatory pathways and associated transcriptional changes genes and cell proliferation genes. The results show that oxidative stress from vanadium exposure causes increased protein glutathionylation in human lung fibroblasts and that functional pathways associated with inflammation and energy metabolism are activated in mouse lung by Vat levels occurring with environmental exposures. Thus, environmental vanadium exposure could be a previously unidentified causal factor in idiopathic pulmonary fibrosis.

**4234 Integrating Metabolic and Transcriptional Responses to Cancer Metabolite hexosamine in Human Lung Fibroblasts**

J. Z. Zhang, M. Prajapati, and T. B. Bartnikas. Brown University, Providence, RI.

Manganese (Mn) is an essential metal to the human body and is necessary for proper physiological function; however, excess manganese is toxic. The proper regulation of manganese levels is dependent on metal transporters. The transporter SLC30A10 is a key metal exporter responsible for moving Mn from the liver to the gallbladder for excretion in the bile. Mutations in SLC30A10 can cause inherited disease of Mn excess, which is characterized by cirrhosis, dystonia, and polycythemia, and patients with the disease phenotype are actually not notably smaller in size. We treated SLC30A10-deficient mouse cells with a gene vector that delivered a functional copy of SLC30A10 via TBG promoter-driven adeno-associated virus (AAV) vectors. The AAV-treated knockout mice had decreased Mn excess in their organs when compared to the saline treated knockout mice, but still had mildly elevated Mn when compared to saline treated wild type mice. The AAV-treated knockout mice also had decreased RBC counts and increased body masses compared to saline treated knockout mice. The RBC counts and body masses of the AAV treated knockout mice were restored back to wildtype levels. Overall, our results suggest that restoration of SLC30A10 in the liver is sufficient to attenuate disease characteristics, and that AAVs are viable treatments for diseases of inherited Mn toxicity.

**4235 Using Adeno-Associated Viral Vectors to Examine the Role of Metal Transporter SLC30A10 in Manganese Toxicity and as a Potential Treatment for Disease**

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The environment contains two toxic forms of inorganic arsenic: arsenite and arsenate. Inorganic arsenic is a highly toxic element that can affect acutely or chronically through foods and drinking water. As reported by the World Health Organization (WHO), groundwater contamination by arsenic has affected at least 140 million people in 50 countries around the world, with most patients suffering from chronic arsenicosis, most remarkably skin inflammation, anemia, and cancer. Arsenic is one of the common symptoms of arsenic poisoning. Erythropoietin (EPO) is a hormone required for hematopoiesis, and suppression of EPO production have not been clarified. Autophagy is also one of the cytoprotective effects and has been reported to be affected by arsenic. In this study, we evaluated the effects of arsenic on MIN6 cells and the effects of low-concentration long-term arsenic (pentavalent inorganic arsenic) treatment on EPO production and autophagy induction in EPO-producing HepG2 cells. The amount of erythropoietin mRNA in harvested cells was measured using real-time RT-PCR. Autophagy was induced by arsenic treatment and EPO production was measured by ELISA. The results showed that functional pathways associated with inflammation and energy metabolism are activated in mouse lung by Vat levels occurring with environmental exposures. Thus, environmental vanadium exposure could be a previously unidentified causal factor in idiopathic pulmonary fibrosis.
4237 Role of Hepatic Hypoxia Inducible Factors in Inherited Disorder of Manganese Excess Due to Slc30a10 Deficiency
M. Prajapati, J. Zhang, and T. Bartnikas. Brown University, Providence, RI.
Manganese (Mn) is a dietary nutrient and essential metal with varied biological functions. In excess, Mn can be toxic. One form of Mn toxicity is reported in patients with mutations in the metal import protein SLC30A10. Patients with SLC30A10 deficiency develop dystonia, liver disease, polyneuropathy, and erythropoietin excess. Though it is assumed that these phenotypes are due to Mn toxicity, the exact molecular mechanisms have yet to be established. In the current study, we aimed to understand the basis of polyneuropathy and erythropoietin excess in Slc30a10 knockout mice (Slc30a10TacKO). Erythropoietin is regulated by hypoxia-inducible factors (HIfs), transcription factors composed of regulated alpha subunits and constitutive beta subunits that mediate the cellular response to hypoxia. We hypothesized that Mn, like other divalent metals such as cobalt, acts as a "hypoxygen mimetic" resulting in excess erythropoietin production leading to polyneuropathy. To test this hypothesis, we used targeted and non-targeted expression of Hif-1alpha and Hif-2alpha in the hepatocytes of Slc30a10 TacKO mice. Deficiency in Hif-2alpha, but not Hif-1alpha, reduced EPO RNA levels, corrected polyneuropathy, and lowered tissue Mn levels. The basis of decreased Mn levels is not clear at this time. Overall, our results indicate that Hif-2alpha is a major contributor to disease phenotypes in Slc30a10 deficiency.

4238 Effects of Cadmium Exposure on Gut Microbiota by Mass Spectrometry–Based Metabolomics: An In Vitro Study
J. Chi, Y. Jin, and H. Gu. Florida International University, Port St Lucie, FL.
Cadmium (Cd) is a heavy metal element frequently used in industrial products such as nickel-cadmium batteries, PVC plastics, and electroplating. Human exposure to Cd through air, water, soil, and food results in toxicity in skeletal, urinary, reproductive, cardiovascular, nervous, and respiratory systems. Previous studies have shown that Cd exposure can cause organ damages and imbalanced gut microbiota; however, the mechanism of Cd toxicity remains largely unknown. In this in vitro study, our central hypothesis is that the interaction between the gut microbiome and host is mainly via metabolites. We selected wild-type Escherichia coli (EC), MG1655 and two probiotics, Nissle 1917 and Lactobacillus rhamnosus, which are known to be repopulated in gut microbiota, cultured in an anaerobic workstation. These bacterial strains were exposed to Cd (cadmium chloride in water) with different concentrations. The OD (600 nm) results showed that Cd significantly decreased bacterial viability for all three of these bacterial strains, in a dose-dependent manner. In addition, targeted metabolomics was used to examine tryptophan (Trp) metabolism, since gut microbiota is a major source of Trp metabolites. We determined 21 differentiative metabolic features that distinguish Cd exposure (p < 0.05). In the principal component analysis (PCA) score plot, there was a clear separation due to Cd exposure, with the high dose groups further away from the control groups. Major metabolic pathways that contributed to the separation included citrate cycle, purine metabolism, pyrimidine metabolism, starch and sucrose metabolism, and arginine biosynthesis. In summary, this study provided potential metabolic biomarkers and a better understanding of dysbiosis of gut microbiota due to Cd exposure, which will be validated in vivo.

4239 Characterization of Cholic Acid Signaling in Novel Low-Bile Acid Mouse Model
Bile acids (BAs) are endocrine molecules essential in the absorption of lipids in the small intestine. BAs are also critical signaling molecules in regulating cholesterol and BA homeostasis via the gut-liver axis. BAs interact with many different nuclear and membrane receptors, including the farnesoid X receptor (FXR) and G protein-coupled BA receptor 1. However, the full functions of individual BAs in vivo remain unclear. BA synthesis is initiated by two key enzymes, CYP7A1 and CYP27A1. We have previously reported on the generation and phenotypic characterization of male and female mice lacking these two key enzymes (double knockout, DKO) by mating Cyp7a1−/− and Cyp27a1−/− mice. Cholic acid (CA) is a primary BA, known as a model, to induce susceptibility to liver injury without known mechanisms. We hypothesize that male and female Cyp7a1−/− and CYP27A1 DKO mice administered CA will have altered BA levels and subsequent changes in molecular pathways involved in BA signaling and homeostasis compared to wild-type (WT) mice. To investigate, male adult WT and DKO mice were fed chow diet containing 0, 0.25 or 0.50% CA for 3 days. Liver clinical pathology was not altered by genotype or treatment. Serum triglycerides were significantly reduced in CA fed WT mice compared to vehicle. Hepatic mRNA expression of genes involved in BA synthesis Cyp7a1 was reduced by CA treatment for WT mice and Cyp27a1 was also reduced in CA treated mice regardless of genotype. Furthermore, mRNA expression of genes in BA conjugation (AcamR, Hsd17b4) were not changed by CA treatment regardless of genotype. Baseline mRNA expression of genes involved in regulation (Shp, Lcn13) in DKO vehicle treated mice were decreased compared to WT vehicle mice and CA treatment led to increases in expression. Hepatic mRNA expression of genes involved in transport (Bsep) increased with CA treatment regardless of genotype. Basal levels of inflammatory genes (CD14, IL-1β, TNF) tended to be higher in DKO vehicle compared to WT vehicle and CA did not lead to synergistic effect. Furthermore, basal level of oxidative stress gene HO-1 was dramatically increased in DKO vehicle mice, CA feeding led to reductions in DKO mice but not WT. We have also measured female DKO mice and trends are similar as those found in male DKO mice, therefore only male data are reported. In summary, this novel low BA mouse model will better understand the effect of CA on BA signaling and will be useful to further characterize BAs, which can be applied to better target mechanistic pathophysiology in liver diseases such as Nonalcoholic steatohepatitis.

4240 Proteomics Reveal In Vivo Effects of Phenobarbital in Mice with Humanized Livers
Xenobiotic activators of CAR (constitutive androstane receptor) may trigger adaptive but also adverse effects in the livers of laboratory rodents. This includes transcriptional induction of drug-metabolizing enzymes, transient proliferation of hepatocytes, and long-term promotion of the growth of liver tumors via a non-genotoxic mechanism. The relevance of CAR-related adverse hepatic effects to humans has been debated controversially. In this study, the chimeric FRG-KO mouse model was used, whose livers are largely repopulated by transplanted human hepatocytes. This allows study of human hepatocytes and their response to exposure to the model CAR activator phenobarbital (PB) in vivo. The mice received a single intraperitoneal injection with 50 mg/kg body weight PB or saline, followed by sacrificed after 72 or 96 hours. Non-repopulated FRG-KO mice were used as additional control. A combination of targeted and non-targeted proteomics approaches was used, and resulting data were merged to generate comprehensive datasets of PB effects on the liver. A novel proteomics workflow was established to allow, for the first time, comparative analyses of the effects of PB on human and murine proteins within an individual sample. Bioinformatics were used for data mining and revealed comparable responses of human and mouse hepatocytes regarding the activation of nuclear receptors and the induction of enzymes of drug metabolism. Contrastingly, the activation of MYC, a key proliferator regulator following stimulation of CAR, was predicted only for mouse hepatocytes, but not for the human cells. This finding was confirmed with analyses of 5-bromo-2'-deoxyuridine incorporation. In conclusion, this study presents a comprehensive proteomics analysis of CAR-dependent effects in human and mouse hepatocytes from the humanized FRG-KO mouse model. Data are in support of the hypothesis that PB induces adaptive metabolic responses in human hepatocytes, but not hepato-cellular proliferation.

4241 Hepatic Transcriptomic Assessment of Sprague Dawley Rats in Response to Dietary PFBS Ingestion
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Per- and polyfluoroalkyl substances (PFAS) belong to a family of manufactured chemical compounds containing multiple fluorine atoms attached to an alkyl chain. Due to environmental and health concerns, there has been a production shift from long-chain PFAS to a new generation of short-chain PFAS over the past two decades. One very common short-chain PFAS in the environment is the Perfluorobutane sulfonic acid (PFBS), which is a four-carbon chain compound based on perfluorobutanesulfonic fluoride (PFBSF) chemicals. There is growing evidence associating adverse health effects and certain liver diseases with environmental factors including exposure to PFBS. The in vivo ingestion of PFBS would induce liver injuries, damage, inflammations, and oxidative stress. To achieve these goals, Sprague-Dawley rats were assigned into three PFBS dietary treatment groups (0, 50, and 100 PPM) for 11 weeks. After this period, the animals were sacrificed, and the serum and liver samples were evaluated for various clinical markers. PFBS exposure resulted in a dose-dependent reduction in body weight compared to the control group but was not statistically significant (P > 0.05). The Total Antioxidant Capacity (TAC) in the exposed group was lower compared to the control. Total protein in rats exposed to high doses declined relative to the control. The study also revealed that ALT levels were dose-dependent. Transcriptomic

4242 Comorbidities of Howler Monkeys and Inflammation:
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Howler monkeys (Alouatta) are highly social primates native to Central and South America. They are known for their loud vocalizations and are considered a keystone species in their ecosystem. Howler monkeys are also known to have a high incidence of inflammatory disorders, which can be attributed to their diet, lifestyle, and genetic predisposition. In this study, we aimed to investigate the comorbidities of howler monkeys and inflammation. To do this, we performed a comprehensive transcriptomic analysis of howler monkey liver tissue samples from a cohort of monkeys with a history of inflammatory disease. The samples were collected from animals housed in a primate facility located in California, USA. The liver tissues were analyzed using gene expression profiling techniques, such as RNA sequencing, to identify genes that were differentially expressed in howler monkeys with inflammatory conditions compared to healthy controls. The results revealed a number of genes involved in inflammatory pathways, including those related to cytokine production, chemokine signaling, and innate immune response. These findings provide insight into the molecular mechanisms underlying inflammatory comorbidities in howler monkeys and suggest potential therapeutic targets for managing these conditions.
4242 Role of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Regeneration after Acetaminophen-Induced Acute Liver Injury

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Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the US and UK. As shown by previous studies, prompt activation of liver regeneration following overdose is critical for survival. For this regeneration to occur, extracellular matrix (ECM) remodeling is imperative. Matrix metalloproteinases (MMPs) play a key role in the turnover of matrix proteins while tissue inhibitors of metalloproteinases (TIMPs) serve to inhibit the MMPs. We have identified a trend relating the balance of MMPs and TIMPs to effects on ECM remodeling in liver injury and regeneration after acute APAP overdose. The APAP incremental dose model was used for this study. Male C57BL/6J mice received either a 300mg/kg (regenerating) dose or a 600mg/kg (non-regenerating) dose of APAP. Samples were collected over a time course of 0 to 96hs to study liver injury and regeneration. qPCR and western blot analyses of liver-relevant MMPs and TIMPs were done to collect preliminary data. Two general patterns of mRNA expression arose at the 300mg/kg dose. In pattern A, MMPs 4, 8, 9, and 14 showed an initial increase in expression at 12-24hrs (injury phase) followed by a decrease at 48hrs (recovery phase). In pattern B, MMPs 2, 19, and 27 showed an increase at approximately 24hrs that continued to rise throughout the recovery phase. TIMP1 displayed pattern A while TIMP2 displayed pattern B, indicating a potential relationship between these proteins and when they are active during liver injury and regeneration. These patterns were similar at the 600mg/kg dose, however, overall mRNA expression tended to be lower compared to the 300mg/kg dose. Western blot analysis focused on the proteins with the strongest expression correlations, which included those between TIMP1 and MMP2 and between TIMP2 and MMP9. The expression patterns seen in the western blot analyses and their relationships to one another at both the 300 and 600mg/kg doses were more complex than what was seen in the qPCR data and will require further study. Preliminary data suggest APAP-induced acute liver injury may affect the careful balance maintained by TIMPs and MMPs in the liver. This disruption has the potential to impact ECM remodeling in a way that negatively influences regeneration after APAP-induced acute liver injury. This work was funded by NIH R01 DK98414.

4243 Disruption of the Mouse Liver Epitranscriptome by Aroclor 1260 Exposure and Diet

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Chronic environmental exposure to polychlorinated biphenyls (PCBs) is associated with metabolic diseases including Non-Alcoholic Fatty Liver Disease (NAFLD) which can be exacerbated by a high-fat diet (HFD). We previously reported that exposure of HFD-fed male mice to a single oral dose Aroclor 1260 (Ar1260, 20 mg/kg), a non-dioxin-like mixture of PCBs, induced more hepatic injury compared to low-fat diet (LFD)-fed mice after 12 weeks. Proteomic and mRNA transcriptome changes were accompanied by increased abundance of the epitranscriptomic mark 2′-O-methyladenosine (Am) and reduced N(6)-methyladenosine (m6A) in the liver RNA from these HFD-fed Ar1260 exposed mice. The goal of the present study was to determine how short-term (12 weeks) PCB exposure affects hepatic AS machinery in HFD-fed male mice with non-alcoholic steatohepatitis (NASH) and identify PCB-induced AS events that may play a role in NASH pathology. We performed integrated analysis of hepatic gene expression and AS in HFD-fed mice exposed to PCBs via oral gavage. C57BL/6J male mice were fed a 42% fat diet (HFD) and exposed to Aroclor1260 (a non-dioxin-like PCB mixture, 20 mg/kg), PCB126 (a dioxin-like PCB, 20 µg/kg), combined Aroclor1260 and PCB126, or vehicle control for 12 weeks. Liver mRNA was isolated and sequenced. REACTOME pathway analyses identified spliceosome and mRNA splicing pathways altered by PCB exposures. We evaluated the abundance of the transcripts of 203 RNA binding proteins in a role in AS regulation and that co-exposure to Aroclor1260 and PCB126 altered the expression of 100 AS factors. We performed replicate multivariate analysis of transcript splicing (mATS) to identify splicing events that were altered by PCBs compared to the HFD control and found that Aroclor1260 + PCB126 exposure resulted in 449 altered AS events in 366 genes. KEGG pathway analysis of these AS events associated with metabolic pathways, xenobiotics, primary bile acid biosynthesis, and fatty acid metabolism, all known to be dysregulated in NASH. The results of this study demonstrate specific hepatic AS regulatory mechanisms are disrupted by exposure to PCBs, contributing to the expression of altered isoforms that may play a role in NALFD progression to NASH. Supported by NIH R21 ES0313510, R21 ES0313510-S1, P30 ES030283, R35 ES028373, R01 ES032189, P20 GM103436 and P20 GM1031326.

4244 Polychlorinated Biphenyls Dysregulate Haptenic Alternative Splicing Machinery by Altering Expression of Splicing Factors in a Mouse Model of Environmental Liver Disease


The liver plays a major role in the detoxification and elimination of xenobiotics and therefore is more susceptible to chemical-induced liver injury. Environmental exposure to polychlorinated biphenyls (PCBs) is associated with Non-Alcoholic Fatty Liver Disease (NAFLD) in human populations and PCBs exacerbate high fat diet (HFD)-induced NAFLD in a mouse model of environmental liver disease. However, the responsible mechanisms for these associations remain to be elucidated. Approximately 95% of human genes generate multiple transcripts through alternative splicing (AS) and disturbances in AS have been shown to cause liver disease. The regulation of AS is mediated by a set of splicing factors with tissue- or disease-specific gene expression and protein activity. The goal of the study was to determine how short-term (12 weeks) PCB exposure affects hepatic AS machinery in HFD-fed male mice with non-alcoholic steatohepatitis (NASH) and identify PCB-induced AS events that may play a role in NASH pathology. We performed integrated analysis of hepatic gene expression and AS in HFD-fed mice exposed to PCBs via oral gavage. C57BL/6J male mice were fed a 42% fat diet (HFD) and exposed to Aroclor1260 (a non-dioxin-like PCB mixture, 20 mg/kg), PCB126 (a dioxin-like PCB, 20 µg/kg), combined Aroclor1260 and PCB126, or vehicle control for 12 weeks. Liver mRNA was isolated and sequenced. REACTOME pathway analyses identified spliceosome and mRNA splicing pathways altered by PCB exposures. We evaluated the abundance of the transcripts of 203 RNA binding proteins in a role in AS regulation and that co-exposure to Aroclor1260 and PCB126 altered the expression of 100 AS factors. We performed replicate multivariate analysis of transcript splicing (mATS) to identify splicing events that were altered by PCBs compared to the HFD control and found that Aroclor1260 + PCB126 exposure resulted in 449 altered AS events in 366 genes. KEGG pathway analysis of these AS events associated with metabolic pathways, xenobiotics, primary bile acid biosynthesis, and fatty acid metabolism, all known to be dysregulated in NASH. The results of this study demonstrate specific hepatic AS regulatory mechanisms are disrupted by exposure to PCBs, contributing to the expression of altered isoforms that may play a role in NALFD progression to NASH. Supported by NIH R21 ES0313510, R21 ES0313510-S1, P30 ES030283, R35 ES028373, R01 ES032189, P20 GM103436 and P20 GM1031326.
metabolite formation after an APAP overdose induces an early translocation of A2BAR from the plasma membrane to the outer mitochondrial membrane, and its translocation depends on JNK activation.

Heavy alcohol drinking induces alcohol-related liver disease, which is initiated from steatosis (fatty liver) to steatohepatitis, fibrosis, and cirrhosis. Ethanol is mainly metabolized by alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1), and catalase. Peroxisomes contain catalase and many oxidases (e.g. peroxisomal fatty acid oxidation rate limiting enzyme acyl-CoA oxidase, ACOX) producing hydrogen peroxide ($H_2O_2$). In peroxisomal matrix, catalase binds oxidases-generated $H_2O_2$ to form catalase-$H_2O_2$ complex, after a second $H_2O_2$ binds to the complex, both $H_2O_2$ will be decomposed to $H_2O$. If ethanol is present, the catalase-$H_2O_2$ complex will bind ethanol instead of a second $H_2O_2$, and the ethanol and $H_2O_2$ will be metabolized to acetaldehyde and $H_2O$, respectively. Previously, we found that peroxisome proliferator WY-14,643 enhanced catalase metabolism of ethanol. In mammalian cells, peroxisomes can be generated de novo requiring a group of proteins called peroxins (encoded by Pex gene) for their assembly and division. PEX16 is one of peroxins critical for the assembly of the peroxisomal membrane and import of peroxisomal membrane protein (PMPs). In patients, PEX16 absence leads to complete loss of peroxisome structure. Here, we report that WY-14,643 mainly induced PEX16 in mice. In liver-specific PEX16 knockout mice (PEX16Alb-Cre), peroxisomal membrane protein PMP-70 was absent. Interestingly, peroxisomal matrix protein catalase was upregulated in the PEX16Alb-Cre mice. Due to lack of peroxisomes, the upregulated catalase in the PEX16Alb-Cre mice was in cytosol and microsomes but not in mitochondria, and the upregulated catalase did not enhance ethanol metabolism, suggesting that peroxisome structure is essential for catalase-mediated metabolism of ethanol. After 25 days of the Lieber-DeCarli liquid ethanol diet feeding, alcoholic fatty liver was developed in PEX16 flx mice (PEX16/+/fl) but not in the PEX16Alb-Cre mice. WY-14,643 (10 mg/kg, in the Lieber-DeCarli liquid diets) induced PEX16, ACOX1 and catalase, enhanced catalase-mediated ethanol metabolism, and ameliorated alcoholic fatty liver in the PEX16/+/fl mice but not in the PEX16Alb-Cre mice. These results suggest that peroxisome structure is pivotal for promoting ethanol metabolism and the development of alcoholic fatty liver.

Nonalcoholic fatty liver disease (NAFLD), a prevalent chronic liver disease, is characterized by substantial variations in severity. In this study, we used a genetically diverse Collaborative Cross (CC) mouse population model to analyze the global transcriptome and clarify the molecular mechanisms involved in hepatic fat accumulation. The level and extent of liver fat was associated with increased plasma thrombin-antithrombin III complexes, decreased plasma prothrombin, and a dramatic reduction in plasma fibrinogen compared to lower APAP doses. Hepatic fibrinogen(ogen) deposits increased independent of APAP dose, whereas plasma fibrinogen(ogen) degradation products markedly increased in mice with experimental ALF. Early pharmacological anticoagulation (+2 hours after 600 mg/kg APAP) limited coagulation activation and reduced plasma fibrinogen. The marked coagulation activation evident in mice with APAP-induced ALF was associated with a coagulopathy detectable ex vivo in plasma. Specifically, prolongation of the prothrombin time and inhibition of tissue factor-initiated clot formation was evident even after restoration of physiological fibrinogen levels. This study suggests that pharmacologic intervention with anticoagulants may fill an unmet need as a model to uncover mechanistic aspects of the complex coagulopathy of ALF.

The liver displays robust repair and regenerative capacity in response to acute injury or partial heptapathy (PHx). PHx induces activation of blood coagulation and hepatic deposition of fibrin(ogen) drives liver regeneration. To date, there is not a clear explanation for the paradoxical observations that both fibrin(ogen) and the primary fibrin(ogen) degrading enzyme plasmin each promote liver regeneration after PHx. Notably, prior studies are limited to plasmino-gen−/− mice, which may fill an unmet need as a model to uncover mechanistic aspects of the complex coagulopathy of ALF.

Patients with acetaminophen (APAP)-induced acute liver failure (ALF) display both hyper- and hypocoagulable changes not necessarily recapitulated in standard APAP hepatotoxicity mouse models. We sought to examine coagulation activation in vivo and plasma coagulation potential ex vivo in experimental settings of APAP-induced hepatotoxicity and repair (300-450 mg/kg) and APAP-induced ALF (600 mg/kg). APAP-induced ALF was associated with increased plasma thrombin-antithrombin III complexes, decreased plasma prothrombin, and a dramatic reduction in plasma fibrinogen compared to lower APAP doses. Hepatic fibrinogen(ogen) deposits increased independent of APAP dose, whereas plasma fibrinogen(ogen) degradation products markedly increased in mice with experimental ALF. Early pharmacological anticoagulation (+2 hours after 600 mg/kg APAP) limited coagulation activation and reduced hepatic necrosis. The marked coagulation activation evident in mice with APAP-induced ALF was associated with a coagulopathy detectable ex vivo in plasma. Specifically, prolongation of the prothrombin time and inhibition of tissue factor-initiated clot formation was evident even after restoration of physiological fibrinogen levels. This study suggests that pharmacologic intervention with anticoagulants may fill an unmet need as a model to uncover mechanistic aspects of the complex coagulopathy of ALF.
offered definitive evidence that neutrophils (or NETs) contribute to experimental APAP-induced liver injury. We determined the role of neutrophils in liver injury and coagulation activation 24 hours after challenge with different doses of APAP (300 or 600 mg/kg) that produce ALI- and ALF-like pathology in mice. Mice were treated 24 hours prior to APAP challenge with rat anti-mouse Ly6G antibody (1A8) and anti-rat IgG K, antibody to induce durable neutrophil depletion. Depletion of neutrophils was successful as demonstrated by the lack of neutrophil accumulation in the liver (live CD45+/CD11b+/CD115/Ly6c-mid). Mice given isotype control antibody and challenged with 300 mg/kg APAP developed marked hepatic necrosis and an increase in biomarkers of coagulation cascade activation. Neutrophil depletion did not affect either liver injury or coagulation activation in mice challenged with 300 mg/kg APAP. Mice given isotype control and challenged with 600 mg/kg APAP developed marked hemorrhage and congestion indicative of vascular injury in addition to hepatic necrosis. Interestingly, compared to mice given 300 mg/kg APAP, hepatic neutrophil accumulation was further increased in mice given 600 mg/kg APAP. Moreover, neutrophil depletion significantly reduced the severity of liver necrosis in mice challenged with 600 mg/kg APAP, although neutrophil depletion did not affect biomarkers of coagulation activity. The results indicate that the role of neutrophils in APAP-induced liver injury depends on the APAP dose. The results suggest that neutrophils drive liver injury by a coagulation-independent mechanism in an experimental setting resembling APAP-induced ALF.

4251 The Role of Tissue-Restricted Fxr Deletion in NASH Development in Mice Z. Henry, S. Gao, S. Mahlia, B. Kong, and G. L. Guo. Rutgers, The State University of New Jersey, Piscataway, NJ.

Non-alcoholic steatohepatitis (NASH) is a condition in which there is excess fat accumulation in the liver, known as steatosis, that is caused by excessive alcohol consumption. NASH is characterized by steatosis, inflammation, hepatocyte ballooning, and necroinflammation. The disease is a major cause of liver-related morbidity and mortality. The role of FXR in NASH development is not yet fully understood. FXR is the major homeostatic regulator of bile acids (BAs) and is known to be involved in the regulation of lipid metabolism and cholesterol homeostasis. The results of this study suggest that the role of FXR in NASH development is not yet fully understood.


The environmental pollutant vinyl chloride (VC) directly causes hepatocellular carcinoma (HCC) at high exposure levels. However, we have shown that lower exposure levels (i.e., < exposure regulation limits) that are currently considered safe and do not directly damage the liver, not only enhance experimental NAFLD but also HCC. Here, we study the mechanisms by which VC exacerbates HCC in an "inside-out" model based on delivery of clinically relevant genes related to the development of human HCC. For liver-specific gene delivery, 9-week-old male C57BL/6J mice with oncogenic Met and mutant-β-catenin (CTNNB1-S45Y), "Sleeping Beauty" plasmids (10 µg) were co-delivered via hydrodynamic tail vein injection. Exposure to sub-OSHA levels of VC (< 1 ppm/d) or control air for 7 weeks (6 days/week, 5 days/week) started immediately following transfection. Additional mice received fenofibrate (PPARα agonist; 50 mg/kg i.p.) for 12 weeks. Tumor burden and size were assessed. Plasma and liver samples were collected for RNA-Seq analysis, and histology. VC exposure by itself caused no evidence of (pre)cancerous lesions. The Met and mutant-β-catenin co-expression in mice caused a few neoplastic foci. In contrast, VC robustly increased tumor size and number in Met-β-catenin mice. Transcriptomic analysis demonstrated that several metabolic processes involved in lipid metabolism were strongly induced. To test whether these results have relevance to human HCC the top changed genes were applied to human ortholog expression in the Liver Hepatocellular Carcinoma (LIHC) sequencing results in the Cancer Genome Atlas (TCGA), that the gene expression changes driven by VC in our mouse models corresponds to a unique HCC phenotype in humans. Upstream regulator analytic predicted a major role for PPARα and SREBP1/2, further supported by IHC, demonstrating that the tumors were PPARα positive. Co-administration of fenofibrate significantly enhanced tumor development in the VC-exposed mice Met-β-catenin, but not in the air controls, indicating that treatment with PPARα responsive VC exposure potentiates tumorigenesis, at least in part, via PPARα and SREBP1/2-mediated pathways. Even low dose environmental exposure of toxicants like VC could serve as disease modifiers for HCC requiring further mechanistic studies in preclinical and clinical settings. This emphasizes also that current safety requirements may be insufficient to account for other factors that can influence hepatotoxicity.


Fatty liver disease can be attributed to different etiologies including alcohol consumption, diet-induced obesity, and exposure to environmental toxicants. Environmental toxicants including persistent organic pollutants like polychlorinated biphenyls have been associated with Toxicant-Associated Fatty Liver Disease (TAFLD). Steatohepatitis in diet-induced obese models is characterized by steatosis and inflammation. While mechanisms of disease including metabolism, endocrine and signaling disruption in the liver have been investigated, there are currently limited studies investigating therapeutic strategies. TADF has similar pathogenesis and disease progression as Non-Alcoholic Fatty Liver Disease (NAFLD). NAFLD/TADF represents a spectrum of disorders ranging from simple steatosis to more severe forms of steatohepatitis and is not yet defined. Several studies have shown to increase susceptibility to NAFLD progression in experimental models, and clinically, zinc supplementation has been applied for treatment of alcohol-associated liver disease. However therapeutic effects of zinc supplementation in NAFLD/TADF are yet to be determined. We therefore developed an in vivo model to initially characterize the effects of zinc supplementation on HFD-induced steatosis without toxicant exposure. We hypothesize that dietary zinc supplementation will be an effective strategy for the treatment of diet-induced NAFLD and this strategy can be potentially applied to mitigate TADF/TASH in the future. To test our hypothesis, 9-week-old male C57BL/6J mice were fed either a control diet (10% fat-kcal) or high fat diet (60% fat-kcal) for 12 weeks. After 12 weeks mice were further grouped into diets containing 30 or 90 mg zinc/4057 kcal, representing normal or high fat diet for 12 weeks. Mice were treated with or without 300 mg/kg zinc sulfate (zinc supplement) for 12 weeks. Plasma and liver samples were collected for lipid, histology, gene expression and metal analysis. Statistics were performed using 2-way ANOVA and Bonferroni correction with significance level of 0.05. 12 weeks of HFD resulted in symptoms of metabolic syndrome: reduced glucose clearance, increased body weight and percent body fat. Eight weeks of subsequent zinc supplementation did not significantly affect these clearance parameters. The expression levels of genes involved in lipid metabolism or their downstream targets. Hepatic zinc accumulation was not different between normal and zinc supplemented diet groups although zinc content showed three-fold increase in zinc supplemented mice. Additionally, neither histology (steatosis and fibrosis) nor liver injury markers such as plasma

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Acetaminophen (APAP) overdose induced liver injury (AILI) is a leading cause of acute liver failure in the US. The temporal course of AILI can be delineated into two phases: injury and recovery. The injury phase occurs in mice from 0-12 hours after the overdose and is characterized by glutathione (GSH) depletion, accumulation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAQI), NAQI-protein adduct formation, activation of c-Jun N-terminal kinase (JNK) and oxidant stress, which culminate in mitochondrial dysfunction, DNA fragmentation and hepatocyte necrosis. A recovery phase follows from 24-72 hours, during which there is hepatocyte proliferation to repopulate the injured liver. While pharmaceutical interventions, such as N-acetylcysteine and fomepizole (4-MP), can prevent injury when given within 2 hours of APAP in mice, there are no therapies that promote recovery. JNJ-26366821 (PEG-TPO) is a novel thrombopoietin (TPO) mimetic peptide. TPO promotes megakaryocyte-erythroid progenitor cell (MEP) differentiation, megakaryocyte and platelet production by acting at the TPO receptor on megakaryocytes and platelets. The TPO receptor is also present on hepatocytes and liver sinusoidal endothelial cells. We hypothesize that PEG-TPO activity at the TPO receptor provides a protective and regenerative effect following liver injury. In this study, we evaluated the extent to which PEG-TPO or 4-MP can provide a protective and regenerative effect by comparing injury and recovery parameters in mice treated with PEG-TPO or 4-MP, and un-treated mice. Plasma ALT levels were significantly reduced by 24 h after APAP and accelerated the onset of the proliferative response which is essential for liver recovery. It's effects are distinct from 4-MP's, which prevents necrotic cell death. While PEG-TPO protected against hepatic necrosis, it did not significantly reduce plasma ALT release, DNA breakage or necrotic area. In contrast, PEG-TPO treatment was beneficial at 24 hours after APAP overdose. These findings indicate that PEG-TPO affects neither metabolism of APAP to NAPQI nor oxidant stress initiation. In addition, liver injury markers such as plasma ALT release, DNA fragmentation and hepatic necrosis were identical across groups, indicating that PEG-TPO does not prevent the induction of AILI. These results were recapitulated later into the injury phase at 12 hours after APAP—PEG-TPO did not significantly reduce plasma ALT release, DNA breakage or necrotic area. In conclusion, PEG-TPO treatment was beneficial at 24 h after APAP overdose. The plasma ALT level in the PEG-TPO group was 3.567 U/L, a 48% drop compared to vehicle treated mice. The PEG-TPO treated group also had smaller necrotic area fraction (16.9% vs 29%) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stained area (12.5% vs 21.6%) than the vehicle treated group. The 4-MP treated group had very similar measurements for these parameters: 3.745 U/L, 16.7% and 9.1% for ALT, necrotic area fraction and TUNEL positive area, respectively. However, PEG-TPO and 4-MP had divergent effects on hepatocyte proliferation, a critical facet of liver recovery after AILI. Cyclin D1 and proliferative cell nuclear antigen (PCNA) are markers of hepatocyte proliferation. Western blotting for cyclin D1 and PCNA showed higher expression in livers of PEG-TPO mice versus vehicle and 4-MP-treated mice. This was confirmed by immunohistochemical staining for PCNA which showed greater induction of PCNA in the hepatocytes bordering the necrotic area with PEG-TPO treatment versus vehicle or 4-MP. These findings indicate that PEG-TPO arrests the progression of AILI by 24 h after APAP and accelerates the onset of the proliferative response which is essential for liver recovery. It's effects are distinct from 4-MP's, which prevents necrotic cell death. While PEG-TPO protects against hepatic necrosis, it does not directly promote hepatocyte proliferation and liver recovery. Therefore, PEG-TPO is a potential novel therapeutic for enhancement of liver recovery after AILI.
of TCDD. To investigate cell-specific changes, single-nuclei RNA-sequencing was performed on livers from male C57BL/6 mice gavaged with 30 μg/kg TCDD or sesame oil vehicle collected at 2, 4, 8, 12, 18, 24 or 72 hrs. Clustering by marker genes identified 10 major liver cell types as well as 3 novel cell (sub)types. Differential gene expression analysis of each cell type identified common and cell specific changes. Dysregulation of the lipid metabolism-related genes Ces1b, Pi3ls3, and Fabp12 was primarily observed in hepatocytes as early as 2 hrs, showing 3.9 -1.8, and 2.1-fold change, respectively. Inflammatory markers were induced across all time points, with Il1r1 occurring primarily in hepatocytes, endothelial cells, macrophages, and cholangiocytes, and Gbp2b occurring primarily in macrophages. Cell-cell interaction inference between all cell types revealed dysregulation of neuregulin (NRG)/ERBB signaling, a pathway involved in cellular homeostasis and subsequent energy metabolism that is dysregulated in Non-Alcoholic Fatty Liver Disease (NAFLD). Plasmacytoid dendritic cells and portal fibroblasts exhibited Nrg1 and 4 ligand interaction that induced Erbb3 and Erbb4 receptors expressed in B cell, cholangiocytes, hepatocytes, hepatocytes stellate cells, neutrophils, and T cells. Accordingly, functional enrichment analysis identified the differential expression of the downstream NRG/ERBB pathway targets including cell cycle regulation and mitogen-activated protein kinase signaling in hepatic stellate cells and endothelial cells, as well as mTOR signaling in hepatocytes. Taken together, these results suggest that early responses to TCDD include a cophenogy of cell-specific intra- and inter-signaling changes that activate diverse pathways in the diverse cell types in the liver that contribute to hepatotoxicity and the emergence of hepatic pathologies. GNC was supported by T32ES007255. This project is funded by RO1ES029541 and the SRF P42ES004911.

2528 Critical Role of MET in Stimulating Liver Regeneration and Restricting Progression of Liver Injury after Acetaminophen Overdose in Mice


Hepatocyte growth factor (HGF) receptor (i.e. MET) is known to be important for hepatocyte proliferation after partial hepatectomy (PH). However, disruption of HGF/MET signaling can be compensated by functionally similar epidermal growth factor receptor (EGF/EGFR) signaling and only combined elimination of both these signaling pathways result in liver failure in the PH model. Role of HGF/MET signal- ing in APAP-induced liver injury (AILI), the clinically-relevant model of acute liver failure, remain unexplored. Unlike PH in a normal healthy liver, AILI is complicated by presence of massive liver injury and inflammation that intricately govern the regeneration vs. regenerative response. Thus, the role of MET in AILI cannot be presumed identical to that known in regeneration of healthy liver after PH. In our previous study, MET was activated dose-dependently after APAP overdose in mice, with much higher activation at high dose of APAP, where animals fail to regenerate spontaneously. To better understand the role of MET in APAP-induced liver injury and regeneration, we eliminated MET in liver via an albumin-CRE system. Wild-type (WT) and liver-spe- cific MET KO mice were administered 300 mg/kg APAP. Liver injury and regeneration- parameters along with underlying signaling pathways were investigated at various time points. Hepatocyte proliferation and liver regeneration were almost completely blocked in MET KO mice. Although, initial liver injury was similar in WT and MET KO mice, severe inhibition of liver regeneration resulted in unrestrained progression of liver injury and failed spontaneous recovery culminating in significant mortality in MET KO mice. Mechanisms that initiate APAP-induced liver injury such as metabolic activation of APAP, early glutathione depletion, APAP-protein adduct formation and JNK activation remain unaltered in MET KO mice. However, there was complete failure of activation of core cell cycle machinery (i.e. phosphorylation of cyclin-dependent kinase inhibitors) in MET KO mice, resulting in impaired liver regeneration. Impaired cell cycle activation was due to inhibition of ERK signaling in MET KO mice. Ingenuity Pathways Analysis (IPA) of RNA-seq data included severe blockage in activation of master regulators of hepatocyte proliferation and viability. Interestingly, there was no compensatory increase in EGFR activation in MET KO mice. In vitro, liver-specific MET deletion caused inhibition of hepatocyte proliferative signaling and failed liver regeneration result- ing in unrestrained progression of liver injury after APAP overdose, which was not compensated by other proliferative signaling pathways.

2529 Studies on Methionine Alleviating Toxic Responses in Liver of Rats Fed High-Cholesterol Diet

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Based on growth studies in animals, L-methionine (Met) was once considered to be the most toxic amino acid. However, recent studies document the beneficial effects of Met. S-adenosyl methionine (SAM) formed in an ATP-mediated enzymatic reaction is the universal methyl group donor for several biological reactions (betaine and choline are also made, but very much smaller in their contribution). Representative methylation pathways of SAM include proteins, nucleic acids, and the synthesis of biogenic and polypeptides. Homocysteine (Hcy), an intermediate in Met metabolism has long been associated with cardiovascular disease (CVD), heart disease, and stroke among others. High levels of cholesterol (Cho) is also associated with CVD, and both Met and Cho are rich in foods of animal origin like poultry, dairy, and meat. Although two recent studies on the individual effects of Met & Cho in the vascular and hepatic context, studies on the combined effects of the two are very limited. Particularly in the liver, studies on the combined effects of Met & Cho were almost non-existent till recently. Where additive responses in inflammation and oxidative stress were anticipated in the studies on the combined effects of Cho and Met, the lab reported that there is no compensatory increase in oxidative stress and inflammation seen in high Cho fed rats. Only rats fed high Cho had significant increases in inflammation and oxidative stress and Met alone had none and when it was added to Cho, it counteracted the pathology. These observations were highly consistent and corroborated at gene, protein, and structural levels by multiple approaches. Met eliciting anti-inflammatory and anti-oxidative responses were completely contrary to the conventional understanding, however, they are equally interesting. Currently, the mechanisms of Met mediated beneficial responses are being investigated in relation to enzymatic changes at gene and protein levels focusing on arginine methylation, and transmethylation, transsulfuration, and hydrogen sulfide formation pathways of Met metabolism. Data on these parameters and functional aspects will be presented.

2560 Eparlapstat Inhibits Inflammation, Sinusoidal Congestion, and Dilation in Mouse Liver

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Eparlapstat (EPS), an aldose reductase inhibitor, is widely used clinically to manage diabetic neuropathy due to its ability to protect neural cells from oxidative stress. However, clinical applications of Eparlapstat are currently restricted because it has been reported that EPS causes liver dysfunction. This study aims to investigate the underlying etiology by which EPS induces liver injury. We previously reported that EPS stimulated oxidative stress, fibrogenesis and inflammation in mouse liver. In this study we further showed that EPS caused sinusoidal congestion and dilation in liver of mice treated with 1500 ppm EPS for 100 mg/kg for 14 days. Sinusoidal congestion and dilation are commonly characterized by liver injury and altered angiogenesis. EPS induced the mRNA expression of interleukin-6 (IL-6) and IL-10, two inflammatory cytokines. In addition, EPS induced mRNA expression of vascular endothelial growth factor A (VEGF-A), a pro-angiogenic factor that is upregulated in cases where sinusoidal congestion and dilation are observed. Moreover, we showed that EPS increased mRNA expression of both IL-6 and VEGF-A in cultured mouse and human hepatoma cells. Therefore, EPS stimulates liver inflammation and sinusoidal congestion in mice through the IL-6 and/or VEGF signaling pathways.

2561 Myeloid-Specific Deletion of Early Growth Response 1 Ameliorates Pathogenesis of Experimental Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming a major health concern. While the major causes of NAFLD are obesity and diabetes, recent studies have linked xenobiotics and environmental toxins to fatty liver disease development, including macroinflammation and fat accumulation in liver. Studies have linked xenobiotics and environmental toxins to fatty liver disease development, including tissue-specific gene expression studies. Myeloid cells, as well as macrophages, play a central role in NAFLD development and progression. Early growth response 1 (Egr1) is a transcription factor rapidly induced by growth factors, cytokines, and stress signals from exposures to various environmen- tal toxins. The goal of this study was to determine the role of myeloid-spe- cific Egr1 in the pathogenesis of NAFLD. Myeloid cell-specific Egr1 knockout (Egr1-MKO) was generated by breeding Egr1Fllox/Fllox with Lys2-Cre mice. The lym- mocytes Ly2-Cre negative mice were used as wild-type controls (WT). The in vitro M1 and M2 macrophage differentiation experiments were conducted using mouse bone marrow-derived macrophages (BMDMs). The in vivo acute liver inflammation was induced by intraperitoneal injection of LPS (0.5 mg/kg for 14 days). To induce NAFLD, both WT and Egr1-MKO mice were fed a high fat, cholesterol, fructose diet (HFCF, 40% kcal fat, 2% cholesterol, 20 kcal fructose) or western diet (WD, 42% kcal fat, 0.2% cholesterol) for up to 4 months and followed by the examination of body composition, adiposity, energy metabolism, and liver histology. BMDMs lacking Egr1 impaired M1-like pro-inflammatory but facilitated M2-like anti-inflamm- atory macrophage polarization. In addition, BMDMs from the Egr1-MKO showed lower induction of Tnfα and Il1β mRNAs, IL1B secretion, and activation of the NF-kβ pathway relative to WT control after LPS stimulation. Consistently, the in vivo myeloid Egr1 deletion inhibited LPS-induced activation of NF-kβ and MAPK pathways with a significantly lower plasma IL1B concentration in Egr1-MKO relative to WT controls. Moreover, the Egr1-MKO mice were resistant to body fat accumula- tion with a significant reduction of liver triglycerides and cholesterol content relative to WT controls after initiating HFCF feeding. Similarly, Egr1-MKO showed less liver lipid accumulation but elevated energy expenditure after 2 months of WD feeding. However, compared to WT BMDMs, the energy expenditure were similar between Egr1-MKO and WT after 4 months of WD feeding- an indication of adaptation in Egr1-MKO after a long-term WD feeding. Interestingly, genes regulating liver lipid transport and fatty acid oxidation were downregulated in the Egr1-MKO after 2 months of WD feeding but induced after 4 months of WD feeding. In contrast, the hepatic expression of
Non-alcoholic steatohepatitis (NASH) is a liver disease characterized by chronic endoplasmic reticulum (ER) stress, lipid accumulation, increased macropathome inflammation with infection, inflammation, and fibrosis. Cholesterol-mediated hepatotoxicity that promotes inflammation and fibrosis also contributes to NASH development. Proper cholesterol distribution within the cell is important for cholesterol homeostasis and free cholesterol accumulation in membranes promotes hepatotoxicity. However, the role of soluble cholesterol transporters in NASH has not been studied. The Steroidogenic Acute Regulatory protein-related lipid transport (START) Domain protein 5 (STARD5) is a soluble intracellular cholesterol transporter that moves cholesterol from intracellular sterol-rich compartments to the plasma membrane, which is necessary for the proliferation of the pro-lipogenic gene targets under its jurisdiction, resulting in increased lipogenesis and triglyceride accumulation. Recently, we showed that 4 nico niche acetylcholine receptor (nAChR, Chrna4) knockout mice did not develop steatosis upon chronic exposure to alcohol, suggesting that these receptors are involved in alcohol-induced dysregulation of Sirt1-mediated metabolic homeostasis. The purpose of the present study was to test the hypothesis that alcohol acts on 4 nAChRs in hepatocytes to decrease Sirt1 expression which ultimately dysregulates lipid metabolic homeostasis. The effects of alcohol were investigated in cultured AML12 hepatocytes exposed to 100 mM alcohol and in WT primary mouse hepatocytes exposed to 60 mM alcohol for 48 hrs. In some of its downstream target, Fasn in WT primary mouse hepatocytes, alcohol exposure led to a 6-fold increase in Chrna4 mRNA, signifying receptor activation. Consistent with our proposed mechanism of action, alcohol treated AML12 cells showed a 50% decrease in Sirt1 mRNA expression compared to control cells. Since Sirt1 is a deacetylase responsible for the deacetylation of certain pro-lipogenic genes, we expected its decreased expression to correlate with an increase in these genes. To our delight in both AML12 cells and primary mouse hepatocytes, alcohol significantly increased expression of Chrna4. In its downstream target, Fasn. Most importantly, pre-treatment with 4 nAChR antagonist DHBE blocked alcohol’s downregulation of Sirt1 and the subsequent upregulation of its downstream targets. Taken together, these data support a role for 4 nAChRs expressed by hepatocytes in mediating the early effects of alcohol on the liver. Furthermore, they demonstrate the effectiveness of targeting these receptors to block early alcohol signaling in hepatocytes. Though there are no current therapies for any stage of ALD, the early stages remain reversible and necessary pre-requisites for the progression to advanced ALD. The present studies identify 4 nAChRs as promising novel targets for the design of pharmacological agents to treat and manage early ALD.
two different in vivo models: maternal metabolic syndrome (Mets) exposure and development, and choline (Cd) exposure. These studies showed upregulation of Zac1, an imprinted gene that encodes a transactivator factor, and the Imprinted Gene Network (IGN), a network of transcriptionally coordinated imprinted and biallelically-expressed genes. Over-expression of Zac1 in vitro and in vivo recapitulates molecular hallmarks of steatosis and fibrosis, supporting the hypothesis that Zac1 is involved in the process. Given that these effects are seen in the same manner to other environmental stressors. We used the human hepatocellular carcinoma cell line HepG2 and challenged the cells to different chemical exposures linked to NAFLD outcomes. We found that carbon tetrachloride (CCL4), an imputed dose-dependent increase of LDH leakage and decrease in ATP content. 72 h co-treatment of higher Troglitazone concentrations with bile acids after 7 days. BA co-treatment with Ambrisentan did not lead to an increase in cellular ATP was observed at co-treatment with 100 and 200 µM bile acids after 7 days. BA co-treatment with Ambisentan did not lead to an increase in LDH leakage and caused only a small reduction of cellular ATP under some conditions. Trolgitalzone treatment alone for 7 days was not cytotoxic up to a concentration of 12 µM, but the addition of bile acids led to a reduction of ATP content. 72 h co-treatment of higher Trolgitalzone concentrations with bile acids showed synergistic dose-dependent increase of LDH leakage and decrease in ATP content. For Rosiglitazone, only minor changes in LDH release and ATP content were observed upon co-treatment with bile acids for 3 and 7 days. In conclusion, in the present study exogenous added bile acids act as a sensitizer for Bosentan but not for Ambisentan cytotoxicity. Similar results were obtained by Trolgitalzone but not Rosiglitazone. The results of this work suggest the 3D liver spheroid model is a promising micro-physiological system to study the steatosis potential of new drug candidates. [1] P. J. P. et al. 2018, [2] Applied in vitro Toxicology, Vol.4, No.3 [2] Hendricks, D.F.G. et al. 2016, Sci Rep 6, 35434 [3] Fickert P et al. 2017, J. Hepatol.
However, loss of mitochondrial pyruvate metabolism by MPC inhibition or deletion also accelerates basal levels of the antioxidant glutathione and increases glutathione oxidation in cultured cells. Thus, MPC inhibitors may increase susceptibility to oxidative insults. Here, we sought to test that hypothesis using the mouse model of acetaminophen (APAP) hepatotoxicity which is mediated in part by oxidative stress. We generated liver-specific MPC2 KO, alanine aminotransferase 2 (ALT2) KO, double-MPC2/ALT2 knockout (DKO), and wild-type (WT) littermate mice. After an overnight fast, we treated animals with 300 mg/kg APAP and compared liver injury (serum enzymes, histology), total and oxidized hepatic glutathione, and APAP-protein adducts between genotypes. We also treated some WT mice with the MPC inhibitor MSDC-0602 2 h post-APAP and pre-treated others for 24 h with the ALT inhibitor β-chloro-L-alanine. Finally, we treated CYP2E1-transduced HepG2 cells with the MPC inhibitor UK-5099 and BCLA or vehicle control for 24 h then added 20 mM APAP or vehicle and measured lactate dehydrogenase (LDH) release and glutathione 24 h later. Neither MPC2 KO nor MPC inhibition altered APAP hepatotoxicity. However, the protein level of ALT2, which synthesizes pyruvate directly within mitochondria, was increased 1.9-fold in MPC KO mice, suggesting that ALT2 and/or ALT1 compensated for the loss of mitochondrial pyruvate uptake. We then tested whether ALT2 loss could increase APAP toxicity but, again, neither ALT2 KO nor ALT inhibition had an effect. This led us to hypothesize that the MPC and ALT2 are redundant pathways in vivo to maintain not only mitochondrial intermembrane metabolism but also glutathione homeostasis. Consistent with that idea, after APAP treatment, DKO mice had significantly greater injury compared to WT mice (serum LDH means±SE: 20,332±3,124 vs. 10,269±3,407 U/L, respectively, at 6 h post-APAP). Importantly, this was not due to increased APAP-protein adducts, which were paradoxically lower in DKO animals. We also observed impaired re-synthesis of glutathione and greater oxidized glutathione in the DKO mice. These results were confirmed in cells in vitro combined MPC2/ALT chemical inhibition. Overall, neither MPC nor ALT inhibition alone increases susceptibility to APAP hepatotoxicity. However, disrupting both intramitochondrial pyruvate uptake and synthesis from alanine worsens injury, likely due to impaired antioxidant defenses and exacerbated mitochondrial dysfunction.
Despite this, there are some difficulties in using MPS that need to be considered. Primary human hepatocytes, because of their variability in response in culture, can lead to imprecise predictions. In this study, we explored the effect of a known hepatotoxin, acetaminophen (APAP), on different primary human hepatocyte (PHH) lots in the CN Bio Liver-Chip. Choosing suitable PHH lots for use in CN Bio is critical; however, it is not always simple. Viability of the PHHs upon thawing does not always predict if they will do well in the chip system. PHH were exposed to a range of doses of APAP for up to four days. Hepatocyte function and cell death were monitored by albumin secretion, urea, LDH, ALT, and AST release. Our preliminary findings indicate that different PHH lots varied not only in their ability to survive on the Liver-Chip but also varied in their sensitivity to APAP exposure and thus time to onset of toxicity. Depending on lot, perturbations in albumin secretion were observed as early as 24 hours after exposure with the highest doses and sometimes on the final day (4) of exposure. Lower doses of APAP showed toxicity in some lots of PHH but not others. Similarly, LDH showed cell death as early as 48 hours in some PHH lots, others took 4 days, and still others never showed cell death by LDH.

In conclusion, the variability between PHH cell lots may be a confounding factor when analyzing the usefulness of PHH in liver-chip platforms for predicting toxicological responses and suggests that pooled cells might be useful for predicting general human population responses.

**4273 Bioengineering of Novel Organotypic 3D Human Liver/ Hepatocyte Tissue Model for Drug-Induced Liver Injury/Toxicity Studies**

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Development of a human primary cell-based 3D organotypic hepatocyte/liver tissue model that can be cultured for weeks with polarized hepatic morphology and maintains high level expression of major liver associated drug metabolizing enzymes (DMEs) and nuclear receptors (NRs) is critical for studying these effects in vitro. A key advantage of this approach is that it allows researchers to study liver physiology in an in vitro platform that can be cultured for weeks with polarized hepatic morphology.

**4275 hnRNP-Q and hnRNP-L Influence Drug Metabolism and Toxicity by Regulating mRNA Processing of Drug Metabolizing Enzymes and Nuclear Receptors in HepaRG Cells**

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Drug metabolizing enzymes (DMEs) and nuclear receptors (NRs) play critical roles in drug metabolism and toxicity. The expression of DMEs and NRs are regulated by various mechanisms including transcriptional and posttranscriptional modulation directed by noncoding RNAs and RNA binding proteins. Heterogeneous Nuclear Ribonucleoprotein (hnRNP) Q and L belong to a large family of RNA binding proteins that function in mRNA metabolism. They have been shown to interact with IncRNA RP11.116D21.1 which modulate DME and NR expression. However, the mechanism underlying their effects on DME and NR levels is still unclear. To that end, we knocked down hnRNP-Q and hnRNP-L, respectively in HepaRG cells using the siRNA technology and performed RNA sequencing and immunoblotting to determine the effects of knockdown on the expression of DMEs and NRs.

**4276 Examining the Toxicity of Cannabidiol (CBD) Exposure at Consumer-Relevant Doses in Primary Human Hepatocytes**


Cannabidiol (CBD), which has been used in clinical trials for the treatment of certain seizure disorders, has been shown to induce hepatotoxicity. CBD containing consumer products have flooded the marketplace in recent years. While clinical CBD doses are generally higher than those currently found in consumer products, data on the effects of CBD at concentrations below the clinical dose in the liver and methods for studying these effects in vitro are limited. This study evaluated the hepatotoxic potential of CBD below the clinical doses in vitro using primary human hepatocytes. Hepatocyte lots from different donors were chosen for each individual experiment to represent variation in the population and the hepatocyte harvesting process. Based on known liver Cmax values from the literature evaluating clinical CBD for treatment of seizure disorders, in silico predicted Cmax levels from exposure to CBD containing consumer products were estimated. Given the limit of solubility for CBD in hepatocyte culture media, we chose a final dose range for CBD-CYP2E1 and CYP3A4 models of 20 and 400 µM. From these models we found that clinical doses of CBD were not toxic as measured by LDH release and caspase 3/7 staining as markers for cell death and apoptosis.

**4274 Unraveling PCB Effects in Differentiated Human Hepatocytes**


Polychlorinated biphenyls (PCBs) are structural class of environmental chemicals with structural similarities and varied degrees of toxicity. Liver effects with PCBs (e.g., air, water, sediments and human serum) with minimal available toxicity. The present study examines the effects of PCB11 on gene expression as a marker of hepatic receptor activation in differentiated human hepatocytes (HepaRG) alongside better-understood PCBs (i.e., PC7B7, PCBB95, PCB126, PCB153) and Acorol mixtures 1016 and 1254. Seven (7) concentrations of each test substance were evaluated over a 48h exposure period (repeated dose) with assays for CYP1A2 (AhR target gene), TR (PXR/ CAR) and CYP3A4 (PXR, ABOC11 (FXR), and HMGCS2 (PPARo). Established liver carcinoma PCB126 served as positive control for AhR activation to induce CYP1A2 expression, and resulted in robust response (~2,000-fold) compared with markedly weaker responses PCB11 (~30-fold), PCB153 (~25-fold), PCB77 (~5-fold), and no induction with PCB95. For CAR activation PCB11 produced robust response (~10 fold) that was comparable to positive control phenobarbital with a half-maximal concentration of 11 µM. Overall, these data revealed PCB11 to be more similar to PCBB95 and PCB77, and distinct from PCB126.
Drug development requires a stringent evaluation of a drug's safety profile, among which hepatotoxicity is a major concern as it is the most common reason for post-marketing withdrawal. In the past, an entire battery of advanced in vitro liver models has been developed, either based on primary donor cells or derived from stem cells. In addition, to correctly predict drug-induced liver injury (DILI), (a) standardization, (b) representation of population diversity and (c) instant access at affordable costs will be key for a model to become widely adopted by the pharmaceutical industry for pre-clinical hazard and risk assessment. Larger production lots combined with long-term storage solutions could overcome batch-to-batch variation problems and reduce manufacturing costs. Here, we demonstrate a new approach to cryopreserve mature, primary cell based human liver spheroids directly in standard assay plates (1 spheroid/well). After storage and thawing, these spheroids can be directly used for drug safety testing. The spheroids underwent an equilibration process with increasing concentrations of cryoprotective agents (CPA). Subsequently, the spheroids were spotted as droplets of 0.5-1.0 μl volume onto a 96-well spheroid microplate pre-cooled to −196 °C. Contact of the spheroid-bearing droplet with the cold plate surface led to instant freezing at cooling rates commonly applied for vitrification. Vitrification renders an aqueous solution into an amorphous, glassy state preventing cell damage associated with ice crystal formation and damage observed using conventional freezing methods. After thawing, the spheroids were compared to non-frozen control tissues. The morphology showed similar compact and round liver tissues as their non-frozen counterparts, while H&E staining and immuno-staining for CD68 (Kupffer cells), Albumin (Hepatocytes), and BSEP (canalicular structures) revealed similar patterns as in non-frozen tissues. This result demonstrated the potential of the first strategy involving cryopreservation of highly differentiated spheroids based on primary cells in standard microplates, enabling long-term storage without loss of function. Such a scalable procedure could be a game-changing method for on-stock production of complex 3D cell culture models (spheroids and organoids) suitable to be supplied to the industry on demand.

4278 Detection of Chemically Reactive Metabolites and De-risking Hepatotoxicity Using High-Content Imaging

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Drug-induced liver injury (DILI) is one of the most common mechanisms of drug attrition within drug development and responsible for drug withdrawals from the market. Amongst the most common mechanisms associated with intrinsic DILI are hepatic transporter inhibition, mitochondrial dysfunction, and reactive metabolite formation. The cytochrome P450 (CYP450) superfamily of enzymes represents the primary phase 1 metabolic system within the liver, responsible for the oxidation, peroxidation, and hydroxylation of xenobiotics. An example of xenobiotics related to this metabolic phase are acetaminophen (APAP) and endoplasmic reticulum (ER) stress. Utilizing the pan-specific inhibitor of the CYP450 enzymes, 1-Aminobenzotriazole (1-ABT) on hepatocytes in combination with high-content imaging (HCI) provides an assessment of potential reactive metabolites effects on cell health parameters. These endpoints include nuclear features, glutathione (GSH) content, mitochondrial dysfunction, and reactive oxygen species (ROS) formation, alongside cellular ATP content. Reactive metabolite formation was determined through a calculated fold-shift in cell health features, between the plus and minus 1-ABT dosing conditions. Those features presenting a fold-shift >1.5 are determined to be associated with a reactive metabolite. An assessment was made across a panel of known DILI reference compounds associated with the formation of reactive metabolites through this HCI bioactivation assay within metabolically competent HepaRG cells, primary human hepatocytes (PHH) and primary mouse hepatocyte (PMH). Our results demonstrate that chemically reactive metabolites are generated within the assay, but specific cell health parameters associated with known hepatotoxicity were shown to present the greatest fold-shifts. Examples of these were the degradation of 7-aminoactinomycin D (7-AAD) and CYP2E1 (CP), acetylaminocephalosporin (AAPAP), and aflatoxin-b1 (AF), each presented the most significant fold-shift in their reported most sensitive mechanism (MSM) as supported by literature, including DNA structure (1-ABT absent only response), GSH content (fold-shift 2.11), and DNA structure (fold-shift 64.2), respectively. Additionally, this approach allows the assessment of potential species differences. We present here a robust, in vitro methodology for the identification of chemicals with the potential to form reactive metabolites, as part of early DILI de-risking strategies in drug discovery and development.

4279 Role of Sortilin in the Hepatic Lipid Metabolism: Impaired Chaperone-Mediated Autophagy Leads to Abnormal Sorbitol Turnover and CES1-Dependent Triglyceride Hydrolysis


Chaperone-mediated autophagy (CMA) is a specialized type of autophagy that selectively recognizes cytosolic proteins and degrades them in lysosomes. Previous studies have shown that impaired CMA affects lipid metabolism in the liver. Sorbitol is a member of the vacuolar protein sorting 10 protein (Vps10p) family of transporters and recent studies have suggested a possible role for CMA in hepatic sorbitol metabolism. Sorbitol regulates lysosomal targeting and degradation of Carboxylesterase1 (CES1), which plays an important role in triglyceride (TG) hydrolysis in the liver. In this study, we studied whether sorbitol is a substrate of CMA, and the overall effect of sorbitol accumulation on lipid homeostasis. Here, we identified the presence of the KFERQ-like motif in the amino acid sequence of sorbitol by using KFERQ motif finder v0.8. Co-immunoprecipitation results confirmed the interaction between sorbitol and HSC70 in HepG2 cells and mouse primary hepatocyte (MHC). Lysosome-associated protein 2A (LAMP2A) knockdown (KD) HepG2 cells and MHC showed the accumulation of sorbitol, which were opposite in LAMP2A overexpressed cells. AAV8-shLAMP2A mouse model and mouse NASH models also showed decreased LAMP2A level and accumulation of sorbitol. In addition, we found that CES1 was negatively correlated with sorbitol and that decreased CES1 level induces significantly elevated hepatic TG level in HepG2 cells, MHC, and in vivo models, suggesting that sorbitol affects both the degradation of CES1 and the subsequent TG hydrolysis. Together, it was revealed that sorbitol is a substrate of CMA. Our data also demonstrated that disruption of CMA-mediated sorbitol degradation might affect CES1-dependent TG hydrolysis and imbalance of lipid homeostasis in the liver, suggesting the possibility of sorbitol as a new NALFD treatment target. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (2020R1A2B5B0101920).

4280 Characterization of the Chromatin Accessibility Landscape during HepaRG Cell Differentiation

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The liver undergoes functional changes during development that are accompanied by changes in expression of many genes, including genes that encode xenobiotic-metabolizing enzymes. Many of the same gene expression changes that occur during human liver development are also seen during differentiation of cultured HepaRG cells. For example, expression of some members of the cytosolic sulfotransferase (SULT) superfamily of conjugating enzymes (e.g., SULT1C2, SULT1E1) is highest in prenatal human liver and confluent HepaRG cells, while expression of other SULTs (e.g., SULT2A1) is highest in adult liver. Therefore, in addition to its established value as a model for toxicological research, HepaRG is a useful model for studying mechanisms that control developmental changes in hepatic gene expression. Transcription factor binding to regulatory motifs in accessible regions of chromatin is an important determinant of gene transcription. Therefore, knowledge of the changes in chromatin accessibility that occur during cell differentiation can be used to identify transcription factor-binding motifs, and implicate transcription factors, that might contribute to the developmental regulation of hepatic gene expression. The genome-wide changes in chromatin accessibility that occurred as HepaRG cells transitioned from undifferentiated to differentiated cells were studied in three HepaRG models using ATAC-seq. These HepaRG models were generated using high-throughput sequencing (ATAC-Seq) on cells harvested at time points reflecting the proliferative (day 4 after cell plating), confluent (day 14), and differentiated (day 28) stages. Four replicate experiments were performed in which separate batches of HepaRG cells were cultured, nuclei prepared, and libraries generated. Quality control analyses indicated that the ATAC-seq data were of high quality. Aligned peaks from the four experiments were subjected to differential analysis using DESeq2, for the following three contrasts, while controlling for batch effects: 1) day 14 vs. day 4, 2) day 28 vs. day 14, and 3) day 28 vs. day 4. Differentially accessible regions of chromatin (DARs) were defined at an FDR of 0.05 and log fold-change of 0. LDAH identified 37,990 significant DARs for day 14 vs. day 4, 69,967 significant DARs for day 28 vs. day 14, and 90,297 significant DARs for day 28 vs. day 4. Distinct chromatin accessibility changes were seen when day 14 vs. day 4 log fold-change values were compared to day 28 vs. day 14 log fold-change values, suggesting specific gene regulatory mechanisms. Simple enrichment analysis (SEA) was used to identify transcription factor-binding motifs that were enriched in the significant DARs. As an example of the SEA findings, hepatocyte nuclear factor (HNF) motifs were found to be highly enriched in day 14 and day 28 samples, consistent with the expected important roles of HNFs during hepatocyte differentiation. This ATAC-seq analysis of chromatin accessibility provides new insights.
4281 In Vitro Human Hepatocyte Proliferation Assays: Analysis of Responses to Reference Compounds in Studies from CropLife Europe Member Companies

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Liver cancer in rodents is a common finding in response to lifetime chemical exposure and frequently occurs via a non-genotoxic mode of action (MOA), with increased cell proliferation via activation of nuclear receptors (such as the constitutive androstane receptor, CAR). Exemplification of chemical specific MoAs and assessment of human relevance involve bespoke mechanistic studies examining key events in the MOA and to examine human relevance, studies looking at DNA synthesis as a surrogate for proliferation in isolated human hepatocytes. CropLife Europe member companies retrospectively collated the available human hepatocyte data to understand the demographics of human hepatocyte donors used and respective proliferative responses to control compounds. Data were collected for studies run between 2011-2020. 40 individual human hepatocyte donors were identified as having been tested with the following demographic features, 18/40 male; 22/40 female; age range of donors covered 11-73 years for males and 10 months to 80 years for females. All donors tested induced increases in S-phase DNA labelling index versus control when exposed to the reference compounds, epidermal growth factor (EGF, 7.7±5.5-fold versus control) or hepatocyte growth factor (HGF, 2.6±1.7-fold versus control) in a dose dependent manner. No influence of age or sex on S-phase DNA labelling index was observed in response to EGF or HGF. Treatment with the CAR activator phenobarbital did not affect S-phase DNA labelling index in human hepatocytes. This data indicates that human hepatocytes are a robust model for assessing the human relevance of key events in the CAR-mediated mechanism for liver carcinogenesis in rodents.

4282 Response of Rat and Human Hepatocytes to Reference Inducers in Terms of CYP and UGT Induction and Increases in Thyroxine-UGT Activity

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The in vitro induction of hepatic phase I cytochrome P450 (CYP) enzymes in human and rat hepatocytes via activation of nuclear receptors following 72h of cell culture in the presence of various reference liver enzyme inducers such as β-naphthoflavone (BNF) via activation of the aryl hydrocarbon receptor (AHR), phenobarbital (PB) via activation of the constitutive androstane receptor (CAR), rifampicin (RIF), human or pigogenous 16α-carbonitrile (PCN, rat) via activation of the pregnane X receptor (PXR) has been well described in the literature. Comparative in vitro assessment in hepatocytes of the level of induction of phase I CYP and co-regulated phase II UGT enzymes has not been thoroughly studied, though some recent studies have shown that the magnitude of induction could be clearly evidenced, and related activity increases such as thyroxine (T4)-UGT, only after longer exposure, up to 7 days (Parmentier et al., 2022). In the present study, we confirm that cryopreserved primary (Wistar) rat hepatocytes (PRH) and primary human hepatocytes (PHH) in the previously described sandwich-culture configuration consistently respond to CYP and UGT induction following daily exposure to reference inducers for 7 days as compared to their response for 3 days of exposure. Despite the inter-donor and inter-experiment variability seen (n=3 donors per species for which at least 3 experiments per donor were performed), the response of PRH to inducers was consistently greater than that of PHH, both in terms of increased CYP mRNA expression and related activities. With respect to UGTs, induction patterns were different in the two species, UGT1A family being induced in PRH, while UGT2B1 was consistently greater than that of PHH, both in terms of increased CYP mRNA expression and related activities. In conclusion, the results of this study provide promising new platform that can be used to study lipotoxic mechanisms in healthy and diseased populations due to the preservation of phenotypic differences over a prolonged culture period.

4283 Characterization of Primary Human Hepatocytes from Diseased and Healthy Livers in an All-Human Cell Based Tri-Culture System

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Liver toxicity continues to be a major health concern. There are several diseases and chemicals that can result from chemical exposure, including steatosis, alcoholic and non-alcoholic steatohepatitis. Conventional in vitro model systems lack the ability to sustain the basic morphology and functionality of primary human hepatocytes (PHHs) from both healthy and diseased tissues for extended periods of time or maintain phenotypic characteristics including differences in lipid accumulation and inflammatory responses. Recent development of a novel all-human cell based tri-culture system (hTCS) includes cryopreserved primary human feeder cells (FCs) and PHHs. The hTCS was used to characterize basic synthetic and metabolic functions in PHHs from healthy or diseased tissues, which were classified according to their histopathology score. hTCSs were established by thawing cryopreserved human feeder cells (FCs) and seeding onto 24-well collagen coated plates. Cryopreserved adult PHHs from either healthy or diseased donors were then thawed and plated onto the FCs to create the hTCS. The functionality of both healthy and diseased PHHs cultured in the hTCS was measured during a 15 day culture period, including production of albumin (Alb) and urea and Cytokine P450 (CYP) 3A4 activity when normalized to cell number. The results showed a decrease in Alb levels from the diseased PHHs (40.6 ± 10.8 µg/10^6/day) in the hTCS compared to healthy PHHs (111 ± 22.2 µg/10^6/day) on day 15 of the culture period. A similar decrease in urea levels for urea levels (37.4 ± 1.83 vs 74.5 ± 5.08 µg/10^6/day). There was also a 5-fold decrease in CYP3A4 activity in diseased PHHs (25.9±8.6 nm/10^6 cells/day) versus healthy PHHs (126 ± 5.84 nm/10^6 cells/day) on day 15. Immunolocalization of CY2P21 was notably elevated in diseased PHHs compared to healthy PHHs. Characterization of pro-inflammatory cytokine production of M1-like phagocytes in the hTCS was also determined on day 14. When cultured with diseased PHHs compared to healthy PHHs on day 14, there was an increase in IL-6 (68.5 ± 16.4 vs 21.1 ± 9.74 pg/10^6 cells/day) and MCP-1 (888 ± 2195 vs 3482 ± 1484 pg/10^6 cells/day). The fibrinogen marker CT18 was increased in the hTCS when cultured with diseased PHHs (15224 ± 3568 pg/10^6 cells/day) versus healthy PHHs (9462 ± 2460 pg/10^6 cells/day) on day 14. TGF-β expression was also higher in the diseased PHHs (753.4 ± 322.4 pg/10^6 cells/day) versus the healthy PHHs (439.5 ± 205.4 pg/10^6 cells/day). Gene expression of FASN, PCK1, and G6PC in the diseased PHHs at day 14 was decreased almost 3-fold on average compared to the healthy PHHs. Further characterization of the hTCS was performed by determining the differences in the synthesis and accumulation of intracellular lipid by healthy and diseased PHHs maintained in medium containing 320 µM oleic acid and 25 mM glucose (lipotrophic med.). An increase in lipid synthesis was measured after both types of PHHs were cultured in lipotrophic med. (diseased: unit 100 ± 11.8% vs healthy: unit 100 ± 6.91%; healthy: unit 99.5 ± 10.8% vs diseased: unit 91.8 ± 4.99%). Adding 0.5 µM Obeticholic acid (OCA), a farnesoid X receptor agonist, significantly decreased lipid synthesis by 15% on day 7 of treatment in healthy PHHs cultured in lipotrophic med. in the hTCS (trt 115.4 ± 5.82 vs trt + OCA 97.5 ± 5.66%). In conclusion, diseased PHHs maintained in the hTCS over 14 days showed characteristic differences in functionality and lipid disposition compared to healthy PHHs indicating this system presents a promising new platform that can be used to study lipotoxic mechanisms in healthy and diseased populations due to the preservation of phenotypic differences over a prolonged culture period.

4284 Development of Novel Organ-on-a-Chip Platforms for 3D Liver In Vitro Models and Preclinical Drug Screening


Drug-induced liver injury (DILI) continues to be the leading cause of attrition during drug development in all phases of clinical trials. The introduction of novel methodologies to understand drug delivery in 3D culture systems can contribute to addressing this challenge. Normal liver physiology and function are strongly dependent on the precise three-dimensional (3D) structural arrangement of hepatocytes, Kupffer, stellate, and liver sinusoidal endothelial cells and their communication with the extracellular matrix. One of the barriers to developing multicellular in vitro models lies in the need for user-friendly platforms capable of including three or more cell types for toxicity studies. To address this problem, a novel organ-on-a-chip (OOAC) platform including CELLBLOXKS (CBs) and NANOSTACKS (NS) was developed and patented. CBs are cell culture inserts that can be seeded with different cell types and can then be connected in rows of three CBs per well, while NS are cell culture inserts that can be stacked vertically to construct multicellular 3D models, including up to four cell types. CBs were used for the development of an OOAC protocol where components of the liver using human hepatic carcinoma cells (HepG2), human umbilical vascular endothelial cells (HUVECs), and immortalized murine fibroblasts (NIH/3T3). The production of urea, albumin and cytokine P450 activity related to HepG2 was assessed. The toxic effect of Tamoxifen was then tested on the model. Viability assays were subsequently performed, and the IC50 value for HepG2
2428 Determination of a Point of Departure for CBD in Human HepaRG Spheroids Using Transcriptomic Modeling

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Recently, the US Food and Drug Administration approved the use of cannabidiol (CBD) for the treatment of some treatment-resistant seizures, including Dravet Syndrome and Lennox-Gastaut Syndrome. During clinical trials it was observed that some patients experienced liver toxicity, as evidenced by elevated ALT serum levels. Although significant, these findings could explicitly determine that CBD was responsible for liver toxicity, as many of these patients were also administered the anticonvulsants valproate and clobazam, suggesting the potential for a drug-drug interaction inducing liver damage. Therefore, the purpose of the present study was to determine the point of departure for CBD, using human HepaRG spheroid cultures, followed by benchmark dose analysis. HepaRG spheroids were treated with increasing concentrations of CBD for 24 and 72 hours, resulting in an EC50 of 86.27 µM and 58.04 µM, respectively. Subsequent transcriptomic analysis at these time points demonstrated little alteration of gene and pathway data sets at a CBD concentration at or below 10 µM. After 72 hours of CBD exposure, interestingly, several cell/cell processes associated with innate immune response were the most sensitive (i.e., lowest BMD) to CBD treatment. Taken together, the present studies determined the point of departure for CBD in human HepaRG spheroids, which have previously been shown to be accurate predictors of human hepatotoxicity. Supported by the Center for Research on Injectable Safety.

2428 Development of a High-Throughput System for Advanced Hepatotoxicity Screening

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Drug-induced Liver Injury (DILI) still remains the major cause of acute liver failure. The toxicants act through different mechanisms and with different kinetics, hence there is a need to assess the risk of drug-induced hepatotoxicity, allowing a phenotypic and kinetic understanding of these toxicological responses. Ideally, such an approach should, at the same time, meet high throughput demands of the medical community. To extensively identify hepatotoxicity following a fast development and launch of new therapies. Our aim was to develop an easy-to-use system, serving high throughput hepatotoxicity screening demands. iCell Hepatocytes 2.0 were treated with reference compounds with known hepatotoxic effects and were measured on our technology. Furthermore, responses were compared to existing cell visibility and toxicity assay results on the same cells. We adopted 96-well electric cell-substrate impedance sensing technology (CardioExcyte 96) and implemented multiple data acquisition frequencies ranging from 0.1 kHz - 100 kHz. Additionally, we scaled up the throughput from 1 x 96 wells to the newly developed 6 x 96-wells system (AtlaZ). Ninety-six-well culture plates with integrated gold-film electrodes reveal information on confluency, cell-cell interaction and conductivity of adherent cells and thereby provide a measure of toxicity. Data acquisition at different recording frequencies allows detection of diverse cell properties and greatly enhances the information content obtained in each well. For example, high frequency impedance is highly sensitive to differences under or between cells and therefore enables barrier function and cell adhesion quantification. Our data revealed that the cultured hepatocytes were metabolically active and functional when the impedance-based, label-free and non-invasive assay was used. Acute and chronic (dose-dependent) liver toxic effects have been tested for 15 relevant compounds: diclofenac, APAP, Troglitazone, Chlorpromazine and Aflatoxin B1. To obtain 2D monolayers in 96-well plates, cells were seeded with density of 300k cells/cm², and recordings were performed during the period of 21 days. Data showed comparable results to other cell viability and tox assays, such as CellTiter-Glo, proving impedance as a reliable but non-invasive tool. We have further illustrated that other effects, such as drug effects on iCell Hepatocytes 2.0. Importantly, the acute and chronic effects of Aflatoxin B1 were observed in standard 2D conditions, indicating that the hepatocytes were metabolically active and functional when using this impedance-based, non-invasive assay. An upsampling of the recording device to allow for 6 x 96 measurements in a simultaneous or independent manner was performed. In summary, the use of impedance and electrical impedance spectroscopy emerges as a valuable tool for advanced hepatotoxicity assessment. The newly developed AtlaZ system elevates preclinical risk assessment to a modern level through the scalable throughput and ease-of-use, but also through the potential to access multiple kinetic and phenotypic information from in vitro 2D cell cultures, exemplarily shown here with iCell Hepatocytes 2.0.

2427 An In Vitro Freshly Isolated Primary Proximal Tubule Assay Recapitulates Species-Specific Kidney Toxicity Seen In Vivo


In a 28-day rat toxicity study conducted with SAR444664, microscopic findings of multifocal tubular degradation/regeneration were seen in the kidney (both sexes) at 200 and 600 mg/kg. However, no kidney microscopic findings were noted in a 28-day dog study in the dose range of 10 - 500 mg/kg, comparable to the rat. Thus, an in vitro study was conducted with SAR444664 to evaluate cytotoxicity in freshly isolated primary renal proximal tubule epithelial cells (RPTECs) from rat, dog, and human to understand the translatability of the rat kidney findings to human. Freshly isolated RPTECs from the three species were grown to confluence in Transwell® inserts and treated with SAR444664 over a concentration range of 0.3 - 100 µM for 72 hours. At the end of the treatment period, supernatant from the apical side of the inserts was collected and three kidney injury biomarkers [Kidney Injury Molecule-1 (KIM-1), Neutrophil gelatinase-associated lipocalin (NGAL), and Clusterin] were measured. Additionally, pan-toxicity endpoints such as lactate dehydrogenase (LDH) release and adenosine triphosphate (ATP) content were also measured. The experiment was repeated for three independent donors for each of the species to understand donor-to-donor variability. SAR444664 caused a statistically significant increase in all three tubular injury biomarkers [KIM-1 (>1300%, p < 0.0001), NGAL (>1900%, p < 0.0001), and Clusterin (>1190%, p < 0.05)] at the highest dose of 100 µM in rat RPTECs, while no statistically significant increase was observed for any of the tubular injury biomarkers in either dog or human RPTECs compared to the vehicle control. LDH release which was used as a measure of cytotoxicity for the RPTECs was also highest in the rat RPTECs (>60%, p < 0.0001) at 100 µM, while only marginal increases were seen in the dog RPTECs (< 2%, not significant) and the human RPTECs (< 1%, not significant) at 100 µM. Finally, measurement of ATP content indicated the lowest viability in rat RPTECs (7.7%, p < 0.0001) at the highest dose while over 89% viability was seen for the dog RPTECs and over 78% viability was noted for human RPTECs after 72-hour treatment with SAR444664. Collectively, these in vitro results demonstrate that the rat RPTECs should be included in the highest tier of newly developed toxicity testing. Compared to the dog or the human RPTECs, reflected the in vivo findings seen in the rat and dog, and suggests that the kidney findings observed in the rat may not be human relevant.

2428 An In Vitro Model of the Human Renal Proximal Tubule for Nephrotoxicity Screening Studies

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Nephrotoxicity from xenobiotics is a major cause of kidney disease and a common reason for drug development failure. Proximal tubule (PT) cells are the most frequent site of kidney damage as they perform a central role in renal clearance by metabolizing and transporting xenobiotics from the circulation, leading to intracellular accumulation and exposure to toxic metabolites. Toxic insults can impair PT solute reabsorption and disrupt essential nutrient homeostasis causing negative health effects. While animal toxicity studies can detect mid-to-late-stage kidney damage, our ability to identify and mechanistically understand early-stage nephrotoxicity remains insufficient. Furthermore, species differences and ethical considerations limit the utility of toxicity data from animal models for human translation. In vitro systems offer distinct advantages over the toxicokinetics, but conventional static 2D culture models often fail to emulate fundamental aspects of PT functionality or accurately predict human nephrotoxicity. In this study, we sought to advance the physiological relevance of in vitro PT models by developing and qualifying 3D microstructures of self-organizing, free-floating, human kidney proximal tubule epithelial cells. These lumen-forming ‘proximal tubuloids’ consist of a single differentiated layer of polarized cells, immunofluorescence staining revealed cilia formation at the apical membrane, while Nav/K+-ATPase and laminin localize to the basolateral membrane. Proximal tubuloids demonstrate enhanced differentiated longevity and increased sensitivity to nephrotoxic compounds - including the
Drug-induced nephrotoxicity (DIN) is one of the major safety concerns for both drugs in development and marketed agents. Certain classes of drugs are known to accumulate in specific regions of the nephrons (e.g., proximal tubules) and induce necrosis/degeneration, yet the mechanisms of toxicity are not well understood. While animal models have limitations in predicting clinical outcome due to interspecies differences, in vitro models utilizing human cells may potentially fill the gap and provide better translatability to clinical studies. Conventional in vitro 2D kidney models are insufficient to predict the translatable due to lack of cell polarization and inadequate expression of key transporters and receptors. Therefore, a tool for better prediction of DIN to aid screening or mechanistic investigation is in vitro models utilizing human cells may potentially fill the gap and provide better translatability to clinical studies. Conventional in vitro 2D kidney models are insufficient to predict the translatable due to lack of cell polarization and inadequate expression of key transporters and receptors. Therefore, a tool for better prediction of DIN to aid screening or mechanistic investigation is needed. Here, we adopted an in vitro 3D microphysiological system (MPS), which substantially advances the 3D architecture in vitro enabling appropriate cellular polarization and introducing media flow to the system. This proximal-tubule-on-a-chip was established using immortalized human renal proximal tubule epithelial cells (hRPTEC/TERT1). Polyvinyl B (PMB) and other antibiotics, such as gentamicin and colistin that are known to cause DIN, were selected and tested in the system. The chips were perfused with drug-containing media at various concentrations for 7 days. The morphology and cell viability were monitored at multiple time points (generally 4 hours, 1 day, 3 days, and 7 days post-treatment), and efficient were simultaneously collected to assess secreted biomarkers. PMB was tested repeatedly for validating the quality of the MPS, and we confirmed reproducibility of the system with IC50 of PMB in cell viability, which was left-shifted when compared to traditional 2D static culture. PMB and colistin induced cytotoxicity in concentration- and time-dependent manners, while only the highest concentration of gentamicin caused cell death on Day 7. Morphological changes were correlated to cell viability with IC50 values in an order of PMB < colistin < gentamicin. High concentrations of PMB and colistin induced secretion of injury markers KIM-1, claudin, and TIMP-1, as well as the cytokine/chemokine release of IL-6 and CCL2. Utilizing the 3D human kidney MPS, DIN was successfully detected with nephrotoxicants at various concentrations depending on the test articles. The secretory biomarker profile informed the degree of injury. In addition, we also leveraged this model to support DIN assessment for internal portfolio molecules. Overall, the 3D kidney MPS has potential for better DIN prediction, improved translation to clinical safety, and study of DIN mechanisms during drug discovery and development.

Utility of an In Vitro 3D Kidney Microphysiological System to Assess Drug-Induced Nephrotoxicity


Chronic kidney disease (CKD) affects 15% of US adults or 37 million people, with over 600,000 patients receiving dialysis. While diabetes, hypertension, and obesity are known risk factors for CKD, over the last 20 years, endemics of hotspots of chronic kidney disease have emerged around the world, disproportionately affecting younger men who do not have these conditions. AFFECTED populations live in tropical, agricultural communities in regions like South Asia and Central America. Since the cause(s) of these endemic nephropathies remain unclear, the condition has been termed chronic kidney disease of unknown etiology (CKDu). In many of these regional CKDu accounts for a majority of instances of renal failure. Several environmental agents have been proposed to contribute to CKDu, including the mycotoxin ochratoxin-A (OTA), one of the most common contaminants in a wide variety of food including wheat, maize, rice, and coffee. OTA causes not only nephrotoxicity but also hepatotoxicity, genotoxicity, and carcinogenesis, however its mechanism of action is largely unknown and controversial. Herein, we hypothesized that OTA may interfere with the interaction of the antioxidant-regulator NRF2 with its cytosolic partner KEAP1, evidenced by the downregulation of numerous oxidative-stress responses transcribed by NRF2. Here, RNA-seq analysis of human proximal tubule epithelial cells (PTECs) revealed numerous differentially expressed genes between cells treated OTA in the presence and absence of NRF2 agonists, sulforaphane (SFN) and tert-butyldihydroquinone (tBHQ) which release NRF2 from KEAP1. NRF2 activators did not recover OTA-induced downregulation of oxidative stress genes, suggesting OTA exposure alters NRF2 nuclear translocation and DNA binding. Transcription factor HIF1α, its heterodimer ARNT, and downstream targets such as PDK1 were also found to be upregulated, indicative of a hypoxic response induction in PTECs due to OTA exposure. Furthermore, several genes involved in mitochondrial fission, fusion, mitophagy and apoptosis were found to be dysregulated. Confocal imaging of OTA-treated PTEC mitochondria corroborated the RNA-seq transcriptional data of mitochondrial dysfunction. Mitochondrial network integrity in these images were assessed using the Mitochondrial Network Analysis (MiNA) ImageJ macro tool, which revealed a significant, 70% decrease cellular mitochondrial signal, 3-fold reduction network branching and a subsequent 3-fold increase in the ratio of puncta (individual mitochondria) to network. Physiological relevant OTA concentrations. This evidence suggests that OTA exposure degrades mitochondrial networks and dysregulates the balance between fission and fusion. Future work will explore regulatory and biochemical mechanisms of OTA-dependent mitochondrial dysfunction, apoptosis, and incorporate organ-on-a-chip systems to model proximal tubule accumulation and renal clearance of OTA. To further explore its role in CKDu water, food and patient plasma, urine and kidney biopsy samples will be examined not only for OTA but for other environmental toxins to elucidate the pathogenesis of this disease.

Effects of Long-Term Electronic Cigarette (E-Cig) Aerosol Exposure and Kidney Health in Mice

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The link between nicotine/cigarette smoke and chronic kidney disease (CKD) is well-established. In contrast, the relationship between E-cigarette (e-cig) use and kidney disease have emerged around the world, disproportionately affecting younger men who do not have these conditions. Affected populations live in tropical, agricultural communities in regions like South Asia and Central America. Since the cause(s) of these endemic nephropathies remain unclear, the condition has been termed chronic kidney disease of unknown etiology (CKDu). In many of these regional CKDu accounts for a majority of instances of renal failure. Several environmental agents have been proposed to contribute to CKDu, including the mycotoxin ochratoxin-A (OTA), one of the most common contaminants in a wide variety of food including wheat, maize, rice, and coffee. OTA causes not only nephrotoxicity but also hepatotoxicity, genotoxicity, and carcinogenesis, however its mechanism of action is largely unknown and controversial. Herein, we hypothesized that OTA may interfere with the interaction of the antioxidant-regulator NRF2 with its cytosolic partner KEAP1, evidenced by the downregulation of numerous oxidative-stress responses transcribed by NRF2. Here, RNA-seq analysis of human proximal tubule epithelial cells (PTECs) revealed numerous differentially expressed genes between cells treated OTA in the presence and absence of NRF2 agonists, sulforaphane (SFN) and tert-butyldihydroquinone (tBHQ) which release NRF2 from KEAP1. NRF2 activators did not recover OTA-induced downregulation of oxidative stress genes, suggesting OTA exposure alters NRF2 nuclear translocation and DNA binding. Transcription factor HIF1α, its heterodimer ARNT, and downstream targets such as PDK1 were also found to be upregulated, indicative of a hypoxic response induction in PTECs due to OTA exposure. Furthermore, several genes involved in mitochondrial fission, fusion, mitophagy and apoptosis were found to be dysregulated. Confocal imaging of OTA-treated PTEC mitochondria corroborated the RNA-seq transcriptional data of mitochondrial dysfunction. Mitochondrial network integrity in these images were assessed using the Mitochondrial Network Analysis (MiNA) ImageJ macro tool, which revealed a significant, 70% decrease cellular mitochondrial signal, 3-fold reduction network branching and a subsequent 3-fold increase in the ratio of puncta (individual mitochondria) to network. Physiological relevant OTA concentrations. This evidence suggests that OTA exposure degrades mitochondrial networks and dysregulates the balance between fission and fusion. Future work will explore regulatory and biochemical mechanisms of OTA-dependent mitochondrial dysfunction, apoptosis, and incorporate organ-on-a-chip systems to model proximal tubule accumulation and renal clearance of OTA. To further explore its role in CKDu water, food and patient plasma, urine and kidney biopsy samples will be examined not only for OTA but for other environmental toxins to elucidate the pathogenesis of this disease.

Ochratoxin Interaction with hOAT4 and mOats: A Tale of Functional Orthologs


Organic anions (OA) are drugs or toxicants that are negatively charged at physiological pH and are typically transported by Organic Anion Transporters (OATs). The human hOAT4 (SLC22A11) is expressed in the apical membrane of renal proximal tubules where it exchanges either organic (e.g., α-ketoglutarate) or inorganic
(e.g., Cl) anionic substrates for an OA. Interestingly, there is no rodent ortholog of hOAT4. However, rodents express Oat5 (Scl22a19) for which there is no human ortholog; it is an anion exchanger that is also localized to the apical membrane of renal proximal tubule cells. The purpose of this study was to determine the functional similarity between mouse Oat5 and human OAT4 in the renal excretion of OAs, including ochratoxin (OTA). Chinese hamster ovary (CHO) cells expressing Scl22A11 or Scl22a19 were used to assess the transport characteristics of radiolabeled ochratoxin (pH 6.5) OTA. To assess the time dependent uptake of OTA, cells were exposed to ~200 nM OTA for 0.5, 2, 5, and 10 minutes. Uptake into OAT4-CHO and Oat5-CHO cells was linear for 10 minutes; therefore 5-minute uptakes were used to provide estimates of the initial rate of transport in subsequent studies of OAT4/Oat5-mediated transport. To determine the kinetics of OTA transport by OAT4 and Oat5, uptake of increasing concentrations of unlabelled OTA (1-1000 µM) were measured into OAT4-CHO and Oat5-CHO cells, and data was analyzed using the Michaelis-Menten equation to generate the apparent affinity (Km) and maximal rate of transport (Jmax). The resulting Km and Jmax values were very similar for both hOAT4 and mOat5 (Km 3.9 and 7.2 µM, respectively, & Jmax 4.4 and 7.7 pmol/106 cells × min, respectively). To assess whether ochratoxin is not only a substrate for uptake (reabsorption) but could also be secreted (efflux) by OAT4/Oat5, we determined the efflux of OTA from preloaded OAT4- and Oat5-expressing cells. Following a 20 min incubation of ~200 nM [3H] OTA (buffer containing 4 mM [NaCl] mannitol to correct for extracellular volume), the cells were brieﬂy aspirated and then exposed to ‘buffer only’ to allow labeled OTA to exit the cells for 3 min. In each case, hOAT4 and mOat5 supported the efflux of OTA. These data support the conclusion that OAT4 and Oat5 are functional orthologs and share selectivity for OTA both for reabsorption and secretion. These data will be instrumental in selecting an appropriate animal model when studying the disposition of anionic drugs and toxicants.

4293 Sugarcane Ash and Sugarcane Ash–Derived Silica Nanoparticles Alter Cellular Metabolism in Human Proximal Tubular Kidney Cells

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Chronic kidney disease of an unknown etiology (CKD) poses a major threat to public health, particularly in developing agricultural communities. Mechanisms underlying the etiology of this disease is lacking, thereby preventing effective therapeutic care. Agricultural workers involved in the harvest of silica-rich crops such as sugarcane are exposed to high levels of amorphous silica and experience elevated rates of CKD, leading to the hypothesis that toxicant exposures from harvest or burning of sugarcane may contribute to the development of CKD. In order to test this hypothesis, we have generated sugarcane stalks consisting of ~200 nm particles and have demonstrated that following burning of sugarcane, nano-sized silica particles are generated. To determine what effect such exposures have on kidney cells, a human proximal convoluted tubule (PCT) cell line (HK-2) was subjected to treatments ranging in concentration from 0.025 µg/ml to 25 µg/ml of sugarcane ash, desilicated sugarcane ash, sugarcane ash–derived silica nanoparticle (SAD SNP)s, or mesoporous pristine 200nm silica nanoparticles. Following 6 to 48 hours of exposure, mitochondrial activity and viability were found to be significantly reduced when exposed to SAD particles at concentrations 2.5 µg/ml or higher. Oxygen consumption rate (OCR) and pH changes suggested significant alterations in cellular metabolism across treatments as early as 6 hours following exposure. While most treatments slightly increased respiration, SAD SNP s were found to inhibit mitochondrial function, reduce ATP generation, increase reliance on glycolysis, and reduce glycolytic reserve. Metabolomic analysis following 24 hours of exposure found several cellular energetics pathways (e.g., fatty acid metabolism, glycolysis, and TCA cycle) significantly altered across ash-based treatments. Of note, the reactive oxygen species (ROS) detection assay showed that a 1.8-fold cisplatin-induced increase in ROS was successfully counteracted by NBMI pretreatment. Additionally, NBMI significantly increased glutathione (GSH) levels in cisplatin-exposed cells. Co-treatment with pre-mixed cisplatin and NBMI resulted in lower cytotoxicity, possibly due to the chelation of cisplatin by NBMI within the mixture itself. NBMI did not protect cisplatin-induced ROS production and cell death in A2780 ovarian cancer cells, indicating that while reducing cisplatin’s nephrotoxic effects, NBMI did not impact its anticancer efficacy. Our preliminary results show that NBMI protects against cisplatin-induced nephrotoxicity by increasing intracellular GSH levels and reducing ROS production. Positive findings from this study would present NBMI as a promising strategy to improve the prognosis and quality of life in cancer patients on cisplatin therapy.

4294 Common Pathways in Human Proximal Tubular Cells Altered by Exposure to Diverse Environmental Agents and Nephrotoxic Therapeutic Drugs

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Primary cultures of human proximal tubular (HT) cells were used as the model system to test hypotheses that exposure to low, physiologically relevant concentrations of diverse chemical agents causes release of proteins, lipids and metabolites into the extracellular space that correlate with specific exposures and cytotoxicity. HT cells were incubated with two environmental contaminants (S-[1,2-dichlorovinyl)-L-cysteine (DCVC) and HgCl2) or three clinically used drugs whose efficacy is dose-dependent: metalloporphyrin (cispatin (CDPP), polyoxin B (Pmox) and tenofuvin disopropyl fumarate (TDF)). Using quantitative mass spectrometry and isobaric tags, several proteins were found to be increased in the extracellular media of HT cells incubated with these chemicals. Both time- and concentration-dependent effects on protein abundance were noted, including several cytoskeletal proteins and multiple mitochondrial proteins. Using high resolution mass spectrometry coupled with ultrahigh performance liquid chromatography, effects of the diverse exposures on low-molecular-weight metabolites were studied showing time- and concentration-dependent perturbations in several common pathways, including amino acid metabolism, fatty acid metabolism, and carnitine shuttle pathways. Importantly, most of the changes in proteome and metabolome could be expected to result in damage that can cause mitochondrial membrane cytolysis, as determined by release of Kidney Injury Molecule-1 (KIM-1) or neutrophil gelatinase-associated lipocalin (NGAL) from exposed HT cells. These findings further support the overall hypothesis and identify multiple biomarkers and altered biochemical pathways associated with early exposure to diverse nephrotoxicants.

4295 Protective Effects of N,N-bis-(2-Mercaptoethyl)Isophthalalimide against Cisplatin-Induced Nephrotoxicity in HK-2 Cells

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Cisplatin is a platinum-containing chemotherapeutic agent commonly used to treat various cancers including ovarian cancer. It is however associated with nephrotoxicity (acute kidney injury and renal failure) as a major dose limitation. Accumulation of cisplatin in the kidney proximal tubular epithelial cells produces oxidative stress, depletes antioxidant reserves, and causes lipid peroxidation leading to inflammation and cell death. Numerous agents have been tested against cisplatin-induced nephrotoxicity but none have been approved for this indication. N,N-bis-(2-mercaptopethyl)isophthalalimide (NBMI) is a lipophilic, non-toxic, heavy metal chelator and antioxidant reported to protect against mercury, lead, and iron-induced toxicity both in vitro and in vivo. It has however never been tested against toxicity associated with platinum present in cisplatin. We hypothesize that NBMI counteracts cisplatin-induced nephrotoxicity without affecting cispla- tin’s antitumor efficacy. In this study, we have investigated NBMI protection against cisplatin-induced nephrotoxicity in HK-2 human kidney proximal tubular cells and A2780 ovarian cancer cells. A cell viability assay revealed concentration-dependent cytotoxicity following a 24-hour exposure to cisplatin (0-500 µM). Specifically, the 25 µM concentration induced significant (35.7%) cell death and was selected for use in the in vivo study. A 24-hour pretreatment with NBMI (10, 25, 50, and 100 µM) provided significant cytoprotection against cisplatin-induced toxicity. The reactive oxygen species (ROS) detection assay showed that a 1.8-fold cisplatin-induced increase in ROS was successfully counteracted by NBMI pretreatment. Additionally, NBMI significantly increased glutathione (GSH) levels in cisplatin-exposed cells. Co-treatment with pre-mixed cisplatin and NBMI resulted in lower cytotoxicity, possibly due to the chelation of cisplatin by NBMI within the mixture itself. NBMI did not protect cisplatin-induced ROS production and cell death in A2780 ovarian cancer cells, indicating that while reducing cisplatin’s nephrotoxic effects, NBMI did not impact its anticancer efficacy. Our preliminary results show that NBMI protects against cisplatin-induced nephrotoxicity by increasing intracellular GSH levels and reducing ROS production. Positive findings from this study would present NBMI as a promising strategy to improve the prognosis and quality of life in cancer patients on cisplatin therapy.
the correlation of Kim1. Our findings also showed that whole kidney responses were less sensitive than OMPT. In conclusion, this LCM-TempO-Seq method revealed a detailed spatial mechanistic understanding of renal injury/regeneration after nephrotoxic exposure and identifies the most representative mechanism-based nephron segment specific renal injury biomarkers.

4299 5-HT₃, Antagonist Antiemetic Drugs Alter Cisplatin Exposure and Risk of Nephrotoxicity L. E. Thompson¹ X. Wen² J. Jorgensen² J. N. Palan¹ C. Kim¹ C. L. Doherty¹ B. T. Buckley¹ E. A. Jaimes¹ M. L. Aleksunes¹ and M. S. Joy¹ ¹University of Colorado Anschutz Medical Campus, Aurora, CO; ²Rutgers University, Piscataway, NJ; ³Rutgers Ernest Mario School of Pharmacy, Piscataway, NJ.

The chemotherapeutic drug cisplatin causes acute kidney injury (AKI) in up to one-third of patients. Previous reports have indicated that risk of AKI is associated with elevated 1) maximum plasma concentrations (Cmax) and 2) area under the plasma concentration vs. time curve (AUC) of platinum (Pt). Ondansetron is a 5-HT₃ antagonist antiemetic that is commonly co-prescribed with cisplatin. Early indications suggest ondansetron may enhance the risk of AKI in rodents and humans based on retrospective clinical studies. However, there has been no prospective evaluation of AKI risk in patients randomized to different 5-HT₃ antagonist drugs.

As part of study NCT03817970, an initial group of patients (n=23) undergoing their first or second round of cisplatin chemotherapy (25-250 mg/m²) were prospectively randomized to one of three 5-HT₃ antagonist antiemetics (ondansetron 8 mg p.o., granisetron 2 mg p.o., or palonosetron 0.25 mg i.v.). Total platinum plasma concentrations were quantified using inductively coupled plasma mass spectrometry (ICP/MS) with LLOQ of 1.0 ng/mL. Noncompartmental pharmacokinetic (PK) parameters were normalized to cisplatin dose and analysis of total platinum exposure was performed using Certara Phoenix™. To assess kidney injury, estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD-EPI creatinine equation and Kidney Injury Molecule-1 (KIM-1) was measured in urine supernatants using an ELISA assay and normalized to urinary creatinine concentrations. Parameters were compared on 5-HT₃ antagonist use/day, one-way ANOVA with Tukey-Kramer post-hoc tests or Pearson correlations using GraphPad Prism (9.4.1). Total Pt plasma Cmax levels were significantly correlated with KIM-1 in urine at 48, 72, and 240 hours following cisplatin and 5-HT₃ antagonist co-treatment (p<0.01). Total Pt plasma AUC over the first two hours (AUC0-2hr) was also significantly correlated with KIM-1 in urine at 48, 72, and 240 hours following cisplatin and 5-HT₃ antagonist co-treatment (p<0.01). Pt concentrations were confirmed by digital caliper measurements. Mice were then dosed with CIS (0, 12.5, 15 mg/kg) or vehicle (saline) 1x/week for up to 4 weeks. Mice were evaluated for overall survival (body weight), survival, and cancer progression (tumor volume, tumor ulceration), and kidney injury (transglomerular filtration rate (tGFR), kidney injury molecule 1 (KIM-1)) ≥1x/week until sacrifice after 4 weeks of CIS treatment. T-test or ANOVA with a Tukey-Kramer post-hoc test were used to assess for differences from baseline to sacrifice based on cancer cells injected and CIS dose. p<0.05 was considered statistically significant. Mice injected with 1 million CMT167 cells experienced the greatest decline in survival due to rapid tumor growth and ulceration (0% at 8 d), often requiring early sacrifice. Mice injected with 50,000 CMT167 cells had the best survival (100% at 13 d). Treatment with at least two doses of CIS (12.5-15 mg/kg) significantly reduced tGFR compared to baseline (p<0.016) and increased urinary KIM-1 levels when compared to baseline (p<0.0012), both biomarkers of the AKI phenotype.

The utility of cisplatin chemotherapy is limited in part by nephrotoxicity, which can be more pronounced with co-administration of drugs to alleviate other side effects including nausea and vomiting. This study sought to determine whether urinary protein biomarkers of subclinical kidney injury could be used to assess novel drug-drug interactions. Adult cancer patients prescribed cisplatin (≥25 mg/m²) were randomized to one of the three antiemetic 5-HT₃ antagonists (granisetron, ondansetron, or palonosetron; NCT03817970). Urine samples were collected at baseline and days 1, 2, 3, and 10 after cisplatin infusion. Three biomarkers (kidney injury molecule-1, KIM-1; t-repellent factor 3, TFF3; and calbindin D28k, CALB1) were measured in urine supernatants using enzyme-linked immunosorbent assays (ELISA) and normalized to urinary creatinine concentrations. Increases in urinary concentrations of all three proteins were observed throughout the 10-day period. KIM-1, a marker of proximal tubule damage, was elevated to a greater extent on day 2 in patients receiving ondansetron (6-2-fold) compared to those treated with palonosetron (2.9-fold, p<0.05) and granisetron (1.4-fold, p=0.08). Conversely, urinary concentrations of TFF3, a protein involved in epithelial repair, was increased preferentially in patients prescribed palonosetron (2.9-fold) and granisetron (5.3-fold) on day 3 which was significantly greater than patients receiving ondansetron (2.1-fold, p<0.05). As a result, there was an inverse relationship between urinary TFF3 and KIM-1 concentrations (r=-0.4, p<0.05) in patients treated with ondansetron suggesting more structural damage and less tubular repair. By day 10, CALB1, a marker of distal tubule injury, trended higher in patients treated with ondansetron compared to patients treated with palonosetron (p=0.02) with little difference compared to patients treated with palonosetron. These data suggest that KIM1 reflects acute kidney injury whereas TFF3 and CALB1 reflect the potential repair mechanisms in patients co-administered cisplatin and ondansetron whereas changes in TFF3 pointed to potential repair mechanisms in patients co-administered cisplatin and granisetron. Little changes in kidney biomarkers were observed in patients receiving palonosetron and cisplatin. Taken together, urinary protein biomarkers can be used to assess novel drug-drug interactions and discriminate differences in cisplatin-induced nephrotoxicity according to tubular location and potential for cellular repair. Supported by R01GM123330, P30CA072720, P20S0505022, ULTR030917.
was quantified in the cell lysates using ICP/MS. Compared to EV cells, platinum concentrations were 58% and 33% higher in hOCT2- and hMATE1-transfected cells, respectively. Pre-treatment of cells with 20µM ondansetron, palonosetron, grani- etron or tropisetron significantly decreased platinum accumulation in hOCT2 (by 52%-68%) and hMATE1 (42%-58%) transfected cells. These data suggest that this cellular transporter model can be refined to test the potential for drug-drug interac-
tions that influence the development of acute kidney injury caused by cisplatin. Supported by KI01 GM123530, P30CA077220, P30ES050522, UL1TR002301.

4301 The Renal Proximal Tubule TXG-MAPr: Safety Assessment Based on Quantitative Gene Network Analysis

Scientific advances in omics technologies and ever-increasing knowledge on human biology render pre-clinical in vivo testing not sustainable in the future. In the kidneys, proximal tubule epithelial cells are the primary target for xenobiot-
ic-induced injury due to increased exposure levels, bilateral transporter-mediated uptake and high oxygen consumption. Through concentration and time course chemical exposure of RPTEC-TERT1 cells using >50 nephrotoxics and reference compounds that cover a wide range of mechanisms of action, and subsequent TempO-Seq whole genome transcriptomics and weighted correlation network analysis, we have established a human RPTEC/TERT1 in vitro kidney TXG-MAPr tool. The TXG-MAPr tool allows user friendly interactive toxicogenomics data interpre-
tation on mechanisms of action and compound activity correlation. Interspecies networks reveal interrelations of nephrotoxic effects observed with prokaryotic cell lines (e.g., E. coli). Analysis of compounds used in the clinic either orally or intravenously. The bisphosphonates zoledronate and pamidronate (PAM) are known for their antiresorptive activity in bone metabolism. Here, in a murine model of phenylhydrazine (PHZ)-induced hemolysis, we demonstrate a dose and time-dependent increase in AKI severity as evidenced by a significant reduction in glomerular filtration rate (GFR), rise in serum creati-
nine, and significant increase in biomarkers of AKI (Kim-1, NGAL) in urine, serum and kidneys of PHZ-treated animals. At 72 hours post PHZ administration, we observed ultrastructural changes indicative of tubular injury, iron accumulation, and increased numbers of TUNEL positive cells in the kidney tubules. Interestingly, these hallmarks of renal injury were associated with an increase in serum cytokines (GCSF, IL-4, IL-6, IL-10, IL-12, Cc12, Cc55, IFN-γ and TNF-α). A mechanism by which this pathobiology is regulated involves activation of the nucleotide-binding oligomerization domain-containing protein 1/2 (NOD1/2). In this regard, kidneys of PHZ-treated mice showed induction of NOD1/2 and phosphorylation activation of RIP2K, which leads to activation of NFkB signaling pathway and transcription of its immune response genes such as IL-6, Ccr1, TNF-α, I30, RelA etc. These studies identify NOD1/2, as an important and critical regulator of hemolysis-induced AKI, and a potential target for therapeutic intervention.

4302 NOD Signaling Regulates Pathogenesis of Acute Kidney Injury

Intravascular hemolysis is a common characteristic of diseases such as autom-
mune hemolytic anemia, sickle cell disease (SCD), paroxysmal nocturnal hemoglo-
binuria, hereditary uremic syndrome, hemoglobinopathy, and surgical procedures. Erythrocyte destruction, during intravascular hemolysis, leads to release of hemoglobin, heme and other pro-inflammatory mediators. These clastogenic molecules are also believed to induce acute kidney injury and hemolytic hemoph-
ocytosis. Here, in a gene model of gphcyd/hydroxyl (PHcy) induced hemolysis, we demonstrate a dose and time-dependent increase in AKI severity as evidenced by

4303 Physiological O2 Influences the Toxicity of ADPKD Drug Candidates in Renal Proximal Tubular Cells

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by the progressive development of fluid-filled cysts in the kidney and is caused by mutations in the PKD1 and PKD2 genes. As the cysts expand, they compress the surrounding tissue, leading to local injury and fibrosis. Consequently, kidney function declines over time, resulting in end-stage renal disease. The genes encode for the PC1/PC2 polycystin complex, which modulates several signaling pathways of the renal epithelial cell. Numerous cellular molecules have been identified as drug targets for ADPKD, but only tolvaptan, a vasopressin receptor 2 antagonist, has been approved for the treatment of the disease. However, side effects of tolvaptan, namely liver toxicity and polyuria, limit its use, and therefore, there is a need for developing drugs that specifically target the cysts. The DRUGtrain consor-
tium aims to find new therapies for ADPKD through drug repurposing. Birinapant, an antagonist of the inhibitors of apoptosis proteins, celastrol, a multitarget drug, rapamycin, an mTOR inhibitor, and salicylic acid, an AMPK activator, reduced the

4304 Prediction of Nephrotoxicity of Nitrogen-Containing Bisphosphonates Using PODO/TERT256 and RPTEC/TERT1 Cells

The past decade of research demonstrated that conventional cultivation of cells cannot recapitulate their physiological environment in vivo. The latter limits the usefulness of in vitro approaches e.g., in drug testing for adverse or off target effects due to limited or lack of predictivity. To overcome this, research has focused on providing cells with conditions that mimic their physiological environ-
ment and optimizing the treatment routine to recapitulate the in vivo situation. For example, atmospheric O2 (21 %), at which nearly all routine in vitro experiments are applied in the clinic either orally or intravenously. The bisphosphonates zoledronate (ZOL) and pamidronate (PAM) as well as salicylic acid were not toxic to the cells, but at physiOx, the cellular metabolism shifted to glycolysis at the highest concentrations. The present study underlines the need for toxicity testing under physOx, as significant differences are observed compared to atmOx. Physiological O2 can help to accurately define the therapeutic window of drug candidates and safely extrapolate the toxicity to the patient.

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Vanadyl (V) is an element widespread in the environment. Kidneys can suffer damage in their function without presenting apparent clinical manifestations. Alterations can occur from exposure to multiple nephrotoxins, such as environmental pollutants. Early renal damage biomarkers (BMDRT) have been suggested to identify the incipient effects of toxic agents in specific nephron regions. Epidemiological studies have evaluated the association of exposure to metals such as arsenic, mercury, lead, and chromium with BMDRT (Pócsí et al., 2022); however, scientific evidence is limited, and many metals have not been evaluated yet. The objective of this study was to assess the association of urinary V concentrations with BMDRT (NGAL, HMOG, and eGFR) in a population of adolescents in the state of Tlaxcala, where a high incidence of chronic kidney disease has been recorded in the young population. A cross-sectional study was conducted in healthy adolescents (n=914), where urinary levels of V and two BMDRT were estimated (NGAL, HMOG). The association of V with BMDRT was evaluated through linear and logistic regression models adjusted for confounders. NGAL, HMOG and urinary V were significantly and negatively associated at urinary levels of V. Our results suggest that V exposure may be related to early tubulointerstitial renal conditions in apparently healthy juvenile subjects. Supported by a grant from Fundación Gonzalo Rio Arronte (FGRA S638).

Role of Oxidative Stress in Folic Acid–Induced Kidney Fibrosis in a Mouse Model

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Kidney fibrosis is a common step during the development of chronic kidney disease after acute kidney injury. Excessive production and accumulation of extracellular matrix during the repair process after repeated injury to the kidney by nephrotoxicants leads to renal fibrosis. Oxidative stress is a commonly observed effect of nephrotoxicants and it has also been implicated in CKD development. The folic acid–induced kidney fibrosis model is an established model to study kidney fibrosis in mice, but the mechanism is poorly understood. Therefore, in this study the role of folic acid–induced oxidative stress in fibrogenic changes was evaluated in mice using a fibrogenic model. C57Bl/6 mice were exposed to folic acid (250 mg/kg) or folic acid in two injection each of 125 mg/kg body weight at the interval of 10 days. Parameters such as animal weight, serum creatinine level, tissue histopathology, and gene expression were used to evaluate the effect of folic acid. Serum creatinine levels, and histopathological data revealed acute kidney injury at 2 days and fibrogenic changes at 21 days. Gene expression analysis at both transcript and protein levels further confirmed the changes in expression of antioxidant, as well as fibrosis and EMT marker genes. In summary, the findings of this study suggest the involvement of folic acid–induced oxidative stress in folic acid–induced kidney fibrosis mouse model. Acknowledgement: This work was partially supported by NIH (NIDDK) grant award #1R15DK121562 to KPS.

Acute Kidney Toxicity in Industrial Metal Coating Painters is Associated with Urinary Exposure Biomarkers of Isocyanates and Epoxies: Challenges in the Era of Global Warming


Occupational exposure as a painter has been classified by IARC as a Group 1 carcinogen, based on the increased risk for lung cancers, urinary bladder cancers, and mesothelioma. The exact carcinogens in paints are not known. Painting of metal structures (such as bridges, pipelines, water tanks, etc.) is a multi-step process and involves completion of sequential tasks over many weeks: surface cleaning using abrasive blasting; priming with an epoxy- or isocyanate-based primer; mid-coating with an epoxy- or isocyanate-based system, followed by a layer of aliphatic isocyanate- or isocyanate-based top coat. In addition to the epoxy or isocyanate component, these formulations (part B) contain additional classes of additives, including solvents, catalysts and crosslinkers, amine hardeners, corrosion inhibitors and nano-fillers. There is a notable paucity of product composition, exposure and biomonitoring data in industrial painters. The primary objective of this study was to examine acute kidney toxicity in industrial painters and association with urinary exposure biomarkers. The motivation for this work originated from field observations of workers’ exposures to complex mixtures, dehydration, signs of heat exhaustion, and the findings of abnormal creatinine in urine that increased notably post-shift. Thirty-six workers (20 in top coating, 9 in mid-coating, and 7 in spray polyurethane foam application for comparison) considered to personal inhalation and skin sampling and donation of two urine samples, one at the beginning and the other the end of the work shift. We assessed personal exposures to oxazolidones, isocyanates, their corresponding urinary biomarkers, as well as exposures to amine hardeners. Each urine sample was analyzed for a panel of six FDA-approved acute kidney injury (AKI) biomarkers: Kidney Injury Molecule 1 (KIM-1, a marker of proximal tubule injury), Cystatin C (Cys-C, a marker of tubular injury), Osteopontin (OPN, a glycoprotein expressed in the ascending loop of Henle and in distal nephrons), Neutrophil Gelatinase-Associated Lipocalin (NGAL, tubular epithelial cell injury), N-acetyl-β-D-glucosaminidase (NAG, marker of renal proximal tubule injury), Clusterin (CLU, marker of renal proximal and distal tubules, glomerulus and collecting duct), as well as urinary fibrogen (marker of chronic kidney injury), total protein, albumin, creatinine, specific gravity, and the standard urinalysis profiling. All exposure and urinary AKI biomarkers were normalized to creatinine and specific gravity prior to statistical analysis. The geometric mean ratios of post-shift to pre-shift values for KIM-1, OPN, and NAG increased significantly post-shift by ~2×, 1.4× and 2.7×, respectively, with topcoat applicators generally exhibiting the greatest increases, followed by midcoat workers, then SPF. Similar (ratios of 1.2-2.0), albeit less pronounced increases were observed for Cys-C, Clusterin and total protein. Moderate positive correlations (Spearman Rho, 0.4-0.7) were observed between several urinary AKI biomarkers. In top coating, KIM-1 and CLU were significantly associated with urinary hexamethylene diamine (HDA, a biomarker of isocyanate exposures), whereas OPN was associated with both HDA and BADGE +H2O (the biomarker of epoxy exposures). The absolute values of KIM-1, OPN, NAG and Cys-C exceeded upper normal clinical values in ~10 to 50% of topcoat and midcoat samples. The data overall provide strong evidence that industrial painters experience acute kidney injury in a dose-dependent manner and their kidney injuries may be compounded by dehydration and heat stress. We will further discuss implications of such data for intervention strategies.
Due to their imperative function, kidneys are meticulously examined during the drug development process for potential drug-induced nephrotoxicity. Proximal tubule (PT) cells are typically utilized for in vitro assessment of nephrotoxicity as these cells are particularly susceptible to toxicity. While traditional in vitro studies utilize two-dimensional (2D) cultured cells, the results have not always been consistent with in vivo and clinical studies. Thus, there is a growing need to explore the three-dimensional (3D) microphysiological systems (MPSs) and their physiologically relevance in vivo. Moreover, establishing 3D MPS platforms comprised of renal cells from pre-clinical species is paramount in order to fill a gap and provide better translatability between pre-clinical and clinical studies. Therefore, we used a 3D MPS platform comprised of human PT cells and, unprecedentedly, rats to interrogate mechanisms of drug-induced nephrotoxicity. Additionally, we investigated nephrotoxicity using 2D PT cultured cells from the same cell lines used in the 3D MPS for cross-platform comparison purposes. All PT cells were exposed to the bisphosphonates: zoledronic acid and ibandronate - medications used for various skeletal disorders, and can potentially cause kidney injury - and subjected to assays to examine cytotoxicity and kidney injury-related biomarkers. We observed that 3D PT cells exhibited cytotoxicity 3 days in rat and 13 days following bisphosphonate exposure in human, demonstrating species-specific sensitivity in the 3D platforms. Cytotoxicity was associated with DNA damage as evidenced by an increased in apoptotic and necrotic cells and in mitochondrial functions after a short-term compound exposure. Importantly, these effects were more prominent after exposure to zoledronic acid when compared to ibandronate, which correlates with previous 2D in vitro studies and clinical findings. In both species, more inflammatory and kidney-injury related biomarkers were secreted from 3D PT cells exposed to ibandronate when compared to zoledronic acid. Clusterin and a tissue inhibitor of metalloproteinases-1 (TIMP-1) were elevated by ibandronate, suggesting their protective effect against ibandronate exposure. The vastly different biomarker profiles between both bisphosphonates indicate that nephrotoxicity is induced via different mechanisms. When comparing sensitivity between 2D cultured and 3D models, zoledronic acid was more toxic in 3D and the kidney injury model was more sensitive as 2D PT cells exhibited cytotoxicity at an earlier timepoint. Unlike the 3D PT cells, there was substantial reduction in biomarker production from 2D human PT cells after short-term bisphosphonate exposure, suggesting 2D PT cells are unsuitable for long-term exposure studies. Overall, our study demonstrates that the 3D PT models have potential for the identification of drug-induced nephrotoxicants.

Renal toxicity remains a major issue in clinical trials and stresses the need for more predictive models fit for implementation in early drug development. Here, we describe the use of a high-throughput, microfluidic platform for the detection of drug-induced nephrotoxicity. A microfluidic platform (Mimetas’ OrganoPlate®) was combined with renal proximal tubule epithelial cell lines (PTEC) and exposed to fluid shear stress. A 12-compound nephrotoxicity screen across multiple laboratories was performed in collaboration with sponsors and the NCSRs. ciPTEC-DAT1 and ciPTEC-DAT1 (Sigmadx) were pre-treated with P-GATEs and then cultured on a 3D layer. The culture exhibited epithelial barrier tightness and drug-transporter interactions were evaluated. Parallel to this, cellular damage and stress were assessed using various read-outs. Finally, gene expression analysis was performed to assess AKI markers. The NPS revealed that a combination of cell viability, LDH and miRNA release were the most predictive readouts in determining nephrotoxicity. Most of the blinded compounds resulted in toxicity detected by at least one of the functional read-outs. NPS provides a reliable standardized and automatable system for efficacious identifying nephrotoxicants and revealing their mode of action.

In drug development it is crucial to detect or prevent unanticipated organ toxicity at an early stage. Integration of toxicogenomic data with histopathology is a useful approach to associate molecular and cellular mechanisms with pathogenesis. In this context we have applied gene co-expression network analysis (WGCNA) on publicly available toxicogenomic rat liver and kidney TG-GATEs datasets and developed an interactive R-Shiny based TXG-MAPr tool to data visualization. Perturbation of WGCNA gene networks (modules) were quantitatively assessed by module eigengene scores (EGs) for each treatment condition, which 2-scores and summarizes the log2 fold change of all genes in a module. These module EGs were statistically associated with pathology phenotypes, providing prognostic information for drug safety assessment. In this way we could link pathogenesis to specific gene co-expression modules that represent cellular processes, including cell cycle, immune response, cell adhesion / cytokine signaling, cell migration, extracellular matrix remodeling and RNA processing. Interestingly, modules that showed the highest statistical association with pathogenesis contain several well-known and novel biomarkers. Using prevalence and association statistics, we showed that several of these rat co-expression modules were also preserved in other test systems, like human tissue, indicating that pathogenic processes, including renal biomarker expression, could translate across species. Finally, new transcriptomic data can be uploaded in the TXG-MAPr tool for visualization of gene network perturbation. We developed a web-based interactive R-Shiny based tool that allows users to visualize the heatmaps and networks and to identify the modules of toxicity by chemical insults. In conclusion, the TXG-MAPr represents an innovation and powerful tool that contributes to drug safety assessment by providing mechanistic understanding of potential adverse drug reactions. This work has received funding from the EU-EFPIA Innovative Medicines Initiative 2 (IMI2) Joint
Undertaking TransQST project (grant number 116030) and eTRANSafe project (grant number 770881). The authors receiving support from the European Union’s Horizon 2020 research and innovation program and EFPA, the EC Horizon 2020 EU-ToxRisk project (grant number 681002), the EC Horizon 2020 RISK-HUNT3R project (grant number 964537) and the Cosmetics Europe and European Chemical Industry Council (CEFIC) Ontology project (project AIRM10).

**4313** Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI MSI) of Lipid Changes in Response to Acetaminophen Overdose in Kidney Tissue

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Acetaminophen (APAP) overdose is a common cause of acute liver and kidney injury. The lack of effective clinical interventions to treat APAP nephrotoxicity limits recovery time and care. In this study, we employed Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) to screen the lipids in the cortex and medulla of rat kidney sections, comparing control and APAP overdose conditions. The maps allow a detailed qualitative analysis of the lipid changes, which can be used as a tool for understanding the metabolic changes associated with APAP overdose in the renal tissue. The results showed significant changes in the distribution of lipids across the cortex and medulla regions, indicating alterations in lipid metabolism. These findings provide insights into the mechanisms of APAP nephrotoxicity and potential targets for intervention.

**4315** Spinosad: A Physiologically Based Pharmacokinetic (PBPK) Model in the Rat and Human Including the Pregnancy Life Stage

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A physiologically-based pharmacokinetic (PBPK) model was developed for the insecticide Spinosad (a mixture of Spinosyns A and D) and built in R programming language. The model simulates dosimetry for rats and humans, including pharmacological and toxicokinetic behavior. In particular, we focused on the differences between spinosyns’ pharmacokinetics in the renal tissue, where they are metabolized and excreted. The predictions of the model were validated against experimental data, demonstrating its ability to accurately simulate the disposition of Spinosad in vivo. The model provides valuable insights into the pharmacokinetics of Spinosad and its metabolites, which can be used to optimize dosing regimens and improve treatment outcomes.

**4314** Preliminary Physiologically Based Pharmacokinetic (PBPK) Modeling and Assessment of Consistency across Published Studies of Perfluorooctane Sulfonate and Perfluorooctanoic Acid Kinetics in Adult Zebrafish

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are known environmental contaminants that are harmful to aquatic organisms, including zebrafish (Danio rerio). However, there is a lack of an integrated, mechanistic understanding of their toxicokinetics in this species. This study presents a preliminary PBPK model of PFOS and PFOA pharmacokinetics in adult zebrafish. The model was constructed using previously published data sets from zebrafish studies. The model provides a framework for understanding the toxicokinetics of PFASs in zebrafish and their potential mechanisms of action. The findings suggest that PFASs can be rapidly absorbed and distributed throughout the body, with potential for accumulation in certain tissues.
Physiologically based pharmacokinetic (PBPK) models describe the disposition of a chemical throughout the body following exposure using parameters that quantify anatomical and physiological features of the body as well as biochemical interactions and processes. Chemical risk assessors use PBPK models for dosimetric calculations in support of risk assessment, but they should first perform quality assurance (QA) review of the models to ensure biological plausibility and correct implementation. We have previously developed and released a model template consisting of a single model "superstructure" with equations and logic commonly found in many PBPK models. Model template users can implement a wide variety of chemical-specific PBPK models by selecting and omitting specific features. QA review of models implemented using the template can be completed more quickly than reviews of conventionally-implemented ("stand-alone") models since the general model equations have already been evaluated and verified, and only the parameters describing chemical-specific model and exposure scenarios need to be reviewed. While the QA review of a template implemented model is faster than that of a stand-alone model, one may ask: does the more complex structure of the model template come with a significant cost in computational speed? We sought to determine how the speed of two stand-alone chemical-specific PBPK models compared to template implementations of the same models. We considered a randomly generated set of 10,000 humans continuously exposed via inhalation to dichloromethane (DCM) or chloroform, and we computed internal dose metrics (including steady-state concentrations of chemical in venous blood and liver tissue and the amount of chemical metabolized) using a stand-alone PBPK model for each chemical and a template implementation of that model. We calculated the time it took to initialize and perform the simulations in total as well as the average time to solve a single initial value problem. Simulations performed using the template implementations of the models were about 4.5 times slower than those using the stand-alone models. The stand-alone models took approximately 8 seconds to compute 10,000 simulations and the template implementations took about 36 seconds. Approximately 70% of the computational time was spent solving the initial value problems in each case. The differences were likely due to the increased number of state equations (38 state variables in the model template compared to 20 for the DCM model or 19 for the chloroform model) and conditional statements used in the model template. While the template implementations of the PBPK models required more computational time than the stand-alone versions of those models, the observed time differences are not excessive on the time scale of human work. Since the model template also improves the consistency, quality, and efficiency of implementing PBPK models, the increased time to run simulations may be justified in many situations.

The thyroid hormones play key roles in physiological processes such as regulation of the metabolic and cardiac systems as well as the development of the brain and surrounding systems. Recent efforts to evaluate potential environmental chemicals for their ability to alter thyroid hormone synthesis, transport, metabolism, and/or function have identified novel chemicals that target key processes in the thyroid pathway. One newly identified chemical, oxyfluorfen, is a dibenzofuran herbicide used for control of annual broadleaf and grassy weeds in a variety of tree fruit, nut, vine, and field crops. Using in vitro high-throughput screening (HTS) assays, oxyfluorfen was identified to be a potent inhibitor of the thyroid sodium-iodide symporter (NIS). To quantitatively assess this inhibition mechanism in vivo, we extrapolated in vitro NIS inhibition data to in vivo disruption of thyroid hormone synthesis in rats using physiologically based pharmacokinetic (PBPK) and thyroid hormone kinetics models. The overall conclusion of this study was that oxyfluorfen is a Category 2 (against in vivo data in rats for the levels of oxyfluorfen in thyroid and serum and against levels of thyroid hormones triiodothyronine (T3) and thyroxine (T4) in serum). The calibrated rat model simulations were within a factor of 3-fold from experimental data. The rat thyroid model was then extrapolated to humans using human in vitro HTS data for NIS inhibition and the chemical hepatic clearance rate in humans. The overall species extrapolation PBPK-thyroid kinetics model can be used to predict dose-response (% drop in thyroid serum levels compared to homeostasis) relationships in humans. These relationships can be used to estimate points of departure for health risks related to a drop in serum levels of TH hormones based on HTS assays, IVIVE, toxicokinetic, and physiological principles. This abstract does not necessarily reflect US EPA policy.
A pharmacokinetic model was constructed and characterized with applications that assessed exposures to adults drinking perfluorooctanoic acid (PFOA) contaminated water. The one compartment pharmacokinetic model incorporates components that account for: age-variable bodyweight; temporal changes in background; and temporal changes for intake dosing. Published data were used to parameterize the model and to generate the time-variable model components. The focus of the exploratory investigations was on historical exposure dose reconstructions that were tailored to site-specific applications; and on characterizing model performance versus measured serum PFOA in impacted communities. One general dosing routine was constructed using contemporary well water data associated with the discovery of the PFOA contamination in site groundwater. Those data were used in the model as a constant water contaminant concentration that was extended back in time to include the previous 5, or 10 years, or several decades. A second dosing routine used decades-long historical time trends that were generated from fate and transport modeling efforts. The use of bottled water by community members was considered as an exposure reduction strategy in some simulations. Modeled predictions for site-specific serum PFOA levels were compared to serum PFOA concentrations that were measured in biomonitoring programs at the sites. In simulations using exposure scenarios that reflect site-specific conditions, most of the model predictions were within a factor of two when compared to PFOA levels measured in the affected communities. The value of incorporating the time-variable background as a component of the predicted PFOA concentrations is demonstrated at several sites. The concordance of the predicted values and the measured serum PFOA concentrations suggests the pharmacokinetic model adequately predicted maternal body burdens of PFOA and that the model could be adapted for use in other settings. The findings and conclusions in this abstract-poster 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.
Fluoxin is the only nonsteroidal anti-inflammatory drug (NSAID) labeled in the US to use in food-producing animals, including cattle and swine. Banamin® Transdermal is a pour-on formulation of fluoxin approved for pain control in beef and dairy cattle, but not for calves and dairy cows of breeding ages or swine. Violative fluoxin residues in edible tissues in cattle and swine have been reported and are usually attributed to non-compliant drug use or failure to observe an appropriate withdrawal time. Thus, the establishment of an adequate withdrawal time for the extra-label use of fluoxin is very important. The primary aim of this project was to develop a physiologically based pharmacokinetic (PBPK) model for fluoxin in cattle and swine to predict withdrawal times after exposures to different therapeutic regimens of Banamin® Transdermal. This model was developed with six compartments: plasma, kidney, liver, muscle, skin, and rest of the body. The model was developed based on a published PBPK model for fluoxin following other routes of exposure, including intravenous, intramuscular, and subcutaneous administrations. Physiology parameter values were updated based on a recent comprehensive review. This model was calibrated with plasma concentration data of fluoxin in cattle, but not for calves and dairy cows of breeding ages or swine. The cardiovascular (CV) system is significantly affected by environmental factors, but risk assessment of environmental chemicals is restricted due to limited data availability. One of the major bottle necks is selection and prioritization of chemicals for further assessment due to lack of adequate animal data. New approach to risk characterization (ARC) methods involving the in vitro bioassay for estimation of exposure and toxic effects, which may offer efficient and comprehensive approaches to replace animal testing and providing strategies to deal with regulatory challenges. In the present study, we adopted an in vitro-to-in vivo extrapolation (IVIVE) workflow using physiologically based pharmacokinetic (PBPK) models to translate concentrations of TCDD to toxicokinetic effects. The in vitro bioassay (HTS) assay relevant to six CV failure modes to human equivalent administered doses (EADs). To evaluate the human relevance of the predictions, these EADs were compared with exposure estimates derived from the US EPA ExpoCast program and in vivo animal study-based points of departure (POD) from the ToxVol database. Geospatial mapping was used to assess the potential risk of exposure for different populations across different counties/regions within the United States. A group of potential cardiac toxic chemicals predicted to have low margins of exposure were identified. Chemicals such as Dibutyl phthalate, Perfluorocarbonic Acid (PFOA) and Bisphenol A (BPA) were identified with overlapping EAD ranges and exposure estimates, particularly within certain geographical hotspots. Incorporation of the spatial distribution of chemicals allows for connections between molecular perturbations and adverse health outcomes across a population, enabling hypothesis generation and prioritization for further testing. Use of HTS assay outcome data in establishing a margin of exposure and POD ratio can advance the step of human health safety evaluation by providing rapid, computationally supported screening-level assessments. This case study utilizes the in vitro bioactivity-based EADs and represents an advancement in the application of NAM-based alternative approaches in human health risk assessment for potentially cardio toxic chemicals.
The compound Tris-(4-chlorophenyl)methanol (TCPMOM; CAS #3010-80-8) is a contaminant of emerging concern due to its unknown etiology and recent detection in biological matrices including marine mammals and human breast milk around the world. We previously showed that exposures to TCPMOM during zebrafish development increased the incidence of structural defects and mortality, including decreased fish length and an increase in yolk sac edema. Here we explore further the developmental toxicity of TCPMOM on the dynamic changes in yolk absorption and overall fish growth by utilizing image analyses, differential equation modeling, and parameter estimation. Zebrafish embryos were exposed to a vehicle control (0.01% dimethyl sulfoxide), 0.5 μM, 1 μM, or 5 μM TCPMOM (n = 20-36 embryos per group) from 0-72hpf post fertilization (p.f.). Endpoints for fish length were quantified using image analyses for mathematical model building and validation. The dynamic changes in yolk absorption, fish length, and their interactions were first established in the control group by building and validating a coupled set of differential equations for the system. Model development and validation establish a nonlinear functional relationship between nutrient absorption and early fish growth. We found that the rate of change in fish length and yolk utilization is logistic, that is the yolk decays rapidly for a period of time before leveling out. The mathematical model was then utilized to investigate the changes under environmental exposure. TCPMOM exposed samples experienced increased yolk absorption as captured by parameters within the mathematical model and a decreased rate of fish growth. As zebrafish contain a finite nutrient source during the developmental stage, these findings suggest the rapid intake of nutrients may be a key contributor to the decrease in overall growth. This study has established a mathematical modeling strategy that explains the relationship between yolk absorption and overall fish growth in a controlled environment and under environmental perturbation. We have found, using parameter estimation, that TCPMOM has a statistically significant impact on nutrient availability in zebrafish. As nutritional perturbation during development has been shown to be associated with cardiovascular and metabolic disease later in life, the developed mathematical model will be a valuable tool in assessing the toxicity of further environmental contaminants in the future.
study is to determine which ML method will best predict the plasma concentration of simulated subject taking various doses of 2AA. In the following experiment, we used various machine learning to determine which algorithm best predicted the data in males and females. Population plasma PK data was generated using a physiologically based pharmacokinetic model with 10-30% variation in parameters with Berkeley Madonna. The time since the first dose was given, time since last dose, dose amount, study number, patient number and dosing cycle was also incorporated into the datafile for each patient. The data for each patient was appended together to represent a single study. Fifty subjects were simulated for each study. Multiple scenarios in which sex (male or female), dose (200-1000mg), and frequency (6-12 hours) were varied for each study. The data file was randomized for training, validation, and test sets for the AI models. Once the data set was split using python based software, the performance was evaluated using the coefficient of determination (R²) for the regression models and the root mean squared error (RMSE) for predictive error. The ML models which performed better than the others tested had an R² value of greater than 0.6. However, the RMSE overall needed improvement. The results will guide further exploration of other model features for the best performing ML methods and algorithms.

4334 Alcohol-Induced Skeletal Cortex Toxicity Is Independent of Oxidative Stress

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Ethanol (EtOH) induces deleterious effects on the quantity and quality of bone in the skeleton. The mechanism of these effects is hypothesized to involve reactive oxygen species (ROS) generated within bone cells. In previous studies we demonstrated that the antioxidant N-acetylcysteine protects against chronic ethanol-induced trabecular bone loss but was ineffective in protection of the cortical compartment. In the current study we subjected wildtype (wt) mice, wildtype mice treated with the superoxide dismutase (SOD) mimic MitoTEMPO, and mice (mCAT) overexpressing human calpastatin in mice to a four-day binge ethanol model previously shown to replicate the chronic negative effects of alcohol. QRT-PCR analysis of femur shaft mRNAs was conducted. mCAT mice overexpressed human calpastatin and higher total amount of calpastatin mRNA than wt mice. Gene expression of osteoblast markers Col1a1 and Smad3 was down regulated and the osteoclast differentiation marker Calr was upregulated (P<0.05) following EtOH exposure in all mouse groups (P<0.05) in the femur shaft. Weight loss in the mice exposed to the EtOH treatment held true in all mouse groups (P<0.001). Serum procollagen 1α1 concentration was determined by ELISA. Oxidative stress was measured by Western blot analysis of carbonylated proteins in liver and femur shaft. Oxidized proteins were significantly elevated by EtOH treatment in liver but not in the femur shaft. Serum concentration of procollagen 1α1 was reduced by EtOH in wildtype and MitoTEMPO mice (P<0.05) but was unchanged in the mCAT mice, suggesting protection of osteoblastogenesis from EtOH’s effects. Our data demonstrate that MitoTEMPO or overexpression of mitochondrial calpastatin do not protect against ethanol effects on gene expression in the femur shaft. Combined with previous data of a similar lack of effect of whole-body calpastatin knock-out mice on the ethanol response, transcriptional effects of ethanol on the femur shaft appear independent of ROS. However, ROS signaling in bone marrow may play a role in reducing systemic concentration of procollagen 1α1. Supported in part by R37AA18282 (NIR).

4335 Examining the Oxidative Stress Effects in Male Rats Exposed to 2-Aminoanthracene In Utero and a High-Fat Diet Three Months After

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that are naturally found in partially burned oil, gasoline, and coal. When large amounts of these compounds are inhaled, ingested, or absorbed through the skin, severe blood, kidney, and/or skin liver problems may result. This chemical also tends to target fat tissue and can cause cancer. 2-aminoanthracene is an example of a PAH and can affect lipid and carbohydrate metabolism and may also cause inflammatory problems in the body. Oxidative stress is caused by an imbalance between production, storage, and detoxification of oxygen reactive species (ROS) in the cells or tissues. Oxidative stress can cause insulin resistance and chronic low-grade inflammation. The goal of the study is to investigate oxidative stress as it relates to diabetes in rats exposed to 2-aminoanthracene (2AA) in utero and a high fat diet later in life. 2AA was administered to pregnant dams to create treatment groups. The offspring were separated into three different groups based upon which group they fell into. Three months after weaning, the remainder of the rats were assigned to regular rat food and a moderately high-fat diet for six more weeks. Adipose tissue from the rat's abdomen was removed along with other tissues and the adipose tissue was homogenized in phosphate buffered saline (PBS). AT protein levels were measured. The results showed significant elevated levels of protein in the treatment groups compared to the control. There was an increase by a factor of ½.

4336 Examining the Effect of Oxidative Stress in Female Rats Exposed to 2-Aminoanthracene In Utero and a High-Fat Diet Three Months after Gestation

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Polycyclic aromatic hydrocarbons (PAH) are carbon-based toxic pollutants composed of two or more fused aromatic rings formed from the incomplete combustion of carbonic substances. PAHs are found in the atmosphere, hydrosphere, geosphere, and biosphere through natural and anthropogenic activities. Exposure can occur through inhalation, dietary ingestion of contaminated foods, and dermal absorption. 2-aminoanthracene (2AA) is part of a larger group of PAHs known as anthracenes. Prior research has shown that 2AA interferes with genes involved in immune response, inflammatory response, lipid, and carbohydrate metabolism through the production of reactive oxygen species (ROS). Oxidative stress is caused by an imbalance between the production of ROS and the detoxifying effects of antioxidants. The goal of this study is to investigate oxidative stress as it relates to diabetes in female rats exposed to 2-aminoanthracene in utero and a high-fat diet later in life. To accomplish study goals, timed pregnant dams were separated into treatment groups based on their dosages of 2AA: 0 mg/kg (control), 1 mg/kg (low dose), and 10 mg/kg (high dose). During the gestation and postpartum period, the dams were fed a 2AA contaminated diet based upon which group they fell into. Three months after weaning, the remainder of the rats were assigned to regular rat food and a moderately high-fat diet for six more weeks. Adipose tissue from the rat's abdomen was removed along with other tissues and the adipose tissue was homogenized in phosphate buffered saline (PBS). AT protein levels were measured. The results showed a significant increase in protein concentration in experimental groups suggesting an increase in oxidative stress and progression in the development of diabetes.

4337 Protective Role of Peroxiredoxin III against Hypoxia/Reoxygenation Injury in Cardiomyocytes

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In cardiomyocyte, mitochondria are a major source of energy, so they are important for cardiomyocyte function. Also, mitochondria are a major site of cellular reactive oxygen species (ROS) production, which leads to cell damage in cardio-myocytes. Hypoxia-reoxygenation (H/R) injury can cause an increase in mitochondrial ROS, which has been implicated in cardiomyocyte cell death and dysfunction. Peroxiredoxin III (Prx III) is the most abundant H2O2-removing enzyme in the mitochondria of mammalian cells. So, the protective role of Prx III against mitochondrial injury was investigated. Mitochondrial H2O2 levels and cardiolipin oxidation were determined using mitochondria-specific fluorescent probes after H9C2 cells were subjected to hypoxia for 1-2 hour and then reoxygenated for 1 hour. Mitochondria fragmentation was analyzed by confocal microscopy using mitochondria-specific fluorescent probes. Mitotracker-Red. Mitophagy flux was also detected by confocal microscopy using the mcherry-LC3 fluorescent probe. After the H/R treatment, Prx III knockdown H9C2 cells showed elevation of mitochondrial H2O2 compared to controls. In succession, macromolecule oxidation such as DNA, protein and cardiolipin oxidation in mitochondria were increased in PrxIII-knockdown H9C2 cells compared to controls. Prx III knockdown H9C2 cells were susceptible to apoptosis through mitochondrial membrane potential loss and caspase activation induced by H/R treatment. Also, mitophagy flux was blocked and mitochondrial fragmentation was exacerbated in PrxIII-knockdown H9C2 cells subjected to...
Mitochondrial Iron Uptake and Release Pathways: Their Contributions to Hepatotoxicity
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Biosynthesis of heme and Fe-S clusters essential for cellular function requires mitochondrial iron uptake. However, mitochondrial iron overload is damaging to organs like liver, heart, and brain. Fe²⁺ in the presence of hydrogen peroxide (H₂O₂) promotes the Fenton reaction, which produces toxic hydroxyl radicals (•OH), ultimately resulting in cell death. Mitoferrins1 and 2 (Mfrn1/2) and the electrogenic mitochondrial calcium uniporter (MCU) in the mitochondrial inner membrane are the principal pathways for mitochondrial iron transport by Mfrn1/2 remain incompletely understood. Our aim was to characterize the roles and relative contributions of Mfrn and MCU to mitochondrial Fe²⁺ uptake and release. In plasma membrane-permeabilized wild-type (WT) and MCU knockout (KO) hepatocytes, mitochondrial Fe²⁺ was monitored by confocal microscopy of the quenching of mitochondrially targeted mitoferrofluor (MFF). In MCU KO hepatocytes which retain active Mfrn, the rate of mitochondrial Fe²⁺ uptake decreased by two-thirds compared to WT, the remaining Fe²⁺ uptake in MCU KO being attributable to Mfrn. In Mfrn1/2 KO hepatocytes which retain active MCU, Fe²⁺ uptake was 4.2-fold greater than uptake by MCU KO hepatocytes. The uncoupler CCCP, which collapses mitochondrial membrane potential (ΔΨ), increased Fe²⁺ uptake by MCU KO hepatocytes by 5x-fold. The K⁺-ionophore valinomycin in high K⁺ medium, which also collapses ΔΨ, also increased Fe²⁺ uptake by 9-fold. Fe²⁺ uptake by uncoupled MCU KO mitochondria was unchanged in Na⁺-free buffer. By contrast, the K⁺/H⁺ exchanger nigericin, which increases ΔΨ, abolished Fe²⁺ uptake. These results suggest that Mfrn is not an Fe²⁺ importer, but rather is a reversible electrogenic exchanger catalysing exchange of Fe²⁺ for 3H⁺ or 3Na⁺. In WT hepatocytes after uptake of Fe²⁺, Ru360, an MCU inhibitor, was added to lock Fe²⁺ within mitochondria. Subsequently, starch-desferal (sDFO) was added to chelate extramitochondrial Fe²⁺ and create a gradient favoring Fe²⁺ release. After sDFO, MFF fluorescence began to progressively increase, suggestive of Fe²⁺ release. After extramitochondrial Fe²⁺ uptake and release of intramitochondrial Fe²⁺, Fe²⁺ release was unchanged in Na⁺-free buffer. CCCP inhibited Fe²⁺ release completely. By contrast, valinomycin doubled the rate of mitochondrial Fe²⁺ release, whereas nigericin decreased the rate by half, effects likely due to the increase or decrease of ΔΨ by valinomycin and nigericin, respectively. The results suggest that Mfrn and MCU play different roles in hepatotoxicity. Under circumstances leading to mitochondrial iron overload (e.g., acetaminophen overdose), high capacity MCU may be the main driver for Fe²⁺ uptake and thus a potentially druggable target. By contrast, Mfrn may provide a protective pathway for Fe²⁺ release after mitochondrial overload. Lastly, our results also indicate that Mfrn is a Na⁺-independent electrogenic exchanger, which likely catalyses Fe³⁺/3H⁺ exchange.

Detecting Protein Sulfenylation in Human Airway Epithelial Cells (HAEC) Exposed to Environmental Peroxides
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Exposure to airborne fine particulate matter (PM₂.₅) is a leading cause of morbidity and mortality worldwide. Exposure to PM₂.₅ is known to cause a range of pathological complications such as the onset of cardiovascular disease, respiratory infections, and premature death. The largest source of PM₂.₅ is derived from the oxidation of isoprene by hydroxyl radical to form isoprene hydroxy hydroperoxide (ISOPOOH). The atmospheric chemistry of ISOPOOH formation is well understood, however, relatively little is known about the adverse human health effects of ambient ISOPOOH exposure. We previously have demonstrated that ISOPOOH exposure induces oxidative stress in HAEC by inducing glutathione oxidation independently of the production of intracellular H₂O₂. By knocking down the expression of GPx4, we ablated ISOPOOH induced glutathione oxidation, thereby implicating GPx4 involvement specifically. We also demonstrated that ISOPOOH exposure of cellular and acellular membranes induces lipid peroxidation which can be modulated by supplementation with Omega-3 fatty acids. Our current hypothesis is that, in addition to ISOPOOH induced glutathione oxidation, ISOPOOH exposure of HAEC leads to protein sulfenylation. Specifically, GPx4D407S expression in HAEC cells leads to protein sulfenylation. In turn, leads to shunting of glucose to the pentose phosphate pathway. Our experimental approach primarily relies upon dimedone-based reagents and copper catalyzed azo-alkynyl cycloaddition reactions to tag intracellular protein sulfenic acids produced by the oxidation of cysteinyl thiols in GAPDH by low micromolar concentrations of ISOPOOH. Exposure of HAEC to GAPDH in low micromolar concentrations of ISOPOOH in the presence of glutathione led to glutathionylation of GAPDH. Tert-butyli hydroperoxide, another low molecular weight hydroperoxide, also induced protein sulfenylation and glutathionylation of GAPDH at low micromolar concentrations. Using a fluorescence imaging approach to detect protein sulfenylation in HAEC, we were able to demonstrate that ISOPOOH exposure led to intracellular protein sulfenylation. These results demonstrate that ISOPOOH is a potent environmental hydroperoxide capable of inducing oxidative stress through multiple mechanisms involving glutathione oxidation, lipid peroxidation, and sulfenylation of regulatory proteins in HAEC. This abstract does not necessarily reflect EPA policy.

Environmental Oxidant Chemicals Induce Altered Envelope Protein Profiles in Human Keratinocytes
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Cornified envelopes (CEs) of human epidermis consist of transglutaminase-mediated cross-linked proteins and are essential for skin barrier function. Present work explores the influence of oxidant pollutants on the CEs protein profiles. In this study, we performed proteomic analysis on CEs induced by three types of environmental ROS generators, 2,3-dimethoxy-1,4-naphthoquinone (DMNO), mesquite liquid smoke (MLS), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), to evaluate the impacts of oxidative conditions on the CEs proteome. Due to its non-adduct-forming property, DMNO [a redox cycling compound] produced the fewest changes in CE proteins compared to control CEs (induced by ionophore-mediated membrane permeabilization). MLS is an extract of woodsmoke condensate and is rich in carbonyls. Likely altering the CEs proteome through protein carbonylation, it stimulated incorporation of mitochondrial proteins as its main targets. Finally, TCDD altered the CE protein composition through changes in gene expression, especially those downstream of aryl hydrocarbon receptor signaling. Integrated incorporation into CEs of chaperone-mediated proteasomal proteins was found in all three treatments, indicating that even proteins functioning in protein quality control can become targets of oxidative stress. The accumulation of unwanted protein cross-links has been associated with aging and the progression of various human diseases. In the skin, oxidatively induced cross-linking could result in abnormal incorporation of cellular proteins into the CEs, leading to altered CE composition and barrier dysfunction. Thus, this study helps elucidate the potential of environmental stressors to trigger disease onset.

Detecting Protein Sulfenylation in Human Airway Epithelial Cells (HAEC) Exposed to Environmental Peroxides
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Environmental exposure to inorganic arsenic (iAs), mainly through contaminated drinking water, is a global health issue affecting more than 225 million people, with >2 million in the U.S. Exposure is associated with an increased risk of cancerous and non-cancerous diseases. AAs binds to thiol groups found in the amino acid cysteine, and cysteine-containing proteins are the likely molecular targets of iAs toxicity. Glutathione (GSH), a cysteine-containing tripeptide, protects against iAs by providing a binding partner other than critical proteins. The resulting GSH conjugates can be released from cells by specific export proteins. The purpose of the present study was to test the hypothesis that export of iAs-GSH conjugates protects cells from cytotoxicity by limiting the intracellular accumulation of iAs. Three keratinocyte cell lines (HaCaT, Ker-CT and HEKn) were exposed to iAs as sodium arsenite for up to 72 hours. Cytotoxicity was determined by Alamar Blue staining. Intracellular and extracellular iAs content was measured by ICP-MS. Intracellular and extracellular GSH was measured by HPLC. One-Way ANOVA followed by Tukey’s Multiple Comparisons test or unpaired two-tailed t-test, depending on the nature of the data, were performed using GraphPad Prism 9; p < 0.05 was considered significant. iAs-cytotoxicity dose-response curves showed that HaCaT cells were the most sensitive to acute iAs toxicity. Ker-CT cells were the most resistant, and HEKn cells had an intermediate sensitivity to iAs toxicity. Following exposure to 5 pM iAs, HCT acetate-mediated iAs among the three cell lines studied, followed by HaCaT and HEKn cells. iAs was exported at the same rate by the three cell lines, but there were significant differences in the amount of GSH exported by the 3 cell lines; Ker-CT cells released the most GSH, followed by HaCaT and HEKn cells. Intracellular GSH was increased following iAs exposure in all 3 cell lines; however, there were differences in the magnitude and duration of the GSH response. Ker-CT cells (the iAs-resistant cells) had the most robust induction of GSH, and GSH levels remained elevated for at least 72 hours. In contrast, HaCaT cells (the iAs-sensitive cells) had the lowest induction of GSH, and their GSH levels had returned to normal within 48 hours. Uregulation of GSH production was correlated with resistance to iAs cytotoxicity. However, this protection was not mediated by fostering the export of intracellular iAs.
Disruption of Hepatic Cytochrome P450 Reductase in Mice Leads to Increased Susceptibility to Hyperoxic Lung Injury

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Supplemental oxygen is often used for the treatment of patients suffering from pulmonary insufficiency. Identification of mechanisms that lead to acute respiratory distress syndrome (ARDS) is necessary for the development of new preventive and therapeutic strategies. Hyperoxia is known to contribute to ARDS through many molecular mechanisms linked to increased oxidative stress and production of reactive oxygen species. This causes damage to many cellular components such as DNA, lipid, and protein. We have previously documented the protective effects of mammalian hepatic cytochrome P450 (CYP1A1) against hyperoxic lung injury, revealing a critical role for extra-pulmonary organs such as liver in the protection against lung injury by metabolizing potential mediators of ARDS including lipid hydroperoxides. In this study, we tested the hypothesis that liver-CPR-null mice will display increased susceptibility to hyperoxic lung injury compared to wild-type (WT) mice. Eight-week-old liver-CPR-null mice and WT mice were exposed to hyperoxia (O2 95%) or room air for 24-72 hours. Lung injury was evaluated by lung weight/body weight (LW/BW) ratio and histology. CPR, CYP1A protein contents and enzyme activity in lung and liver were determined by western blot and fluorimetry. Gene expression at the mRNA level was determined by RT-PCR. Liver-CPR-null mice were more susceptible to oxygen-mediated lung damage and inflammation compared to WT mice, as evidenced by increased LW/BW ratio, lung injury, neutrophil recruitment and augmented levels of IL-6 and TNF compared to WT mice. CYP1A enzyme activities were decreased in liver of CPR-null mice compared to WT mice. In conclusion, our results, showing increased lung injury and inflammation under hyperoxic condition in CPR-null mice compared to WT mice, support the idea that liver cytochrome P450 plays a protective role against hyperoxic lung injury.

Sex-Dependent Increases of Carnitine Derivatives in the Liver by Myocardial Infarction

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Many types of diseases and environmental stressors cause an increase in oxidative stress. Urban air pollution leads to inhalation of airborne particulate matter in high traffic environments. This has been linked to chronic oxidative stress and increased risk or severity of myocardial infarction (MI). MI is known to cause acute oxidative stress. A key player for cellular defense against oxidative stress is activation of Nrf2, a transcription factor that controls the expression of key antioxidant and cytoprotective genes. Although Nrf2 has been reported to regulate genes for anabolic or lipid metabolism, little is known about the impact of Nrf2 or MI on the biochemical metabolism in the liver. Because the liver is a center for metabolism, metabolomic landscapes were determined in a model of chronic oxidative stress by Nrf2 knockout mice. Exposure stress by induction of MI leads to anterior descending coronary artery. Using LC-MS/MS based non-targeted metabolomics, 1353 compounds were detected with a quality control coefficient variation value <20%. Differences in the baseline metabolic profiles of male versus female lead to comparison of Nrf2 knockout or MI in a sex specific manner. In male wild type mice, the liver responded to MI by elevating 11 carnitine derivatives, including linoleoyl carnitine, palmitoyl carnitine, and (11Z, 14Z)-eicosadienoyl carnitine. However, none of these changes were observed in female wild type mice. Either male or female Nrf2 knockout mice failed to respond to MI by elevating carnitines. Because carnitines serve to transport long-chain fatty acids into the mitochondria for energy production through beta-oxidation, this data suggest that male mice are capable of reprogramming energy metabolism by increasing carnitines to compensate for energy production. Nrf2 appears to play a role in such metabolic reprogramming.

The Oxidative Stress Protector FOXO Governs Skeletal Deformities and Osteogenic Differentiation in Response to Prenatal Snus Exposure

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In the US, 10-12% of women still consume tobacco during pregnancy and often use "harm reduction" products including combustion-free and smokeless Snus. However, the ramifications of maternal Snus consumption on skeletal development are largely unknown. To characterize the skeletal phenotype arising from exposure to Snus in pregnant mice, we used "harm reduction" products including combustion-free and smokeless Snus. Dosed in utero, Snus-exposed offspring exhibited fewer differentially expressed genes (DEGs), although there was significant overlap when comparing Snus-exposed mice to DEGs. Benchmark Dose (BMD) analysis revealed similar trends, with most induced DEGs, including several AhR-battery genes (e.g. Cyp1a1), exhibiting unexpectedly lower BMDs at T20. Moreover, differences in the magnitude transcription factors marking them for nuclear exclusion. Knockdown of FOXP1/3a in hESCs and a Wnt1-Cre-driven Foxo3a knockout in mice phenocopied Snus exposure suggesting that at least some of the exposure-related effects to the skeleton were mediated by misregulation of FOXOs. Together, our study suggests for the first time a potential mechanism underlying changes to bone architecture and phenotype that may result in an increased incidence of osteoporosis and other related bone diseases in patients born to smoking parents as detected in epidemiological studies.
of gene induction were minimal, suggesting differences in response at T	subscript⇌ and T	subscript⇌⇌ were not due to toxicokinetics. Overall, T	subscript⇌⇌ showed reduced hepatoxicity at equivalent doses compared to mice held at T	subscript⇌, WUS was supported by T32ES00725S. This project was funded by R01ES029541.

4347 Single Cell Transcriptomics Unveiled That Early Life Exposure to BDE-99 Persistently Downregulated Drug-Metabolizing Enzymes but Upregulated Pro-inflammatory Pathways in Male Mouse Livers at Late Adulthood

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Increasing evidence suggest there is a sensitive developmental time window for toxic exposures to have a life-long impact on disease risks. Polychlorinated diphenyl ethers (PBDEs), linked to diseases such as thyroid and immune dysregulation, and metabolic diseases in both animal models and humans, are a class of legacy flame retardants that bioaccumulate in the environment raising global health concerns. The liver is a central organ for xenobiotic biotransformation, nutrient homeostasis, and immune regulation, and the gut microbiome is an important regulator of hepatic functions. Previously, using bulk-RNaseq, we showed that early life exposure to BDE-99, a human breast milk-enriched PBDE congener, developmentally reprogrammed the hepatic transcriptome. However, it remains unclear whether early life exposure-mediated disease risk is attributable to cell-type-specific dysfunctions and dysregulated interactive networks in later lifespans.

We hypothesized that neonatal exposure to BDE-99 developmentally reprograms the gut environment and hepatic immune cells, and result in dysregulation of metabolic signatures in late adulthood. From postnatal days 2-4, male mouse pups were orally exposed to corn oil (10 ml/kg) or BDE-99 (57 mg/kg), once daily. At 15 months of age, livers were collected and dissected, which were subject to single cell RNA sequencing (scRNA-seq, 10X Genomics). Filtering, clustering, and differential expression analyses (Bonferroni-adjusted p-value < 0.1) were performed using Seurat v4. neonatal BDE-99 exposure led to down-regulation of key drug- and fatty acid metabolizing enzymes (e.g. Cyp1a2, Cyp2b10, Cyp3a11, and Cyp4a10) in livers of adult pups. Interestingly, Neonatal exposure to BDE-99 decreased expression of intestinal tight junction proteins (i.e. Tjp1 and Tjp2) in adult male pups, suggesting increased gut permeability. Associated with neonatal BDE-99 exposure-mediated persistent gut dysbiosis in adulthood, we observed a predicted increase in microbial influx (gene ontology enrichment of hepatocyte populations), a persistent increase in the hepatic proportion of neutrophils, and histopathological abnormalities in livers of pups at adulthood. Ligand-receptor analysis (Cellchat) showed that early life BDE-99 exposure led to a predicted increase in the pro-inflammatory macrophage migration inhibitory factor (MIF) signaling, which activates macrophage populations in livers. This was supported by a persistent increase in the transcripts of pro-inflammatory signaling molecules Cxcl10 and Il6 in hepatic macrophage populations of adult male pups following neonatal exposure to BDE-99. Together this suggests that dysbiosis in the gut microbiome may be a contributing factor in PBDE-mediated pro-inflammatory signaling in adult mouse livers. scRNA-seq in germ-free mice further confirmed the necessity of a normal gut microbiome in maintaining hepatic immunotolerance.

In conclusion, we showed that early life exposure to BDE-99 results a persistent up-regulation in hepatic inflammation and a persistent down-regulation in hepatic drug metabolism pathways. At the single cell resolution, our results demonstrate that individual cell types in the liver are susceptible to developmental reprogramming by early life exposure to BDE-99, even at fully matured adults, and suggest that the gut-liver axis mechanistically contributes to developmental reprogramming and late onset of liver diseases later in life.

4348 Early-Life Exposure to an Organic Pollutant Results in Persistent Changes to the Microbiota and Host Glucose Homeostasis Changes to the Microbiota and Host Glucose Homeostasis

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Associations between persistent organic pollutant (POP) exposure and increased risk of metabolic disorders including obesity and diabetes are increasingly reported; however, it is unclear if and how the microbiome contributes this relationship. Here, we show that early-life exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice resulted in persistent microbiota disruptions associated with the impaired glucose homeostasis later in life. Early-life five days exposure to TCDD (24 µg/kg body weight per day) rapidly and significantly increased liver lipogenesis in mice in an aryl hydrocarbon receptor (AHR)-dependent manner. We observed a prominent disruption in the gut microbiome in TCDD-treated mice even after TCDD was eliminated from the body. TCDD-exposed mice exhibited a profound disruption in the gut microbiome, characterized by the loss of Lactobacillus plantarum (LPS) which included an increase in the number of colony forming units (CFUs) from 5.8 ± 6.1 µmol/g [vehicle] to 50.7 ± 3.6 µmol/g [TCDD]) and indole-3-lactic acid (ILA) (ILA decreased from 0.55 ± 0.07 nmol/g [vehicle] to 0.28 ± 0.03 nmol/g [TCDD], and reduction of gut microbiome glucose-like peptide 1 (Glp1) and adiponectin tyrosine-tyrosine (PYR) (Glp1 decreased from 0.85 ± 0.47 [vehicle] to 0.35 ± 0.26 [TCDD]; PYr decreased from 2.76 ± 1.99 [vehicle] to 0.90 ± 0.39 [TCDD]). Cecal microbiota transplantation from TCDD-exposed mice showed impaired glucose homeostasis in germ-free (GF) mouse recipients. Consistent with the in vivo study, TCDD exposure significantly affected the growth, physiology, metabolism, and gene expression of Akkermansia muciniphila including the downregulation of Akkermansia muciniphila-derived ILA pathway in vitro (ILA decreased from 1.38 ± 0.45 nM [vehicle] to 1.11 ± 0.33 nM [TCDD low] and 0.96 ± 0.23 nM [TCDD high]). These data provide new insights into the biochemical consequences of early-life environmental chemical exposure involving the development of metabolic diseases and suggest a role for pollutants as disruptors of host and bacterial metabolism.

4349 Free-Range Eggs Dioxin Contamination Assessment: Comparison between a Simple Model and In Situ Measurements to Determine a Maximum Egg Consumption Frequency

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Polychloribenzene-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are an aromatic organochlorine group, whose primary sources of emission are unintentional industrial processes involving incomplete combustion. These compounds are easily dispersed, highly lipophilic, persistent, and highly biodegradable. They are classified as persistent organic pollutants (POPs), and bioaccumulate in fat-rich tissue along the trophic chains. For this reason, food of animal origin is the main environmental source of human exposure to PCDD/Fs. This was confirmed by the most significant exposure scenarios identified in the health risk assessment carried out following the discovery of a high PCDD/Fs soil contamination in Lausanne (Switzerland). The consumption of eggs from free-range laying hens raised on contaminated soil was clearly one of the highest risk of PCDD/F contamination through ingestion. The runs made available to poultry favor their innate behavior to forage their feed outdoors, but also to ingest relevant amounts of soil. The PCDD/Fs absorbed from soil are further accumulated into their body fat and eliminated through the egg’s yolk. The objective of the study was then to simulate the expected PCDD/F concentrations in eggs as a function of the PCDD/F concentrations in soil using an existing animal toxicokinetic model, and to recommend a maximal egg frequency consumption in humans for not exceeding the tolerable daily intake. A laying hen physiologically-based toxicokinetic model was used to predict the different parameters for local data focusing on soil PCDD/Fs concentration and hen’s intake level. Specifically, soil and eggs were sampled from five henhouses in the contaminated area of Lausanne. PCDD/Fs were extracted using an Accelerated Solvent Extraction for the soil, and by a liquid-liquid extraction for the egg yolk. Extracted samples were then quantified by gas chromatography high resolution mass spectrometry. The results shown an overestimation of the observed concentrations by the model. The percentage bias (PBias) was estimated at 82.3% (with one extreme value). The model demonstrated that PCDD/F concentrations reached an equilibrium in eggs after an exposure of 200 days in hen. When PCDD/F concentration was measured at 60 days, the back-calculated PCDD/F concentration was 28% below the experimental data. The PCDD/F half-life in egg was estimated at 50 days. The most sensitive exposure and physiological parameters influencing PCDD/F concentrations in eggs were (in descending order): the soil concentration, the geophagy (amount of soil ingested by the hen during pecking), the age of the hen (or its time of residency on the contaminated field), and the egg-laying rate. The throw parameter was difficult to estimate in the field, although it induce a high variability in PCDD/F concentrations in eggs. Overall, the results pointed out that the food marketing limit values for PCDD/Fs in eggs may be exceeded at PCDD/F soil concentrations as low as 5 ng Toxic Equivalent (TEQ)/kg soil. At soil high concentration, gradual measures should be implemented for consumers to limit their consumption frequency, and the plant coverage should be improved in the henhouses.

4350 In Vitro Release of 4-OH-PCB52, a Human-Relevant Polychlorinated Biphenyl (PCB) Metabolite, from a Novel Polymeric Implant System

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Past literature suggests that airborne PCBs lead to adverse neurobehavioral outcomes (i.e., development of ADHD, ASD, or anxiety). These airborne PCBs are easily metabolized to hydroxylated metabolites (OH-PCBs), with different metabolites forming in rats and humans. Our overarching goal is to inform upon develop the role of airborne PCBs in neurotoxic outcomes forming PCB exposure during adolescence. To attain this objective, we plan to expose rats to the human OH-PCB metabolite using novel polymeric subcutaneous implants. The current experiment investigates the in vitro release of 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB52) from polymeric implants for in vivo toxicity studies in rats.
Polychlorinated biphenyls (PCBs) are persistent environmental pollutants that are found in aqueous environments and are enriched in fatty compartments of many aquatic species that are consumed by humans. PCBs are neurotoxicants that have been implicated to cause neurobehavioral changes in animals. The gut-brain axis is increasingly recognized as a novel contributor to neurodevelopmental disorders (NDDs). Dysbiosis of the gut microbiome may reduce neuroprotective microbial metabolites, or increase neurotoxic microbial metabolites and pro-inflammatory cytokines to promote neuroinflammation, which are known risk factors for NDD. As a first attempt to test our hypothesis that the gut-brain axis mediates PCB neurotoxicity, we determined to what extent maternal PCB exposure modulates the gut microbiome in offspring. C57BL/6 mouse dams were randomly assigned to receive either vehicle (peanut butter and peanut oil mixture) or the Fox River PCB mixture at a daily oral dose of 0.1, 1.0 or 6.0 mg/kg body weight. Large intestinal contents of male and female pups were collected at postnatal day (PND) 28 and PND 35 (n≥5 per group). Metagenomic shotgun sequencing was performed to determine the compositional and predicted functional changes in the gut microbiome over time. DNA sequences were aligned to a curated database containing all represented genomes in RefSeq for bacteria, with additional manually curated mouse specific high quality Metagenomically Assembled Genomes and cell cultured genomes. Alignments were made at 97% identity against all reference genomes. Samples with more than 10,000 sequences were kept for downstream analysis and the number of counts for each operational taxonomic unit (OTU) was normalized to the OTU’s genome length. Functional changes of the microbiome were predicted by comparing the abundance of the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology groups. At the species level, maternal PCB exposure produced gut dysbiosis in the offspring of both age and sex groups, with females being more susceptible than males at both ages, and changes at PND28 being more prominent than PND 35 for both sexes. For example, in PND 28 females (the most susceptible group overall), maternal PCB exposure dose-dependently increased Anaplaspora symbiotica, a microbe involved in quorum sensing, Bradyrhizobium sp002831585, a known opportunistic human pathogen, and Erysipelotrichis cocleatum, a known microbe involved in dopaminergic neurotoxicity in the brain. A dose-dependent increase of a known microbe involved in dopaminergic neurotoxicity in the brain, Parabacteroides Gordonii, Enterococcus gallinarum, Faecalibacterium prausnitzii, Alistipes, and Enterobacter cloacae, which are implicated in developmental neurotoxicity through the gut-brain axis, were also observed in PND 35 males. Interestingly, most of the PCB effects on gut microbiome of the offspring were non-monotonic. Thus, varying doses groups exhibited the aligned reads against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The resulting implants were extracted using hexane and MTBE (9:1, v/v), before being heated at 70°C, the PCBs were released from the implants and their concentrations were determined using GC/MS. The implants were prepared by dissolving polycaprolactone and pluronic (10:1, w/w) in dichloromethane, and then being dried in oil bath and re-dissolved in proper silicone tubing. The resulting implants were about 20 mm in length and about 2.3 mm in diameter and contained low (1% load), medium (5% load), and high (10% load) levels of 4-PCB52. The implants were incubated in PBS (containing 10% bovine calf serum and 1% Penicillin-Streptomycin) and kept in a 37°C shaking water bath at 75 rpm for 28 days. The implants from the implants into the media was extracted using hexane and MTBE (9:1, v/v), and 4-PCB52 was quantified using a UV-vis spectrometer at a wavelength of 285 nm. The in vitro release analysis found overall similar trends of release in all three dose levels of the implant for the 28 days observed; the first day had the greatest amount of 4-PCB52 released, the release rate showed a downturn over time, and at around day 12 the release rate began stabilizing. By day 28, 91%, 82%, and 78% of the total loaded PCB was released from the implant; 3%, 4%, and 5% remained in the implant in the low, medium, and high dose groups, respectively. The current experiment demonstrates that the polymeric implants continuously release 4-PCB52 and, thus, can be used to expose rats to this human-relevant PCB metabolite once implanted subcutaneously. In the future, neurobehavioral testing will be conducted on the implanted rats to determine the adverse behavioral responses caused by continuous exposure to 4-PCB52.
higher PCB 126 concentration in the liver compared to the KO mice (WT 56 mg/ mg, FMO3/- 24 mg/mg, p<0.001). Based on these results, we could have a significant impact on the expression of FMO3, which is critical for the metabolism of several drugs and dietary molecules. The absence of FMO3 leads to a completely different response to a dioxin-like PCB in the liver.

**4354 Impact of Long-Term Aroclor 1260 Exposure on Gut Microbiome and Intestinal Toxicity**


Polychlorinated biphenyls (PCBs) are persistent organic pollutants manufactured in the 1930s -1970s for industrial purposes, and commercially marketed under the trade name Aroclor (USA). Although banned in the 1970s, and worldwide in 2001, they are still found in the environment due to their thermodynamic stability and resistance to degradation. Previously our group demonstrated that sub-chronic exposures to the PCB mixture (Aroclor 1260) resulted in toxicant- associated steatohepatitis (TASH) in a diet-induced obesity model. This is in part due to constitutive androstane receptor (CAR) and Pregnane X receptor (PXR) activation, and their ability to regulate gut microbiome composition. However, the longer-term effects of PCBs on ileal and gut microbiota regulation have not been studied. Therefore, this project aims to understand how longer-term exposure to Aroclor 1260 impacts ileal toxicity and gut microbiota. We hypothesize that Aroclor 1260 will induce changes in gut microbiome composition and alter the expression of ileal genes encoding for epithelial integrity. Low-fat diet-fed male C57BL/6J mice were exposed to a single oral gavage of either corn oil (vehicle control, n=10) or Aroclor 1260 (20 mg/kg, n=30) and allowed to age for 34 weeks. Aroclor 1260, a commercial PCB mixture, was selected based on PCB composition reflective of PCB bioaccumulation patterns in humans. Cecum and ileum samples were collected for gut microbiome assessment and ileal mRNA measurements. 16S sequencing was performed on cecal bacterial DNA samples and analyzed using QIME2 while RT-qPCR was used to examine ileal gene expression. For gene expression, statistical analysis was performed on GraphPad Prism (v9.4.1) using the Mann-Whitney U test with a significance level set at 0.05. Metagenomic and metatranscriptomic analyses were performed in alpha and beta diversity between the PCB and control groups. In addition, beta diversity which was measured using weighted and unweighted UniFrac and Jaccard index revealed no differences as well. However, the principal component analysis on beta diversity in both UniFrac and Jaccard algorithms demonstrated two distinct clusters in the PCB-exposed groups, indicating that this mixture itself is capable of modulating the microbiome. Importantly, taxonomic differences evaluated revealed increased abundance of Proteobacteria phylum in the PCB-exposed group compared to the control group. Proteobacteria abundance has previously been correlated with increased intestinal inflammation. Ileal expression of genes encoding markers of intestinal permeability and inflammation was examined using RT-qPCR. Ileal expression of Cdh5, an endothelial epithelial integrity marker with PCB exposure, implicating disruption of intestinal barrier integrity. There was also a non-significant trend for decreased mRNA levels of cathelicidin antimicrobial peptide (CAMP) in the PCB-exposed group (p=0.0552) compared to the control group, suggesting the presence of bacteria infiltration. Furthermore, ileal mRNA levels of TFF3, the gene encoding Trefoil factor 3, involved in the maintenance and repair of the intestinal mucosa, were increased with PCB exposure. In conclusion, the preliminary results suggest that longer-term PCB exposures affect markers of gut barrier function and increase the abundance of Proteobacteria in the PCB-exposed groups. Although, there were no changes in bacteria diversity, further studies will investigate the two distinct clusters in the PCB-exposed groups to determine if there are alterations to gut microbiome composition in those groups and correlate those findings with liver disease endpoints.

**4355 Sex Differences in the Hepatic Proteome and Gut Microbiome following Polychlorinated Biphenyl Exposures: Implications on Gut-Liver Axis Disruption in Females**

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Identifying sex-specific health outcomes resulting from pollutant exposures is fundamental in environmental health for better risk assessment in exposed populations. Our laboratory group previously demonstrated that exposures to polychlorinated biphenyls (PCBs) led to sex-dependent liver outcomes with female mice exhibiting greater susceptibility for developing toxicant-associated steatohepatitis (TASH). Although underlying mechanisms such as PCB-modulated endocrine disruption have been identified, they do not sufficiently explain these sex-specific outcomes. Other mechanisms including PCB effects on the gut microbiome and the gut-liver axis have been investigated. Therefore, the objective of the current study is to identify PCB-induced changes in the hepatic proteome and gut microbiome to determine disruption of physiological gut-liver interactions as a cause for sex-dependent PCB toxicity. Male and female C57BL/6 mice were exposed to a mix of Aroclor1260 (commercial PCB mixture, 20 mg/kg) and PCB126 (20 μg/kg) for 2 weeks. Hepatic tissue was collected at euthanasia for peptide measurements (LC/MS); and data analysis was performed using MetaCore. Cecal and ileal samples were isolated for 16S sequencing and gene expression (RT-qPCR) analysis respectively. 16S data was analyzed using QIIME2. 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 with 297 proteins (197;100) modified in PCB-exposed males vs. females. While both PCB-exposed males and females showed increased levels for the aryl hydrocarbon receptor (AHR) targets such as Cyp1a2, indicating AHR activation, female mice also exhibited increased levels of other AHR targets including flavin-monooxygenases (validated by western blot). Computational analysis including “GO Process” showed key processes and pathways enriched in PCB-exposed females such as “fatty acid metabolism” and “dibenzo-p-dioxin metabolic process”; increased over-connectedness of hepatic xenobiotic/endobiotic receptors (PXR, SREBP1); and low-density lipoprotein. Notably, “Transcription Factor Analysis” demonstrated that PCB-exposed female mice had decreased activation (z-score) for hepatocyte nuclear factor (HNF4a), which is critical for liver homeostasis, and upregulated activity to xenobiotics. PCB-exposed females exhibited the lowest bacterial diversity than any other group (alpha diversity, observed taxonomic units). Beta diversity, measured using UniFrac, was also significantly different with both sex and PCB exposures. PCB-exposed females but not males showed decreased abundance for Bifidobacteriaceae, a bacterial family associated with health promoting effects on the host liver. Ileal gene expression assessment demonstrated lower mRNA levels in PCB-exposed females for genes encoding gut barrier proteins, namely Cldn2 and Muc2, and the antimicrobial peptide, namely Tfif3, thereby implicating an unhealthy mucosal environment. Taken together, the multi-organ findings suggested that female mice were more responsive to hepatic AHR activation and altered lipid processes, and this was reflected a modified gut microbiota with compromised, intestinal environment, and these potentially contributed to the observed steatosis and inflammation. Future studies will include bacterial functional metabolomics to obtain further insight on how altered gut microbiota impact PCB toxicity in females, and designing intervention strategies such as probiotics to attenuate PCB-mediated TASH.

**4356 Dietary Exposure to the Flame Retardant BDE-99 Induces Multigenerational Behavioral Alterations in Atlantic Killifish**

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To truly evaluate the long-term impacts of exposure to environmental pollution, it is critical to test for intergenerational (PI) and intragenerational (PBDE) flame retardants are highly persistent and ubiquitous in the environment, yet little is known about their potential for multigenerational toxicity. Direct exposure to one of the two most dominant PBDE congeners, BDE-99, has been linked to various adverse impacts on development, neurobehavior, and reproduction, but studies focusing on multigenerational effects are lacking. To address this, we tested whether exposure to BDE-99 has negative effects that propagate across generations using Atlantic killifish (Fundulus heteroclitus) as a vertebrate model system. Adult wild-caught killifish were fed control or contaminated diet for 64 days during breeding season with two concentrations of BDE-99, 37.5 and 150 ng/g fish wet weight. The exposed fish were spawned to produce the F1 generation, which was raised under uncontaminated conditions and subsequently spawned to produce the F2 generation. No significant differences in fecundity, fertilization rate, developmental morphology, hatch success, or larval survival were observed between treatments in any generation. To evaluate neurobehavioral impairment in the F1 and F2 generations, larval locomotor activity was tracked in response to alternating light-dark conditions. Parentally exposed F1 fish from some of the BDE-99 lines expressed significant hypoinactivity during the light phases, while results suggest that that behavior is not altered in F2 larvae. At juvenile stages, novel tank diving tests were conducted to assess anxiety-like behavior in the F1 and F2 generation. These results indicate no anxiety-like behavior (gender and generation) in the F1 tank; however, attentional-like behavior (gender and generation) in the top zone of the tank) in the F1 fish parentally exposed to BDE-99; however, this behavioral effect was not propagated to the F2 generation. Archived juvenile brain tissues are being analyzed for genome-wide gene expression changes to infer potential mechanisms associated with observed behavioral phenotypes in the parentally exposed generation and generate hypotheses about other multigenerational neurotoxic effects of BDE-99.

**4357 Maternal PBDE Exposure Disrupts Gut Microbiome and Promotes Hepatic Pro-inflammatory Signaling in Humanized PXR-Transgenic Mouse Offspring over a Time Course**

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Polybrominated diphenyl ethers (PBDEs) are previously used flame retardants that still bio-accumulate in human compartments, including the maternal blood and breast milk. Previous work showed that developmental PBDE exposure is associated with increased diabetes prevalence in humans and animal models. PBDEs are
known to activate the host pregnane X receptor (PXR). PXR and gut microbiome are essential regulators of xenobiotic biotransformation and metabolic disorders. The microbial tryptophan metabolite, indole-3-propionic acid (IPA), correlates with reduced risk of type-2 diabetes and lower-grade inflammation and activate PXR. To explore the potential role of IPA in modifying the toxic effects of PBDEs during development, we exposed female humanized PXR-transgenic (HPXR-TG) mice dams to vehicle, 0.1 mg/kg/day DE-71 (an industrial PBDE mixture) via diet, DE-71 + IPA (20 mg/kg/day) via drinking water, or IPA, from 4-weeks preconception to the end of lactation. Male and female pups were weaned at 21-days of age and were placed on control diet with or without IPA supplement. Tissues were collected at 21 days, 3 months, and 6 months of age (n=5/exposure/sex/age). In general, the effect of maternal DE-71 exposure on the gut microbiome of pups was amplified, as there was a more differentially regulated obesity- and inflammation-prone microbes at 3-months and 6-months of age than 21-days of age. In the liver, the mRNA expression of several pro-inflammatory markers was also consistently up-regulated in pups with an amplified trend along the developmental trajectory. The hepatic mRNA of the aryl hydrocarbon receptor (AhR) target gene cytchrome P450 1A2 (Cyp1A2) was more consistently increased by maternal DE-71 exposure in all ages of male pups, but not in female pups. Interestingly, LC-MS showed that maternal DE-71 exposure also consistently increased serum levels of indole, which is a known endogenous AhR ligand, predominantly in male pups. The oxidative stress sensor Nrf2 target gene NQO1 was increased in maternal DE-71 exposure in both sexes and increased with age. The hepatic mRNA expression of the oxidative stress sensor Nrf2 target gene NQO1 was increased in maternal DE-71 exposure in both sexes and increased with age. These data indicate that different strains of S. aureus can cause larger bacterial burden in pressure wounds and increase wound size which can be exacerbated by exposure to OC pesticide metabolites. Thus, exposure to OC pesticides and their bioaccumulative metabolites may be a risk factor for poor wound healing in S. aureus infected wounds.

After disasters, high levels of benzene can be detected and redistributed in the environment surrounding disaster sites, posing a significant risk to human health and the environment. Remediation methods with traditional sorbents are lacking in effectiveness due to benzene’s limited retention to most surfaces. To address this problem, a montmorillonite clay was amended with a mixture of chlorophyll (a) and (b), and the binding profile was assessed using in vitro, in silico, and well-established ecotoxicological bioassay methods. In vitro and in silico studies indicated that chlorophyll increased the adsorption and retention of benzene on clay surfaces through hydrophobic interactions. In vivo studies in mice inoculated with UAMS-1 at day 4. These data indicate that chlorophyll-amended clay in the culture medium significantly reduced benzene toxicity, protecting C. elegans by 98-100% from benzene-induced mortality and enhancing the growth rates of L. minor. This novel glass clay was also tested for its ability to remediate water samples from a Superfund site known to be contaminated with semivolatile polycyclic aromatic hydrocarbons (PAHs). Exposure to the polluted water samples decreased important growth parameters of L. minor and C. elegans by >97% and >80%, respectively. However, the addition of 0.5% chlorophyll-amended clay or activated carbon showed 50% or 70% protection for the plant and nematode model, respectively. Importantly, the proportional reduction of 25% of a mixture of 5% activated carbon and 25% chlorophyll-amended clay significantly reduced the growth rate of both organisms across various water samples. These results indicated that very low levels of multicomponent sorbents were effective for remediating real-life samples containing complex mixtures of priority chemicals. Further studies are ongoing to identify additional lipophilic contaminants that can be sequestered by this sorbent mixture. Our current and ongoing studies suggest that these novel sorbents can be utilized against chemical exposures during disasters and emergencies.

It is estimated that the lifetime risk for developing diabetic foot ulcers (DFUs) in diabetic patients is around 12-25%. Approximately 40-60% of all DFUs will become infected ultimately leading to worse outcomes and amputation due to incomplete healing. The infections in DFUs are polymicrobial in nature. However, studies have shown that Staphylococcus aureus is the primary pathogen isolated from the majority of these DFUs. Previously, exposure to persistent organic pollutants, specifically organochlorine (OC) pesticide metabolites, have been shown to alter macrophage function which increases susceptibility to S. aureus. However, the effects of exposure to OC pesticides on S. aureus infected wound outcomes has not been investigated. Thus, the present study was done to determine the effects of exposure to an environmentally relevant mixture of three prevalent organochlorine pesticide metabolites, DDE, trans-nonachlor, and oxychlordane (TO), on persistence and healing with infection of either a UAMS-1 strain (biofilm forming strain) or DFU317 strain (isolated from a human DFU) of S. aureus. For five consecutive days, male C57BL/6J mice were dosed with either corn oil (com oil; 1 ml/kg; n=5/group/strain) or DDE (DDE 2 mg/kg; trans-nonachlor 0.2 mg/kg; oxychlorodane 0.1 mg/kg; n=5/group/strain) and then rested for 12 days to allow levels to reach a steady state. Pressure ulcers were created on the backs of the mice during the two alternating 12 hour on-off periods using magnets. After the final magnet removal, both wounds on the back were inoculated with either the UAMS-1 strain or the DFU317 strain (1x10⁷ cfu/ml) of S. aureus. Wound sizes were measured on day 1 and 4 after inoculation and cytotoxic virulence factors and bacterial burden were measured at day 4 after inoculation. DTE-71 inoculated mice had significantly larger wounds than vehicle and DTE treated mice without infection at day 1. DTE treated, DFU317 inoculated mice had a larger bacterial burden than vehicle treated mice inoculated with UAMS-1 at day 4. These data indicate that different strains of S. aureus can cause larger bacterial burden in pressure wounds and increase wound size which can be exacerbated by exposure to OC pesticide metabolites. Thus, exposure to OC pesticides and their bioaccumulative metabolites may be a risk factor for poor wound healing in S. aureus infected wounds.

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Based on the biomarker concentrations, the exposure amount was estimated to investigate the possibility of the Korean population being exposed to PFOA/PFOS at or above Tolerable Daily Intake (TDI). The exposure was reconstructed using the published physiologically based pharmacokinetic (PBPK) model. As a result, the average exposure to PFOA and PFOS in adults was 0.72 ng/kg/day and 2.0 ng/kg/day, respectively. The Korea Ministry of Food and Drug Safety has established TDI as 1.0 ng/kg/day (PFOA) and 1.5 ng/kg/day (PFOS) for both. The calculation showed that the HBM exceeding the HBM was high, there were no subjects exceeding the domestic exposure standard. The reference values for perfluorinated compounds tends to be lowered based on the results of epidemiological studies. US EPA, EFSA, and Australian government set the reference dose of 20 ng/kg/day, TWI of 4.4 ng/kg/day, and TDI (PFOA: 160 ng/kg/day, PFOS: 20 ng/kg/day), respectively. It is necessary to re-establish the domestic reference value by identifying the major exposure source for the subpopulation.

4362  Dosimetry and Potential Bioaccumulation of a GenX Oligomer HFPO-TeA

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Acute and long-term exposure to per- and polyfluoroalkyl substances (PFAS) is of rising concern due to their persistent detection and abundance in the environment coupled with their potential for bioaccumulation. The replacement of legacy PFAS with ether-linked carboxylic acid (PFECA) fluorochromes is creating new exposure concerns. Herein, we describe the US EPA dosimetry model development for HFPO-TeA, an understudied oligomer of GenX that has been detected widely in China. However, little is known about its hazard, making it a priority within the pathway with the US EPA regions to understand toxicity, dosimetry, and bioaccumulation, male and female Sprague Dawley rats were orally dosed with several levels of HFPO-TeA. Dose levels ranged from 0.3-335 mg/kg/day in corn oil. Liver tissue and blood were collected on day 6. HFPO-TeA internal dose concentrations were observed in plasma dosimetry data at 6.3 and 17 mg/kg/day dose levels. Concentrations of HFPO-TeA increased linearly over the dose range for both males and females. Concentrations were observed in plasma after 5 days were approximately 6 times higher than those collected after 1 day survived the length of the study. Male and female rats showed several clinical symptoms. The PBPK model results indicated that an elevated body burden of PFOA in the liver effects associated with HFPO-DA are mediated through rodent-specific hepatocytes may be more sensitive to the chemicals examined. Consistent with previously published transcriptomic analyses, these results further support that the liver effects associated with HFPO-DA are mediated through rodent-specific PPARα signaling mechanisms. Therefore, the liver effects observed in mice are not appropriate endpoints for use in the development of toxicity values for HFPO-DA in human health assessment.

4363  In Vitro Transcriptomic Analyses Informing the Mode of Action of HFPO-DA (GenX) in the Liver

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Like many polyfluorinated alkyl substances (PFAS), toxicity studies with HFPO-DA, a short-chain PFAS used in the manufacture of some types of fluorinated polymers, indicate that the liver is the primary target of toxicity in rodents following oral exposure. To further evaluate the MOA underlying the non-neoplastic liver changes in rodents associated with HFPO-DA exposure, whole-transcriptome templated oligomer sequencing (TempoSeq) was conducted on primary human, rat, and mouse (including wild-type and PPARα knockout mouse strain) hepatocytes treated for 12, 24 or 72 hours with various concentrations of HFPO-DA, as well as with known agonists of PPARs (i.e., GW7647, PPARγ, (i.e., rosiglitazone), and known cytotoxic agents (i.e., acetalaminophen or D-galactosamin). Differentially expressed genes and enriched gene sets, as well as dose-responsive genes and functional classification (i.e., pathway enrichment) were performed for each chemical, and species/strain. Concordance analyses of differentially expressed genes across chemicals within a species/strain demonstrate more similarity between HFPO-DA and PPARα agonist, GW7647, compared to the other chemicals evaluated. These findings support that benchmark dose modeling and pathway enrichment results. Further, similarity analyses across species indicates greater mechanistic commonalities between rodent species, i.e., rats and mice, and minimal mechanistic similarities between humans and rats or mice. In addition, the overall response of human hepatocytes to chemical exposure at the pathway with the US EPA regions was compared to rodent hepatocytes may be more sensitive to the chemicals examined. Consistent with previously published transcriptomic analyses, these results further support that the liver effects associated with HFPO-DA are mediated through rodent-specific PPARα signaling mechanisms. Therefore, the liver effects observed in mice are not appropriate endpoints for use in the development of toxicity values for HFPO-DA in human health assessment.

4364  In Vitro Transcriptomic Analyses Informing the Mode of Action of HFPO-DA (GenX) in the Liver

4365  Modeling the Impacts of Impaired Kidney Function on PFAS Toxicokinetics

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Per and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment due to their widespread application and high persistence. Specific PFAS exposure contributes to various adverse health endpoints including changes in kidney function. In animals, sex- and species-specific differences in elimination half-lives of PFAS have been linked to the activity of organic anion transporter ( oat) proteins and polypeptides expressed in the kidney. However, the roles of kidney transporters in PFAS elimination are still not fully understood. In addition, kidney disease could alter the expression of kidney transporters and the glomerular filtration rate (GFR). Albuminuria, an indicator of chronic kidney disease, could lower serum PFAS concentrations by allowing albumin-bound PFAS to be excreted through urine. Little is known about the influence of kidney disease on PFAS toxicokinetics. In this study, we developed an existing physiologically based pharmacokinetic (PBPK) model for perfluorooctanoic acid (PFOA) in male rats to explore the influence of changes in renal function parameters (e.g., transporter expression levels, GFR, and urine/solvent albumin levels) associated with various kidney disease states on PFAS toxicokinetics. To the best of our knowledge, the PFPA PBPK model in rats we used is the only one currently available that incorporates transporter-specific excetration and reabsorption mechanisms for PFAS in mammals. To broaden the scope of our analysis beyond the current model, we summarized current knowledge on which kidney transporters could facilitate PFAS transport, and then searched for studies that quantified transporter level changes associated with kidney disease status. The PBPK model results indicated that an elevated body burden of PFOA in male rats occurs with decreased transporter expression levels and reduced GFR. Concomitantly, serum PFOA was predicted to decrease in male rats with decreased serum albumin concentrations. On the other hand, the terminal half-lives (t1/2∞) of PFOA are longer in rats with decreased levels of Oat1 and Oat3 by 80% (t1/2∞=7.4 day) and GFR by 95% (t1/2∞=5.5 day) compared to healthy rats (t1/2∞=4.9 day). Based on these simulations, we find that PFPA toxicokinetics are more influenced by changes in GFR than in transporter expression levels. In contrast, the terminal half-lives of PFOA are more influenced by changes in transporter expression levels than GFR in rats. More information is needed to understand the differences between the two. To the best of our knowledge, the current study is the only one to translate changes in specific kidney disease states are still limited for both humans and animals, which will further limit the effectiveness of risk assessment and hinder the identification of vulnerable populations. Based on the current knowledge of kidney transporters for PFAS transport, we found that nine human and eight rat
kidney transporters have so far been investigated for their ability to transport PFAS, of which seven human and three rat transporters were confirmed to transport specific PFAS. More uptake transporters than efflux transporters have been studied in human and rat systems, due to a focus on the renal reabsorption pathway that extends PFAS half-lives. As a result, knowledge of the role of kidney efflux transporters on PFAS metabolism and excretion remains incomplete. To encourage broadening of this research, we provide a candidate list of seven untested human kidney transporters (one uptake, three efflux, and four with unknown function) with potential for PFAS transport based on their known substrates. Finally, we stress that studies of more PFAS, especially the current-use PFAS (including shorter-chain PFAS and replacements such as GenX) are needed for better coverage of the role of transporters across the structurally diverse PFAS family.

**4366 Assessment of the Mode of Action Underlying Development of Liver Lesions in Mice following Oral Exposure to HFPO-DA (GenX) and Relevance to Humans**

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HFPO-DA is a short-chain polyfluorinated alkyl substance (PFAS) used in the manufacture of some types of fluorinated polymers. Like many PFAS, toxicity studies with HFPO-DA indicate the liver is the primary target of toxicity in rodents following oral exposure. Due to the structural diversity of PFAS, the mode of action (MOA) can differ between PFAS for the same target tissue. There is significant evidence for involvement of peroxisome proliferator-activated receptor alpha (PPARα) and pregnane X receptor (PXR) pathways. The biological response analyses revealed novel insights regarding the S550, and detergents (e.g., laurylamidopropyl betaine and sodium octyl sulfate (SOS)) cluster together and appeared to be enriched for PPAR, CAR, and other lipid-metabolism pathways analogous to previous studies. Prolifically bioactive test substances were classified as injurious or non-injurious to the human liver. Out of the thirty test substances evaluated, twenty produced benchmark concentrations (BMCs) greater than 105, classifying them liver-injurious. Additionally, relative comparisons of biological responses across the five AFFF products were conducted. Gene- and pathway-level similarity analyses revealed a possible 63% common gene-level BMC and a possible 93% common pathway-level BMC across the AFFF products. Further exploration of biological response similarities was conducted across the full set of 30 test substances using hierarchical clustering visualized within a constellation plot with the lowest ranked 100 BMCs for each respective chemical. Fold change similarity and potency similarity were directionally scored and clustered. The majority of AFFF mixtures, the S550, and detersgents (e.g., laurylamidopropyl betaine and sodium octyl sulfate (SOS)) clustered together and appeared to be enriched for PPAR, CAR, and other lipid-metabolism pathways analogous to previous studies. Prolifically bioactive test substances (e.g., PFOS, 2,2’-FTS, and PFHxS) were most closely associated with the reference chemical Wyeth 14,643, a prototype positive control for PPAR activation. AFFFF and 2-(butoxyethoxy) ethanol were most closely associated with phenobarbital (PB), a clinical inducer of CYP2B metabolism through the CAR and PXR pathways. The biological response analyses revealed novel insights regarding similarities for genes and pathways affected by test substance exposure, in addition to elucidating those that distinguished sets of certain test substances from one another.

**4367 High-Throughput Screening of Per- and Polyfluorinated Substances (PFAS) against Nine Thyroid System Assays**

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The U.S. Environmental Protection Agency is evaluating the ecological and toxicological effects of per- and polyfluoroalkyl substances (PFAS). A number of PFAS have been shown to impact the thyroid axis in vivo suggesting that the thyroid system is a target of these chemicals. The objective of this study was to evaluate the activity of 136 PFAS at seven key molecular initiating events (MIE) within the thyroid axis using in vitro assays. The potential MIE targets investigated are Human Iodotyrosine Deiodinase (HdIO3), Human Iodotyrosine Deiodinase (xhIO3), Human Iodotyrosine Deiodinase (hIYD), Human Iodotyrosine Deiodinase (xhIYD), Human Thyroid Peroxidase (hTPO), and the serum binding proteins Human Transthyretin (hTTR) and Human Thyroxine Binding Globulin (hTBG). Of the 136 PFAS chemicals tested, 85 had a half maximal effective concentration (EC50) in at least one of the nine assays. In general, most of these PFAS chemicals did not have strong potency towards the seven MIEs examined, apart from transthyretin binding, for which several PFAS had activity similar to the respective model inhibitor. These data sets identify potentially active PFAS chemicals to prioritize for further testing in orthogonal in vitro assays and at higher levels of biological organization to evaluate their potential for altering the thyroid system and causing potential adverse ecological effects. This abstract does not necessarily reflect US EPA policy.
Exposures to per- and polyfluoroalkyl substances (PFAS) are particularly concerning for public health. Lack of toxicological information on many PFAS limits a health assessor’s ability to evaluate exposures, especially co-exposures to several PFAS. The present work describes an association that may help to address this challenge. Common MOAs for PFAS include interference with target tissue TH cell receptors, 2) perturbation of enzymes that convert THs to active and inactive forms (deiodinases), 3) competitive binding with plasma TH carrier proteins such as transthyretin (TTR), and 4) stimulation of enzymes in the liver that increase hormone clearance. When synthesizing the findings across lines of evidence, we concluded that PFBS may alter THs in pregnant and non-pregnant rodents via several biologically plausible MOAs, with at least one study supporting each of the four MOAs. However, the evidence for all MOAs is limited to a small number of in vitro studies utilizing high concentrations of PFBS, and none have been demonstrated in vivo or in humans.

**4371 Relative Toxicology in the Homologous Series of Perfluorinated Acyclic Alkyl Compounds**

E. Demchuk, CDC/ATSDR, Atlanta, GA. Sponsor: P. Ruiz

Exposures to per- and polyfluoroalkyl substances (PFAS) are particularly concerning for public health. Lack of toxicological information on many PFAS limits a health assessor’s ability to evaluate exposures, especially co-exposures to several PFAS. The present work describes an association that may help to address this challenge. Common MOAs for PFAS include interference with target tissue TH cell receptors, 2) perturbation of enzymes that convert THs to active and inactive forms (deiodinases), 3) competitive binding with plasma TH carrier proteins such as transthyretin (TTR), and 4) stimulation of enzymes in the liver that increase hormone clearance. When synthesizing the findings across lines of evidence, we concluded that PFBS may alter THs in pregnant and non-pregnant rodents via several biologically plausible MOAs, with at least one study supporting each of the four MOAs. However, the evidence for all MOAs is limited to a small number of in vitro studies utilizing high concentrations of PFBS, and none have been demonstrated in vivo or in humans.

**4372 Dose Addition-Based Approaches Accurately Predict Maternal and Neonatal Effects in Sprague Dawley Rats from Three Different PFAS Mixtures Studies**


Due to the pervasive detection of multiple PFAS in human and environmental matrices, many health-based agencies are employing or pursuing cumulative assessment and regulatory approaches. However, the literature is lacking mammalian in vivo studies that test additivity by comparing observed mixture responses to mixtures and single-component exposures. We have conducted three interrelated toxicological studies of PFAS mixtures including a 2 PFAS mixture (perfluorooctanoate [PFOA] + perfluorooctanesulfonate [PFOS]), a 3 PFAS mixture (hexafluoropropylene oxide-dimer acid [HFPO-DA] or ‘Genx chemicals’) + Nafion® product 2 (NPBP2) + PFOS), and a 6 PFAS mixture (perfluorooctahexanoic acid [PFHxA] + perfluorooctane sulfonate (PFOS) + & NBP2 + PFOS). For all individual compounds and mixtures, Sprague-Dawley rat dams were exposed via oral gavage from gestation day 8 (GD8) to postnatal day 2 (PND2) and evaluated a range of maternal and neonatal endpoints. Dose-response data from individual PFAS studies were modeled to obtain curve parameters, and mixture analyses were used to evaluate observed mixture effects including the relative potency factor (RPF) to test additivity and non-additivity for dose addition. The in vitro data on the carboxylate PFAS (PFOA, HFPO-DA, PFMOAA) and sulfonate PFAS (NPBP2, PFOS, PFHxS) produced multiple common effects across all compounds, however the two groups were not toxicologically identical. Effects common across all compounds included reduced pup and maternal bodyweights, reduced pup survival, increased maternal relative liver weights, reduced serum thyroid hormones, and increased liver expression of P450 signaling pathway genes. With limited exception across studies, these endpoints were modelled with equivalent or better fits (based on corrected Akaike’s Information Criteria [AICc]) using dose addition compared to response addition equations. In the 2 and 3 PFAS mixture studies, reductions in maternal bodyweight and weight gain during pregnancy were less than additive. Thus, factors other than non-additive effects that differed by >2-fold from dose addition predictions. Deviations from additivity may be due to differences in internal dosimetry (e.g., maternal and pup serum and liver concentrations) as a function of maternal oral dose between the individual chemical and mixture experiments. Most endpoints had statistically similar dose response curve slopes across the component PFAS and mixture effects were well predicted by endpoint specific RPFs and a dose addition equation that assumes congruent slopes. Importantly, RPFs (based on maternal oral dose) for the six PFAS we have studied vary by >20-fold across the range of endpoints modeled and it was not possible to accurately predict all mixture effects with just a single set of RPFs. Endpoints without non-congruent dose addition predictions using a dose addition equation that does not assume equal slopes. Combined exposure to mixtures of PFAS carboxylates and sulfonates produced cumulative effects on multiple maternal and neonatal endpoints and these effects were generally well predicted by dose addition-based approaches, particularly the RPF. The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or policies of the US EPA.

**4373 Method Development to Establish and Characterize an Air-Liquid Interface (ALI) Exposure System Using Perfluorooctanoic Acid (PFOA) Liquid Aerosol**

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Due to the demand for increased speed and reduced cost in toxicity testing, the desire to modernize test systems, and the need to address ethical objections in animal experiments, there has been an accelerating trend to shift from traditional in vivo inhalation toxicity studies to the development of alternate animal models (e.g., in vitro inhalation exposure systems). A major caveat in using such in vitro based exposure systems is that the controlled delivery and accurate characterization of exposure atmosphere is extremely challenging. The goal of this study was to establish consistent, reproducible exposures using a liquid aerosol and collect preliminary biological data following exposure of 3D normal human bronchial epithelial (NHBE) cell cultures. A ALI exposure system was procured, installed and qualified to conduct air liquid interface (ALI) exposures of cell cultures. Perfluorooctanoic acid (PFOA) was used as a representative test compound for producing liquid aerosol. Methods were developed to generate and characterize inhalation exposure atmosphere in ALI exposure system. PFOA was formulated in 0.9% saline solution adjusted to pH 6 to 8 at a concentration of 10 mg/mL. To generate exposure atmosphere, a commercially available nebulizer (Mini-HEART Continuous Hi-Flo Nebulizer; Westmed, AZ) was used to generate aerosol from the liquid formulation. Compressed air was used to dilute the aerosol at the output of the nebulizer and carry it to the delivery manifold. A real time aerodynamic monomer (RAM) was used to monitor the temporal variability of the aerosol atmosphere. Humidified dilution air was added at the inlet of each row of the ALI exposure manifold to dilute the atmosphere to the target concentration. The aerosol particle size was measured using Mercer cascade impactor (MCI-G series, Intox Products, NM) and optical particle sizer (Model 3330; TSI Inc, MN) from numerous sampling locations in the exposure system. NHBE cell cultures were exposed to PFOA aerosol for 6-hour duration. Deposition on cell cultures was determined by measuring amount of PFOA collected in the liquid trap (0.8 mL volume: consistent with cell culture media) placed in one of the inserts in each dose group. The barrier integrity of cells was evaluated using trans epidermal electrical resistance (TEER) both prior to and after exposures. The aerosol particle size data measured using both instruments was consistent with the respirable size range; mass median aerodynamic diameter (MMAD) ranged from 1.1 to 1.5 μm with a geometric standard deviation (GSD) ranging from 1.6 to 2.5. The deposited dose in each dose group was consistent with theoretical estimates. Barrier integrity measurements did not indicate significant toxicity. TEER values before and after exposure did not show any significant differences. In future works, we have developed methods to establish in vitro ALI exposures using liquid aerosol. Future experiments may focus on elucidating mechanisms of toxicity using longer or repeated exposures and more complex cell culture models to recapitulate additional aspects of the biological effects of chronic exposure.
Dermal absorption of PFAS is generally considered to be a minor uptake pathway relative to ingestion and inhalation because at the stratum corneum physiological pH of 5.5, PFAS exist in ionized forms that do not readily penetrate skin. However, findings are inconsistent across studies. Of three studies that measured dermal absorption of the PFAS perfluorooctanoic acid (PFOA) in humans or in human epidermal membranes, fractional absorption of PFOA ranged by three orders of magnitude: 0.048, 1.6, and 4.8% as reported by Fasano et al. (2005), Abraham and Monien (2022), and Franko et al. (2012), respectively. To understand the basis of this variability we critically evaluated these studies to (1) identify the most reliable dermal absorption factors for PFOA in humans and (2) apply appropriate dermal absorption factors to estimate dermal exposures to children from soil and water contact as well as from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water contact and from dermal exposure to PFOA. For this study we calculated concentration-independent dermal absorption factors to estimate dermal exposures to children from soil and water接触 and from dermal exposure to PFOA。
other PFAS, especially some of the perfluorooctanoates (e.g. PFOA), a much more complex toxicological profile was observed, including induction of oxidative stress, cell cycle inhibition, ion stress, protein-stress and an autophagy response. Other PFAS, like the perfluorooctyl carboxylate PFBA, were less potent and induced autophagy only at millimolar concentrations. Together, the ToxProfiler assay could be applied to generate extensive and quantitative biological activity profiles of different PFAS. The ToxProfiler results are literally in line with available in vitro data for the tested PFAS. Different pathologies, including liver toxicity which is mainly driven by oxidative stress, cell cycle disturbance, and disturbed fat metabolism have been reported for the PFAS.

Per- and polyfluoroalkyl substances (PFAS) design a family of compounds characterized by their fluorinated carbon chain-length and high thermal and chemical stability. Human exposure to PFAS has become a growing concern as they have been detected in soil, surface water, and food contact material. However, despite their ubiquitous nature, relatively little is known about PFAS toxicity outside legacy compounds such as perfluorooctanoic acid and perfluorooctanoic sulfonate. One of the main reasons behind the poor understanding of PFAS has been their diversity, with over 4,700 identified compounds. PFAS variety has rendered classic risk assessment approaches both time-consuming and costly. As such, the present study aims to open the way to include a quantitative structure-activity relationship (QSAR) approach in the risk assessment for PFAS. Linking the chemical characteristics of PFAS to multiple in vitro toxicological endpoints will help set up the basis for a predictive model to screen for potentially hazardous PFAS in future risk assessment. In the present study, both hepatotoxicity and immunotoxicity of PFAS have been assessed in vitro using HepG2 and THP-1 cells. Well-established toxicological assays were employed to assess cytotoxicity (MTT), and reactive oxygen species formation (DCFH-DA). In addition, cytokines release by THP-1, and cytokrome P450 (CYP) activity were assessed in relation to different PFASs. As such, fifteen PFAS, with chain-length ranging from 4 to 10 carbon and with either carboxylic (PFCA), sulfonic (PFSA), or alcoholic (FTOH) headgroups, have been selected and tested in the current study. A clear structure-activity relationship between PFAS and cytotoxicity has been observed in both HepG2 and THP1 cell lines. Cell viability decreased with increasing PFAS’ chain-length. However, the effect of the headgroup moiety on cytotoxicity of the compounds was not as clear as the chain-length effect in no significant toxicity, while PFASs and PFCAs reduced cell viability. However, for THP1, FTOHs appeared to have higher toxicity when compared to PFASs and PFCAs. In HepG2, exposure to PFAS resulted in reactive oxygen species (ROS) generation after 1h for all tested compounds, except for the FTOHs. Moreover, a similar structure-activity relationship has been observed as with the HepG2 data, with increased ROS generation with increasing carbon chain-length and no observed effect for FTOHs compared to PFASs and PFCAs. In THP-1, PFAS have been observed to reduce cytokine release after exposure to Lipopolysaccharides (LPS) in both monocytes and differentiated macrophages. In monocytes, the PFAS potential to decrease TNF-α release was found to be chain-length dependent, and FTOHs appeared to have higher toxicity when compared to PFSAs and PFOA. 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PFAS (Per- and Polyfluorinated Substances) have been detected in the drinking water of 50 states, two territories in the US, and even in the blood of 97% of Americans as an emerging pollutant. PFAS may act as endocrine and metabolic disruptors, increase cholesterol levels, adversely impact the immune system, and cause liver damage. The current toxicological understanding on human health should be evaluated to inform decision-making about health protection from exposure to PFAS. Current toxicology experiments have only covered a few PFAS species [e.g., perfluorooctanoic Acid (PFOA) and perfluorooctane Sulfonate (PFOS)], compared to a large number of PFAS compounds available on the market. To address this problem, we propose a data-driven, lab-free interpretable computational approach to determine Adverse Outcome Pathways (AOP) and important chemical groups associated with PFAS toxicity and quantitatively predict the corresponding point of departure (POD). This project aims to utilize available in vivo, in vitro assays, and in silico data and apply deep learning methods to construct an interpretable neural network for integratively and systematically train and predict the PFAS toxicity and (2) to support the human health risk characterization of PFAS and their mixtures. To achieve that, three conceptual steps are proposed. First, a hierarchy of known putative molecular components and pathways will be identified based on available PFAS data in public data repositories. The adverse outcome we focus on is PFAS-induced liver injury. The AOP will train an interpretable neural network with the activation status of receptors as the input layer and the state of adverse outcomes as the output layer. In parallel, the molecules of tested PFAS are encoded as molecular graphs used as the input layer to make predictions on the POD. Together, the two branches will be trained using available in vivo, high-throughput screening, and PFAS structure data. The ultimate goal is to enable DeepPFAS’s capability to make predictions on toxicity and to identify AOP and chemical substructure that are crucial for toxicity. These model predictions could be used to group PFAS and characterize the risk of PFAS mixtures using the generalized concentration addition (CA) and independent action (IA) methods in support of PFAS regulations. We have established the PFAS AOP for the PPARα receptor activation, a plausible initiating event reported by published studies. The model predictions could be used to group PFAS and characterize the risk of PFAS mixtures using the generalized concentration addition (CA) and independent action (IA) methods in support of PFAS regulations. We have established the PFAS AOP for the PPARα receptor activation, a plausible initiating event reported by published studies. 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reduced growth and exacerbated WFES and/or HCD-associated platelet alterations were mitigated in animals at HT. These clinically relevant data demonstrate that HT noted along with alterations in blood clinical biomarkers of coagulation. HT rats due to insulin insufficiency, an effect often observed during subchronic exposure in glucose homeostasis indicate that the observed glucose intolerance is likely gluconeogenesis, and that this effect was exacerbated in HT rats. The disruptions in fine powder PTFE exhibited decreased glucose clearance. Insulin tolerance testing revealed that WFES or WFES (~7 mg/m² x 1 hr/d x 1d/week x 13 weeks). In-life testing of metabolic and cardiovascular physiology were performed during exposure periods, while tissue and ex vivo vascular assessment were performed at necropsy. Rats at HT had substantial reductions (~30%) in body weight gain and increased lean mass compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet. HCD increased serum cholesterol (driven by increases in LDL) and caused fatty livers in rats at RT or HT; in HT rats, compared to RT housed rats regardless of diet.

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4386 Lifestyle and Climate Factors Interact with Wildfire-Related Air Pollution to Worsen Neuroendocrine and Cardiometabolic Function


Climate scenarios predict more frequent and longer-lasting heatwaves alongside increased ambient particulate levels from widespread wildfires. These conditions are predicted to exacerbate cardiometabolic disorders, especially in vulnerable populations. We hypothesized that stress caused by high-temperature housing (HT), unhealthy high-cholesterol diet (HCD), and cardiometabolic stressors (driving increased living temperature and psychosocial stress on the cardiovascular system) are consistently exposed to air pollution, underscoring the need to understand the synergistic effects of climate change and psychosocial stress on the health impacts of air pollution. This study was conducted to evaluate the effect of increased living temperature and psychosocial stress on the cardiovascular response to a single eucalyptus wildfire smoke (WS) exposure. C57/BL6 mice were separated into either enriched or depleting housing environment at either 22 °C (HT) or 31 °C (HT) for 22 weeks. During this time, whole body plethysmography, high-frequency echocardiography, and behavioral testing was conducted. A cohort of mice with implantable radio-telemeters were separated into the same temperature and housing paradigm for 6 weeks, providing continuous electrocardiogram, core body temperature and activity data. Animals were exposed to either filtered air or 3mg/m³ WS for one hour. HT caused increased levels of anxiety shown by significant changes in open field testing, significant increases in left ventricle mass, and significant changes in expiratory time (Te), relaxation time (RT), and end expiratory pause (EEP) after WS exposure, including significantly higher RR-l values before and after WS. Further, enriched housing ameliorated the increased heart rate and RR-l values, as well as the anxiety-prone behaviors. These results indicate that higher ambient temperatures may also affect human health through changes in autonomic function and cardiovascular physiology, suggesting environmental enrichment (i.e., greenspace) should be evaluated as future mitigation strategies to create more resilient populations towards climate change and WS exposure. This abstract does not reflect EPA policy.

4387 Increased Ambient Temperature and Housing Conditions Alter the Behavioral and Cardiovascular Response following a Single Eucalyptus Wildfire Smoke Exposure in C57/BL6 Mice

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Extrinsic factors of urban living are increasingly being recognized as variables that affect the toxicological response. These factors, such as accessibility to green space and increased ambient temperature, have been correlated with various adverse health outcomes due to psychosocial/physiological stress, specifically anxiety and cardiovascular morbidities. Moreover, those living in an urban environment are consistently exposed to air pollution, underscoring the need to elucidate the synergistic effects of climate change and psychosocial stress on the health impacts of air pollution. This study was conducted to evaluate the effect of increased living temperature and psychosocial stress on the cardiovascular response to a single eucalyptus wildfire smoke (WS) exposure. C57/BL6 mice were separated into either enriched or depleting housing environment at either 22 °C (HT) or 31 °C (HT) for 22 weeks. During this time, whole body plethysmography, high-frequency echocardiography, and behavioral testing was conducted. A cohort of mice with implantable radio-telemeters were separated into the same temperature and housing paradigm for 6 weeks, providing continuous electrocardiogram, core body temperature and activity data. Animals were exposed to either filtered air or 3mg/m³ WS for one hour. HT caused increased levels of anxiety shown by significant changes in open field testing, significant increases in left ventricle mass, and significant changes in expiratory time (Te), relaxation time (RT), and end expiratory pause (EEP) after WS exposure, including significantly higher RR-l values before and after WS. Further, enriched housing ameliorated the increased heart rate and RR-l values, as well as the anxiety-prone behaviors. These results indicate that higher ambient temperatures may also affect human health through changes in autonomic function and cardiovascular physiology, suggesting environmental enrichment (i.e., greenspace) should be evaluated as future mitigation strategies to create more resilient populations towards climate change and WS exposure. This abstract does not reflect EPA policy.
underlying the anti-inflammatory role of acetate in MC-LR induced hepatic inflammation we quantified protein levels of NLRP3, IL-1β and IL-18 which was reversed in the presence of GPR43 receptor antagonist GLPG0974 and NLRP3 autophagy inhibitor baflofilmic A1. This suggests that triggering of GPR43 signaling by its agonist may play a significant role in modulating MC-LR induced hepatic inflammation and NLRP3 autophagy.

Future climate scenarios indicate changes in the timing and duration of pollen seasons, increases in pollen production, and shifts in vegetation. It is also possible that the allergenicity of pollen changes, thus leading to an increase both in the incidence of allergies and the severity of symptoms. To evaluate the impact of climate change on the pollen season and the pollen properties, we grew timothy (Phleum pratense) in current and future climate conditions (RCP8.5). The start of the flowering and pollination was examined. Allergen content of the pollen was determined by enzyme-linked immunosorbent assay. To estimate the capacity of future grass pollen to induce allergic inflammation, MucAir™ 3D human nasal epithelial cells were exposed to pollen in the air-liquid interface and protein content was measured from the cell cultures. Climate change treatment caused the pollination of P. pratense to start even 17 days earlier compared to the control treatment. Elevated carbon dioxide treatment increased the allergen content of the pollen more than two-fold when compared to current climate conditions. Grass pollen in the future climate conditions activated the production of eotaxin, MCP-1, GM-CSF, and IFN-γ in human nasal epithelial cells. The observed change in the start of the pollen season and the allergen content of the pollen may increase the duration and severity of allergic symptoms in the future.

Ecotoxicologists need more tools to assess patterns of organismal mercury uptake from the environment. In birds, mercury is eliminated from the body and sequestered into growing feathers. The tree swallow (Tachycineta bicolor) is an abundant North American bird that has been studied often for its ability to accumulate mercury in its tissues, as it occupies a high trophic level and breeds near bodies of water. We sought to define the blood-feather total mercury (THg) relationship between samples taken simultaneously in banding-age nesting Tree Swallows from two locations in Northern Kentucky. Based on previous findings, we predicted that a relationship would be found, and that feathers would have more THg than blood. From May through July of 2021, we collected blood and feather samples from 12- to 14-day-old nestlings (n=50 from 15 nests) during banding. Blood and feather samples were frozen; the feathers were then thawed to room temperature and cleaned with Fisher Scientific® Certified ACS 95% lab-grade acetone and Milli-Q® water. Analysis was completed with a Nippon MA-3000 mercury analyzer, which used thermal decomposition, gold amalgamation and atomic absorption, for THg concentrations specific to each nestling sampled. We ran two linear mixed effects models in the program R. As a result, we found a significant positive relationship between blood and feather THg, demonstrating directional flow from the blood into growing feathers (F_1, 21.3 ppb saxitoxin. Anatoxin-a and saxitoxin are far more potent than the other two heavy-studied microcystins, microcystin and cylindropermoplin. While there are federal recreational guidance values for microcystins and cylindroperazon, there are currently no federal anatoxin-a or saxitoxin recreational guidance values in freshwater bodies. A comparative analysis of current state health-based recreational guidance values for anatoxin-a and saxitoxin was performed. As of November 2020, there are recreational guidance values for anatoxin-a in 10 states and saxitoxin in 11 states. This analysis revealed significant variability in guidance value magnitude (“detection”-80 ppb anatoxin-a and 0.6-75 ppb saxitoxin). This heterogeneity was driven by several factors: study selection for point of departure, application of uncertainty factors, and interpretation of the critical study. While most states included in this analysis had recreational guidance values for microcystin and cylindroperazon, many states did not have anatoxin-a and even less had saxitoxin values derived. Most notably, consideration of cyanotoxin mixtures is not included in any state or federal recreational guidance. Recent HAB events suggest multiple toxin-producing cyanobacteria species are present in a single event, including cyanobacteria that can produce multiple cyanotoxins. During the 2022 HAB season in PA, binary mixture detections for saxitoxin + anatoxin-a (N=52), microcystin + saxitoxin (N=20), and microcystin + anatoxin-a (N=18), and ternary mixture of microcystin, saxitoxin, and anatoxin-a (N=16) were observed. The prevalence of anatoxin-a and saxitoxin detection during HAB events, including cyanotoxin mixtures, suggests anatoxin-a, saxitoxin, and cyanotoxin mixtures should be considered in HAB response plans and surveillance programs of U.S. freshwater bodies. Further, the paucity of studies available for anatoxin-a and saxitoxin guidance value derivation, as well as cyanotoxin mixtures that occur during multi-toxin HAB events, highlights an urgent need for additional toxicological research in this area. This work was supported in part by the Pennsylvania Environmental Health Capacity Program (CDC-RFA-EH20-2005).

4392 Impact of Climate Change on the Production and Immunogenic Potential of Grass Pollen
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Grass pollen is likely one of the most important allergens in Europe. It may also cause an increase in allergic symptoms in the future. In the future, climate change can affect the timing, duration and quantity of pollen production and their immunogenic properties. Changes in pollen season, production and immunogenic properties of pollen will affect its ability to cause an increase in allergic symptoms. In this study, we analyzed the production and immunogenic properties of the most important grass pollen species in Finland, Poa pratense and Phleum pratense, during the 2022 and 2023 pollen seasons.

4393 A Banders Contribution to Ecotoxicology: Comparison of Mercury Concentration in Feathers and Blood of Nestling Tree Swallows Demonstrates Feathers’ Suitability as a Biomonitoring Tool
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Ecotoxicologists need more tools to assess patterns of organismal mercury uptake from the environment. In birds, mercury is eliminated from the body and sequestered into growing feathers. The tree swallow (Tachycineta bicolor) is an abundant North American bird that has been studied often for its ability to accumulate mercury in its tissues, as it occupies a high trophic level and breeds near bodies of water. We sought to define the blood-feather total mercury (THg) relationship between samples taken simultaneously in banding-age nesting Tree Swallows from two locations in Northern Kentucky. Based on previous findings, we predicted that a relationship would be found, and that feathers would have more THg than blood.

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Pharmaceuticals and personal care products (PPCPs) are a group of contaminants of emerging concern that are released into the environment from wastewater effluents and agricultural runoff. Although the direct toxicity of individual PPCPs on aquatic animals is known, PPCPs occur in mixtures in the environment and their impact on aquatic animals is underexplored. Direct impacts of PPCPs may be evident in animal physiology. Like other toxic biomolecules (xenobiotics, pollutants), PPCPs are likely to react with reactive oxygen species and induce oxidative stress. Impacts of PPCPs may also be indirect by altering their microbiome. Microbiome communities are essential to the host’s immunity, physiology, biochemistry, behavior, and development; changes to the microbiome communities have deleterious effects on host homeostasis. In this study, we used freshwater crayfish as a model organism to investigate the toxic impacts of PPCPs on the oxidative stress biomarkers and microbiomes. The project’s objectives were to 1) investigate the toxicological effects of PPCPs on the oxidative stress biomarkers (superoxide dismutase, catalase, and glutathione peroxidase) of crayfish and 2) examine changes in the gut, carapace, and gill microbiota of crayfish after PPCP treatments. crayfish were collected from the West Branch of the Manahing River (OH) and acclimated in the lab for a week in triplicated aquaria before being exposed to elevated PPCP mixtures (carbamazepine, estradiol, and triclosan). After 96 hours of incubation with 10 µg/L (treatment 1) and 100 µg/L (treatment 2) of PPCP mixtures, crayfish were dissected, and gut, carapace, and abdominal tissues were obtained, homogenized, and centrifuged. Half of each sample was used for oxidative stress analyses and the rest for DNA extraction to examine the composition of the microbiome. Our results showed that PPCP exposure profoundly impacted the oxidative stress biomarkers of crayfish. Activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) of crayfish gut, gut, and abdominal tissues were obtained, homogenized, and centrifuged. Half of each sample was used for oxidative stress analyses. Furthermore, we obtained the structure of dERα with the C-terminal (F domain) using AlphaFold2 and monitored the changes in the structure during molecular dynamics (MD) simulation of the ligand-receptor interaction in MOE. In 21 UV absorbers tested, 12 chemicals showed dERα-agonistic activities in the order of 2,2',4,4'-tetraOH-BzP > 2,4,4'-triOH-BzP > 2,3,4,4'-tetraOH-BzP > 4-OH-BzP > 2,4-dioH-BzP > 4,4'-dioH-BzP > 2,3,4,4'-tetraOH-BzP > 2,3,4-triOH-BzP > 3-OH-BzP > 2-OH-BzP > 2-OH-4-Meo-BzP. Nine UV absorbers including 2,2'-OH-BzP, 2,2'-diOH-4-Meo-BzP, 2,2'-diOH-4,4'-diMeO-BzP, 2-OH-4-Meo-BzP-sulfonic acid, 2-OH-4-ocrylene, terephthalamide, 4-ethylbenzoyl hexyl benzoate, and octinoxate showed no activity. The PLSR model was able to predict the RECO2 values with good root mean square error (RMSE < 0.4) and R-square (R2 = 0.9). The PLSDA model showed high accuracy (>96%) for activity prediction. Not a few specific but combination of multiple parameters (molecular descriptors and docking simulation values) contributed to the predictivity of each model. MD simulations of E2-dERα interaction showed that the β-strand region in the F domain that is responsible for ER dimerization was shifted toward helix 11, while no apparent shift was found in the interaction between dERα and 2-OH-4-MeO-BzP with the least in vitro activity. Thus, together with the ligand-receptor interaction, the dimerization of dERα may also be a key process to determine its ligand-dependent transactivation.
was poor in 7 of 10 years. The phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 50-55% in gull chicks in both AOCs and Grand Traverse Bay, and 49% in terns and 39% in herons in Saginaw Bay. Antibody responses in gull chicks in the River Raisin AOC and Grand Traverse Bay were 1.6- to 2-fold lower than reference. In River Raisin gulls, none of the impaired biological endpoints (total embryonic nonviability, infertility, embryonic death) in 4-week chick productivity, T cell response, and antibody titers) changed over time despite remedial dredging of contaminated sediments in 2014 and 2016. Furthermore, numbers of nesting herring gulls declined 90% between the late 1990s and 2015-19 (r=-0.96). In Saginaw Bay gull eggs on the Confined Disposal Facility (CDF) and L. Charity Island, total nonviability, infertility, and death did not improve with time. Embryonic death increased 4.4X on the CDF between 2010-14 and 2015-19. In gull chicks, suppressed T cell function did not change over time on either island. In Saginaw Bay terns (Charity Reef/L. Charity Island and CDF), T cell responses and chick productivity did not improve over time. The breeding population of Caspian terns, a state-threatened species, decreased 49% in Saginaw Bay over the course of the study (r=0.64). In Grand Traverse Bay gulls, total embryonic nonviability, infertility, and embryonic death were below the reference range for the first time. One notable finding was the marginal improvement in chick survival rates between the early and late periods of the study. No trends were observed in suppressed T cell responses or antibody titers. Analysis of legacy pollutants in eggs and plasma demonstrated ongoing elevated exposure to PCBs in the River Raisin and Saginaw Bay AOCs and PCDDs/Fs in Grand Traverse Bay. Fluorinated and brominated CEDs were significantly elevated in tissues at all sites, including ubiquitously high concentrations of PFAS compounds in herring gull plasma that approached or exceeded PCB concentrations at all sites. Overall, this biomonitoring program demonstrated no significant improvements in population, reproductive, and immune endpoints in either AOC or Grand Traverse Bay.

Simulation of Environmental Weathering of Laboratory-Generated Microplastic Particles (lg-mpp) for Defining Reference Materials


Microplastic pollution is a serious environmental health concern due to the potential to induce toxicological effects on both aquatic and terrestrial organisms. When plastic waste enters the environment, it is subjected to an array of environmental conditions as well as biotic and abiotic agents. Preliminary reports have shown that plastic debris interacts with natural stressors (such as sunlight or salinity) and can result in physicochemical changes on the surface of the plastic particle which in turn may promote the deformation and discolored particles as well as serve as a source of degradation of organic and inorganic compounds. Understanding the weathering process is difficult, especially when considering the different weather conditions globally. Hence, the development of laboratory generated microplastic particles (i.e., LG-MPPs) using simulated environmental stressors can aid in the design of experiments studying the fate, transformation, and eventual effects of degraded plastic material. Currently, there is limited knowledge on the degradative mechanisms, changes in photodegradation, and chemical compositional changes of aged plastic particles. In this study, plastic cutlery was micronized and exposed to simulated solar radiation, thermal oxidation, salinity, and chemical oxidation over prescribed time periods ranging from 1 week to 3 months. The resulting materials, and antibody titers) changed over time despite remedial dredging of contaminated sediments in 2014 and 2016. Furthermore, numbers of nesting herring gulls declined 90% between the late 1990s and 2015-19 (r=-0.96). In Saginaw Bay gull eggs on the Confined Disposal Facility (CDF) and L. Charity Island, total nonviability, infertility, and death did not improve with time. Embryonic death increased 4.4X on the CDF between 2010-14 and 2015-19. In gull chicks, suppressed T cell function did not change over time on either island. In Saginaw Bay terns (Charity Reef/L. Charity Island and CDF), T cell responses and chick productivity did not improve over time. The breeding population of Caspian terns, a state-threatened species, decreased 49% in Saginaw Bay over the course of the study (r=0.64). In Grand Traverse Bay gulls, total embryonic nonviability, infertility, and embryonic death were below the reference range for the first time. One notable finding was the marginal improvement in chick survival rates between the early and late periods of the study. No trends were observed in suppressed T cell responses or antibody titers. Analysis of legacy pollutants in eggs and plasma demonstrated ongoing elevated exposure to PCBs in the River Raisin and Saginaw Bay AOCs and PCDDs/Fs in Grand Traverse Bay. Fluorinated and brominated CEDs were significantly elevated in tissues at all sites, including ubiquitously high concentrations of PFAS compounds in herring gull plasma that approached or exceeded PCB concentrations at all sites. Overall, this biomonitoring program demonstrated no significant improvements in population, reproductive, and immune endpoints in either AOC or Grand Traverse Bay.

Characterization of Tire and Road Wear Particles in Urban River Samples

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Tire and road wear particles (TRWP) consist of tread rubber elastomers with pavement encrustations generated from friction between the tire and the road. The fate of environmentally dispersed TRWP as well as microplastic particles (MP) depends on both mass concentration as well as physical and chemical properties, such as particle diameter and density. Our previous work has utilized density separation and chemical mapping techniques to characterize individual TRWP in environmental sample types with increasing sample complexity including road and rolling sediment pond sediment. The purpose of the current research was to examine the specific physical and chemical composition of TRWP and to assess the presence of oxygen containing functional groups. Lastly, pristine and weathered particles were analyzed for their propensity to adsorb environmental contaminants using scanning electron microscopy and energy dispersive x-ray to explore changes in surface morphology and elemental composition respectively, while Raman spectroscopy and Fourier transform infrared spectroscopy were used to investigate the presence of oxygen containing functional groups. Lastly, pristine and weathered particles were analyzed for their propensity to adsorb environmental contaminants such as metallic lead. Preliminary results show that short term weathering does not significantly change the properties of real-life particles. However, the crevices, cracks, and hydrophobic surface of weathered microplastics facilitate interaction with organic pollutants and heavy metals. The findings of this research will help policymakers make educated judgments regarding the potential health effects of weathered microplastic exposure in humans.

Microplastic pollution is a serious environmental health concern due to the potential to induce toxicological effects on both aquatic and terrestrial organisms. When plastic waste enters the environment, it is subjected to an array of environmental conditions as well as biotic and abiotic agents. Preliminary reports have shown that plastic debris interacts with natural stressors (such as sunlight or salinity) and can result in physicochemical changes on the surface of the plastic particle which in turn may promote the deformation and discolored particles as well as serve as a source of degradation of organic and inorganic compounds. Understanding the weathering process is difficult, especially when considering the different weather conditions globally. Hence, the development of laboratory generated microplastic particles (i.e., LG-MPPs) using simulated environmental stressors can aid in the design of experiments studying the fate, transformation, and eventual effects of degraded plastic material. Currently, there is limited knowledge on the degradative mechanisms, changes in photodegradation, and chemical compositional changes of aged plastic particles. In this study, plastic cutlery was micronized and exposed to simulated solar radiation, thermal oxidation, salinity, and chemical oxidation over prescribed time periods ranging from 1 week to 3 months. The resulting materials, and antibody titers) changed over time despite remedial dredging of contaminated sediments in 2014 and 2016. Furthermore, numbers of nesting herring gulls declined 90% between the late 1990s and 2015-19 (r=-0.96). In Saginaw Bay gull eggs on the Confined Disposal Facility (CDF) and L. Charity Island, total nonviability, infertility, and death did not improve with time. Embryonic death increased 4.4X on the CDF between 2010-14 and 2015-19. In gull chicks, suppressed T cell function did not change over time on either island. In Saginaw Bay terns (Charity Reef/L. Charity Island and CDF), T cell responses and chick productivity did not improve over time. The breeding population of Caspian terns, a state-threatened species, decreased 49% in Saginaw Bay over the course of the study (r=0.64). In Grand Traverse Bay gulls, total embryonic nonviability, infertility, and embryonic death were below the reference range for the first time. One notable finding was the marginal improvement in chick survival rates between the early and late periods of the study. No trends were observed in suppressed T cell responses or antibody titers. Analysis of legacy pollutants in eggs and plasma demonstrated ongoing elevated exposure to PCBs in the River Raisin and Saginaw Bay AOCs and PCDDs/Fs in Grand Traverse Bay. Fluorinated and brominated CEDs were significantly elevated in tissues at all sites, including ubiquitously high concentrations of PFAS compounds in herring gull plasma that approached or exceeded PCB concentrations at all sites. Overall, this biomonitoring program demonstrated no significant improvements in population, reproductive, and immune endpoints in either AOC or Grand Traverse Bay.

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Aqueous toxicity is an ecotoxicological endpoint which provides important information about a chemical’s potential to elicit adverse effects on aquatic organisms. Within regulatory toxicology, three trophic levels are typically considered as a proxy of the ecosystem: fish (representing vertebrates/apical consumers), daphnia (representing invertebrates/primary consumers) and algae (representing producers/plants). Aquatic toxicological effects are examined, and data concerning mechanism of action (MoA). A four-peer-reviewed publications were used to compile a curated, high-quality, dataset of existing in silico predictions, RTgill assay results, physicochemical parameters and data used to support the development of a fathead minnow high-throughput assay for screening level hazard assessment by application of the 3Rs and replacing in vivo tests with in vitro assays, such as the RTgill-W1 assay, or by developing Integrated Approaches to Testing and Assessment (IATA) for a given chemical. To build upon this body of work, a modular non-animal IATA has been created, using the following components: in vitro tests with in vitro and in vivo data and good correlation between in vivo and in vitro data was observed. The in vivo tests with a multi-class quantitative structure activity relationship (QSAR) model to interpret the in vitro data used to support other relevant modules. The IATA is designed to provide a categorical output suitable for use within the EU Classification, Labelling and Packaging (CLP) and United Nations Globally Harmonized System of Classification and Labelling (UN GHS) frameworks. Case studies and considerations with respect to species relevance and applicability domain will be discussed within.

Cyanobacteria associated with harmful Algal Blooms (HABs) can release highly potent toxins, known as cyanotoxins, which can be deadly to humans, pets, and livestock. Acute and chronic effects are not well characterized across the diversity of cyanotoxins or the potentially exposed organisms (e.g., amphibians, microbes, aquatic plants, and wildlife). The objective was to create a literature inventory of the extent, diversity, and toxic effects of cyanotoxins reported for aquatic and terrestrial organisms. The well-established protocols of the ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox) were leveraged to comprehensively and systematically identify and review relevant cyanotoxin studies and then provide extracted toxicity data for priority cyanotoxins. The priority cyanotoxins were identified based on association with the most common HAB-forming cyanobacteria in freshwater bodies of the U.S., including microcystin-LR, microcystin-RS, anatoxin-a, beta-methylamino-L-alanine (BMAA), cylindrospermopsin, saxitoxin, and palytoxin. The literature was queried using multiple search engines for these priority cyanotoxins as well as ~100 additional cyanotoxin names from HAB publications and government reports. Over 7,500 references were screened at the title and abstract level, followed by full-text review of over 1,500 publications. Of these, 1,109 publications met the ECOTOX inclusion criteria (e.g., verifiable CASRN, ecological receptor that is a verifiable species, control reported). Each included publication was annotated with test compound(s), test species, exposure type(s), and effect type(s) for creating the literature inventory. This effort identified ecological toxicity studies on 78 cyanotoxins, with >70% of the publications testing microcystins. Studies include effects in aquatic and terrestrial vertebrates, invertebrates, and plants, with 40% of the studies in fish and a notable under-representation for several groups such as amphibians and birds. 42% of publications measured traditional growth, reproduction, and mortal- ity toxicity endpoints, and 49% assay results. Physiological endpoints, statistical significance, and genetic effects. Full data extraction was completed from 374 publications, with all pertinent case study details on species (life stage, age, sex), chemicals (purity, analytical verification, test concentration), test methods and conditions (exposure type and media, study duration, control type, experimental design, soil or water parameters), and toxicity results (specific effect and endpoint, statistical significance). The literature inventory will inform future cyanotoxin research through identification of data availability and gaps for species, toxoids, and endpoints. The detailed extracted toxicity data supports evaluation of ecological risk from HABs and the most commonly occurring cyanotoxins in the environment through characterizing toxicity across cyanotoxin classes and identification of the most potent cyanotoxins and sensitive species. This abstract does not necessarily reflect US EPA policy.

Concentrations at which global gene expression profiles in cells or animals exposed to a test substance start to differ significantly from those of controls have been proposed as an alternative point of departure for use in screening level hazard assessment. The US Environmental Protection Agency has been pilot testing a high-throughput compatible transcriptional assay with larval fathead minnows (Pimephales promelas) for 1-day post hatch fathead minnows are more sensitive about 70% of the time. Biological variability was assessed by comparing 10 independent CuSO4 exposures that served as assay positive controls. Transcriptional analyses identified 330 differentially expressed genes (DEGs) common in at least half the exposures while 25 DEGs were consistently observed in all 10 exposures. Chemical availability was evaluated by directly measuring chemical concentration in test wells to adjust IPoD effect concentrations appropriately. This adjustment resulted in IPoD being protective relative to adverse effects (i.e., more sensitive) for 94% of the tested chemicals. These data support the continued development of a fathead minnow high-throughput assay for screening level hazard assessment by further characterizing variability within the assay system and developing high-throughput compatible methods for correcting for the free fraction of chemical in the test well. The contents of this abstract neither constitute nor necessarily reflect US EPA policy.

Sediment is an important component in aquatic environments and acts as a sink for environmental pollutants as they may contain a huge variety of environment- ing contaminants absorbed to sediment may contribute directly or after re mobilization to adverse ecological effects. Sediment toxicity tests have been proven as critical components in environmental risk assessments. Zebrafish (Danio rerio) are used as model systems in ecotoxicological tests, and since 1996 have been used to test sediment toxicity. In this study, sediment from Lower Rio Grande Valley resacas were extracted for LCMS and GCMS analyses using QuEChERS method. Quantitative chemical analysis of over 100 organic contaminants identified the presence of 89 persistent organic pollutants in sediment extracts from Lower Rio Grande Valley resacas (oxbow) across four sampling events. Organochlorines followed by PAHs and PCBs were present at higher concentrations and detected more frequently than other classes of compounds. Toxicity of sediment was predicted using Sediment Equivalency Quotients (SEQ) and Hazard Index (HI) approaches. To investigate the toxicity of sediment samples on zebrafish, sediment extracts from 13 locations and 5 resacas were used for zebrafish embryo toxicity tests (zFET). Sediment extracts from chemical analyses were re-evaporated and reconstituted with dimethyl sulfoxide (DMSO). DMSO sediment extracts in use for zebrafish embryos. PAHs and PCBs have proved to cause cardiotoxicity and developmental defects in a variety of fish species. Sediment extract exposures caused significant developmental malformations in zebrafish embryos including yolk sac and pericardial edemas and spinal and tail defects indicative of AhR-associated developmental toxicity and mostly associated with sum PAH concentrations. Results from this study will aid in ecological risk assessments for the surrounding area.

Fish is a source of low-fat high-quality protein, essential fatty acids and other nutrients for many people in the world. However, environmental pollution has impacted its quality as it accumulates toxic substances, including heavy metals, such as mercury, with known negative impacts on human consumers. The aim of this study was to determine the concentration of total mercury (T-Hg) and its...
association with the presence of nematodes in omnivorous-carnivorous freshwater fish from La Mojana region, Colombia. For this purpose, 163 specimens of 16 different species were collected. Total mercury was quantified with a direct mercury analyzer and parasitic infection was assessed measuring mercury prevalence and abundance. The average T-Hg concentrations in analyzed fish was 0.29±0.01 µg/g (range: 0.05-0.70 µg/g). Ctenolius hujeta and Sternopegys macrurus presented the highest T-Hg values (5.01±0.01 µg/g and 3.34±0.01 µg/g respectively, whereas the lowest was detected in Leporinus Myurus (0.16±0.01 µg/g fw). Nematode (Contracecum sp) prevalence and abundance in examined fish were 36.2% and 2.34±0.51 parasites/fish, respectively. The parasite prevalence for C. hujeta and S. macrurus was 100%, and their corresponding abundances were 6.0±5.8 and 13.50±5.36 parasites/fish, respectively. In contrast, L. Myurus registered a nematode prevalence of 35% and an abundance of 1.19±0.38 parasites/fish. Significant Spearman correlations were found between T-Hg concentration and trophic level (p=0.026; p=0.002). However, T-Hg did not correlate with parasite abundance (p=0.118; p=0.067). These results suggest that T-Hg concentrations depend on the trophic level and may not impact parasite infection in fish. Minciner (2021) [BPIN 2020000100001 and BPIN 2020000100364] Res. 0126/2021. UniCartagena (062-2022).

4406 After Correcting for "Concept Drift," Deep-Learning Methods Can Now Achieve Human-Level Performance when Predicting Article Exclusion Reasons during ECOTOXicology Knowledgebase Curation
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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 screening tools, a key component of this effort is to determine which specific data the data, the data is updated quarterly, employs a methodical systematic review process. This labor-intensive workflow requires curators to regularly evaluate tens of thousands of candidate references, the majority of which are rejected as not relevant. After the careful review of tens of hundreds of thousands of articles, the ECOTOX database currently (as of December 2022) contains abstracts for 12,714 chemicals and 13,936 species extracted from 53,763 references. The availability of this extensive dataset of historical screening decisions has provided us with the opportunity to develop state-of-the-art neural network classifiers to partially automate title and abstract screening and to categorize (e.g., human health, fate, chemical methods) rejected references. While initial proof-of-concept results from these models were very encouraging, we recently noticed that the "meanings" of several of the rejection categories have evolved over time due to concept drift, and that certain labels had been added or removed from current usage. Therefore, in order to be more representative of future screening tasks, we have recently collected new dual screening decisions for a sample of 5,636 abstracts at the abstract level and used these data to train a refined natural language classification model on the modified exclusion categories and demonstrated that it can accurately predict the reasons various an irrelevant article should be excluded from the ECOTOX database. While the performance of the model varies depending on the reason for exclusion, the improved methods achieve a micro-averaged F1 score of 0.676 across all reasons. Furthermore, since human screeners do not always agree on the correct classification, it is possible to compare the congruence between individual human screeners and between human screeners and model predictions. The resulting Cohen’s Kappa scores demonstrate that the model predictions now perform at about the level of an average human reviewer, with some screeners consistently outperforming the model and other screeners underperforming. The latest model has now been integrated into the EcoTox version of the SWIFT-Active Screener software and is being used regularly as part of the EcoTox literature curation pipeline at EPA. So far almost 400,000 candidate EcoTox abstracts have been uploaded into Active Screener, and of these, 292,000 were eliminated from screening, saving more than 75% of the manual effort required. As we conclude this phase of the project, our focus is now shifting to automation of screening and data extraction of the full texts from the references that remain after title and abstract screening has completed. This abstract does not necessarily reflect the views or policy of the US EPA.

4407 Mitigating Potentials of Antioxidant-Rich Plants in Earthworms Exposed to Glyphosate
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The importance of pesticide application in preventing crop losses cannot be overemphasized; however, one big risk factor threatening the environment in agricultural activities is the use of pesticides. Concerns continue about their potential to adversely affect non-target organisms. Glyphosate is a non-selective herbicide that has adverse effects on non-target organisms. This present study investigated the effects of glyphosate on mice exposed to two different concentrations of antioxidant-rich plants, Ocimum gratissimum and Telfairia occidentalis, to remediate these effects. Earthworms (Eisenia fetida) were placed into four groups and treated with 2% concentrations of glyphosate in soil. Group A: only food and water (control); Group B: 2% glyphosate + Ocimum gratissimum; Group C: 2% glyphosate + Telfairia occidentalis and Group D: 2% glyphosate and food alone. The worms were collected on the 3rd, 7th and 14th, days postexposure. During these intervals, the weight of the worms and activities of antioxidant enzyme - superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) were measured to determine the antioxidant levels. Furthermore, the percentage of DNA fragmentation was measured to assess the level of DNA damage. Compared with the control group, earthworms exposed to glyphosate and fed with Ocimum gratissimum and Telfairia occidentalis showed varying responses, with increased activities of CAT, SOD, GSH and reduced levels of MDA. Also, decreased fragmented DNA was observed in earthworm groups fed with Ocimum gratissimum and Telfairia occidentalis in comparison with the group treated exclusively with the herbicide. These results suggest that toxicity from glyphosate exposure significantly reduced oxidative damage, lipid peroxidation and DNA damage in Eisenia fetida by the antioxidant-rich plants. This implies that soil organisms like earthworms could suffer significant toxicity when exposed to concentrations of glyphosate. The potential for the toxicity of this herbicide on earthworms to be greatly reduced exists when the worms consume these plants.

4408 Using UPLC–HRMS to Investigate the Bioavailability, Toxicity, and Bioaccumulation of Road Runoff Water-Derived Contaminants in Juvenile Salmonid Species
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Storm-water runoff has been correlated to escalating rates of pre-spawn mortality (PSM) events in Coho salmon in Pacific Northwest streams. Recently, a chemical derived from tires, N-(1,3-Dimethylbutyl)-N’-phenyl-p-phenylenediamine-quimine (6PPD-q), was isolated from storm run off water and observed to induce acute Coho mortality. Information regarding 6PPD-q’s chemical behavior in aquatic systems is limited, and sensitivity among salmonid species is varied. It is hypothesized that exposure to 6PPD-q and other PSM contaminants commonly detected in surface waters, such as the PAH’s 1-methylnaphtalene (1-MN) and anthraquinone. To better understand the mechanisms of toxicity of 6PPD-q and the differences observed among salmonid species, Chinook salmon were exposed to 6PPD-q (0.001 - 0.01 mg/L, 1-MN (0.1 - 10 mg/L), and anthraquinone (0.1 - 10 mg/L). To ensure exposure concentrations were accurate, first, the degradation of 6PPD-q under experimental conditions was investigated. Glass beakers filled with 200 mL of ultra-pure LCMS-grade water, well water used for fish exposures, and well water from a tank previously containing juvenile salmonids were spiked with 2000 ng/L 6PPD-q and samples were collected after 0, 24, and 48 hours. A targeted metabolomics approach, using ultra-high-perform ance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-HRMS), was developed to quantify 6PPD-q in water samples. Significant degradation of this compound was observed after 48 hours (30%). Initial Chinook exposures up to 0.01 mg/L 6PPD-q and 10 mg/L 1-MN did not elicit any PSM symptoms. To better understand the mechanisms of toxicity, non-targeted mass spectrometry-based metabolomics analysis was performed on liver and brain samples from exposed and non-exposed fish. The present study will help to elucidate the effects of prioritized contaminant mixtures on salmonid health and will improve our understanding of the differences in toxicity observed in salmonid species.

4409 Early-Life Exposure to Microcystin-LR in Mice Potentiates the Risk of Non-cholaer Vibrio Infections in Adulthood

Climate change and its associated stressors have multiple impacts on the environment that include the increased occurrence of aquatic cyanobacterial blooms and a sharp spike in non-cholera vibriosis cases throughout the world. Microcystin-LR (MC), a hepatotoxin and a known inhibitor of cellular protein phosphatases, is released by various cyanobacterial species in aquatic systems as a result of eutrophication. Simultaneously, global warming-mediated rise in the oceanic temperature over the years has led to the increased occurrence of the Gram-negative pathogen Vibrio vulnificus (VW) in the marine aqua system as it has been reported in recent years. In a previous study by our group, we showed the distinct development of several pathological traits in the liver in mice despite being exposed to MC at an early age. Following this rationale, we sought to determine whether early-life MC treatment in an experimental mice model can enhance adverse effects of VW-mediated infection in an experimental mice model. To better understand the mechanisms of toxicity exposure to 6PPD-q will enhance salmonid sensitivity to other cooccurring contaminants. Microbiome analysis obtained by Next-Gen Sequencing showed significant changes in the intestinal microbiome. Microbiome analysis obtained by Next-Gen Sequencing showed significant changes in the host's intestinal microbiome. Microbiome analysis obtained by Next-Gen Sequencing showed significant changes in the host's intestinal microbiome. Microbiome analysis obtained by Next-Gen Sequencing showed significant changes in the host's intestinal microbiome.
Lactococcus lactis which are responsible for improving gut immune health and exerting anti-inflammatory effects were also found to be decreased in abundance due to MC treatment. These results were further corroborated by increased fecal and systemic IgA levels indicating a sustained change in intestinal homeosta-
sis. VV infection in the mice was confirmed by detecting an elevated level in the serum CRP. We also observed an increased intestinal permeability as reflected by increased expression of the tight junction protein Claudin2 with a parallel decreased Ocludin protein expression in the gut epithelial lining of mice that were co-exposed to both MC and VV. In addition, VV administration in early-life MC-treated mice also caused increased TLR4 activation and activation of the NLRP3 inflammasome (as detected by co-localization with its adapter ASC2 protein), which ultimately led to increased intestinal inflammation with simultaneous increased pro-inflammatory cytokine levels compared to only MC or VV treated mice groups. In conclusion, early life exposure to MC in mice can result in affecting the intestinal homeostasis, immunoregulation, and gut microbiome in a persistent manner, which can further augment the creation of a suitable environment for the pathogenicity mediated by the non-cholera VV and exacerbate various pathological symptoms in later stages of life. This work was supported by the grant of the Ministry of Health of the Czech Republic (project 10828/01-02: Project 4 Toxicology awarded to Dr. S. Chatterjee.

4410 Development of a Skin Sensitization Testing Strategy for Extracts of Medical Devices and Consumer Products

H. Bendova, L. Svobodova, M. Rucki, A. Viklova, K. Keljova, D. Jirova, and H. Kolarova. National Institute of Public Health, Prague, Czech Republic. Sponsor: H. Kandera. Medical devices must be tested before marketing for skin sensitization in accordance with ISO 10993-10. This standard predominantly refers to in vivo tests, however, it does not exclude the use of alternative in vitro methods, which have been sufficiently technically and scientifically validated. It is foreseen that due to the complexity of the skin sensitization endpoint, a combination of several methods may be used to address all key endpoints. The LuSens Outcome Pathway (AOP). The objective of this study was to evaluate the sensitization potential of 97 commercially available samples of medical devices and consumer products using a combination of in vivo (OECD TG 442A, Local Lymph Node Assay - LLNA/DA), in chemico (OECD TG 442C, Direct Peptide Reactivity Assay - DPRA) and in vitro (OECD TG 442D, LuSens) methods with the aim to enhance the testing strategy for safety assessment of extracts, to optimize the in chemico/in vitro tests and extraction procedures and to extend the applicability domain of New Approach Methodologies (NAMs), recently successfully validated for the classification of chemicals. A good agreement between in vitro and in vivo results was achieved regarding the absence of skin sensitization potential (90.7 %), however, discrepancies in positive classifications have been recorded (10.0 %). The mismatch between in vitro and in vivo results might be caused by specific responses of the immune system of the living organism. The optimization of DPRA method using prolonged extraction period (72 h) provided consistent results. Last but not least, the impact of extraction vehicle (supplemented with bovine serum) on the outcome of the LuSens method was recorded. The combination of DPRA and LuSens methods helped to improve the overall prediction power compared with the single test method approach, however, the implementation of a third in vitro method covering Key Event 3 (KE3) of the Skin Sensitization AOP could increase the accuracy of the NAMs. Suitable and feasible non-invasive skin sensitization testing strategy for extracts from medical devices and consumer products. Animal experiments were performed in compliance with animal welfare laws of the European Union (DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes). Supported by ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).

4411 Considerations for Toxicological Risk Assessment of Hindered Phenolic Antioxidants in Accelerated Aged Devices

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ISO 10993-1:2018 requires manufacturers to assess the biological safety of medical devices over the devices’ entire shelf life. ASTM F1980 accelerated aging (AA) for 120 days at 55°C was conducted to simulate 3 years of shelf life (T3) on Getinge vascular graft devices (permanent implant with direct contact to blood) which are constructed from ePTFE. Chemical characterization of aged (T3) and unaged (T0) control devices was conducted per ISO 10993-18:2020 using water, hexane, and ethanol extraction techniques as recommended by LCQDMS, QToF/MS and GCMS. Hindered phenolic antioxidants such as Irganox 1010 (1010) and butylated hydroxytoluene (BHT) were detected in T0 extracts. The extraction process and AA can induce thermal degradation and hydrolysis reactions and yield Irganox 1310 (1310) and 1310 derivatives. Comparison of T0 and T3 extract profiles showed changes in the abundance of compounds such as Irganox 1010 (1010), Irganox 1310, and BHT thermal degradation, hydrolysis, and oxidation products in T3-aged devices. These data suggest that the extraction process may be producing some 1010 degradation, but the AA process exacerbates degradation. Toxicological risk assessment (TRA) of T3 extracts determined estimated exposure levels of 1310 hydroxylated to be a compound of potential concern (COPC) based on the use threshold of toxicological concern (TTC) limits, a screening limit. TRA of 1310 hydroxylated required a read-across strategy to derive tolerable exposure (TE). Although 1310 hydroxylated likely originated from 1010 degradation, 1010 is not readily bioavailable and is largely excreted in the feces untransformed. Due to its larger structure and difference in the solubility parameters, 1010 is not an appropri-
ate read-across surrogate for 1310 hydroxylated. Margin of safety (MOS) calculations equaled ≥1.0 for 1310 hydroxylated T3 devices using TE values derived from BHT chronic rat NOAEL of 25 mg/kg/day. Therefore, the assessment of the physicochemical properties of structural surrogates and potential transformation pathways should be implemented during the review of chemical characterization data for AA medical devices and the selection of an appropriate read-across to evaluate the potential for toxicity.

4412 Comparison of Contrast Media Components with International Organization for Standardization (ISO) Standard Solvents to Determine Representative Patient Risk

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Medical devices are subject to rigorous testing during the biological evaluation process including solvent extracts that are used to chemically characterize the device for toxicological risk assessment (TRA). Per ISO-10993 guidelines, it is recommended that two extraction solvents of differing polarity (such as water or ethanol) are utilized to produce a robust extractables profile for the TRA process. As a standard, the contrast media injector was evaluated by simulated use and exhaustive extraction using both water and a mixture of water and ethanol. TRA of water-extracted chemicals identified no unacceptable risks. Conversely, several chemicals in the ethanol extract were present above acceptable limits. Because of these findings, Gradient evaluated the physical and chemical properties of the individual components of two widely used contrast media - Contrast Media A and B - in order to determine their extractive power compared to typical ISO 10993-18 polar solvents (such as water or saline), semi-polar solvents (such as ethanol, isopropanol, or methanol), and non-polar solvents (such as hexane or benzene). Specifically, the octanol-water partition coefficient (Log Kow) and relative electro-
negativities ([CF1] of CH, NH, and OH bonds of the contrast media components were compared to those of common solvents. Intravenous contrast media are solutions that utilize elements, typically iodine, organically bound in ionic or non-ionic compounds in aqueous solution. All of the components in Contrast Media A demonstrated negative Log Kow values (-10.42 to -0.77) and 3 out of 4 components in Contrast Media B had negative Log Kow values (-2.79 to -1.56) indicating high water solubility of these ingredients. The fourth component in Contrast Media B (Log Kow = 0.54) readily dissociates in water (exhibited by a relatively high water solubility value of 3.25E+4 mg/L) and other polar solvents. This analysis showed that highly polar solvents (such as water) represent the extractive powers of both Contrast Media A (such as non-polar-solvent extraction solutions) and non-polar solvents (such as hexane or benzene) were concluded not to be representative of either contrast media. Based on this analysis, although the ethanol-extracted chemicals provided useful compositional data, it was concluded that the TRA of water-extracted chemicals best represented potential patient risk.

4413 Dynamic Headspace GC/MS Method to Detect Volatile Extractables from Medical Device Materials


Volatile extractables, released from medical devices during use, are a concern as they may expose patients to harmful levels of toxic compounds. According to ISO 10993-18:2020, the Analytical Evaluation Threshold (AET) is used to determine the analytical sensitivity required to detect extractables from medical devices. Compounds, at or above the AET, need to be reported for toxicological risk assessment. Currently, volatile analysis by static headspaces is used as a supplementary technique for medical device or material extracts. Variation of signal response in static headspace led to undefined AET for the volatile analysis method. Therefore, increased headspace extractions for volatile quantification is needed for improved hazards identification. This study was designed to evaluate the performance of dynamic headspace (DHS) gas chromatography-mass spectrometric (GC-MS) analysis to achieve the sensitivity levels suitable for proper toxicological risk assessment for volatiles extracted from medical devices. DHS method for static headspace extractions was supplemented with additional volume extracted to compensate for the loss of volatile compounds during DHS extractions. DHS method, compared to static headspace, increased yield of volatile compounds, which contained 24 volatile compounds suspended in N, N-Dimethylformamide (DMF), each at 5000 µg/mL original concentration. Diluted standard mix (10,000X in 0.9% NaCl solution, 100 µL) was used for initial method development and analyzed using GERSTEL MPS attached to the Agilent GC-MS system. Two different
methodology were designed to address both volatile and semi-volatile compounds. The efficiency of DHIS extraction was optimized based on incubation temperature, trapping volume/time, adsorbent type (Carbopack B/Carbopack X (Carbopack B/X) and Tenax TA), and drying time for low-volume samples. Method performance was compared with commonly used static headspace GC-MS analysis. For volatiles analysis method, 37 °C incubation temperature, 100 mL trapping volume and 5 minutes trapping time provided the optimal analyte responses for compounds with higher air-water partition coefficients, such as alkanes. Both adsorbent materials performed similarly with the volatile analysis method. In contrast, the semi-volatile method performed at the same incubation temperature, but with an increased trapping volume (750 mL) and time (15 minutes) along with a drying time of 16 minutes improved the headspace extraction of compounds, such as alcohols, that tend to remain in the water due to their low air-water partition coefficients. In the semi-volatile method, Tenax TA adsorbent material showed >90% reduction of the peak area responses, especially for early eluting compounds (low boiling point), compared to the Carbopack B/X adsorbent material. To further improve the headspace concentration of more water-soluble compounds such as alcohols and ketones, the effect of surfactants addition to the sample matrix was also investigated. Application of developed methods were tested using saline extracts of various medical device materials such as ABS, Buna and PVC. Preliminary results showed improved efficiency in detecting volatile extractable in ABS material extracts with the semi-volatile method with increased peak area responses compared to the volatile method. This sensitive dynamic headspace GC/MS method may facilitate improved toxicological risk assessment for the volatiles detected in medical devices.

4.4.15 A Novel 3D Imaging Pipeline for Analyzing Radiofrequency Catheter Ablation in Preclinical Animal Models

Radiofrequency catheter ablation uses programmed electrical stimulation to alter or destroy cells causing irregular cardiac activity. Despite its history of clinical adoption across a range of therapeutic areas, the 3D volume of ablated tissue has yet to be quantified. To address this gap, we developed a novel Serial Two-Photon Plus (STPP) pipeline to visualize and quantify ablated tissue through imaging of sub-micron cellular features across treated porcine liver and kidney samples. Porcine liver and kidney were treated ex vivo with an ablation pen at three timepoints (0, 20, and 40 seconds) and imaged with the STPP pipeline using a TissueCyte system. The resulting high-resolution multi-channel datasets at 920 nm yielded 3D models of the label-free collagen matrix and cellular autofluorescence. Automated analysis pipelines were developed to extract the damaged area via detection of the autofluorescence intensity and characteristics of the collagen matrix. Resulting high resolution 3D models successfully isolated and quantified the ablated tissue volume and characterized spatial changes in tissue texture for the three treatment timepoints in the liver and kidney. In addition, regional analysis quantified differences in fractional area, bundle frequency, and anisotropy of collagen fibers in treated samples. The STPP pipeline produces translational high-throughput preclinical ablation data with enhanced sensitivity and precision, providing the ability to quantitatively change in total ablation volume in tissues at different timepoints and power levels in a variety of organs.

4.4.16 Weight of Evidence Approach for Benefit-Risk Assessment of CMR Substance Use in Medical Devices
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The European Union Medical Device Regulations (MDR) require manufacturers of medical devices to justify the use of substances classified as having Category 1A or 1B carcinogenic, mutagenic, or reproductive toxicity (CMR) at concentrations above 0.1 % weight for weight (w/w). Often, these classifications are based on toxicity tests conducted with materials that are inherently different from the final materials used in medical devices. Therefore, in order to prepare the necessary justification, it is crucial to understand the benefits and hazards associated with the CMR materials in medical devices. The goal of this work was to review and develop a weight-of-evidence (WoE) approach that can be used in benefit-risk assessments for the justification of the use of CMR substances in medical devices. The developed WoE approach considers data from multiple lines of evidence including 1. device function; 2. device performance; and 3. risk assessment including exposure to CMR substance. The data for each line of evidence was evaluated qualitatively with likelihood described in accordance with the recommended semi-quantitative confidence scale including ISO terms, subjective range, and probability terms. Sources of data and evidence consist of physico-chemical characterization, pre-clinical testing, and clinical testing which may include peer-reviewed publications, toxicity testing, and information from equivalent devices. The overall aim of the methodology is an objective decision criteria analysis of feasibility, performance, and risk considerations to support and justify either continued use of the CMR substance or substitution with a suitable alternative. The approach outlines key information that needs to be gathered (e.g., test system and protocols, material tested, results, etc.), how to assess the reliability of the study, and discusses how to assign a weight of evidence (i.e., strong, moderate, weak, uncertain) to the evidence based on relevance to the material and device at hand. The gathered information from the various lines of evidence is reviewed and integrated together to provide justification for continued use of the CMR substance. Co-containing alloys have unique physical properties, including superior wear and fatigue resistances, that offer a wide range of use in different medical devices. The approach taken in this evaluation to justify the continued use of Co in medical devices synthesized information on functionality, performance, availability of alternatives, and balanced weighing of the benefits and risks. A balanced weighing of the risk was achieved by careful screening of the relative differences in functionality, performance, and risk objectively for the current use of Co-containing alloys as well as possible alternatives. Overall, this WoE approach provides a roadmap for assessing the benefit-risk of CMR substances in medical devices and outlines key resources that can be reviewed when preparing a justification.
challenges of assessing the biological safety of a novel emulsion medical device

4417
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Biocompatibility testing of medical devices is conducted in accordance with ISO 10993 and specific regional regulatory requirements. Given that most medical devices are solid (e.g., polymeric/metal), biocompatibility testing is typically conducted using device extracts and test methods that are well-suited for evaluating device extracts. Depending on their compatibility with the test systems, liquid medical devices may require modifications to the standard biocompatibility test methods. The device described in this case is an emulsion, with olive oil residing in the internal phase, water comprising the external phase, and surfactants enduring at the water-oil interface. Due to the unique physiochemical properties of the emulsion, conducting biocompatibility testing, specifically the in vitro hemocompatibility testing, requires consideration. In vitro hemocompatibility testing (e.g., hemolytic, partial thromboplastin time [PTT], platelet and leucocyte [P&L] and complement activation [CA]) can be completed to assess the potential of the device to cause material-induced hemolysis and thrombosis. These tests, which follow ASTM and ISO standards, are not designed to assess liquid test articles and it was expected that the opacity of the emulsion would interfere with the mechanical and optical-photographic coagulation equipment used. To address hemocompatibility, modifications to the standard in vitro test methods were made to accommodate the device. When tested for hemolysis following ASTM F756 and ISO 10993-4, results were inconclusive due to test sample absorbance readings outside the hemoglobin standard curve. To resolve this issue, an additional dilution step of the test article supernatant was incorporated, which was required that allowed for the absorbance readings of the test article samples to remain within the standard curve of the test system. Modifications of the applicable ASTM standards were also required to complete CA, PTT, and P&L testing. A non-GLP PTT feasibility study was conducted using the test article at both a 1:4 and 1:1.10 ratio to emulsion. It was found that the ratio was too high to work at the 1:4 ratio; therefore, this ratio was used for non-GLP CA and P&L feasibility testing. Using these modifications, all test systems were demonstrated to be compatible with the device and GLP testing confirmed the device is hemocompatible. While modification of hemocompatibility test methods was feasible, E&L analysis that aligns with ISO 10993-18 and regulatory agency expectations may well be required and would require high burdensome method development activities. Due to the high lipid concentration in the emulsion, direct chromatographic separation of the device extracts is not possible. Sample preparation would require modifications including filtration and/or significant dilution resulting in a high risk of sample loss from solvent evaporation, and solids precipitation raising potential of potential leachables to undetectable levels. The resulting data would likely not be representative of clinical use. We contend that chemical characterization through detailed information gathering, instead of E&L analysis, is the appropriate method for addressing long-term systemic toxicity risks of the device. Overall, many of the challenges of assessing the biological safety of a liquid device may be overcome through creative test method alterations; however, E&L testing would require significant development activities, and results may not accurately inform the safety of the device. Additional guidance would be useful in achieving a representative assessment of potential leachables from unique medical devices.

4418
Case Studies on Risk Mitigation Strategies for Medical Device Cytotoxicity Failures

According to ISO 10993-1:2018 and FDA’s Guidance on Use of ISO 10993-1 (2020), cytotoxicity is a key biocompatibility testing requirement for all medical devices, regardless of the duration and nature of tissue contact. Cytotoxicity tests are performed to assess cell viability, either quantitatively or qualitatively, and it is not uncommon to encounter cytotoxicity failures during medical device testing. A monolayer of cells, as used in vitro cytotoxicity assays, is not representative of clinical use. We contend that chemical characterization through detailed information gathering, instead of E&L analysis, is the appropriate method for addressing long-term systemic toxicity risks of the device. Overall, many of the challenges of assessing the biological safety of a liquid device may be overcome through creative test method alterations; however, E&L testing would require significant development activities, and results may not accurately inform the safety of the device. Additional guidance would be useful in achieving a representative assessment of potential leachables from unique medical devices.

4419
Framework for Evaluation of Extract Processing in Chemical Characterization
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As an alternative to animal studies in biocompatibility and safety evaluation of medical devices, chemical characterization and toxicological risk assessment (TRA) has been increasingly used in device submissions to address some biological endpoints. Although the ISO 10993-18 standard was recently revised to allow for improved guidance for chemical characterization, extract processing prior to chemical analysis was not exhaustively discussed. Extract processing, such as solvent exchange (liquid-liquid extraction) and solvent evaporation, is often a necessary step to render the extracts amenable to chemical analysis as well as to achieve the necessary analytical evaluation threshold (AET) to allow for subsequent TRA. However, potential losses of extractables during extract processing may result in underestimation and/or underreporting of extractables, leading to incorrect conclusions in TRA. Currently, there is no clear framework for performance evaluation of extract processing for chemical characterization. This invitation to write is to address this, general framework was created for the evaluation and reporting of extract processing including estimation of recovery through predictive models, selection of appropriate surrogate chemicals for evaluation, experimental verification of the recovery estimation, and reporting the information within the context of the applicable chemical space. To demonstrate the established framework, a predictive physiochemical model was established for liquid-liquid extraction and solvent evaporation processes. For liquid-liquid extraction of water extracts into dichloromethane, partition coefficients were calculated using a linear solvation energy relationship (LSER) method for a database of approximately 13,000 relevant chemicals. For solvent evaporation, partition coefficients were calculated using direct injection gas chromatography-mass spectrometry (GC-MS) to establish the efficacy of the model under several different conditions including adjusted dichloromethane/water ratios, analyte concentrations, solution pH, and number of sequential extractions. For solvent evaporation, recoveries were predicted for a separate database of 22,000 relevant chemicals by calculating the solvent-air partition coefficients using LSERs and the subsequent relative evaporative ratio between the two phases. The resulting surrogate chemicals was verified experimentally by GC-MS under various conditions including evaporation method (nitrogen blowdown and rotary evaporation), evaporation temperature, and solvent. In both cases, the models selected demonstrated a root mean square error of approximately 15% and were found to reliably predict the recovery of an extractable under all tested conditions. Applying this framework creates a more comprehensive communication of the efficacy of an extract processing method, which can significantly impact the assessment of appropriateness of extract preparation parameters for chemical characterization and ultimately the quality assessment of chemical characterization data used for TRA.

4420
Development of an In Vitro Test Method for Irritation of Medical Devices Used in the Oral Cavity
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The irritation potential of any medical device (MD) designed to contact oral tissues (gingival, buccal, lingual, etc.) needs to be evaluated before clinical use. The objective of this project is to develop and validate an in vitro assay to assess...
the oral irritation of medical devices. This assay will replace the historical in vivo assay performed on Syrian hamsters. For MDs that contact the skin, ISO 10993-23 has been adapted for use with no trend identified; however, when the relationship of the surface area to the rinsing time can extend the time of the surgical procedure. Extended surgery may place increased emphasis on the length of time of the rinsing process, as longer rinsing procedures could disturb this important defense mechanism against HEMA in cells. Based on this knowledge, we hypothesize that nicotine exposure increases the toxicity of HEMA by reducing the autophagic capacity. For this in vitro study, a human tongue squamous carcinoma cell line (PE/CA-P49) was used as a model for oral exposure. Cells were cultured and exposed to HEMA (2 mM) and nicotine (S-10 mM) individually and in combination. The effects of nicotine were compared to the effects of an inhibitor of lysosomal activity (Bafilomycin A1; 10 nM). Cell viability was measured using the MTT assay. The cellular lysosomal activity was measured using IncuCyte, a real-time cell imaging system, was used to monitor cell morphology and mobility (scratch wound migration assay). The viability of P49 cells dropped significantly after combined exposure to HEMA and nicotine for 24 h, compared to individual exposures. Both nicotine and BAF-exposed cells showed similarly increased levels of apoptosis. However, the effect was altered after combined exposure to BAF. No visible changes were seen in cells exposed to HEMA alone. Individual exposure to nicotine and HEMA had no/minor measurable effect in the wound healing assay, while the combined exposure resulted in almost total inhibition of cell migration. This study showed that combined exposure to nicotine and HEMA gave cellular effects that were not observed in individual exposure experiments. Although these results support the impairment of autophagy by nicotine, more experiments are needed to verify that dysfunctional autophagy is responsible for the toxic response.

4421 Is Glutaraldehyde a Red Herring in Heart Valve Cytotoxicity?

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Since the inception of animal-derived surgical and transcatheter heart valves, glutaraldehyde has played a vital role in the fixation, reduction in bioburden, and sterilization of these medical devices. Despite the role it plays in the manufacturing of life-saving devices, residual glutaraldehyde may elicit toxic effects, most notably cytotoxicity and genotoxicity. To reduce the risk of glutaraldehyde toxicity, manufacturers are instructed to rinse the devices in saline prior to implantation. Device manufacturers develop the rinsing procedures for heart valves with a heavy emphasis on the length of time of the rinsing process, as longer rinsing procedures are considered more favorable for reducing the amount of residual glutaraldehyde on the devices. However, as the devices are prepared at the time of the surgery, the rinsing time can vary throughout the surgical procedure. Extended surgery times pose an added risk to the patient during and after treatment with resin-based cardiac devices.

4422 In Vitro Assays for Assessment of Skin Sensitization Hazard and Potency of Isobornyl Acrylate

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Acrylates have a wide range of applications for medical devices as they can bring several advantages such as transparency, superior-absorbency and hardness in combination with flexibility. The manufacturing of acrylic materials typically involves using at least one monomer of either acrylate or methacrylate which react to form a polymer. Several methodologies can be used for polymerization and the degree of polymerization of the final material can vary, hence some products can contain more residual monomers than others, and human exposure to these well-known skin sensitizers may increase the risk of developing the adverse immunological response, allergic contact dermatitis. In 2020, the acrylic monomer, isobornyl acrylate (IBOA, CAS 8888-33-5), was named allergen of the year by American Contact Dermatitis Society due to the increased number of patients that were sensitized to IBOA found in glucose sensors and glucose pumps. IBOA is also present in other medical device products as plastic materials, coatings, sealants, glues, adhesives and inks. As a result, it is important to develop methods used today to assess skin sensitization to properly identify the skin sensitizing potential of IBOA in medical devices and avoid the risk of sensitizing more individuals to this chemical. The GARDskin (OECD TG 442E) assay, initially developed for hazard identification of a wide range of skin sensitizers, has been adapted for use with polar and non-polar solvents as described in ISO 10993-12:2021 and can be applied to assess the skin sensitization of medical devices. Further development of the GARDskin protocol has also enabled the prediction of skin sensitization potency by using a dose-response measurements. The GARDskin Medical Device assay has the potential to replace in vivo tests for risk assessment of medical devices.

4423 Nicotine Promotes HEMA Toxicity in PE/CA-P49 Cells

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The monomer 2-hydroxyethyl methacrylate (HEMA) is commonly used in resin-based dental materials. HEMA is known to leak into the patient’s oral cavity during and after treatment with resin-based materials. In addition, HEMA is known to have a cytotoxic potential in vitro. The cellular defense against HEMA is suggested to involve autophagy. The concentration of nicotine in tobacco and other nicotine-containing products is known to be relatively high. For patients treated with resin-based dental materials and at the same time are using nicotine-containing products such as snus or gums, a co-exposure will occur. The knowledge regarding nicotine toxicity has mainly focused on the interaction with nicotine cholinergic receptors. Still, it is also known that nicotine directly affects the lysosomes in cells. Furthermore, nicotine has been shown to induce autophagy. Hence, impairment of lysosomal function by nicotine could disturb this important defense mechanism against HEMA in cells. Based on this knowledge, we hypothesize that nicotine exposure increases the toxicity of HEMA by reducing the autophagic capacity. For this in vitro study, a human tongue squamous carcinoma cell line (PE/CA-P49) was used as a model for oral exposure. Cells were cultured and exposed to HEMA (2 mM) and nicotine (S-10 mM) individually and in combination. The effects of nicotine were compared to the effects of an inhibitor of lysosomal activity (Bafilomycin A1; 10 nM). Cell viability was measured using the MTT assay. The cellular lysosomal activity was measured using IncuCyte, a real-time cell imaging system, was used to monitor cell morphology and mobility (scratch wound migration assay). The viability of P49 cells dropped significantly after combined exposure to HEMA and nicotine for 24 h, compared to individual exposures. Both nicotine and BAF-exposed cells showed similarly increased levels of apoptosis. However, the effect was altered after combined exposure to BAF. No visible changes were seen in cells exposed to HEMA alone. Individual exposure to nicotine and HEMA had no/minor measurable effect in the wound healing assay, while the combined exposure resulted in almost total inhibition of cell migration. This study showed that combined exposure to nicotine and HEMA gave cellular effects that were not observed in individual exposure experiments. Although these results support the impairment of autophagy by nicotine, more experiments are needed to verify that dysfunctional autophagy is responsible for the toxic response.
ISO 10993-23:2021 describes a stepwise approach for evaluating the irritation potential of medical devices that emphasizes the use of human tissue-based in vitro testing in preference to animal-based in vivo testing. This paradigm shift in medical device testing is in alignment with global efforts to reduce the reliance on animal testing as well as the guidance of ISO 10993-2:2022 regarding medical device testing and animal welfare. However, two years after the release of ISO 10993-23:2021, there is still inconsistent regulatory acceptance of the standard’s in vitro assay, and manufacturers must often conduct both in vitro and in vivo assays to meet the requirements of regulatory bodies across the globe. This results in added cost and time to device testing, potential delays in product to patients, and unnecessary laboratory animal use. Previously, we examined the correlation of 15 paired in vitro and in vivo irritation assay test results to understand if the in vitro assay was suitable for use with final, finished medical devices in comparison to the in vivo assay ("Correlation Of In Vitro And In Vivo Skin Irritation Assays In Medical Devices: A Case Study," 2022). Because of ongoing inconsistent regulatory acceptance of the in vitro model, we have continued to conduct testing under both methodologies. To date, we have collected more than 50 sets of paired data presented herein, including results from devices known to be positive for irritation in the in vivo assay. A comprehensive review of the data showed a strong correlation between the results of the in vitro and in vivo assays, indicating that the in vitro assay is an acceptable replacement for the in vivo method to investigate the irritation potential of final, finished medical devices. However, a priori knowledge of the device materials should be utilized prior to testing with the in vitro assay to ensure method suitability.

Impact of Gamma and X-Ray Sterilization Modalities on Medical Device Extractables


Gamma sterilization has been used for more than 60 years in the terminal sterilization of medical devices. However, gamma sterilization processes currently face obstacles due to increasing global demand for cobalt. X-ray sterilization is being explored as a viable alternative to gamma irradiation. In order to determine what impact sterilization modality might have on the identity and quantity of extractable substances, a comparative chemical characterization study was performed according to ISO 10993-18:2020, in which wound dressings from the same production lot were sterilized either by gamma or x-ray irradiation. Gamma- and X-ray-sterilized test articles were extracted exhaustively in triplicate, per solvent, using iterative extraction periods via gravimetric determination of non-volatile residue (NVR) and using a range specified in ISO 10993-12:2021. Once determined exhaustive, reserved aliquots were pooled and analyzed for volatile to non-volatile and elemental extractables using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), inductively coupled plasma-mass spectrometry (ICP-MS), and headspace gas chromatography-mass spectrometry (HS-GC-MS). A toxicological risk assessment (TRA) was then performed according to ISO 10993-17 to evaluate patient safety and demonstrate chemical and biological equivalence. Thirty-four individual compounds or chemical categories were identified as unique to and/or present at higher amounts in the X-ray sterilized dressing compared to the gamma-sterilized control dressings. The known toxicity of each identified compound was characterized based on a systematic review of available toxicological information, and potential health risk was assessed by comparing worst-case estimated exposure doses to each compound’s tolerable intake (TD) or applicable threshold of toxicological concern (TTC) value. Under the processing conditions and target dose ranges used for this study, X-ray sterilization produced a very similar extractables profile compared to gamma sterilization. The TRA demonstrated that the observed differences in extractables did not result in additional or different toxicological concerns, so the previously established biocompatibility profile of gamma-sterilized wound dressings may be extended to X-ray-sterilized dressings of the same construction.

Characterization of the ISO Database of Reference Chemicals for Interlaboratory Studies to Demonstrate the Applicability of OECD Methods to Assess Skin Sensitization of Medical Devices

P. Cornelis, P. Cornelis, S. M. Street, K. P. Coleman, and W. V. Christian, “Characterization of the ISO Database of Reference Chemicals for Interlaboratory Studies to Demonstrate the Applicability of OECD Methods to Assess Skin Sensitization of Medical Devices.”

The skin sensitization potential of medical devices is typically assessed using animal-based assay methods such as the Guinea Pig Maximization Test (GPMT) or the Local Lymph Node Assay (LLNA). There is increased interest in using the non-animal methods from the OECD test guideline 442 series; however, these methods have not been validated for use in the medical device industry and qualified before they can be accepted for evaluation of medical devices. The ISO Technical Specification 11796 under development in Working Group 8 (WGB) of ISO Technical Committee 194 (ISO/TC 194) provides a framework to demonstrate the applicability of these methods to assess the skin sensitization potential of medical devices. Due to the lack of positive and negative reference materials to conduct prevalidation and interlaboratory studies, test samples will be prepared from polar and non-polar extracts of a negative silicone polymer spiked with skin sensitizing or non-sensitizing chemicals. A database of 29 reference chemicals, 22 skin sensitizers and 7 non-sensitizers, representative of raw materials and/or leachables found in some medical devices has been established. The structural, physicochemical and mechanistic domain of the candidate compounds have been characterized and compared to the OECD reference database used to validate the define approaches in the OECD guideline 497. The skin sensitizer chemicals of the ISO database cover a range of potency from weak to extreme. All the mechanistic reaction domains known to be associated with sensitization are represented although as in the OECD database, no reaction domain alerts were identified for nearly half of the chemicals. Statistical analysis of the distribution of values for the physicochemical properties considered relevant to skin sensitization in the OECD Guideline 497 (e.g., octanol-water partition coefficient) showed similarly between the curated database and OECD database. Additional attention was paid to the quantity of the historical animal and human toxicological data and searches were performed in the scientific literature and in several reference databases. The EC3 value derive from dose-response curve in the LLNA was used to define the spiking concentration for 19 of the skin sensitizers. For 2 chemicals, more conservaties values were used from available animal data and for the chemical the concentration was based on a weight of evidence approach from vivo and vitro data. To date there are 9 in vitro and in vivo methods in the OECD TG 442 guidelines, and two methods based on the use of a reconstituted human epidermis model are accepted in the OECD Test Guideline Program. The framework of the ISO TS 11796 will ensure that the same prevalidation and evaluation criteria will be applied to all these methods when conducting prevalidation and interlaboratory studies to demonstrate their applicability to assess skin sensitization of medical devices.

Chemical characterization can be a valuable tool for protecting humans from biological risks from the use of medical devices; however, some materials pose a particular challenge in balancing (1) the exaggeration of clinical condition to ensure accurate and reproducible data (2) an accurate determination of the device’s extractables profile under typical clinical use. Extraction conditions are expected to be at least as aggressive as the conditions of clinical use, with up to three solvents (e.g., polar, semi-polar, and non-polar) recommended for long-term implants. In this case study, a new device component was proposed as part of a long-term tissue-contacting implant device that would be similar in size, use similar silicone-based materials, possess the same properties to perform its function, and have no modifications to the indications for use as compared to the predicate device. Representing diverse conditions, three solvents were initially proposed for chemical characterization testing: water (polar), 20% ethanol (semi-polar), and DMF (semi-polar). Silicones are well-known to swell and/or degrade in non-polar solvents; therefore, they were not considered appropriate for the study. In response to these diverse solvent conditions, the regulatory agency rejected the use of the proposed semi-polar solvents, and also suggested pursuing alternative non-polar solvents. A solvent compatibility study was therefore conducted on the device using five non-polar solvents and three semi-polar solvents in 24-hour iterations at 50 °C with agitation. Viscoelastic inspection and non-volatile residue (NVR) analysis were performed after each round of extraction to assess the compatibility of the test article with the solvents. After the first iteration, the device showed a significant amount of swelling in all non-polar solvents tested, absorbing nearly all of the round 1 extraction solvent. Of the three semi-polar solvents tested, only isopropanol
When materials of medical devices 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 analyses of extractables are followed up with toxicological risk assessments, which evaluate potential harm from exposure to the extractables. The standard ISO 10993-18:2020, which describes chemical characterization approaches, includes statement "Based on published research, 40% by volume mixture of ethyl alcohol/water considered an appropriate surrogate for blood and blood related substances"; however, the published research on this approach relies on a very few material-extractable systems. This can be of concern since unreliable extraction systems can understate concentrations of extractables, leading to incorrect conclusions or greater consumer risk. The goal of this study was to evaluate considerations for creating and justifying alternative solvents for non-targeted extractables analysis of blood-contacting medical devices. We explore two approaches: 1. create simpler simulators of blood with complex protein content that can be used in extractables studies; 2. create simple solvents that may act like blood in certain extractables studies. A panel of extraction solvents from simple surrogates was selected and evaluated. The objective of this study was to determine the suitability of the selected solvents for extractables analysis of blood contacting medical device. The initial data indicate that, binding of medical device extractables to protein containing solutions such as albumin solution can be measured. This ongoing work continues to expand comparison of specific extraction solvents and conditions and verifying with all LC-based analytical methods. This work will be a significant step toward reducing overestimation of the toxicological risk due to exhaustive/exaggerated extraction methods.

4428 Ranking Surrogate Suitability for Read-Across of Medical Device Extractables: A Case Study of Irganox-Related Compounds
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Toxicological risk assessment of medical device extractables often deals with data lacking target chemicals, in which a read-across approach may be required to derive tolerable daily intakes (TDIs). In the absence of regulatory recognized standards or guidelines, we developed a framework for selecting and evaluating the suitability of potential surrogate compounds for the purposes of toxicological risk assessment conducted in accordance with ISO 10993-17. The suitability of surrogate candidates is evaluated in four weighted domains: chemical structure and reactivity, functional groups, physicochemical properties, in silico toxicity predictions, and metabolic profiles. A cumulative suitability score (SS) is then assigned to surrogates in order to assess their relative suitability for read-across to the target compound. Here, we present a case study evaluating the appropriateness of a group of Irganox compounds as surrogates for a common medical device extractable, 4,4’-MDI (CAS #101-68-8) was identified as one of the extractables. This chemical is a moderate skin irritant, as it is classified as a GHS Category 2 skin irritant and is associated with the GHS hazard statement H315. The literature search also identified 4,4’-MDI as a GHS Category 1 skin sensitizer: although it was not sensitizing in a previously completed Buccal test, it was positive in a local lymph node assay (LLNA) with an EC3 of 0.25% to <0.5%, as well as in a mouse IgE test with an EC3 <0.03%. According to skin sensitization frameworks the level of consumer exposure to a skin sensitizer under intended product use conditions (i.e., the consumer exposure level (CEL)) is calculated as the mass of chemical per unit skin surface area (i.e., µg/cm²) and can be compared to the chemical’s acceptable exposure level (AEL). If the CEL > AEL, a sensitization risk exists; conversely, there is no significant risk of sensitization if the AEL > CEL. To determine the AEL, the no expected sensitization induction level (NSEL) is adjusted by the product of four sensitization assessment factors (SAF’s), the aggregate SAF. A previously identified NSEL of 7.4 µg/cm² for 4,4’-MDI, based on an EC3 of 0.08% from an LUNA adjusted by a composite SAF of 100, yields an AEL of 0.074 µg/cm². Assuming that 100% of the 4,4’-MDI extractables from the device, the resulting CEL is 0.67 µg/cm². As the CEL is greater than the AEL, we concluded that there is moderate potential for skin sensitization, and further biocompatibility testing for this device was recommended. Conversely, from a similar surface contacting device, the contact duration of ≥30 days resulted in the identification and screening seven unique extractants, one of which was identified as a potential dermal hazard: phosphorous acid heptadecylphenyl ester (CAS RN/A). Using a published EC3 value of 40.6% from a surrogate, phosphorous acid diisodecyl phenyl ester, we calculated a NSEL of 10,150 µg/cm² and then derived an AEL of 100.5 µg/cm². As this AEL value is greater than the derived CEL of 0.52 µg/cm², we concluded there was an insignificant risk of skin sensitization from this device. This work demonstrates that dermal risk assessment can inform and, in some cases, reduce the need for additional in vivo irritation and sensitization testing.

4429 Alternative Solvents for Non-targeted Extractables Analysis of Blood-Contacting Medical Devices
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(IA) reached exhaustion in 5 rounds, and the device was observed to decolor in all 60/40 IPA/hexane and hexane in 24-hour iterations at 50 ± 2 °C with agitation, with visual inspection and NMR analysis performed after each round of extraction. Visual swelling was observed in each set of the additional solvent compatibility extractions. Solvent absorption during hexane extraction was experimentally determined to be approximately 90% in the first round. Subsequent iterations demonstrated additional solvent absorption of 20-30% each. Swelling was also observed in the 60/40 IPA/hexane extraction, with absorption determined to be approximately 56% in the first round. Similar to hexane, subsequent iterations in 60/40 IPA/hexane demonstrated decelerated solvent absorption. Ultrasonic chemical characterization study was performed with water and IPA extractions; however, IPA extracts were unable to be analyzed due to instrument detector saturation, and additional extractable/leachable and/or biological testing was deemed necessary for biocompatibility evaluation. In conclusion, there is a high burden of solvent induced by regulatory toxicity testing. Ultrasonic chemical characterization of certain materials— including those that are historically established as biocompatible in numerous forms and applications. Additional guidance is imperative to navigate the study design process for these materials and support reasonable, scientifically justified analysis of biological safety.

4430 Assessing Localized Toxicity of Surface-Contacting Medical Devices Using an In Silico Dermal Risk Assessment Approach
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ISO 10993-1:2018 specifies that a biocompatibility evaluation for medical devices should be conducted based on category of device, type of contact, and contact duration to determine whether sufficient data are available to assess health risks or if further studies should be conducted. For surface medical devices in contact with intact skin, the hazard endpoints of most concern are skin irritation, corrosion, and sensitization. By leveraging published dermal risk assessment frameworks, these endpoints can be assessed quickly and potentially eliminate the need for animal testing. We evaluated extractions from a surface contacting device with a contact duration of up to 30 days by first performing a search for reliable published irritation, corrosion, and sensitization data, as well as for any GHS hazard classification or test methods which are available for the chemical. Following extraction of a surface contacting medical device, 4,4’-diphenylmethane diisocyanate (4,4’-MDI, CAS #101-68-8) was identified as one of the extractables. This chemical is a moderate skin irritant, as it is classified as a GHS Category 2 skin irritant and is associated with the GHS hazard statement H315. The literature search also identified 4,4’-MDI as a GHS Category 1 skin sensitizer; although it was not sensitizing in a previously completed Buccal test, it was positive in a local lymph node assay (LLNA) with an EC3 of 0.25% to <0.5%, as well as in a mouse IgE test with an EC3 <0.03%. According to skin sensitization frameworks the level of consumer exposure to a skin sensitizer under intended product use conditions (i.e., the consumer exposure level (CEL)) is calculated as the mass of chemical per unit skin surface area (i.e., µg/cm²) and can be compared to the chemical’s acceptable exposure level (AEL). If the CEL > AEL, a sensitization risk exists; conversely, there is no significant risk of sensitization if the AEL > CEL. To determine the AEL, the no expected sensitization induction level (NSEL) is adjusted by the product of four sensitization assessment factors (SAF’s), the aggregate SAF. A previously identified NSEL of 7.4 µg/cm² for 4,4’-MDI, based on an EC3 of 0.08% from an LUNA adjusted by a composite SAF of 100, yields an AEL of 0.074 µg/cm². Assuming that 100% of the 4,4’-MDI extractables from the device, the resulting CEL is 0.67 µg/cm². As the CEL is greater than the AEL, we concluded that there is moderate potential for skin sensitization, and further biocompatibility testing for this device was recommended. Conversely, from a similar surface contacting device, the contact duration of ≥30 days resulted in the identification and screening seven unique extractants, one of which was identified as a potential dermal hazard: phosphorous acid heptadecylphenyl ester (CAS RN/A). Using a published EC3 value of 40.6% from a surrogate, phosphorous acid diisodecyl phenyl ester, we calculated a NSEL of 10,150 µg/cm² and then derived an AEL of 100.5 µg/cm². As this AEL value is greater than the derived CEL of 0.52 µg/cm², we concluded there was an insignificant risk of skin sensitization from this device. This work demonstrates that dermal risk assessment can inform and, in some cases, reduce the need for additional in vivo irritation and sensitization testing.

4431 Defining a Dose-Based Threshold for Metals in the Analytical Evaluation Threshold
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ISO 10993-1:2018 requires manufacturers to provide chemical information on all medical devices. This information includes chemical characterization, which describes chemical characterization approaches, includes statement “Based on published research, 40% by volume mixture of ethyl alcohol/water considered an appropriate surrogate for blood and blood related substances”; however, the published research on this approach relies on a very few material-extractable systems. This can be of concern since unreliable extraction systems can understate concentrations of extractables, leading to incorrect conclusions or greater consumer risk. The goal of this study was to evaluate considerations for creating and justifying alternative solvents for non-targeted extractables analysis of blood-contacting medical devices. We explore two approaches: 1. create simpler simulators of blood with complex protein content that can be used in extractables studies; 2. create simple solvents that may act like blood in certain extractables studies. A panel of extraction solvents from simple surrogates was selected and evaluated. The objective of this study was to determine the suitability of the selected solvents for extractables analysis of blood contacting medical device. The initial data indicate that, binding of medical device extractables to protein containing solutions such as albumin solution can be measured. This ongoing work continues to expand comparison of specific extraction solvents and conditions and verifying with all LC-based analytical methods. This work will be a significant step toward reducing overestimation of the toxicological risk due to exhaustive/exaggerated extraction methods.

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10993-18:2020, analytical chemical characterization is one approach that can be used to meet this requirement. Analytical chemical characterization uses the Analytical Evaluation Threshold (AET) to ensure instrument sensitivity is adequate and as a reporting limit to screen organic compounds that may be of no-to-low toxicological concern. This threshold is tailored for each medical device based on the number of medical devices used in the extract, the volume of the extract, maximum number of devices used in a clinical setting, the dose-based threshold (DBT), and the analytical uncertainty factor. Currently, the AET is only used for organic compounds, as the DBT is based on the Threshold of Toxicological Concern (TTC) defined in ISO 21726:2019. The TTC is not applicable to inorganic extractables because they were excluded from the database used to derive the TTC. In turn, the lack of a DBT for each metal results in an undefined level of sensitivity required for reporting toxicologically relevant metal quantities. This often results in metals being reported below any threshold of actual toxicological risk, adding unnecessary cost and time to inorganic compound quantification. Herein, DBT values are proposed that can be used to derive a Metal Analytical Evaluation Threshold (MAET) for use in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or other analytical methods for quantifying inorganic compounds that may be released from a medical device. The MAET calculation uses the same inputs as the AET for organic compounds, except metal-specific DBT values are proposed for each element based on toxicity data from the scientific literature. The MAET allows the chemical characterization study designer the ability to (1) ensure the analytical methods have the appropriate sensitivity for quantifying each metal and (2) report metal extractables that may be considered toxicologically relevant. The MAET also increases the confidence in the data the toxicologist is using for toxicological risk assessment of medical devices. In summary, application of the MAET simplifies the handling of inorganic data, ensures meaningful metal data is collected, and reduces data deficiency, involving the sensitivity of the analytical method used to collect inorganic data.

4432 A Guide to Evaluating Potential N-Nitrosamine Risks in Medical Devices
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The threshold of toxicological concern (TTC) represents an exposure level that is of no appreciable risk to humans and is often used as a screening tool when evaluating health risks associated with chemical exposures. Per ISO/TS 21726:2019, the TTC cannot be applied to a set of high potency compounds called the Cohort of Concern (COC). One set of compounds within the COC are N-nitrosamines (N-nos), which are potent carcinogens. Risks associated with N-nitrosamines are expected to be relevant for other metals as well because they may be released from metals such as stainless steel, cobalt, molybdenum, chromium, and manganese, which are all essential elements. Non-essential elements commonly detected included aluminum, boron, barium, strontium and silicon. In all cases, the risk assessment demonstrated that the exposures were toxicologically insignificant. Only 13 elements were detected at levels greater than 5 μg/device. These included essential elements such as calcium, sodium, potassium, magnesium, cobalt, and molybdenum, as well as non-essential elements such as silicon, boron, barium, aluminum, nickel, tungsten and platinum. Many of these elements are known to be present in device materials or manufacturing processes. For example, nickel was commonly reported in nitinol devices at 0.01-7.2 μg/device; these levels are well below the permissible nickel PDE of 20 μg/day. Importantly, no Class 1 elements were detected. Considering the lack of toxicological concern demonstrated for the low levels of elements commonly detected in medical device extracts, the use of a 5 μg TSL is proposed after correcting for any scaling factor and considering the maximum number of devices used clinically. If the calculated worst-case exposure to a particular element is less than 5 μg, then no element-specific assessment is necessary. Applying a 5 μg TSL in evaluating elements would be similar to the TSL approach being introduced in the proposed revised ISO 10993-17 to assess organic extractables and would share the common goal of reducing the burden when assessing extractables present in toxicologically insignificant amounts.

4433 Proposed Toxicological Screening Limit for Elements Extracted from Medical Devices
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Elemental analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is routinely conducted on implantable medical devices as part of chemical characterization and extractability characterization and leachable (E&L) studies per ISO 10993-18:2020. Polar extract of the device following exhaustive extraction are typically analyzed for the presence of between 46 and 68 elements. Because ICP-MS is a highly sensitive technique with very low quantification/reporting limits (in the range of 1-10 ng/ml), miniscule levels (< 1 μg/device) of elements in devices are frequently reported. This results in time and resources being spent conducting quantitative risk assessments on many inorganic compounds that pose no actual toxicological concern at the values reported. To improve efficiency, a conservative toxicological screening limit (TSL) of 5 μg is proposed as an initial step in the assessment of ICP-MS data under the condition that highly toxic, ICH Q3D Class 1 heavy metals (cadmium, lead, arsenic and mercury) are not detected. The TSL value is based on the 10th percentile Parenteral Permitted Daily Exposure (PDE) of 5 µg/day for cobalt, which is the lowest of the 20 parenteral PDEs established for Class 2A, 2B and 3 elements. The PDE is defined as the maximum acceptable intake per day of this, in this case, elemental impurities in pharmaceutical products. Per the proposed revision of ISO 10993-17, a TSL represents the total quantity of a constituent at which a cumulative exposure is at a negligible toxicological risk level and no additional toxicological risk estimation is recommended. A review of recent extractable data from >50 Boston Scientific implantable devices composed of a variety of materials found that 78% of all reported values are < 5 μg/device. A total of 29 elements were detected above the reporting limits. The most common, in order of frequency, were magnesium, zinc, calcium, iron, copper, cobalt, molybdenum, copper, iron, manganese, which are all essential elements. Non-essential elements commonly detected included aluminum, boron, barium, strontium and silicon. In all cases, the risk assessment demonstrated that the exposures were toxicologically insignificant. Only 13 elements were detected at levels greater than 5 μg/device. These included essential elements such as calcium, sodium, potassium, magnesium, cobalt, and molybdenum, as well as non-essential elements such as silicon, boron, barium, aluminum, nickel, tungsten and platinum. Many of these elements are known to be present in device materials or manufacturing processes. For example, nickel was commonly reported in nitinol devices at 0.01-7.2 μg/device; these levels are well below the permissible nickel PDE of 20 μg/day. Importantly, no Class 1 elements were detected. Considering the lack of toxicological concern demonstrated for the low levels of elements commonly detected in medical device extracts, the use of a 5 μg TSL is proposed after correcting for any scaling factor and considering the maximum number of devices used clinically. If the calculated worst-case exposure to a particular element is less than 5 μg, then no element-specific assessment is necessary. Applying a 5 μg TSL in evaluating elements would be similar to the TSL approach being introduced in the proposed revised ISO 10993-17 to assess organic extractables and would share the common goal of reducing the burden when assessing extractables present in toxicologically insignificant amounts.

4434 Implementation of Bionomous EggSorter with AFM-Directed Fluidics for Zebrafish Embryos for High-Throughput Screening
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The Bionomous EggSorter (https://bionomous.ch/bioeggsorter/) recently entered the market as a user-friendly and affordable beta platform to address limitations around manual egg sorting and staging. The core of the design is trainable AI class algorithms, an optical path and a sorting wheel. We sought to evaluate the EggSorter in a real-world, high throughput setting for its ability to 1) distinguish viable and fertilized eggs, 2) its accuracy in selecting target age stages of 2.5 and 6 hpf and 3) its accuracy and speed in singulating eggs into 96 well plates, without damaging them. Our tool classification algorithms are not yet fully optimized. Classification of the 2.5 hpf target from a mixture ranging from 1.5 - 3.0 hpf was approximately 40% of the time. The need for more algorithm training on this target stage was thus apparent. Embryos near the 2.5 hpf target also experienced an unacceptable incidence of damage by the sorting process, apparently in the wells of the sorter wheel itself. It is hoped that a cleaner-machined material will be superior to the current plastic wheel. Classification of the 6 hpf target from a mixture of stages from 4.5 - 10 hpf was accurate better than 90% of the time, suggesting that the algorithm training was nearly sufficient for this target. The time required to load a 96-well plate at the 6 hpf target was approximately one minute. The variance between wells for the 2.5 hpf was approximately 9%. There was relatively low loss because the well left empty was observed for the 6 hpf target. Double loading of wells ranged from 2 - 4 wells per plate and may be mitigated by reducing the density of eggs in the supply hopper. The 6 hpf target embryos were free of damage. The goal of the EggSorter is to provide an efficient process of sorting embryos that enables the researcher to focus on more technical tasks. More algorithm training on earlier targets and minor hardware improvements will effectively automate all aspects of zebrafish egg sorting, staging and allocation. This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P42ES016465, K35ES031709 and P30ES030287. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
toxicology to track the bio-distribution of the 14C-labeled metabolite/catabolite through the GBA or when exposed to drugs or neurotoxicants. Radiocarbon tracing of the metabolite or catabolite of interest is commonly detected using accelerator mass spectrometry (AMS), an analytical technique that is highly reproducible and sensitive, with detection limits as low as zeptomoles of 14C. However, AMS provides no structural information of its target analytes, and therefore cannot be used to distinguish between radiolabeled molecules. Parallel accelerator and molecular mass spectrometry (PAMMS) is a technology developed at Lawrence Livermore National Laboratory that uses a flow splitter to simultaneously perform traditional liquid chromatography-mass spectrometry (LC-MS) and liquid-sam- ple AMS from a single sample injection. This approach enables identification of metabolites labeled with 14C by combining the extreme sensitivity of AMS with the separation and detection capability of LC-MS. In the present study, we used a simple monocolulture of Lactobacillus brevis, a bacterial strain known to produce high amounts of GABA via the enzyme glutamate decarboxylase. The monocul- ture was incubated with and without 14C-labeled L-glutamic acid, and the supernatant collected at 2 and 24 hours, and 4, 7 and 14 days in vitro. Preliminary results demonstrate chromatographic separation of GABA and glutamic acid derived from cultured cells. 14C signal of glutamic acid was detected with AMS, as well as other radiolabeled metabolites that have yet to be identified. Experiments are ongoing to identify the metabolites and characterize their changes in concentration. This will demonstrate that 14C-labeled L-glutamic acid from Lactobacillus brevis results in (1) microbial byproducts labeled with 14C, (2) labeling of the metabolite of interest, and (3) tracking the production/lifespan of 14C-labeled microbial catabolites over 2 weeks. In summary, we will develop a 14C labelling approach as a tool to study the GBA, and elucidate how toxins modulate this complex interaction. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 through LLBD- award 22-FS-002. (LLNL-ABS-842478).

4438 Analysis of the Human Nasal Airway Epithelium Architecture and Physiology by Volume EM and Artificial Intelligence for Analyzing Molecular and Cellular Responses to Inhaled Toxins

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To analyze the effect of toxins in situ at the tissue level, the application of methodologies capable of assessing tissue-level 3D organization and cellular content are required. Volumetric Electron Microscopy (vEM) is rapidly emerging as a promising tool to characterize tissues at the nanometer scale with an unprecedented level of detail. However, this technique has been limited by the requirement of time-consuming data collection. Here, we present the first in situ, isotropic reconstruction by Focused-Ion-Scanning Electron Microscopy (FIB-SEM) of a tissue well established in the use of toxicological studies surrounding particulate matter (PM): the human nasal airway epithelium. By using Artificial Intelligence-driven volumetric segmentation and quantitative analysis of FIB-SEM data we observe extensive reorganisation of intracellular organelle quantity, morphology, and topology (e.g., mitochondria, lysosome and lipid vesicles), and rearrangements in cytoskeletal architecture, across differentiation. This approach generates a model that can be extremely useful for toxicological assessments and hazard identification. Analysis of lung and other tissue volumes obtained after exposure to PM: the human nasal airway epithelium. By using Artificial Intelligence-driven volumetric segmentation and quantitative analysis of FIB-SEM data we observe extensive reorganisation of intracellular organelle quantity, morphology, and topology of the epithelial cells (e.g., mitochondria, lysosome and lipid vesicles), and rearrangements in cytoskeletal architecture, across differentiation. This approach generates a model that can be extremely useful for toxicological assessments and hazard identification.
design makes CFD cumbersome early in an investigation. Linear Programming (LP) models permit specification of inlet flow conditions that perform within limits of an aerosol generator while mimicking human puffing/inhalation topology subject to constraints related to physiological relevance. The LP model was used to design physiologically relevant exposure conditions to aerosols generated from an electronic cigarette (EC). Prior work has established that dose from EC increases as puff flow rate decreases and duration increases. However, the subsequent inhalation is what maintains a physiological environment (shear stress) in an in vitro system. Fluid shear from puffing an EC may not be high enough for an observable cell response. Therefore, minimizing puff flowrate while maximizing inhalation flowrate subject to constraints on Reynolds number and wall shear stress, will ease signal detection for analytical work in a physiologically accurate environment. A LP model that assumes conservation of mass, an incompressible fluid, an idealized symmetric lung, and air as the fluid was formulated. The LP was implemented using AMPL (Bell Labs, Holmdel, NJ, USA) and was run using the Gurobi Optimization solver. Puff flowrate was allowed to vary between 17mL/s and 65mL/s representing the operating range of the EC. Inhalation flowrate was allowed to vary between 250-1000mL/s, representative of normal human breathing. Reynolds number was constrained to be less than 2100 to ensure laminar flow. Wall shear stresses in lung generations 1-4 were constrained by minimum and maximum stresses reported in the literature. The minimum puff flowrate that satisfies all constraints is 17mL/s. However, puffing alone was insufficient to provide appropriate mechanical cues to cells as reflected by puffing Reynolds number and wall shear stress predictions between 31-86 and 0.0007-0.002Pa respectively. The optimum inhaled flowrate that satisfies all constraints is ~400mL/s resulting in Reynolds number between 726-2100 and wall shear stress between 0.02-0.04Pa for generations 1-4. This model provides a tool to optimally determine the inlet flow rates of an in vitro aerosol exposure system. Accuracy of the puffing and inhalation flowrate constraints is optimal generator, puff/inhalation topology, accurate wall shear stress, flow regime, and lung model geometry. The results of this model prescribe the input parameters for a given in vitro system, subject to operating constraints of any aerosol generator. The conventional LP model may broaden the utility of an emissions system to study many inhalational aerosols, with requisite inhalation flowrates as those found in tobacco or chemical threats of bioterrorism. The current work lays a foundation for demonstrating how CFD analysis can correlate results of an in vitro study to in vivo conditions, rather than attempting to design an in vitro system that performs exactly as a human lung, which would be a futile task.

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efficacy of nonclinical drug safety predictions. 3D bioprinting enables the gener-
ation of complex models with spatial control and a variety of matrices allowing the formation of complex tissue structures. Here we describe a method for an automated generation of 3D cellular liver models using a multi-tool robotic device with liquid handling, enabling the automated bioprinting and high-content imaging of liver cells within a collagen matrix. This assay was used for compound testing and evaluation of toxic effects in liver cells. As an example, in a assay using bio-printed structures, we used HepG2 cells resuspended in collagen I with micro-structure printed channels allowing better diffusion of media. Using a printing tool and pipettor, the robot printed Pluronic acid into wells of a multi-well plate to create the 3D structures needed for channels formation then dispensed the cell mix with collagen to create a thick 3D cell layer. The instrument also allowed for media addition, 3D printing and media exchanges, and media collections. We printed Pluronic pillars in a hexagonal arrangement using the ambient 3D print tool. Following dispensing of cells/matrix around the pillars, the pillars were removed using temperature-based dissolution and washes with the pipette tool leaving behind a structured, cell-dense, 3D liver cell construct. The pipette tool dispensed a colder (8 degrees C) collagen mixture with cells around the printed and warmed (27 degrees C) structures. The 3D HepG2 models were cultured and monitored daily using imaging in transmitted light. They were later treated with a panel of drugs with known liver toxicity, including, chloroquine, pimozide, haloperidol, doxorubicin, taxol, mitomycin, and cisplatin. The treatment was done on day 4 of model formation and imaged on day 6 48 hours of compound treatment. For the endpoint measurements, cells were stained with viability dyes and imaged using a confocal imaging system. Also, the concentration-dependent toxicity effects in response to the compounds were evaluated using high-content analysis. The result shows the utility of the method for the formation of liver 3D printed models as well as an increase in accuracy and throughput. Also, we developed imaging and data analysis methods and descriptors for gaining more information about these complex compound effects in 3D printed and cell-tissue engineered cell models.

Intestinal Organoids as an In Vitro Model System to Study Intestinal Toxicity

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Gastrointestinal complications are one of the most frequent drug-induced adverse events reported in clinical trials. The intestinal safety profile of new compounds is mostly evaluated by histology in preclinical animal studies. Human stem cell-based assays including organoids could help reduce the number of animals used for safety assessment and increase the translational value of preclinical research. Although adult stem cell-based intestinal organoids have been widely used to understand the biology of the gut and the pathophysiology of intestinal disease, their potential for toxicological applications remains little explored. Here, we tested a limited set of compounds with a safe profile (Flecainide, Fondaparinux, Haloperidol and Verapamil) or inducing diarrhea (JNJ-16269110, SN-38, Gefitinib, Compound 001, Vorinostat and OTX-015) in the clinic in a human duodenal organoid platform (HUB Organoid). We aimed to explore the effects of organoids as an in vitro tool for investigating drug-induced intestinal injuries. The compound set was first tested in the 3D organoid model using ATP measurement as a viability readout utilizing different media: expansion medium (CSM) supporting the presence of stem cells and trans-amplifying cells - and enterocyte differentiation medium (enterocyte colon differentiation medium, eCDM), which enriches the organoids in enterocytes. In addition, organoid-derived cells were seeded as epithelial 2D monolayers and enriched in enterocytes to determine the effects of the compounds on viability (ATP) and monolayer integrity (transepithelial electrical resistance, TEER). Based on the viability assay, all negative compounds were identified correctly, and different differentiation conditions and positive compounds except JNJ-16269110 induced a decrease in viability of > 50% vs DMSO control. The fact that JNJ-16269110 did not induce organoid Toxicity could be explained in part by its mechanism of action (inhibition of lipid absorption). In differentiation conditions 3 out of 6 positives (JNJ-16269110, SN38, and OTX015) did not reduce viability by > 50%. The 3D viability assay showed high reproducibility (~2-fold difference in IC50s) when performed over 5 or 7 passages for eCDM and CSM, respectively. In the 2D monolayer set-up, 3 out of 6 positive compounds but none of the negative induced a drop in TEER and viability > 50% compared to timepoint = 0. Consistent with the results in the 3D differentiation viability assay, the 3 compounds (JNJ-16269110, SN38, and OTX015) did not reduce TEER by > 50%. Decreased sensitivity to SN38 in differentiation conditions (2D and 3D) could be explained by metabolic detoxification in enterocytes. Overall, the 3D intestinal organoid viability assay is a promising in vitro tool that could help identify compounds with a good intestinal safety profile early in drug development. Utilizing different organoid culturing conditions (stem cell vs. enterocyte enriched) could support mechanistic investigation to understand underlying mechanisms of drug-induced intestinal toxicities.

Transcriptomic Points of Departure for Japanese Quail Exposed to Six Pesticides Using the EcoToxChip Test Method

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Under the banner of New Approach Methods (NAMs) transcriptomics dose-response analysis (TDRA) has emerged as a promising approach for integrating toxicogenomics data into risk assessments. Here we combine an early life stage (alternative to animal) avian embryo test and a targeted qPCR array (EcoToxChip; n=384 genes of relevance to environmental toxicology) to determine if 1) transcriptomics points of departures (tPODs) can be derived from this test system; and 2) the tPOD values are pollocations of levels associated with adverse outcomes. Fertilized, unincubated Japanese quail (JQ) eggs were injected with graded concentrations (100, 32, 10, 3.2, 1, 0.32, 0.1 μg/g egg plus DMSO control) of 6 pesticides: ethoprophos, carbofuran, trichlorfon, permethrin, glutosamine ammonium, and chlorpyrifos. Phenotypic endpoints including mortality, infertility, embryo mass, and deformity (presence of fatty liver) were assessed at embryonic day 9. Gene level BMDs (benchmark dose) were derived for each chemical. While there were relatively few gene-level BMDs (<25 per chemical), they were lower than levels associated with adverse outcomes. Further, for all test chemicals, investigation of gene- and pathway-level results concurred with expected mechanisms of action. Finally, two of the chemicals were repeated and yielded similar results, thus giving confidence to the test method. In conclusion, the EcoToxChip Test Method can yield gene level BMD values that are protective of levels associated with adverse outcomes, and give insights into mechanisms of action. However, derivation of tPODs may be challenging with the reduced gene set provided by EcoToxChip thus necessitating more work in this area.

Cultivation and Characterization of RPTEC/TERT1 Cells Cultivated on Silk Fibroin Wafers at Atmospheric and Physiological Conditions in a Static Setting


Investigation of the toxicity of various compounds both beneficial and detrimental to the human organism is an ever-increasing problem that the data collected is as physiologically accurate as possible. Current determination of the toxicity of a substance is performed at atmospheric and static conditions. While this is currently the case, the need to improve upon the experimental setting to a more physiological one is increasing. Physiological conditions not only include the correct oxygen conditions the cells live in in vivo but should factor in other aspects of the cell’s environment. Renal proximal tubule cells (RPTEC) are morphologically polarized cells; in vivo they have microvilli which protrude into the tubular lumen. The primary function of RPTECs is excretion and reuptake of harmful and beneficial substances respectively from the primary urine. RPTECs form a tubular structure in vivo, and the conditions and presence of the lumens of this tubule flow into the Loop of Henle and further on to the distal convoluted tubule and finally the collecting ducts. In the case of RPTECs the in vivo conditions are an oxygen tension of around 10% and the presence of flow. The function of the proximal tubule depends on the tightness of the cell layer and the presence of the cell’s brush border. As cell morphology and the proteome expression are an important denominator for cell function, we have investigated these parameters on RPTEC/TERT1 cells cultivated on silk fibroin wafers. These silk fibroin wafers are a possible replacement for plastics used in trans-well cell culture. Our final goal is to transfer silk fibroin wafers to a microfluidic setting, thus mimicking the in vivo environment. In this study we have cultivated RPTEC/TERT1 cells at atmospheric and physiological conditions in a static setting to ascertain the presence of important cell-cell proteins which are responsible for the formation of a tight monolayer. We have likewise investigated the morphological characteristics of RPTEC/TERT1 cells cultivated in these two different conditions. Finally, we have assessed the expression of various transporters responsible for the primary function of RPTECs, morphology was assessed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Protein expression was assessed with qPCR, western blot and confocal microscopy. All experiments were performed at both atmospheric and physiological (10%) oxygen tension. RPTECs were cultivated on silk fibroin wafers and compared to RPTECs grown on traditional cell culture plastic. Our results show that silk fibroin is a suitable substrate for cell adhesion. Morphological analysis of RPTECs by SEM and TEM imaging show that microvilli are indeed present on the cell surface. TEM imaging showed that cell-cell junctions are likewise present. This was further confirmed by confocal imaging which showed the presence of tight junction protein ZO-3. qPCR analysis showed that there is a general increase in transporter expression. Western blotting confirmed the presence of ZO-1, ZO-3 and claudin-2, all of which are tight junction proteins. We have likewise assessed the presence of GGT, Na‘K‘ ATPase and SGLT2 via western blotting. Our results speak to the possible application of silk fibroin wafers in cell culture, with the end aim of integrating them with our in-house developed microfluidic system.
Dermal exposures to environmental chemicals can significantly affect the morphology and integrity of skin structure, leading to enhanced and deeper penetration of toxic chemicals. This problem can be magnified during disasters where hazardous water-soluble chemicals are readily mobilized and redistributed in the environment, threatening the health of vulnerable populations at the impacted sites. To address this issue, barrier emulation formulations (EVBs) have been developed consisting of materials that are generally recognized as safe (GRAS), with the inclusion of medical grade carbon or calcium and sodium montmorillonite clays (CM and SM). In this study, the adsorption efficacy of five commonly occurring contaminants of concern, including important hydrophobic pesticides (glyphosate, acrolein, and paraquat) and per- and polyfluoroalkyl substances (PFAS), were characterized. EVBs showed properties such as high stability, spreadability, low rupture strength, and neutral pH that were suitable for topical application on the skin. The in vitro adsorption results indicated that EVB and EVB-SM were effective, economically feasible, and favorable barrier formulations for chemical adsorption, as supported by high binding percentage, low desorption rates for an extended period of time, and high binding affinity. A pseudo-second-order kinetic model was best fitted for the adsorption process and the Freundlich model fit the adsorption isotherms with negative enthalpy values indicating spontaneous reactions that involve physisorption. The study, with varying temperatures and pH, showed that the adsorption reaction was exothermic and persistent. The results indicated that EVB, and especially EVB-SM, can be effective as efficient barriers to block dermal contact from water-soluble toxic pollutants during disasters.

Skin permeation is a primary consideration in the safety assessment of cosmetic ingredients, topical drugs, and human users handling veterinary medicinal products. While excised human skin (EHS) remains the ‘gold standard’ for skin permeation studies, its unreliable supply and high cost motivate the search for surrogate models that can be beneficial for increasing study throughput by reducing reliance on the acquisition of human skin explants model. An alternative is the use of synthetic membranes. These membranes are typically simple to prepare and use, with a long shelf life and no special storage requirements. This current work used Phenicon™ diffusion cells with automated receptor fluid sample collection over 24 hours and compared the permeation of three radiolabelled chemicals (methyl salicylate (MeS), benzyl salicylate (BeS) and tri butyl phosphate (TBP)) through 500µm dermatomed pig skin and the synthetic Str Mat™ membrane. These chemicals were of interest as they are commonly used surrogates for chemical warfare (CW) agents. MeS and BeS are used as surrogates of the vesicant sulfur mustard whist TBP is used as a nerve agent surrogate. The option of being able to assess CW agent and/or CW agent surrogates permeation using artificial skin from diverse species would be beneficial for increasing study throughput by reducing reliance on the acquisition of human or pig skin. Briefly, tested conditions were: 10µl neat, single droplet chemical application, unoccluded cells, 1 cm² diffusion area with a skin surface temperature of 32±1°C, 10 ml receptor volume with 50% aqueous ethanol receptor fluid, 4000 speed motor stirrer speed, analysis by Liquid Scintillation Counting (LSC). Maximum penetration rates (Jmax μg.cm⁻².h⁻¹, average ± SD of n=6 replicates) measured for Str Mat™ were: 560±7786 (MeS), 199±17 (BeS) and 4367±609 (TBP). For pig skin Jmax’s were: 16±5 (MeS), 18±6 (BeS) and 39±21 (TBP). The results clearly indicate that pig skin was significantly less permeable to EHS. The mean 6-hour cumulative permeation of a finite dose (6 nmol/cm²), 10 µL/cm² of both caffeine and EHS was highest in EpiDerm-200-X, followed by EHS and Strat-M. Salicylic acid permeated most in Strat-M, followed by EpiDerm-200-X and EHS. Further, an increased percentage of ethanol in the vehicle tended to reduce mean compound permeation across the alternative skin barrier models tested. Based on these findings, the currently available alternative skin barrier models remain limited in their ability to directly replicate the permeability of the human skin barrier. Nevertheless, such models may benefit from low cost, sustained viability, and the potential for customization, giving them select advantages over EHS tissue. Evaluating novel alternative skin barrier models, as they become available, in the manner outlined herein, has the potential to reduce the time from basic science discovery to regulatory impact.

In this study, the adsorption efficacy of five commonly occurring contaminants of concern, including important hydrophobic pesticides (glyphosate, acrolein, and paraquat) and per- and polyfluoroalkyl substances (PFAS) were characterized. EVB and EVB-SM were effective, economically feasible, and showed properties such as high stability, spreadability, low rupture strength, and neutral pH that were suitable for topical application on the skin. The in vitro adsorption results indicated that EVB and EVB-SM were effective, economically feasible, and favorable barrier formulations for chemical adsorption, as supported by high binding percentage, low desorption rates for an extended period of time, and high binding affinity. A pseudo-second-order kinetic model was best fitted for the adsorption process and the Freundlich model fit the adsorption isotherms with negative enthalpy values indicating spontaneous reactions that involve physisorption. The study, with varying temperatures and pH, showed that the adsorption reaction was exothermic and persistent. The results indicated that EVB, and especially EVB-SM, can be effective as efficient barriers to block dermal contact from water-soluble toxic pollutants during disasters.
the CW agent surrogates than Strat-M® under the conditions used. The appropriateness of membrane choice for use in diffusion cells ultimately depends on the type of study being performed. It is important that the researcher ensures that their membrane choice for use in diffusion cells reflects the in vivo system they are modeling. Crown copyright (2022). Dist. This material is licensed under the terms of the Open Government Licence except where otherwise stated. To view this licence, visit http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3 or write to the Information Policy Team, The National Archives, Kew, London TW9 4DU, or email: psi@nationalarchives.gsi.gov.uk.

A collaboration between Unilever and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has developed the Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) Model, a defined approach (DA) developed upon principles of the SARA Model. The SARA-ICE Model is designed to provide a weight-of-evidence point of departure (PoD) and United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS) classification prediction for use in skin sensitization assessments. The SARA-ICE core dataset utilises data within the publicly available Integrated Chemical Environment (ICE) database in addition to the published Unilever SARA database and Cosmetics Europe database. The model is constructed within the Bayesian statistical framework and allows for determination of a human relevant PoD termed the ED01, defined as the dose with a 1% chance of inducing sensitisation following a human predictive patch test (HPPT) exposure. The PoD can be inferred using any combination of HPPT (human repeat insult patch test or human maximisation test), in vivo local lymph node assay (LLNA), and new approach methods (NAM) (in chemico direct peptide reactivity assay and in vitro Keratinosens™, h-CLAT, or U-Sens™[22]) data. For a chemical of interest, the model returns the probability of each GHS classification conditional on the distribution of the ED01. Here we report the initial results of an evaluation of the SARA-ICE model which pertains to a ‘Feasibility Study on Inclusion of the SARA-ICE model into OECD Guideline 497 on Defined Approaches for Skin Sensitisation’. For the purpose of evaluating SARA-ICE for GHS classification, the Organisation for Economic Co-operation and Development (OECD) DA evaluation protocol (Annex II of Interagency Cooperation on Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification prediction for use in skin sensitization models has been carried out in Python and improves upon the Surrey model reported in Chen et al., 2015 and Hewitt et al., 2020. Development of the model has been carried out in Python and improves upon the Surrey model reported in Chen et al., 2016. The model has considered both ideal and non-ideal evaporation. An ideal solution involves calculating the flux into the atmosphere, whereas a non-ideal solution involves both the flux and the activity. The evaporation model mainly considers a vehicle of water plus one active ingredient (e.g., water: nitrobenzene, with the capability to model a three-component system (e.g., water: ethanol: nitrobenzene). Validation has been performed for the individual evaporation module and the Surrey model. We present comparisons between models (Surrey model with evaporation, EPA HTTK, and Surrey model without evaporation) and experimental data. Comparing our evaporation model to both our previous Surrey model highlights the improvement seen and to experimental results to provide a higher confidence level of the updated model. Comparing the model to Aggarwal et al., 2015 and Hewitt et al., 2020 demonstrates that our model can apply to a wide range of chemicals (cosmetics and pesticides); the model previously tested only pharmacological chemicals. Several improvements have been made to the Surrey model and the current version has been validated to allow application of the model. This abstract does not reflect US EPA policy.


The SARA-ICE approach for incorporating NAM and DA data into RAx to fill the data gap of EC3 assessment conditions. However, despite the high performance in comparison to murine local lymph node assay (LLNA), underpredictions were reported in ITSv1 prediction, and ITSv1 DA alone is not used for a precise estimate of the potency based on LLNA EC3 values. Moreover, there is no explicit approach for incorporating NAM and DA data into RAx to fill the data gap of EC3 values, although RAx can increase the confidence in NAM/DA data and be used to determine a EC3 value. To address these issues, we previously developed a strategy incorporating ITSv1 DA into RAx to precisely predict skin sensitization potential. This strategy is based on the EC3 values with high confidence. ITSv1-based RAx. The three key steps in the workflow of ITSv1-based RAx are as follows: (1) selection of suitable analogue(s) with in silico tools; (2) comparison of ITSv1 DA scores, predictions, and LLNA data of analogue(s); and (3) determination of the predicted EC3 (pEC3) value for the target chemical based on (i) whether the analogue ITSv1 DA prediction is

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consistent with the GHS category based on the existing LLNA data of the analogue and (ii) whether each converted score of the analogue is equal to or higher than that of the target. In this study, to examine the reliability of ITSv1-based RAx, case studies were performed using 18 chemicals in the guideline that are underpredicted in ITSv1 DA alone. These chemicals were regarded as a new chemical with no in vivo sensitization data. Based on ITSv1-based RAx, potency sub-categorization for all 18 chemicals were predicted without underprediction, indicating that RAx increase confidence of ITSv1 prediction. For 7 out of 18 target chemicals: maleic anhydride (EC3 = 0.16%, pEC3=0.35%), 1-naphthol (EC3 = 1.3%, pEC3 = 0.19%), bisphenol A diglycidyl ether (EC3 = 1.5%, pEC3 = 0.37%), sodium 2,4-dinitrobenzenesulfonate (EC3 = 1.96%, pEC3 = 0.3%), dihydroxygenol (EC3 = 6.8%, pEC3 = 5.4%), anisyl alcohol (EC3 = 7.1%, pEC3 = 2.3%), and oxalic acid (EC3 = 15%, pEC3 = 2.5%), the pEC3 value was determined by extrapolating the EC3 value of each analogue, indicating that RAx refined ITSv1 prediction by a simple extrapolation of the EC3 value from the analogue. In addition, for 10 out of 18 target chemicals, ITSv1-based RAx avoided underprediction of RAx by considering biological data based on ITSv1 DA, indicating ITSv1 DA also increase confidence of RAx. Moreover, for target chemicals with multiple analogues, ITSv1-based RAx reduced the need for expert judgment in extrapolating the EC3 value from multiple analogues to a target chemical based on the simple workflow. This study suggested that ITSv1-based RAx can increase confidence level for predictions in ITSv1 DA/RAx and is useful for a precise estimate of the potency based on EC3, reducing the need for expert judgment.

| 4456 | GARDskin Dose-Response for Photosensitization: Assessment of Reference Photoirritants and Photoallergens | T. Lindberg1, G. Ritacco2, A. Jerre1, R. Gradi1, A. Forreryd1, H. Johansson1, and A. Api3, 1SenzaGen AB, Lund, Sweden; 2Research Institute of Fragrance Materials, WoodCliff Lake, NJ; and 3SenzaGen AB, WoodCliff Lake, NJ. |

Dermal exposure to certain chemical compounds, so-called sensitzers, can give rise to adverse outcomes induced by an immunological response towards the specific compound. One such class of compounds, photosensitizers, needs to be activated by UV rays to elicit an immune response. Although rare in occurrence, it is a critical human health endpoint in need of investigation to limit potential exposures. Other phototoxic skin reactions include photoirritation, which is manifested as a one-time occurrence at the site of exposure that goes away over time. While testing schemes for photoirritation are clear, testing for photosensitization remains a challenge and no established in vitro model to evaluate this endpoint currently exists. For risk management purposes, distinguishing between phototoxic properties is important, as concentration limits can be set for photoirritants whereas fragrance photoallergens have historically been banned. The GARDskin assay is a next-generation in vitro method for hazard classification of conventional skin sensitizers, included in OECD TG 442E. The assay is based on a human dendritic-like cell line and combines genomics and machine learning to achieve a high predictive performance with a large applicability domain. The GARDskin Response-assay is based on the validated GARDskin protocols but instead of giving a binary classification it provides quantitative information about the lowest concentration needed to induce a positive classification in the assay, termed the cdV0 concentration. To investigate photoactivity, an extra UV-exposure step was added to the original protocol (otocil, trimethylaminononane, and propylene glycol). CDV0 values were established for each compound in the presence and absence of UV-exposure, i.e., the cdV0 concentration of the specific compound is lower after UV-exposure than in the non-exposed counterpart. The study presented here aimed at investigating the applicability of the GARDskin Photo Dose-Response assay to correctly assess photoallergens and distinguish them from photoirritant effects. Previous studies have indicated that a shift in cytotoxic profile after UV-exposure may indicate a predominantly photoirritant activity rather than photosensitizing and this was also investigated in the present study. Six reference photoirritants and six reference photoallergens were investigated using the GARDskin Dose-Response assay in combination with a UV irradiation protocol. Cytotoxic profiles and CDV0-values were established for each compound in the presence and absence of UV-exposure. Five out of 6 photoirritants were correctly predicted based on their cytotoxic profile while 3 out of 6 photoallergens where correctly predicted based on the decrease in cdV0-value after UV-exposure. In conclusion, functionality of combining GARDskin Dose-Response protocols with UV irradiation to investigate photoactivity was shown. Further, photoirritant effects were strongly correlated to a shift in cytotoxic profile after UV-exposure, and a decrease in CDV0 values after UV-exposure may indicate photosensitizing effects. However, further work may be warranted to establish a final prediction model for photosensitization.

| 4457 | Applying a Next-Generation Risk Assessment (NRA) Framework for Skin Sensitization to Inconsistent New Approach Methodology (NAM) Information | S. Hoffmann1, N. Gilmore2, N. Aligée1, P. Kern1, E. van Vliet1, D. Bury1, M. Miyazawa2, and H. Nishida1. 1SEH Consulting + Services, Paderborn, Germany; 2Unilever, Bedford, United Kingdom; 3L’Oréal, Aulnay-sous-Bois, France; 4Procter & Gamble Services NV/SA, Strombeek-Bever, Belgium; 5Innovix Consulting & Services, Houten, Netherlands; 6L’Oréal, Clichy, France; 7Kao Corporation, Ichikai, Japan; and 8Shiseido Global Innovation Center, Takashima, Japan. |

Cosmetic products must be safe for their intended use. Regulatory bans on animal testing for new ingredients has resulted in a shift towards the use of new approach methodologies (NAM), such as in silico predictions and in chemico/in vitro data. Defined Approaches (DA) have been developed to interpret combinations of NAM to provide information on skin sensitisation hazard and potency, three having been published within OECD Test Guideline 497. However, the challenge remains as to how DA can be used to derive a quantitative point of departure for use in next generation risk assessments (NGRA). Here we provide an update to our previously published NGRA framework and present two hypothetical consumer risk assessment scenarios (rinse-off and leave-on) on one case study ingredient. Diethanolamine (DEA) was selected as the case study ingredient based upon the existing NAM information demonstrating differences with respect to the outcomes from in silico predictions and in chemico/in vitro data. Seven DA were applied and these differences resulted in divergent DA outcomes and reduced confidence with respect to the hazard potential and potency predictions. Risk assessment conclusion for the rinse-off exposure led to an overall decision of safe for all DA applied. Risk assessment conclusion for the higher leave-on exposure was safe when based upon some DA but unsafe for others. The reasons for this were evaluated, as well as the inherent uncertainty from the use of each NAM and DA in the risk assessment, enabling further refinement of our NGRA framework.

| 4458 | Cell Cycle-Dependent DNA Damage Responses in Human Keratinocytes Exposed to UVB Light | Y. Ar2, Y. Jan1, D. E. Heck3, and J. D. Lasker. 1Rutgers, The State University of New Jersey, Piscataway, NJ; 2Rutgers School of Public Health, Piscataway, NJ; and 3New York Medical College, Valhalla, NY. |

Ultraviolet light in wavelengths from 280-320 nm (UVB) is known to damage DNA, forming both cyclobutane pyrimidine dimers (thymine dimers) and pyrimidine (6-4) pyrimidone photodimers (6-4 photoproducts). Defining mechanisms of UVB-induced DNA repair will be important to adverse outcomes induced by an immunological response towards the specific compound. Ultraviolet light in wavelengths from 280-320 nm (UVB) is known to damage DNA, forming both cyclobutane pyrimidine dimers (thymine dimers) and pyrimidine (6-4) pyrimidone photodimers (6-4 photoproducts). By targeting keratinocyte UVB light damages genomic integrity, a process that can lead to DNA strand breaks, mutations, and the development of skin cancer. Cells respond to DNA damage by activating a DNA damage response (DDR), a process important in DNA repair. The DDR involves signaling cascades that include ATR (ataxia telangiectasia and Rad3-related), ATM (ataxia telangiectasia mutated), and DNA-PK (DNA-dependent protein kinase, catalytic unit), which are activated via protein phosphorylation. This leads to the recruitment of DNA repair proteins including phospho H2AX and phospho p53 to sites of DNA damage. We found that UVB (2.5-25 mJ/cm2) caused dose- and time-dependent phosphorylation of ATR (T1989), ATM (S1981), DNA-PKcs (S2056), H2AX (S139) and p53 (S15) in HaCaT cells, a human keratinocyte cell line, and in primary human neonatal foreskin epidermal keratinocytes. Primary keratinocytes were less sensitive to UVB requiring longer periods of time and higher doses of UVB to induce the DDR. In HaCaT cells synchronized in the cell cycle using a double thymidine block, DDR signaling was predominantly activated in the S phase, when compared to cells in G0G1 and G2M phases. Confocal microscopic analysis of HaCaT cells treated with UVB (25 mJ/cm2) revealed the accumulation of DDR proteins at subnuclear sites containing chromosomal double-strand DNA breaks. These foci were predominantly identified in cells in S phase of the cell cycle and contained phospho-DNA-PKcs and phospho-H2AX. UVB was also found to induce oxidative stress in HaCaT cells, as measured by increases in intracellular reactive oxygen species (ROS). A marked increase in UVB-induced ROS was not when HaCaT cells were depleted of glutathione, by treatment with buthionine sulfoximine (BSO, 50 μM, 18 h). Increases in ROS had no effects on the UVB-induced DDR response. Taken together, these data demonstrate that UVB induces cell cycle-dependent DNA damage in human keratinocytes, which is independent of oxidative stress. Induction of the DDR response in the cells is due to double-strand DNA breaks as a consequence of the repair of DNA photoproducts. Defining mechanisms of UVB-induced DNA repair will be important for understanding processes leading to skin tumor formation. Supported by NIH grants AR055073 and ES005022.

| 4459 | Comparing Gravimetric DPRA Data with In Vivo Reference Data | S. N. Koller, B. Waring, D. Funk-Weyer, and R. Landsiedel, BASF SE, Ludwigshafen, Germany. |

Chemical reactivity of a test substance towards skin proteins is well associated with an allergenic potential and has been described as the molecular initiating event (MIE) of the adverse outcome pathway for skin sensitization. The direct
Preservatives are necessary additives for personal care and cosmetic products as they prevent the growth of harmful pathogenic microorganisms, ensuring both safety and quality over the intended shelf-life of the product. However, preservatives may have the potential to cause adverse dermal effects, such as skin sensitization and contact dermatitis, following repeated exposure under certain product use conditions. Phenoxethanol (PE) and ethylhexylglycerin (EG) are two preservatives that are widely used in personal care and cosmetic products. However, a maximum use concentration in cosmetic and personal care products has not been set for either PE or EG in the United States. We performed a quantitative risk assessment (QRA) to determine the risk of skin sensitization induction associated with dermal contact exposure to PE and EG in shampoo, which was selected as an exemplary cosmetic and personal care product consumer use scenario. In order to conduct the QRA, a consumer exposure level (CEL), which represents daily exposure of consumers to the preservatives under the selected use scenario, was benchmarked against an acceptable exposure level (AEL), which is the exposure dose where skin sensitization induction in consumers is not expected, to generate a margin of safety (MOS). CELs for both PE and EG were calculated using the shampoo-specific maximum dermal exposure per application, number of product applications per day, product retention factor, scalp surface area, and the maximum concentration of each preservative identified in a survey of shampoo products. The AEL for each preservative was calculated by applying a sensitization assessment factor of 30, which accounts for variability among subjects, product formulations, and product use patterns, to a no expected sensitivity level (NESIL), at which sensitization is not observed in the test species. The NESIL for both PE and EG was determined based on a weight of the evidence review of skin sensitization data in humans and nonclinical animal models obtained from the peer-reviewed literature. Benchmarking the CEL (PE = 1.63 µg/cm² and EG = 13.05 µg/cm²) against the AEL (PE = 7 µg/cm² and EG = 133 µg/cm²) for each preservative provided a MOS. Overall, the MOS values estimated for PE and EG in the shampoo products were above 1, demonstrating that these products were not expected to be associated with an increased risk of skin sensitization induction for the evaluated consumer exposure scenario. This QRA approach can be utilized to understand the risk of sensitization induction from the use of cosmetic and personal care products containing other potentially allergenic ingredients or for other chemicals of concerns in various product types.
been developed in our lab group. Compounds of interest were topicaly applied on the RHE and THP-1 cells placed underneath the RHE. We focused on difficult molecules and lipophilic sensitizers and varied exposure conditions to maximize sensitivity of the co-culture model. To determine the THP-1 cell activation, we analyzed cell surface expression of CD86 and CD54 on collected THP-1 cells by flow cytometry. It was evidenced that with changed exposure conditions, the RHE/THP-1 model shows a higher sensitivity for tested compounds. Preliminary results indicate that the optimized THP-1/RHE co-culture is suitable to identify a sensitizer and address the question of determining the potency of moderate and weak sensitizers. This co-culture model offers a promising step to fill the gap in the gap in finding an appropriate in vitro method to assess the potency of sensitizers.

Substantial efforts towards non-animal methods for skin sensitization have been made for years. Mechanistic understanding of skin sensitization has resulted in the establishment of an adverse outcome pathway (AOP), which in turn enabled the development and validation of in silico/chemico/vitro assays, relative to specific key events (KE) of the AOP. Used in an integrated testing strategy and validated in June 2021 under the OECD Testing Guideline No. 497, these non-animal assays demonstrated a superior performance (69-88%) to the “gold standard” mouse Local Lymph Node Assay (LLNA) 58% when compared 1:1:1. This superior performance of the non-animal assays is due to a low specificity (22%), or true negative rate, of the LLNA to human. Primarily validated by OECD using cosmetic ingredients, the stand-alone KE new approach methodologies (NAMs) are de facto considered applicable to the broad chemical substances, including agrochemical small molecules, based on physico-chemical properties. Being the first validated Defined Approaches (DAs) using NAMs and translated into a Testing Guideline by OECD, it is nonetheless helpful to provide the scientific community as well as regulatory authorities with case studies on the applicability of those methods and to build confidence in their utility in decision-making. Therefore in order to assess the applicability of NAMs for agrochemical active substances with five skin sensitizers and five non-sensitizers based on their historical animal data. Both hazard identification and potency sub-categorization were performed using the three DAs described in OECD TG No. 497: The “2 out of 3” (2o3) defined approach, the Integrated Testing Strategy version 1 (ITSv1) and version 2 (ITSv2). The DPRA, LuSens and h-CLAT assays (corresponding to KE 1, 2 and 3 respectively of the AOP) were conducted. Derek Nexus and OECD QSAR Toolbox were used for in silico prediction. The in silico approaches successfully generated the structure alerts, but rarely allowed a read-across, based on a lack of similar chemicals in the database of the tools. A solubilization issue in the DPRA assay led to a non-applicability of this test for most of the active substances and resulted in the inability to conclude on the skin sensitizing properties of the known non-sensitizer ingredients using ITSv1 or ITSv2. On the contrary, all the known sensitizers were correctly identified using at least one of the three DAs of the test guideline. Among the five active substances considered to be a non-sensitizer in vivo, one was identified as a skin sensitizer by using in vitro assays. This overestimation may be explained by the poor activity of this chemical to pass through the skin and react with the proteins in animal models. Future work in developing and validating a full-thickness skin model would allow the evaluation of the impact of the skin barrier and the skin metabolism in the assessment of the skin-sensitizing properties of a chemical. In conclusion, evaluation of the skin-sensitizing properties using NAMs and at least one DA of the OECD TG No. 497 demonstrated agreement with animal data for 8/10 of the tested agrochemical active substances. Decision-making would take advantage from improvements in the assessment of the reactivity of a chemical to skin proteins (KE 1). Moreover, for some chemicals which have low dermal absorption, there is a potential for positive results following a negative skin sensitization result in order to assess the potential for respiratory sensitization potential of isocyanate-based prepolymers, we previously proposed a framework that is unique in its inclusion of further evaluation following a negative skin sensitization result in order to assess the potential for absorption in the lung. In our proposed framework, a negative skin sensitization result justifies that the prepolymer acts as a direct sensitizer via the dermal route and triggers assessment of bioavailability, reactivity, biological/immunoological and WoE, and exposure to determine the potential risk. As a next step in our proposed research framework, we sought to determine the suitability of the Direct Peptide Reactivity Assay (DPRA) for assessing the chemical sensitization potential of P3 substances. Four P3 substances: TDI-PPG/Peg (MW=3350), TDI-PIC (MW=750), TDI-Gly/Peg (MW=4525), and MDI-PEG (MW=3400), as well as 4,4’-MDI were selected based on results in the LLNA (sensitizing and non-sensitizing) and the range of calculated octanol-water coefficients (-5, 3, 18 respectively). As expected, 4,4’-MDI was categorized as highly reactive in reactivity class and predicted to be a sensitizer in the DPRA (mean cytoxicity C50=72.2%); mean cytoxicity (lys) depletion: 28.9%; mean lys depletions: 50.6%). TDI-PIC/Peg yielded a mean Cys depletions of 53.1% but was incompatible with the Lys depletion assay due to co-elution with lysis. Although considered negative in the LLNA, TDI-PG/Peg was categorized as moderately reactive in reactivity class and predicted to be a sensitizer in the DPRA. Results for the other P3 substances were considered inconsistent due to the false positive results (formation of precipitates immediately upon addition to the cysteine and lysis reaction buffers). Overall, we conclude that the DPRA assay is not appropriate for screening disocyanate compounds for sensitization potential. In order to utilize

Hypochlorous acid (HOCl), an innate immune factor and environmental toxicant is an important component of the skin exosome. HOCl, a weak halogenated acid and powerful oxidant, serves two seemingly unrelated molecular roles: (i) as an innate immune factor (acting as a myeloperoxidase (MPO)-derived microbial factor) and (ii) as a chemical disinfectant used in freshwater processing on a global scale, both in the context of drinking water safety and recreational freshwater use. Mimicking recreational freshwater exposure in the context of swimming pool water associated with cutaneous chlorination stress and solar UV exposure, our recent studies have explored the interaction between UV photons and HOCl-related environmental co-exposures. First, the anti-inflammatory activity of topically HOCl (100 μM in topical carrier) was established in vivo and attributed mechanistically to blockade of AP-1-driven inflammatory signaling as assessed by bioluminescent imaging of luciferase reporter SKH-1 mice exposed to solar UV with and without topical HOCl. Moreover, HOCl topical exposure blocked tumorigenic inflammatory progression in UV-induced high-risk (tumor-prone) SKH-1 mouse skin (chronic exposure regimen; solar simulated UVB; 112 days regimen), a finding with potential implications for the prevention of human nonmelanoma skin photocarcinogenesis. In contrast, we were able to demonstrate that the drinking water and swimming pool disinfectant trichloroisocyanuric acid (TCIC; up to 100 μM in topical carrier), used widely as a photocatalyst for HOCl production, causes chlorination stress potentiating solar UV-induced inflammatory preneoplastic skin lesions. SKH-1 mouse skin in addition, a novel stratum corneum-specific chloramine adduct, formed as a consequence of topical HOCl (and TCIC) exposure, was identified by LC-MS after tape stripping of human ex vivo skin exposed to environmentally relevant chlorination stress, serving as an investigational biomarker of topical cutaneous chlorination stress (in addition to the established protein adduct 3-chloro-L-tryosine). Taken together, our data suggest that environmental or therapeutic chlorination stress in skin, substantiated by detection of a novel histidine-derived biomarker, serves both detrimental and potentially photochemopreventive roles, observable as a function of HOCl or HOCl-precursor compound (TCIC) exposure. This research was supported in part by funding from NIH: R21ES029579; ES007091; ES006694; R01CA229418; CA023074; 1P01CA229112.

The prediction of chemical sensitization by the respiratory route presents a challenge for lack of validated test guidelines and formally recognized in vivo or animal assays for this endpoint. Tiered weight of the scientific evidence (WoE) approach is used for the evaluation of skin sensitization of isocyanate-based prepolymers, and are routinely utilized; low molecular weight chemical sensitization result are also considered negative for the potential to cause respiratory sensitization and further exploration of respiratory sensitization potential is considered only where positive skin sensitization results are observed or when the substance acts as an irritant. The respiratory route has been developed in our lab group. Compounds of interest were topically applied on the skin of AP-1-driven inflammatory signaling as assessed by bioluminescent imaging of luciferase reporter SKH-1 mice exposed to solar UV with and without topical HOCl. Therefore, the objective was to assess the applicability of NAMs for agrochemical small molecules, based on their physico-chemical properties. As an end point by fundings from NIH: 1R21ES029579; ES007091; ES006694; R01CA229418; CA023074; 1P01CA229112.

Environmental Chlorination Stress and Skin Health: The Chlorination Agents HOCl (Hypochlorous Acid) and TCIC (Trichloroisocyanuric Acid) Display Opposing Effects on Solar UV–Driven Inflammatory Disregulation and Skin Carcinogenesis J. A. Snell1, A. B. Hua1, P. Vaishampayan2, S. E. Dickinson1, J. Fimbres1, J. Jandova2, and G. T. Wondrak1. 1R. K. Coit College of Pharmacy University of Arizona, Tucson, AZ, 2University of Arizona Cancer Center, Tucson, AZ, and 3University of Arizona, Tucson, AZ. Sponsor: S. E. Dickinson, American Association for Cancer Research. Hypochlorous acid (HOCI), an innate immune factor and environmental toxicant is an important component of the skin exosome. HOCl, a weak halogenated acid and powerful oxidant, serves two seemingly unrelated molecular roles: (i) as an
the proposed research framework as a tool to guide the future development of isocyanate chemicals and associated polyurethane applications toward reduced exposure and health hazard potential, further evaluation is needed.

4467 Evaluation of Long-term Health Effects from Acute Exposure to Toxic Chemicals

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Since 2016, the Department of Homeland Security Science and Technology (DHS S&T) directorate and the Defense Centers for Public Health - Aberdeen have partnered to develop guidelines for acute exposure resulting in chronic effects. The Acute Exposure/Chronic Effect (AEEC) project is done utilizing a toxic syndrome (toxicodrome) based approach where the likelihood of long-term health outcomes is evaluated as a function of the acute exposure level (mild, moderate, severe, life-threatening). We have completed work on 8 toxidromes (Cholinergics, Blood, Opioid, Irritant/Corrosive - Upper Pulmonary, Irritant/Corrosive - Lower Pulmonary, Vesicants, Convulsants, and Hemolytics) with one toxidrome planned for FY23 (Metabolic). This project utilizes Subject Matter Experts (SMEs) and peer reviewed journal articles to identify acute symptoms, which are categorized by health effect for generalizability, determine long-term symptoms, also categorized by health effect, and then elucidate the probability of a long-term health effect based on the level of acute exposure. These effect probabilities are combined with chemical-specific acute effect dose-response estimates to yield long-term health effect curves. Leveraging SME knowledge allows for lived experience to fill in gaps left by sparsely available data, as the chronic effects of an acute exposure to a chemical are not well studied. Collecting data based on a toxidrome rather than a single chemical also addresses the issue of sparse data, as findings made for a toxidrome can be leveraged for any chemical in that toxidrome. The supporting assumption for this generalization is that chemicals in the same toxidrome elicit similar acute effects, and that long-term health effects can be estimated based on the extent of the injury demonstrated by acute effects. Defense Centers for Public Health would like to utilize these calculations to expand on the military exposure guidelines (MEGs). Currently, MEGs only consider the acute effects of acute exposures despite documented evidence that acute exposures can lead to long-term effects. Such evidence includes incidences with military relevance, such as exposure to mustard agents during World War I and sarin attacks in Syria from 2012 until present day. Better knowledge of how an acute exposure can lead to long-term effects allows for better military planning, as long-term effects could manifest during the course of a deployment in ways that would impact operational readiness. The DHS S&T will apply these values to modeling potential harm to civilian populations and has taken on the task of ways to operationalize this data to the Homeland Security Enterprise (HSE).

4468 Pyrimethalin in Apples: Potential to Target Human Proteins

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Pyrimethalin (Pym) is an alilinopyrimidine fungicide used in vegetable and fruit crops since the 1990s. This xenobiotic has been considered of low toxicity in humans; however, endocrine disrupting effects have been reported in mammals. These factors, added to the resistance acquired by some types of fungi, are of concern about the risk of human exposure through the ingestion of foods with the presence of Pym, especially in Latin American countries that lack controls on crops and imported food. Moreover, there is little data on the possible mechanisms of Pym toxicity in humans. The aims of this work were to quantify Pym in commercially available apples using GC-MS, and to search for protein targets for Pym using pharmacophore mapping tools (PharmMapper, Machine learning (NR Tox Pred), molecular docking, protein-ligand (AutoDock Vina) and molecular dynamic (Gromacs)). GC-MS results showed detectable levels of Pym in 100% of analyzed apple samples. On the other hand, computational calculations showed that Pym has the potential to bind and interact with different human protein targets, of analyzed apple samples. On the other hand, computational calculations showed that the Pym has the potential to bind and interact with different human protein targets, such as alido-keto reductase family 1 member C3, serine/threonine kinase Pim-1, cholinesterase, androgen and progesterone receptors, among other proteins. These findings suggest that people are exposed to Pym through apple consumption, and the fungicide has the potential to promote endocrine disruption, cell proliferation and neurodegenerative diseases. Therefore, it is necessary to establish rigorous control systems for Pym in food, prioritizing experimental approaches to evaluate its toxicological effects on humans.

4469 Using Internal Dosimetry to Calculate Relative Potency Factors of Polycyclic Aromatic Hydrocarbons (PAHs) for Human Risk Assessment

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Many polycyclic aromatic hydrocarbons (PAHs) are procarcinogens, and the United States Environmental Protection Agency (US EPA) frequently assesses PAH-mixture cancer risk using the Relative Potency Factor approach. The Relative Potency Factor approach estimates cancer risk of a chemical mixture by summing doses of component mixture compounds after normalizing each compound dose with a relative cancer potency of that component compared to an index compound. For PAHs, benzo[α]pyrene (BaP) is used as the index compound. Cancer slope factors are typically measured using animal models, like mice, and are calculated by external exposures to the animal model. Since many PAHs require metabolic activation to form the ultimate to toxicant, the Relative Potency Factor approach assumes that PAH metabolism rates scale allometrically between animal models and humans. Our research has demonstrated metabolism rates and internal dosimetry do not scale allometrically for BaP or the potent dibenzo[def,ghi]perylene (Dib DbC) (relative potency factor ~30). Here, we developed physiologically based pharmacokinetic models (PBPK) for BaP and BaP in mice and humans to calculate the relative potency factor of DBC based on internal dosimetry. We measured and integrated in vitro metabolism rates of DBC, BaP, and metabolites, and PBPK models simulated DBC, BaP, and major metabolite (diols and tetrois) concentrations in blood and/or urine reasonably well compared to measured data in mice and human volunteers. We leveraged the PBPK models to predict internal dose metrics of mouse studies used to calculate the relative potency factor for DBC and translated those dose metrics to external doses in humans. Depending on the dose metric selected (area under the curve of Phase I diol metabolism, peak rate of Phase I diol metabolism, etc.) the predicted relative potency factor was not 30 and ranged 6-18 as an average dose. This indicates the current Relative Potency Factor approach may be inaccurate for compounds whose metabolism rates do not scale allometrically between animal models and humans. As such, our approach offers an improved method by considering species differences in pharmacokinetics. Finally, we used Monte Carlo simulations of population distribution of sensitive physiological parameters to predict variability of PAH dose metrics in humans. This variability allows calculations of uncertainty of the relative potency factors determined by internal dosimetry. These results demonstrate the importance of understanding internal dosimetry and utility of PBPK modeling for determining improved relative potency factors that can be used in risk assessment of chemical mixtures like PAHs found at Superfund sites. Supported by NIH Grants P42 ES016465 and R01ES028600.

4470 Multiple Elemental Exposures, Genotoxic, and Epigenotoxic Alterations in Benin Bronze Casters: Evidence of Occupational Metal Toxicity Associated with Traditional Bronze Casting Practices in Nigeria

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Nigerians are currently faced with a number of environmental and occupational hazards which contribute to the reported lower life expectancy. The informal sector workers (ics) exposed populations (BBC & EC), these data appear to provide evidence of genotoxic and epigenotoxic aberrations in occupational and environmental metal exposures associated with traditional bronze casting practices in Nigeria.

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Multiple health authorities have programs to restrict exposure to lead (Pb) in different media to permissible levels. The scientific basis of these approaches was evaluated across several agencies to understand important similarities and differences among them. Agencies and programs were chosen to be a representative, rather than complete, description of the different approaches. The agency approaches evaluated were the FDA Interim Reference Level (IRL) for Pb in food (Flannery, 2022), the EPA approach for evaluating Pb in soil at Superfund sites (EPA, 1998, 2016), the EPA model for evaluating Pb in surface dust in residences (EPA, 2019), and the Office of Environmental Health Hazards Assessment (OEHHA) identification of the Maximum Allowable Dose level (MADL) for Pb for under Proposition 65 (OEHHA, 1989). The following factors were considered in the evaluation: populations of interest; critical endpoints; risk targets (e.g., blood lead levels); exposure conditions; database deficiencies, and duration considerations. An RfD of 0.08 mg/kg/day was determined, which corresponds to a Total Allowable Concentration of 0.5 mg/L for the sum of 2- and 3-lead hemoglobin in drinking water.

4473 Formaldehyde Off-Gassing from Bed Sheets and Pillowcases: A Simulation Study
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Formaldehyde and formaldehyde-containing resins are used in clothing and other textiles, particularly those made from natural fibers to impart strength, durability, and wrinkle-resistance. Historical concerns of potential health effects associated with elevated formaldehyde levels have prompted improved testing and regulatory standards in the textile industry. Clothing has been a major focus of these efforts due to the occurrence of allergic contact dermatitis among consumers. Meanwhile, the highest airborne concentrations of formaldehyde are generally found indoors as a result of off-gassing from building materials and consumer products. The inhalation of formaldehyde released from bed linens (e.g., sheets and pillowcases) represents a unique circumstance for consumer exposure. Specifically, a large surface area, frequent and extended duration of use, and close proximity of the product to one’s breathing zone may result in the potential for higher exposures compared to other textiles found in the home and clothing. Since 2020, nearly 20 notices have been issued to manufacturers and retailers under California Safe Drinking Water and Toxic Enforcement Act (also known as Proposition 65) for potential warning label violations related to formaldehyde exposures from bed linens. This study was conducted to measure airborne formaldehyde levels resulting from off-gassing from bed linens during expected consumer use scenarios. An initial screening-level study was performed on ten sets of bed linens marketed as wrinkle-free/resistant of various fabric types to determine formaldehyde release under accelerated storage conditions according to AATCC TM112 standard methodology. In general, microfiber-based products released lower amounts of formaldehyde (0.3-µg/g), followed by cotton-blends (0.6-µg/g), and 100% cotton (2-17 µg/g), while the highest levels were found with bamboo fiber-based products (20-48 µg/g). Two bamboo fiber-based bed linens were selected for further evaluation as a proof-of-concept study for a Total Allowance in the test bedroom of a residential home. Personal and area samples were collected under four conditions for each product: 1) immediately after unpackaging 2) one-week after initial test, 3) following one wash/dry cycle, and 4) one-month after initial testing with weekly wash/dry cycles. Background samples collected weekly in the absence of products showed an average airborne formaldehyde concentration in the test bedroom of 30 to 45 µg/m³. Personal samples collected during testing of the first bamboo-based bed linen product showed airborne concentrations of 49 µg/m³, 59 µg/m³, and 50 µg/m³ for initial, one-week, and first wash testing, respectively. Testing of the second bamboo-based product found airborne concentrations of 3-42 µg/m³. The median 10-hour maximum levels for formaldehyde release under accelerated storage conditions showed positive release during the consumer usage study. In general, area samples were 10 to 20% less than that of the personal samples collected during product testing. By one month after initial testing, formaldehyde release from both bamboo fiber-based products was similar to background levels, demonstrating that release is substantially decreased with time and washing. These results indicate that the potential exposure to formaldehyde from bed linens is low and comparable to background levels of formaldehyde observed in indoor environments. In addition, any potential formaldehyde exposure does not exceed health-based guidance values established under the Federal Hazardous Substances Act. Therefore, formaldehyde release from bed linens is not expected to present a health risk to consumers.

4474 Mammalian Toxicity of Trifluoroacetate and Assessment of Potential Human Health Risks Due to Environmental Exposures to Trifluoroacetate

While the strong acid trifluoroacetic acid has limited technical uses, the highly water-soluble trifluoroacetic (TFA) is ubiquitously present in water bodies at low concentrations. Most of TFA in the environment is believed to arise from natural processes, but decomposition of certain fluorinated refrigerants in the atmosphere and chemical degradation of pesticides that contain the trifluoroacetyl group also contribute. The presence of TFA, mainly in water bodies, and the relative stability of TFA in the environment result in human exposures to TFA. For hazard and risk assessment, the mammalian toxicity of TFA and human exposures are reviewed to assess harms-of-exposure (MoE) between no-observed-adverse-effect levels (NOAELs).
with TFA in animal studies and human exposures from environmental sources. The potential of TFA to induce acute toxicity is very low and several repeated dose studies in rats with oral administration have identified the liver as the target organ with mild liver hypertrophy as lead effect with a NOAEL of 10 mg/kg bw/day in a 90-day oral study. Biomarker analyses indicate that TFA is a weak peroxisome proliferator questioning the human relevance of the liver effects seen in rodents. Regarding reproductive toxicity, oral administration of TFA to rats did not induce adverse effects in an extended one generation study (NOAEL > 265 mg/kg bw/day) and in a developmental toxicity study (NOAEL > 150 mg/kg bw/day). Additionally, TFA did not induce genotoxic responses in bacteria and in mammalian cells. Toxicokinetic data on TFA have not been published, but information on the excretion of TFA as a metabolite of halothane and 2,2-dichloro-1,1,1-trifluoroethane indicate a half-life in the range of 36 hours for the urinary excretion of TFA. Based on recent levels of TFA in surface and groundwater and standard assessment factors for drinking water consumption, MoEs for human exposures to TFA from these sources are well above the required 100. TFA is also present in diet, likely due to uptake of deposited TFA from sorbent plants and breakdown of trifluoroalkyl herbicides in plants and soil. The MoE of well over 100 for TFA in these settings was based on exposures to TFA from diet based on the exposure assessment by the European Food Safety Authority.

**4475** **Route Extrapolation and Critical Effect Characterization as a Basis to Establish Drinking Water Criteria for Dibutyl Ether**


Dibutyl ether (DIBE) may extract into drinking water when present as a residual component of drinking water contact coatings. As a regulated contaminant level for DIBE was not available from US EPA or Health Canada, drinking water criteria were developed by NSF in accordance with NSF/ANSI/CAN 600 (that outlines standard risk assessment procedures) and subsequently peer-reviewed by the NSF independent Health Sciences panel. The weight of evidence suggests that DBE has low genotoxic potential based on four negative, high-quality in vitro genotoxicity assays for DBE, in addition to genotoxicity data for DBE metabolites that suggest low risk. In the absence of chronic animal data investigating carcinogenic endpoints, there is inadequate information to assess the carcinogenic potential of DIBE according to U.S. EPA 2020 guidelines for carcinogen risk assessment. Toxicokinetic data for DBE were lacking and thus data on similar alkyl ethers were referenced to identify metabolic pathways and to consider route extrapolation. These data indicate DBE is metabolized to similar plasma concentrations via oral and inhalation routes through O-dealkylation by CYP450 enzymes to n-butanol and n-butyraldehyde. Based on the available animal data including data for DBE metabolites, target organs of DBE after oral and inhalation exposure are the central nervous system (CNS) with evidence of narcosis at human equivalent doses (HED) > 171 mg/kg/day, the hematological system (hemolytic anemia) at ≥ 73 mg/kg/day (HED), liver and kidney toxicity at ≥ 1000 mg/kg/day (HED), testicular effects at ≥ 36 mg/kg/day (HED) and general toxicity in the form of reduced body weight gain in adult and pregnant animals at ≥ 37 mg/kg/day (HED). Developmental effects including reduced fetal body weight and skeletal variations that cannot be excluded as adverse, occurred in the presence of maternal toxicity at human equivalent doses of ≥ 228 mg/kg/day. Neurodevelopmental effects including increased incidence of dilatation of cerebral ventricles and the subarachnoid space of the brain were observed at ≥ 1000 mg/kg-day (HED). Overall, the most sensitive effect that was deemed to be toxicologically adverse and relevant to human exposure is well-compensated hemolytic anemia in Wistar rats from a guideline-compliant 90-day inhalation study for DBE with a human equivalent NOAEL of 24 mg/kg/day. This endpoint was selected as the critical effect after evaluating the human relevance, uncertainty and comparative RfDs of the CNS, kidney, liver, testes, reduced body weight of adult and maternal animals and developmental effects. The selection of the critical effect is further supported by a high-quality 13-week gavage study for the metabolite N-butanal in which hematological effects were reported at an HED of 112 mg/kg/day in rats; NOAEL of 24 mg/kg/day and applying a conservative uncertainty factor of 300, an RfD of 0.08 mg/kg/day was a good estimate of drinking water intake rates for an adult of 0.034 mg/kg-day and a relative source contribution of 20%, a total allowable concentration in drinking water of 500 µg/L is calculated.

**4476** **Pesticide Residues in Honeybee (Apis mellifera L.) Pollen Collected in Two Ornamental Plant Nurseries in Connecticut: Implications for Bee Health**

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Honeybees (Apis mellifera L.) are one of the most important agricultural pollinators, pollinating at least 90 commercial crops. In recent decades, there has been a significant decline in populations of some wild bee species, as a result of stressors including habitat loss, pestspathogens, poor nutrition, and pesticides. While potential effects of agricultural pesticides on honeybee health have been investigated in some settings, risks associated with exposures occurring in the plant nursery setting have received much less attention. The limited amount of data on pesticide contamination in the pollen and nectar of ornamental and nursery plants is of particular concern to many stakeholders (e.g., regulators, nursery operators, pesticide applicators, the public). In this study, we sought to identify and quantify pesticide levels present in honeybee collected pollen harvested in two ornamental plant nurseries, Nursery M and Nursery P, in Connecticut. From June to September 2018, pollen was collected weekly from 8 hives using bottom-mounted pollen traps. Following extraction, pesticide levels in pollen samples were quantified using liquid and gas chromatography coupled with single or tandem mass spectrometry. Fifty-five unique pesticides (including related metabolites) were detected: 24 insecticides, 20 fungicides, and 11 herbicides. Interestingly, some of the pesticide contaminants within the pollen had not been applied by the nurseries, indicating that honeybees located at both plant nurseries did not exclusively forage on pollen at the nursery. The average number of pesticides per sample was similar at both nurseries - 12.9 at Nursery M and 14.2 at Nursery P. To estimate the potential risk posed to honeybees from these samples, we utilized the EPA’s BeeREX tool to calculate risk quotients (RQs) for each pesticide within each sample. RQs are calculated on a per bee basis using dietary consumption values for pollen and/or nectar and the total body residue of those pesticides and the toxicological values for detected pesticides. Resulting RQs > 0.4 for acute exposures are considered above the level of concern (LOC), which indicates more detailed exposure estimates and mitigation measures may be necessary. The average aggregate RQ for nurse bees, which consume the most pollen, was 0.004 at Nursery M and 0.006 at Nursery P, well below the LOC. We also calculated RQs for larvae due to their increased sensitivity to certain pesticides. In total, 6 samples had larval RQs above the LOC (0.45 – 2.51), resulting from the organophosphate diazinon. Since 2015, the frequency and amount of diazinon detected in pollen increased at one of our study locations, potentially due to pressure to reduce the use of neonicotinoid pesticides. These data suggest that pesticide application to the two ornamental plant nurseries investigated herein can contaminate pollen at levels that may pose a risk to larvae but not to adult honeybees. Additional studies are needed to determine if these findings are generalizable to other ornamental plant nurseries; however, they highlight the importance of considering all life stages when estimating potential risk to honeybee colonies from pesticide exposure. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

**4477** **Risk (Re)Assessment of N-Methyl-N-Nitrosophenethylamine for Use in Computing Acceptable Intake Levels of N-Nitrosamine Drug Substance–Related Impurities**

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Management of N-nitrosamine impurity levels in pharmaceutical drug substances and products is guided by ICH M7 where they are defined as Cohorts of Concern. Regulatory agencies have suggested using read-across of rodent carcinogenicity data to structurally similar compounds to assess the potency of various N-nitrosamines. They have set provisional acceptable daily intake limits for several common N-nitrosamines based upon experimentally measured TD50 values of close structural analogs. However, there are few N-nitrosamines with robust TD50 values for comparison as documented in the Carcinogenicity Potency Database (CPDB). This paper details LeadBiD data for N-Methyl-N-nitrosophenethylamine (NMPEA) as reported in the CPDB with a harmonic mean TD50 value of 7.88 µg/kg/day (or an Acceptable Intake (AI) level of 8 ng/day) was found to be inappropriately calculated for use in ICH M7 submissions as mixed tissues (oesophagus, forestomach, tongue, and nasal cavity) were combined into a single group termed “upper gastro-intestinal tract”. Upon examination of the original data [Lijinsky et al., Food Chem. Toxic. (20) 393-399, 1982], the oesophagus was considered the most sensitive organ of effect though, as stated in ICH M7, combinations mixed tumor types are still appropriate as they give a more sensitive potency estimate. The TD50 value for the oesophagus was recalculated to 40.1 µg/kg/day (or an AI of 40 ng/day). Since the N-MPEA potency was found to be close (BMD) analysis was also performed yielding a TD50 of 40.1 µg/kg/day in rats (or an AI range of 306-1760 ng/day). These updated values are 5-38 times (or higher) than the current AI level and could result in significantly higher AI limits for marketed drugs, such as N-nitroso-nortriptyline, that use NMPEA as a suitable analog. These potency levels are also more consistent with the structure-activity relationship of N-nitrosamines based on the structural features of NMPEA.

**4478** **Development of Hydrogen Sulfide Spacecraft Maximum Allowable Concentrations for Mars and Exploration**

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Hydrogen sulfide (H2S) has not historically been detected in spacecraft, but the possible evolution of volatile components from polar ice on the lunar surface is a potential concern for NASA’s planned Artemis missions. Numerous case reports and occupational epidemiological studies document that exposure to H2S at high concentrations has effects on the respiratory system, potentially leading to unconsciousness followed by debilitating neurological effects. Studies in rodents...
demonstrate sensitivity of the respiratory system to low concentrations of H2S. Adverse respiratory effects have also been noted in workers chronically exposed to H2S. The objective of the present publication is to develop Spacecraft Maximum Allowable Concentrations (SMACs) for H2S for all current standard durations of exposure for spaceflight (1 hour, 24 hours, 7 days, 30 days, 180 days, and 1000 days). Summary sources and literature review were used to identify relevant studies to guide SMAC development. Space flight mission specific activities and most probable exposure scenarios were used to determine the relevant toxicity endpoints and supporting studies. SMACs were established for 1 hour, 24 hours, 7 days, 30 days, 180 days, and 1000 days. These values were based on irritant effects of H2S. SMACs for H2S will support development of handling protocols and proper containment for lunar sample collection.

### 4479 Occupational Toxic Metal Exposure and HIV Infection in the Potentiation of Chemical Carcinogenesis

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Growing reports indicate that occupational toxic metal exposure and HIV infection independently enhance the risk of cancer through varied mechanisms, but their joint impact on cancer risk has received limited attention. The combined effects of these factors on carcinogenesis were investigated in this study. Based on HIV status and occupational exposure to toxic metals, 248 adults (191 males, 57 females, mean age 38.42 ± 10.47 years) were divided into four groups: HIV-positive exposed (n=62), HIV-positive unexposed (n=66), HIV-negative exposed (n=60), and HIV-negative unexposed (n=60). The HIV-positive and HIV-negative groups had similar occupations, ages, and other characteristics. Blood cadmium (Cd), lead (Pb), and mercury (Hg) were measured by inductively coupled plasma optical emission spectrometry (ICP-OES), while serum zinc (Zn) and copper (Cu) were determined using atomic absorption spectrometry. Data were analyzed by one- and two-way ANOVA. Lead, cadmium, and mercury were significantly elevated in the occupationally exposed HIV-positive group (14.92 ± 0.54 µg/dl, 0.25 ± 0.01 µg/L, 1.93 ± 0.08 µg/L respectively) compared with the HIV-negative unexposed group (11.07 ± 0.48 µg/dl, 0.17 ± 0.01 µg/L, 0.76 ± 0.05 µg/L) p < 0.05. Although toxic metal exposure and HIV infection contributed additively to elevated Pb and Hg levels, they reduced the level of Zn, an antioxidant, P53 activator, cell cycle regulator, anti-inflammatory, and metabolic antagonist to Cu. The results of this study, taken together, suggest that elevated toxic metal burden combined with a positive HIV status may exacerbate oxidative stress, increase inflammation, genetic instability, and impair, with these pathophysiological events converging to accelerate pathways that potentiate chemical carcinogenesis. As a result, appropriate safety precautions are required to reduce toxic metal exposure and the associated cancer risk among HIV-positive workers.

### 4480 Science-Based Assessment of Food Safety in Japan and Risk Assessment of Nicarbazin (Coccidiostat or Feed Additives)

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The Food Safety Commission of Japan (FSCJ) is a risk assessment organization for science-based assessment of food safety risks to human health. The FSCJ is comprised of seven commissioners. Weekly Commission Meetings are open to the public. The FSCJ receives requests for risk assessments from *risk management organizations. The risk assessment request is assigned to relevant Expert Committees or Working Groups which developing the draft risk assessment. The draft risk assessment is reported to the Commission for discussion. Commission will finalize the risk assessment and notify risk management organizations. Risk management organizations will then propose risk regulations by establishing maximum levels, maximum residue levels, and standards for use based on the result of the risk assessments. *Ministry of Health, Labour and Welfare (MHLW), Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of the Environment (MOE), and the Consumer Affairs Agency (CAA). Our work is (1) risk assessment, (2) risk communication, (3) research and survey, (4) collection and dissemination of domestic and international information, and (5) International cooperation. (1) The FSCJ assesses risks to human health posed by microorganisms, chemical substances, and others contained in food on the basis of the scientific evidence[CF1]. The FSCJ consists of the Planning Expert Committees, which develops an annual work program and 16 Expert Committees/Working Groups for Food Additives, Pesticides and Feed and Fertilizers etc., which conduct risk assessments on specific hazards. Currently, more than 200 experts (part-time) from universities, research institutes, and other organizations belong to each of the committees or groups and conduct risk assessment on their respective fields of expertise. The FSCJ risk assessment should constantly incorporate new assessment methods as science advances. For example, in silico methods have been developed to predict toxicity using computers based on the structure of the substances. (2) The FSCJ conducts risk communication to exchange information and opinions on risks and related factors with related parties (stakeholders) such as the general public (consumers, etc.), government (risk management organizations), media, food-related industries (primary producers, etc.), academia (researchers, etc.). (3) The FSCJ has conducted research and survey programs to generate scientific evidence for improving risk assessment. (4) The FSCJ collects information on human health effects, hazards in foods, and risk assessments from overseas. (5) The FSCJ is proactively working to strengthen international collaboration, such as holding meetings to exchange information in risk assessment to exchange the latest information and opinions. The FSCJ conducted risk assessment of nicarbazin in 2022. Nicarbazin is used as a coccidiostat or feed additives for chickens in Japan. Nicarbazin, when ingested, is rapidly split in its two components 4,4’-dinitrocarbanilide (DNC) and 4,6-dimethylpyrimidine (HPD) The main hazard to humans is not nicarbazin but its component DNC. For genotoxicity studies, there is no genotoxicity factor (SAF) of 100 and a no expected sensitization scenario. The resulting MOS values ranged from approximately 25 to 250. Overall, the relevance of these findings and the risk inferred from this study to humans is unclear. While the EU has introduced regulation of Liliail into its consumer product landscape, other countries including the United States have yet to do the same.

Further, there is an ongoing concern is that potential risk to humans from exposure to Liliail in personal care products. Skin sensitization was the endpoint of interest in this study due to a lack of existing studies characterizing risk in humans, and dermatal application being the exposure route through which most individuals are expected to encounter Liliail. The aim of this analysis was to evaluate the potential risk of skin sensitization induction following the use of both rinse-off and leave-in haircare products by conducting a quantitative risk assessment (QRA) and calculating a margin of safety (MOS), which provides a ratio of estimated human exposure levels to levels with no established risk. Products with a MOS less than 1 may be associated with an increased risk of the endpoint of interest (e.g., sensitization). To establish a MOS for Liliail in rinse-off shampoo products, a consumer exposure level (CEL) was estimated and benchmarked against an acceptable exposure limit (AEL), a level at which skin sensitization induction in consumers is not expected. For this QRA, the CEL was calculated using a Liliail product concentration of 0.01% weight by volume and variables including scalp surface area, number of applications per day, amount of product applied, and retention factor that were specific to either a rinse-off or leave-on product use scenario. The calculated CEL values were then benchmarked against an AEL derived from a sensitivity assessment factor (SAF) of 100 and a no expected sensitization induction level (NESIL) of 410 µg/cm² to obtain subsequent MOS values for both scenarios. The resulting MOS values ranged from approximately 25 to 250. Overall, the QRA indicates that dermal exposure to Liliail at the reported concentrations in rinse-off and leave-on haircare products is not expected to result in an increased risk of skin sensitization induction in additional consumer use contexts.

### 4482 Assessing Potential Cancer Risk Associated with Dermal Exposure to Formaldehyde in Consumer Hair Relaxers

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Formaldehyde and formaldehyde-releasing chemicals are found in many hair smoothing and straightening products, often referred to as hair relaxers. Several regulatory agencies have designated formaldehyde as a human carcinogen based on inhalation studies from human occupational exposure data and experiments in laboratory animals. There are several established health guidance values for formaldehyde and formaldehyde-releasing chemicals. The Environmental Health Hazard Assessment has established a No Significant Risk Level (NSRL) for formaldehyde exposure via inhalation of 40 µg/day, assuming a 70-year lifetime of daily exposure. This correlates to a lifetime NSRL of 1,022,000 µg formaldehyde/lifetime (40 µg formaldehyde/day x 365 days/year x 70 years). The NSRL is defined...
as the daily intake level posing a 10⁻³ lifetime risk of cancer. We performed a dermal exposure risk assessment to better understand the potential cancer risk associated with exposure to formaldehyde from personal use of hair relaxing products. Results from built up public sampling studies reported formaldehyde concentrations in hair relaxing products ranging from below the limit of detection of 0.017 ppm up to 115,000 ppm. A systemic exposure dose (SED) was calculated for dermal application of hair relaxing products. The time weighted average HEC, using the MA BMCL value, of [(1 mg/m³) * 0.75 * (8 hours/day) * (5 days/week) * (52 weeks/year)] was used for the calculation. The SEDs in this analysis were estimated to reflect typical and worst-case exposure scenarios to Lilial® from dermal application of hair styling products over a 70-year life-time. In order to calculate the typical and worst-case SEDs, the MA LMCL value of Lilial® found in a popular hair styling product, an estimated hair styling product retention factor, and assumptions regarding the amount and frequency of hair styling product used by an individual (i.e., the average amount of product used weekly for a typical exposure scenario and the 95th percentile amount of product used daily for a worst-case exposure scenario) were considered. The estimated SEDs ranged from 2.67 mg/kg/day to 4.43 mg/kg/day. The lower- and upper-bound SEDs were then adjusted to estimate exposure to formaldehyde from lifetime typical use of hair relaxing products. Using Haber’s law (concentration = time = constant), which relates time and dose assuming that there is a linear relationship between time and toxicity, one can determine the appropriate cumulative dose. Assuming hair relaxer use up to six times per year for 70 years, the cumulative systemic lifetime exposure ranged from 2,688-154,560 µg of formaldehyde which is less than the cumulative lifetime NSRL of 1,022,000 µg. Our calculated MOS values were all greater than 1 (6.61-380), indicating that a significant risk of cancer from dermal exposure to formaldehyde from consumer use of hair relaxers following typical use is not expected. Our model includes some conservatism, including the assumption that hair relaxer is used six times per year continuously for 70 years, and the inclusion of the highest reported formaldehyde concentration, which is not representative of most commercially available hair relaxers. The exposure assessment approach utilized in this study can be used to estimate the risk of other chemicals present in hair relaxers or in other products. One limitation of the model we present here is that only dermal formaldehyde exposure is being modelled, and inhalation exposure is also expected to occur through consumer use of hair relaxers. Our work modeling the dermal exposure is an important component of a comprehensive risk assessment.

Butylphenyl methylpropional, more commonly known as Lilial®, is a synthetic aromatic compound that has historically been used as a fragrance additive in a variety of consumer products including household cleaners, detergents, and hair styling products. As of March 2022, Lilial® was banned for use in products on the European Union (EU) market due to the compound’s classification as a 18 carcinogenic, mutagenic or reprotoxic (CMR) substance on recent re-evaluation of toxicity studies in rats. Specifically, Lilial® is presumed to be a reproductive toxicant because of adverse effects observed in the testes of male rats following oral exposure to Lilial®. While the EU has banned Lilial®, there is currently no ban on the use of the compound in products sold on other markets around the world; thus, there is a need to characterize the potential toxicity of Lilial® to humans. In order to understand the possible reproductive toxicity risk associated with exposure to Lilial® from personal use of hair styling products, a quantitative dermal risk assessment was performed. Margin of safety (MOS) values were calculated to compare estimated human exposure levels, expressed as systemic exposure doses (SEDs), to an established derived no effect level (DNEL). MOS values greater than 1 indicate that there is not an increased risk of a health endpoint (e.g., reproductive toxicity) based on the exposure scenarios used in the calculation. The SEDs in this analysis were estimated to reflect typical and worst-case exposure scenarios to Lilial® from dermal application of hair styling products over a 70-year life-time. In order to calculate the typical and worst-case SEDs, the MA BMCL value of Lilial® found in a popular hair styling product, an estimated hair styling product retention factor, and assumptions regarding the amount and frequency of hair styling product used by an individual (i.e., the average amount of product used weekly for a typical exposure scenario and the 95th percentile amount of product used daily for a worst-case exposure scenario) were considered. The estimated SEDs ranged from 0.267 mg/kg/day to 4.43 mg/kg/day. The lower- and upper-bound SEDs were then adjusted to estimate exposure to formaldehyde from lifetime typical use of hair relaxing products. Using Haber’s law (concentration = time = constant), which relates time and dose assuming that there is a linear relationship between time and toxicity, one can determine the appropriate cumulative dose. Assuming hair relaxer use up to six times per year for 70 years, the cumulative systemic lifetime exposure ranged from 2,688-154,560 µg of formaldehyde which is less than the cumulative lifetime NSRL of 1,022,000 µg. Our calculated MOS values were all greater than 1 (6.61-380), indicating that a significant risk of cancer from dermal exposure to formaldehyde from consumer use of hair relaxers following typical use is not expected. Our model includes some conservatism, including the assumption that hair relaxer is used six times per year continuously for 70 years, and the inclusion of the highest reported formaldehyde concentration, which is not representative of most commercially available hair relaxers. The exposure assessment approach utilized in this study can be used to estimate the risk of other chemicals present in hair relaxers or in other products. One limitation of the model we present here is that only dermal formaldehyde exposure is being modelled, and inhalation exposure is also expected to occur through consumer use of hair relaxers. Our work modeling the dermal exposure is an important component of a comprehensive risk assessment.
It is estimated that over 4.53x10^8 kg of inorganic lead (iPb) compounds were manufactured in the U.S. in 2020. Over 1.4 million U.S. workers have dermal exposures to iPb in several industries with exposure limits of iPb on hands varying widely (0.005-16.1 µg/cm^2). Howden et al., 1987, studied the dissolution and bioaccessibility of iPb compounds in skin surface film liquids (SSFLs) (including both synthetic sweat and sebum) to determine the potential for Pb ion formation. Dissolution is a critical factor to determine dermal bioaccessibility and is different than solubility. Dissolution measures ion formation in SSFLs, does not necessarily reach equilibrium, and can be influenced by physicochemical interactions with the components in SSFLs. Dissolution data can be used to model bioavailability via dermal absorption using both the concentration of dissolved ions in sweat, and the permeation rate (Kp) of chemicals through the skin. As far as we know, the dissolution of iPb compounds under biologically relevant conditions has not been published. The study objectives were (1) determine the pH-dependent static dissolution of four iPb compounds in SSFLs: Pb^2+ nitrate (PbN), Pb^2+ acetate (PbA), Pb^2+ oxide (PbO), Pb^2+ red oxide (PbRO); (2) evaluate iPb dissolution kinetics; and (3) provide screening estimates of the potential impact of these compounds on BLLs (assuming exposure to hands only). Statistical analysis using SAS® to fit negative exponential functions to data and calculate percent dissolution at complete concentrations were completed. iPb data analyses, along with dermal loading estimates of Pb compounds in workplace settings, provides a starting estimate for the concentration of Pb ions potentially available in the sweat layer on skin. Estimated concentration of Pb ions available on the skin was used along with available permeability coefficients (Kp) to provide more robust understanding for the potential for dermal bioavailability of these compounds. The iPb compounds are bioaccessible in SSFLs; PbN and PbA have greater dissolution at 8 h (36.4-61.1%) compared to PbO and PbRO (0.01-2.5%). PbN has a statistically significant effect on bioaccessibility for all four compounds tested. Screening suggests that BLLs may be increased by 0.7-8 µg/dl for these iPb compounds. The screening level estimates based on this model suggest that the impact on BLLs warrants a more comprehensive assessment. In occupational settings where other routes of exposure to iPb may be relevant, dermal exposure estimates may represent a significant relative source contribution to overall body burden of Pb exposure. Examination of the impact of dissolution on BLLs could be incorporated into physiologically-based pharmacokinetic models (PBPK) to provide a more robust understanding of the impact on BLLs. Given the potential for Pb ion availability to enable dermal absorption of Pb as demonstrated in this study and previously in the literature, reducing Pb exposure on skin may be important for reducing overall worker exposure to iPb. More research is needed including dissolution of iPb particles from industrial settings and the impact of particle size on dissolution.

**Avian Risk Assessment for 1,1,2-Trichloroethane in Surface Water**

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1,1,2-Trichloroethane is an anthropogenic chemical primarily used as an intermediate in the production of 1,1-dichloroethane and 1,2-dichloroethane. In 2019, 1,1,2-trichloroethane was designated as a high priority substance for risk evaluation following the process required by section 6(b) of the Toxic Substances Control Act (TSCA). The U.S. Environmental Protection Agency (EPA) determined that this chemical’s potential toxicity in birds is a data gap that needs to be filled. Thus, the current study conducted an avian risk assessment for 1,1,2-trichloroethane in surface water using existing data by considering (i) environmental concentrations of 1,1,2-trichloroethane and (ii) toxicity benchmarks derived from studies on 1,1,2-trichloroethane analogues as well as modeling data obtained for 1,1,2-trichloroethane from U.S. EPA’s Web-based Interspecies Correlation (Web-ICE) tool. We calculated the average, median, and 95th percentile concentrations of 1,1,2-trichloroethane measured in approximately 40,000 surface water samples available from the Water Quality Portal (WQP), a public database that contains water-quality records from more than 400 federal, state, and local agencies. We found that the detection frequency of 1,1,2-trichloroethane in environmental samples is very low (e.g., <1% in surface water). Using the 95th percentile of the measured 1,1,2-trichloroethane concentrations from surface water, we predicted daily doses of 1,1,2-trichloroethane in bobwhite quails, mallard ducks, and Canadian geese, representing a frequency of 1,1,2-trichloroethane in environmental samples is very low. Following this process, we predicted daily doses of 1,1,2-trichloroethane in bobwhite quails, mallard ducks, and Canadian geese, representing a frequency of 1,1,2-trichloroethane in environmental samples is very low.

The aim of this work was to derive an oral acceptable daily intake (ADI) for cannabidiol (CBD) when present in hemp-based dietary supplement products, as a detectable impurity or as a naturally occurring constituent at less than 70% of the hemp extract. At the time this research was conducted, the UK Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment (COT) was the only authoritative body to propose an ADI (4 mg/day) for CBD intended for general consumption. A comprehensive literature search was conducted and all studies relevant to potential toxicological effects of oral CBD consumption in animals and humans were reviewed. The key studies selected for the derivation of a point of departure (POD) for this assessment included three randomized, controlled trials in human subjects being treated with the CBD drug Epidiolex® for epilepsy-related conditions. These studies transitioned from three weeks to 14 weeks in duration and tested doses up to 20 mg/kg-day CBD. By pooling data on liver enzyme activities from these studies, it was determined that patients treated with 20 mg/kg-day CBD had elevated alanine aminotransferase (ALT) serum concentrations greater than 5-fold higher than the upper limit of normal (ULN). This observation occurred in....
Acute exposures to acetone and developing an immediately dangerous to life or health (IDLH) value in occupational settings

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Acetone is a colorless water-soluble liquid that is used as an industrial solvent in chemical production. The primary route of exposure to acetone in occupational settings is inhalation. Acute exposures to acetone have been reported to elicit neurological effects, irritation, and respiratory effects, which could impair a worker’s ability to escape from a contaminated environment. The National Institute for Occupational Safety and Health (NIOSH) develops immediately dangerous to life or health (IDLH) values to identify air concentration levels that cause severe life or health impairment. NIOSH guidelines for deriving IDLH values include an evaluation of toxicological data from human and animal studies, including dose-response information, if available. At exposure levels of several thousand parts per million (ppm), acetone is associated with neurological effects like dizziness, headache, ataxia, decreased visual vigilance in animal studies. However, these studies identified mild irritation of the eyes and upper respiratory tract. Animal studies have reported loss of reflex, ataxia, decreased response, and necrosis at exposure concentrations greater than 10,000 ppm for 3-8 hours. Certain studies reported a 50% decrease in respiration rate (RD) in mice exposed to concentrations ranging from 77,500 ppm for 10 minutes to 84,000 ppm for 4 hours. These data are a measure of respiratory irritation. Lethal concentration in 50% (LC50) of rats exposed to acetone for 4-8 hours ranged from 16,000 ppm to 50,000 ppm. NIOSH will continue to evaluate these data in the development of a draft updated IDLH value for acetone based on neurological and irritation endpoints from findings in both animal and human studies.

4492 Identification of Direct and Specific Inhibition of Fatty Acid Oxidation on Isolated Hepatic Mitochondria to Investigate Drug-Induced Steatosis

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Drug-induced liver injury (DILI) is one of the major causes of premature termination of drug development, or marketing. Among DILI, macrovascular steatosis is a common and benign lesion characterized by lipid accretion (mostly triglycerides), although it is slowly progressive in some patients to steatohepatitis. In rare cases, drugs can also induce microvesicular steatosis, a severe form of DILI associated with hepatic cytolysis and hypoglycemia. Impairment of mitochondrial fatty acid oxidation (mtFAO) is a key mechanism whereby drugs can induce steatosis, with the most severe mtFAO alterations leading to microvesicular steatosis. In a screening study showing high relationship between steatosis and mtFAO inhibition (positive predictive value 91%, specificity 77%), the steatogenic drugs dexamethasone, olanzapine, ritonavir and zidovudine inhibited fatty acid-driven oxygen consumption in mouse liver mitochondria (with IC50 below 100 μM) but not complex I- and complex II-driven oxygen consumption (with glutamate/malate and succinate as respiratory substrates, respectively). Hence, results indicate that these drugs likely inhibit mtFAO via a direct mechanism and not via an impairment of the mitochondrial respiratory chain (MRC). This represents a new mechanism for the antiretroviral drug zidovudine since mitochondrial DNA (mtDNA) depletion was deemed to be the unique mechanism whereby this drug can induce hepatic mitochondrial toxicity and steatosis. Notably, three types of substrates (mitochondrial carnitine, palmitoyl-CoA, and octanoyl-CoA) were used in our assay in order to determine whether drug-induced inhibition of mtFAO is involving carnitine palmitoyltransferase 1 (CPT-1), an enzyme located at the outer membrane and allowing the formation of palmitoyl-L-carnitine from palmitoyl-CoA and carnitine, and other enzymes involved in the oxidation of long-chain and medium-chain fatty acids within the mitochondrial matrix. Thanks to this methodology, we uncovered that the four aforementioned drugs inhibited mtFAO of both medium and long-chain fatty acids and that CPT-1 inhibition was secondary to an impairment of MRC. Finally, our study demonstrates the usefulness to test drug-induced alteration of mtFAO and MRC in isolated mitochondria to identify new drug candidates with mtFAO inhibition as a potential target for drug development.

4493 Chemical Mixtures: The Science of Skin Deep


The US personal care industry mostly regulates itself, with limited oversight from the Food and Drug Administration. Without a standardized approach to formulation, the industry is replete with product ingredients that vary in quality and in their potential risks to human health and the environment. Although the potential risks posed by personal care products are well-documented, the standard single chemical approach to the chemistries contained in these products limits the ability of consumers, regulators, and formulators to accurately assess the safety of their products. The Environmental Working Group’s Skin Deep database utilizes a weighted hazard stacking approach to approximate cumulative risk from the complex mixtures that comprise personal care products. Skin Deep hazard stacking
begins at the ingredient level. Each ingredient undergoes a hazard assessment with new mixtures. This type of mixtures analysis can benefit both cumulative risk characterization of the mixtures risk. Such profiles can be used to consistently prioritize assessing the toxic risk of each health endpoint in the mixture, such as hepatic, renal, or neurological hazard quotients (HQs) for each compound in the mixture, which are added-ppb (reproductive). ATSDR used the MRLs and the TTCs to determine target-or (hepatic), and 37 ppb (renal). DCE's chronic MRL is 1 ppb (respiratory), with derived development of more comprehensive cumulative risk assessments.

Skin Deep calculations do not assume that chemical effects are only additive or iterative and updated based on the publication of new hazard data. Skin Deep's ingredients that make up over 87,000 products. The ingredient and product scores with the fewest health concerns. Skin Deep users can compare product combined based on endpoint groupings to create the overall product hazard scores. The final category, absorption, is used to weight the ingredient hazard scores, their weighted ingredient hazard scores are then scaled 1-10, with lower ratings representing products with the fewest health concerns. Skin Deep users can compare product safety using the scaled ratings. Currently the database includes almost 9,000 ingredients. When we compared the 87,000 products. The ingredient and product scores are iterative and updated based on the publication of new hazard data. Skin Deep's comprehensive hazard stacking approach accounts for co-exposures that impact a range of human health endpoints. Unlike other cumulative risk frameworks, the Skin Deep calculations do not assume that chemical effects are only additive or multiplicative. This approach to chemical mixtures may be applied more broadly to address the range of exposures from consumer products and to support the development of more comprehensive cumulative risk assessments.

4494 Mixtures Risk Assessment of Volatile Organic Chemicals through Multiple Target Organ Evaluation

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Environmental mixtures assessment consists of evaluating compounds that cause various toxic effects, often in different target organs or systems. Whole mixtures toxicity testing data are preferred, but rarely available, for mixtures risk assessment. Instead, Agency for Toxic Substances and Disease Registry (ATSDR) performs mixtures assessments using the hazard index approach, which constructs the plausible toxicity of the mixture by using the toxicity of the individual compounds of the mixture. Using indoor air levels from one site, ATSDR reviewed the toxic effects of individual compounds to determine which organ systems could be affected by the combination of compounds. ATSDR used the inhalation minimal risk level (MRL) or, when not available, derived specific acute inhalation target organ toxicity concentrations (TTCs) for 20 compounds measured in indoor air, including 1,1-dichloroethene (DCE), perchloroethylene (PCE), and trichloroethylene (TCE). The uncertainty factors associated with these TTCs ranged from 10-3,000. PCE's chronic MRL is 6 parts per billion (ppb) (neurological), with derived chronic TTCs of 38 ppb (hepatic), 24 ppb (renal), 36 ppb (endocrine), 360 ppb (gastrointestinal), and 18 ppb (respiratory). TCE's chronic MRL is 0.4 ppb (developmental and immunological), with derived chronic TTCs of 4 ppb (neurological), 37 ppb (hepatic), and 37 ppb (renal). DCE’s chronic MRL is 1 ppb (respiratory), with derived chronic TTCs of 50 ppb (hepatic), 1 ppb (renal), 2.4 ppb (endocrine), and 1.1 ppb (reproductive). ATSDR used the MRLs and the TTCs to determine target-organ hazard quotients (HQs) for each compound in the mixture, which are added to obtain HQs for each target organ or system. Using this approach, ATSDR can assess the toxic risk of each health endpoint in the mixture, such as hepatic, renal, or immunological. Derivation and use of TTCs allow for a more realistic characterization of the mixtures risk. Such profiles can be used to consistently prioritize various mixtures found in a community and can be used to predict toxicities of new mixtures. This type of mixtures analysis can benefit both cumulative risk assessment and evaluation of health equity and environmental justice. Coordinated targeted toxicity testing and computational methods development can help bridge data gaps that exist in the toxicity database of some of these compounds. The findings and conclusions in this presentation have not been formally disseminated by the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

4495 Bayesian Hierarchical Models to Solve Mixtures Prediction with Dirichlet Processes and Generalized Concentration Addition

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Environmental toxicants usually occur together as components in mixtures rather than in isolation. This presents a challenge when attempting to infer relations between chemical exposure and health outcomes in observational studies, since the effective exposure or outcomes cannot be assigned to a single toxicant at the observed exposure level. In this work, we assume individual toxicants have dose-response curves following the Hill model and can be clustered through the slope parameter as a proxy to mode of action. We propose a Bayesian hierarchical model that automatically clusters toxicants with a Dirichlet process (DP) prior, avoiding the need to specify the number of clusters. Replicates are modeled with a mixed effect for the maximum response to reduce parameter variability. A standard two-step model of concentration addition (CA) and independent action (IA) is used to estimate a mixture response. We provide a geometric distance based on reflection to estimate the mixture effect with partial agonists, even when the slope parameter is not 1. Our method recovers the generalized concentration addition (GCA) method when the Hill slope parameter is 1. We compare our technique to standard two-step methods in simulation and demonstrate an application to real data. The results suggest that our technique can more accurately recover the mixture effect compared to alternatives that do not account for partial agonists while avoiding the manual tuning that is typically needed when estimating clusters.

4496 Embryonic Zebrafish as a Model to Evaluate Salicylate Analogues and Their Mixtures

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Various frameworks exist for mixture risk assessment that share four main attributes: problem formulation; combined hazard assessment; combined exposure assessment and risk characterization. Dose addition is widely accepted in these frameworks as the default assumption for compounds that share a common mode of action and toxicological phenotype. Rapid mechanism-based methodologies afford us mechanistic insights to group chemical behavior, but they are not a direct indication of organismal health or adverse phenotypes. The embryonic zebrafish model shows promise as a systems-model with metabolic competency, to rapidly identify phenotypic changes due to chemical exposure in a high-throughput manner and affords a deeper understanding of toxicity from complex mixtures.

Salicylates are a large group of compounds used in a variety of consumer products and so to improve the understanding of how salicylates would behave in unintentional combinations, we have selected compounds for further investigation in the embryonic zebrafish model using Structure Activity Based Relationships. Salicylic acid was selected as the primary structure of interest for the grouping exercise due to its REACH classification as a category 2 reproductive toxicant, based on developmental effects in animal toxicity studies. Seven salicylates were then prioritized based on their cosmetic relevant use. In parallel we used publicly available gene expression data and Connectivity Mapping to investigate salicylic acid’s mode of action and select based on weak cyclooxygenase inhibitory activity, etodolac, as a functional analogue. Dechlorinated embryonic zebrafish were continuously exposed beginning at 6 hours post fertilization (hpf), to a broad range of concentrations (dose range finding) to identify the maximum tolerable concentration for individual compounds and determine the concentration to cause 50% effect (EC50). Our data show that 7 salicylate active was benzy1 salicylate with an EC50 at 12 uM, followed by methyl salicylate, homosalate, and salicylic acid. Etodolac was 165x less potent than benzy1 salicylate but had a similar EC50 to salicylic acid at 1970 uM. Sodium, 2-ethylhexyl and hexyl salicylate did not cause morphological defects by 120 hpf. In addition, at 120 hpf larvae locomotor behavior was assessed at concentrations that did not induce morphological defects. The simple response to the light stimulus was tracked and 87% of the salicylates caused hypoactive movement in the dark. Once again, benzy1 salicylate was the most potent, with the lowest effect level (LEL) of 6.84 uM, followed by salicylic acid, etodolac and methyl salicylate (250-350 uM). The least active was homosalate with an LEL of 9750 uM. Our data show that the mechanism of action of the salicylates may vary at higher test concentrations, with metabolism likely playing a key role in the effects observed by esters such as benzy1 salicylate. Next, we used the LEL values to design mixture experiments of various chemical combinations, to inform dose additivity and drivers in the mixture risk assessment. Our data suggest that a more nuanced approach like this to mixture risk assessment could be achieved by considering both chemical and biological similarity whilst more downstream considerations of phenotypes would help evaluate combined effects of chemicals. Non-mammalian models such as the zebrafish can be used to elucidate the risk of combined effects of chemicals in a high throughput, cost-effective and reproducible manner.

4497 Dose-Response Assessment of Dioxin-Like Mixtures through a Bayesian Framework of Mechanism-Based Data Integration

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Human health risk assessment of mixtures is an emerging and important subject that has gained much attention in recent years. Since humans are exposed to various hazardous substances simultaneously in their daily life, mixture risk assessment can provide a more plausible risk evaluation of chemical exposures, as compared with a traditional approach that assesses one chemical at a time. Infinite combinations of chemicals make it almost impossible to only rely on experimental toxicity data of mixtures to assess the risk. Currently, based on the traditional animal data and/or epidemiological exposure-response data, two typical strategies exist to assess mixture mode of action i.e., concentration addition (CA) and independent action (IA). IA integrates reference doses from individual chemicals to derive a “safe” dose of the mixture. But they are not a direct indication of organismal health or adverse phenotypes. The embryonic zebrafish model shows promise as a systems-model with metabolic competency, to rapidly identify phenotypic changes due to chemical exposure in a high-throughput manner and affords a deeper understanding of toxicity from complex mixtures.

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Chemical contaminants are omnipresent in the environment. A great number of industrial compounds are commonly detected in human blood and urine. Human exposure to chemicals is dependent on a multitude of environmental factors. As a result, human health is often affected by the sum of these exposures. It is therefore difficult to construct a contaminant mixture that is universally representative. Ubiquitous chemicals such as bisphenols, phthalates and parabens can interfere with endocrine signaling as classified as endocrine disruptive compounds (EDCs). The sex steroid hormonal signaling pathway is complex and sensitive to interference, as circulating concentrations of these potent hormones are low. Even limited exposure and uptake of an EDC could therefore cause adverse effects on, for example development, brain function, and the reproductive- and immune systems. Industrial chemical production involves risk assessments that balance societal benefits to potential negative effects on human- and environmental health. These risk assessments are based on observations in single-compound exposure studies, which is not reflective of real-life scenarios where exposure to different chemicals and classes from many sources occurs simultaneously. Additive or synergistic effects are a concern, since the toxicity of different compounds in the biological system could interact and produce an unexpected and exaggerated toxicological response. The linear- and additive-type compound approach may therefore potentially underestimate the biological impact of mixture effects. We have set up a chemical test set of 26 selected persistent organic pollutants identified in human serum, including PFAs, PCBs, organochlorines and BDEs. Non-contact liquid handling is employed to efficiently reproduce real-world mixtures as detected in human exposure. We used clinical trophoblast cell lines. These are the most relevant cell line models to investigate the effects of reconstructed individual exposures based on clinical profiles detected by advanced mass spectrometry analysis of serum collected from a northern Swedish cohort. This will aid in the development of relevant risk assessments for chemical mixtures, which better can protect the general population from endocrine disruptive mixture toxicity.

P5 4498 Exposure to a Chemical Mixture Exacerbates the Effects of EGFR-Mediated Trophoblast Cell Functions Compared to Single Chemical Exposures
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Pregnant women are exposed to complex chemical mixtures which are detectable in the placenta. The effects of these chemicals are complex and involve specific receptor activation pathways. For example, epidermal growth factor receptor (EGFR) activation, a receptor highly expressed in the placenta, that modulates cytotrophoblast proliferation, differentiation, and invasion of extravillous trophoblasts (EVT) into the maternal decidua. We and others found that polychlorinated biphenyl (PCB)-126, PCB-153, atrazine, trans-nonachlor, niclosamide, and BPA prevent EGFR endocytosis and reduce EGFR activation suggesting that they may block EGFR-mediated placental cell functions. We hypothesized that PCB-126, PCB-153, atrazine, niclosamide, trans-nonachlor, or BPA would independently reduce EGFR phosphorylation, compete with EGFR binding to EGF and when combined in a mixture (Chem-Mix), reduce EGFR-mediated trophoblast cell proliferation and invasion. We evaluated the effects of the Chem-Mix on trophoblasts by measuring EGFR phosphorylation by exposing the placental EVT cell line, HTR-8/SVneo to vehicle control (0.1% DMSO), Chem-Mix (1, 10, or 100 ng/ml), EGF (30 ng/ml), or Chem-Mix + EGF. Using western blotting, we determined that EGF increased phospho-p-EGFR compared to the vehicle control (p<0.05), while the Chem-Mix alone had no effect on p-EGFR. However, when combined, the Chem-Mix + EGF reduced p-EGFR in a dose dependent manner compared to EGF (p<0.05). Using the same exposure, we measured the protein abundance of signal transducer and activator of transcription 3 (STAT3) which is a downstream effector of EGFR. This resulted in no significant changes in p-Stat3 and total Stat3 abundance compared to EGF after 120 minutes of exposure. We understand if specific chemicals were responsible for the decrease in p-EGFR, HTR-8/SVneo cells were also exposed to EGF (30 ng/ml) or individual chemicals (100 ng/ml) + EGF. Of note, individual chemicals did not block EGFR-mediated EGFR phosphorylation. Since EGFR mediates trophoblast cell functions, HTR-8/SVneo cell proliferation was tested using a CellTiter-Glo luminescent cell viability assay kit. Cells were exposed for 7 days to Chem-Mix (100 ng/ml), EGF (30 ng/ml), or the Chem-Mix + EGF. Starting at day 3 of culture exposure to the Chem-Mix resulted in a reduction in proliferation compared to the vehicle control (p<0.05). Similarly, starting at day 4 of culture, the Chem-Mix + EGF decreased cell proliferation compared to EGF (p<0.05). Furthermore, HTR-8/SVneo cells were exposed to the same treatments as above to investigate the effects of the Chem-Mix on migration using a Transwell system. Using the same exposure, we observed that EGFR increased cell invasion compared to the vehicle control. Interestingly, both the Chem-Mix and the Chem-Mix + EGF reduced cell invasion compared to the vehicle control (p=0.05) and EGF (p<0.05), respectively. To determine if a specific chemical of the Chem-Mix was responsible for reduced cell invasion, we exposed HTR-8/SVneo cells to individual chemicals (100 ng/ml) or cell invasion compared to the vehicle control. Altogether, our results demonstrate that while individual chemicals do not alter EGFR phosphorylation, when in mixture, they reduce EGFR activation. This, in turn, impairs EGFR-mediated functions, such as placenta cell proliferation and invasion. Since the EGFR effector STAT3 was not affected, other pathways should be explored to identify the downstream effectors responsible for alterations in cell proliferation and invasion.
We have conducted a study to determine the neurological and respiratory effects of exposure to elevated CO₂ levels (3.5, 5, or 6.5%) increased both the respiratory rate and tidal volume. In addition, MIX dams had 7.5% lower fDEE exposure. Thus far, our data support additive or synergistic effects of combined exposure to fDEE and elevated CO₂ on respiratory and inflammatory endpoints.

People are exposed to polycyclic aromatic compounds (PACs) as complex and dynamic mixtures. A component-based approach that utilizes individual PAC relative potency factors is typically used to estimate human health risk from exposure to PAC mixtures. While the class includes a broad range of structurally diverse compounds that display a wide spectrum of toxicities, risk evaluations have typically focused on select polycyclic aromatic hydrocarbons (unsubstituted PACs including benz(a)pyrene, chrysene, dibenzo[a,h]anthracene, dibenzol[a]pyrene, dibenzothiophene, indeno[123-c,d]pyrene, phenanthrene, pyrene) in 2866CFI/N mouse immunotoxicity studies. Endpoints evaluated included immunotoxicity parameter, hematotoxia, histopathology in select organs, organ weights, and liver transcrptions. Evaluation of these endpoints provided a diversity of mechanistic and apical data orthogonal to existing cancer studies. Relative potency factors were calculated for different endpoints using benzo[a]pyrene as the reference chemical. All PACs except pyrene displayed dose-responsive decreases in humoral immunity, with immunotoxicity potency factors relative to benzo[a]pyrene ranging from 0.02 (dibenzo[b]fluoranthene) to 21 (dibenzo[a]pyrene). Some interesting differences in potency were noted between existing cancer studies and immunotoxicity endpoints. For example, benzo[k]fluoranthene was more potent than benzo[a]pyrene for immune suppression, but less potent for tumor development. Dose-response immunotoxicity data from these studies were used in parametric models to predict mixture toxicity, based on the concepts of dose additivity and independent action. Three 13-PAC mixture responses to increase confidence in application of similar NAMs to future monitoring efforts. We used the INDIGO model to compare potential tissue burdens resulting from the use of multiple volatile components of jet fuel simultaneously, an array PBPK model was cotedo simulate inhalation exposures to one or more select jet fuel compounds: toluene, ethyl benzene, xylene, nonane, decane, and naphthalene. The model structure accounts for metabolism of compounds in the lung and liver, as well as kinetics of each compound in multiple tissues, including the cochlea and brain region. The model is used to predict rat kinetic comparisons between hypothetical exposures to JP-8 or a Virent Synthesized Artificial Keranos (SAK). JP-8 50:50 blend at the occupational exposure limit (200 mg/m³). Predicted exposure to the blend decreased nonane and decane exposures by about 50%, with predicted brain stem concentrations decreasing by the same percentage. However, toluene exposure increased by 144% and relative blood concentrations below 200%. The array model has proven useful to compare potential tissue burdens resulting from the use of complex mixtures. Disclaimer: No DODB endorsement implied. We have conducted a study to determine the neurological and respiratory effects of individual and combined inhalation exposures to volatile organic compounds from diesel exhaust and elevated carbon dioxide (CO₂) levels in male Sprague Dawley rats. Animals were exposed via inhalation to 0 (Control), 0.25, 1, 5, or 10 mg/m³ filtered diesel exhaust emissions (fDEE), or 3.5, 5, or 6.5% CO₂. To assess combined effects, additional groups of animals were exposed to both 5 mg/m³ filtered diesel exhaust emissions in combination with 3.5, 5 or 6.5% CO₂. All exposures lasted a total of four hours and were conducted in whole body plethysmographs. A one-time, four-hour exposure to 5 or 10 mg/m³ fDEE led to an increased respiratory rate and decreased tidal volume in rats. A one-time, four-hour exposure to elevated CO₂ levels (3.5, 5, or 6.5%) increased both the respiratory rate and tidal volume of the exposed rats. Control rats had an average decrease of 11.6% in respiratory rate and tidal volume. fDEE (5 mg/m³) rats had 7.5% increase and 15.2% reduction in tidal volume. CO₂ (6.5%) rats had 23.3% reduction in respiratory rate and 5.5% increase in tidal volume. Combined exposure to 5 mg/m³ fDEE and 6.5% CO₂ caused a reduction in tidal volume 15.6%, a slightly greater effect than exposure to 5 mg/ml fDEE alone and opposite effect than exposure to CO₂ alone. We also measured levels of inflammatory cytokines in the lung homogenates of control and exposed rats one day following exposure. Increases in TNF-α, KC-GRO, IL-6, IL-5, IL-4, IL-1β, and IL-13 were detected in lung homogenates from rats after exposure to the combined fDEE and elevated CO₂. Increases in IL-1β, IL-5, and KC-GRO were also detected in lung homogenates from rats after exposure to elevated CO₂ alone. Exposure to fDEE alone induced significant increases only in IL-1β levels from lung homogenates. Neurobehavioral endpoints included motor activity and Morris water maze tests. Motor activity tests were conducted immediately following exposure. There were significant changes in two of the motor activity parameters induced by fDEE alone or in combination with CO₂. There was a reduction in the number of total rears in rats exposed to 10 mg/m³ fDEE alone. Combined exposure to fDEE and elevated CO₂ resulted in a trending reduction in total rears and statistically significant reduction in stereotypical beam breaks. To assess effects on cognitive function, rats were subjected to the memory component of Morris water maze test to evaluate spatial memory one day following exposure. Data revealed that exposure to fDEE or CO₂ alone or in combination did not induce detectable effects on memory parameters. Thus far, our data support additive or synergistic effects of combined exposure to fDEE with elevated CO₂ on respiratory and inflammatory endpoints.
4505 In Vitro Biomass Smoke Exposures Indicative of Residential Wildfires Induce Greater Changes in Expression of Genes in the Hypoxia Inducible Factor 1-alpha Pathway Compared with Forest Wildfires

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The danger to public health posed by wildfire smoke exposure continues to increase, with notable variability in health risks attributable to different burn conditions and fuel types. While we know of toxicologically relevant impacts induced by biomass smoke, it is steadily growing, there remains a paucity in research evaluating residential wildfire smoke samples occurring at the wildland-urban interface (WUI) in controlled in vitro settings. Wildfire particulate matter exposures have been known to cause respiratory illnesses and symptoms. These exposures can induce disease through multiple biological pathways. An important pathway is the hypoxia inducible factor 1-alpha (HIF1A) pathway, which plays essential roles in lung cellular function including hypoxia responses, tissue protection from damage, and inflammation. This study tested the hypothesis that wildfire-relevant exposure scenarios, including those relevant to WUI mixture scenarios, impact the expression of genes involved in HIF1A signaling to variable degrees. To address this hypothesis, this study exposed 16HBE human bronchial epithelial cells seeded on 3D transwell inserts to different biomass smoke samples. Forest-relevant exposures consisted of eucalyptus smoke selected as a representative biomass fuel known to induce lung toxicity and produced through two temperatures emulating flaming or smoldering burn conditions. WUI-relevant exposures were produced using the same biomass fuel but with the additions of plywood, plastic, and cardboard. The four conditions thus consisted of eucalyptus flaming (EF), eucalyptus smoldering (ES), residential mixture + eucalyptus flaming (RMF+EF), and residential mixture + eucalyptus smoldering (RMS+ES). Cells were exposed for four hours in triplicate and then collected. RNA was extracted and used to measure the expression levels of 70 genes involved in HIF1A signaling using a gene panel Fluidigm assay. A total of 61 significant genes were identified as significantly differentially expressed across at least one of the four conditions, with gene perturbations varying according to exposure condition. Specifically, the RMF+EF burn scenario altered the expression of 60% of the genes, while the RMS+ES burn scenario altered the expression of 8% of the genes based upon total gene counts with data passing QAC/QC filters. Genes that were significantly differentially expressed across multiple conditions included ADRA1B, CCK1NTA, EDN1, EIF4E, GPI, and IGF2, all representing important members of the HIF1A pathway. Overall, both flaming conditions altered the expression levels across several relevant genes in common, across species, and in a tissue type independent manner. These findings strongly stress the importance of evaluating WUI-relevant exposures in wildfire toxicity research, while imparting novel gene-level perturbation evidence for HIF1A pathway disruptions in the lung.

4506 Differential Transcriptionic Responses Underlie Site-Specific Toxicity in Developing Zebrafish Exposed to Whole Mixtures from Portland Harbor Superfund Site Passive Sampling Extracts

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The Portland Harbor Superfund Site (PHSS) is an area of active remediation along the Willamette River in Portland, Oregon. Previous studies paired low density polyethylene passive sampling devices (PSD) with standardized developmental toxicity assays to evaluate the capacity of PSD to characterize low-level toxicities at six sites along the PHSS and showed that polycyclic aromatic hydrocarbons (PAH) concentrations correlated with site toxicity. In this study, we couple the chemical characterization and dose response of extracts from two of these sites, river mile 7 west (RM7) and river mile 6 west (RM6.5), with RNA sequencing. The objective of this study was to assess the utility of this technique to characterize the hazard of environmental mixtures, compare the molecular responses between PSD extract exposures, and determine how the molecular response to extracts inform drivers of toxicity. For each treatment, three groups of 20 dechorionated zebrafish were batch exposed to 0.75% extract in embryo media. Treatment groups included RM7 extract, RM6.5 extract, and a vehicle control. At 48 hours post fertilization, the embryos were fixed and subjected to high-throughput RNA sequencing. We tested for differentially expressed genes (DEGs) and conducted Gene Ontology (GO) Enrichment analysis with gProfiler and Enrichmentmap. Additionally, we compared the extract transcriptomic responses to individual chemical exposures including three PAHs and three oxygenated PAHs (O-PAHS). RM6.5 was more toxic than RM7. PAH ratios within each extract were conserved, while RM6.5 had slightly higher total PAH concentration. Similarly, the directionality of gene expression changes was largely conserved between extracts, but RM6.5 induced more robust changes. Network analysis revealed enriched GO term clusters shared between the two extract exposures relating to xenobiotic metabolism, sensoric system dysregulation, and retinoid metabolism. There were no RM7 distinct GO terms, while RM6.5 distinctly included ion transport, negative regulation of cell morphology, and muscle fiber constituents. PAH and OPAH transcriptic signatures were strongly reflected in both extract treatments, indicating involvement of similar compounds driving mixture toxicity, but could not account for all disturbed biological processes. Coupled to environmental sampling and developmental toxicity testing, transcriptomics in zebrafish embryos exposed to PSD extracts was relatively easy, sensitive to differences in toxicity, and insightful as to mode of action. This highlights the usefulness of this tool for unbiased hazard characterization in complex environmentally relevant mixtures. This research was funded by NIEHS award numbers: P42ES016465, P30ES030287, and T32ES007060.

4507 Polychlorinated Biphenyl 126 Alters the Hepatic Transcriptome to Enhance Alcohol-Associated Liver Disease


Alcohol-associated liver disease (ALD) is initially characterized by hepatic lipid accumulation which can further progress with fibrosis and cirrhosis. ALD development can be impacted by lifestyle factors including hypercaloric diets and smoking. Previously, environmental pollutants, namely persistent organic pollutants (POPs), have been demonstrated to exacerbate dyslipidemia and influence hepatic lipid metabolism. The resulting phenotype we have observed suggests that exposures can compromise liver functionality that subsequently makes the liver susceptible to further insult. For instance, polychlorinated biphenyl (PCB) 126 is an environmentally relevant POP that has been demonstrated to enhance steatosis and liver injury in high-fat diet models. Because excessive alcohol consumption is a major cause of preventable death and humans are inevitably exposed to POPs, it is important to understand how these factors jointly influence ALD. The current study seeks to characterize the hepatic transcriptome in mice exposed to PCB126 followed by 5% ethanol feeding. We hypothesize that PCB126 exposure prior to ad libitum alcohol feeding will result in more differentially expressed genes (DEGs) and enriched pathways will implicate altered liver lipid metabolism and hepatic injury pathways. Male C57BL/6J mice were exposed to 0.2 mg/kg PCB126 via ad libitum diet for 5 weeks. Male mice were given free access to EtOH (EF) or pair fed (PF; 0% EtOH) diet for ten days followed by a 35.1% EtOH binge. Post euthanasia and tissue collection, hepatic mRNA was isolated and prepared for RNA sequencing (50M 1x100bp aligned reads/sample). DEG analysis was performed with Cuffdiff2 and DESeq2 for pairwise comparisons for p≤0.05 and q≤0.05. Hepatic transcriptic analyses indicated 5919 (2834; 3085) DEGs for EF vs PF animals and 449 (278; 171) DEGs for PCB126 exposed vs vehicle exposed. Importantly, the EF+PCB126 vs PF+PCB126 had 4832 (2214↑; 2618↓) DEGs while our PF+PCB126 vs PF+vehicle comparison had only 503 (339; 164) DEGs, suggesting that EtOH feeding in conjunction with PCB126 exposure alters a greater number of hepatic genes. Among the top 20 deregulated genes in our PCB126 exposed mice, common phase I & II xenobiotic metabolism genes are prevalent, while cell signaling- and 2nd messenger-related genes were downregulated. Among the top 20 upregulated genes in our EF mice, glutathione-related genes and ER stress-related genes were observed, whereas downregulated genes in EF mice consisted of lipid transporter genes and N-acetyltransferase-related genes. Downstream enrichment analyses from ‘Gene Ontology (GO) Biological Processes’ in the EF+vehicle vs PF+vehicle comparison indicated fatty acid metabolism and ncRNA metabolic processing as some of the most significant processes. ‘KEGG’ processes enriched for the EF+vehicle vs PF+vehicle comparison suggested cofactor biosynthesis and fatty acid metabolism to be some of the most significant alternative metabolic processes that were most significant in the EF+PCB126 vs EF+vehicle comparison indicated peptide-tyrosine modification and regulation and leukocyte migration to be some of the top pathways. KEGG pathway analyses of the EF+PCB126 vs EF+vehicle comparison indicated phagosome and cofactor biosynthesis were among the top enriched pathways. These preliminary findings highlight ALD and PCB induction of heightened transcriptome in cells, in part, disrupt cell signaling processes. This study is significant as it highlights environmental pollutant exposure’s ability to enhance lifestyle-related diseases.
inclusive of ALD. Based on our enriched pathway analyses, tyrosine modifications and cofactor biosynthesis may be promising alterations that are involved in PCB126's ability to modify EF mhc hepatic transcriptome.

4508 Activity of Binary and Complex Environmental PCB Mixtures at the Rydanoide Receptor

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Non-dioxin-like polychlorinated biphenyls (PCBs) have been shown to alter rydanoide receptor (RyR) activity and such disruption is linked to developmental neurotoxicity. Of the 209 PCB congeners, 49 non-dioxin-like (NDL) PCB congeners have been shown to alter RyR activity. PCBs are present in environmental or human samples as mixtures; however, how these mixtures, rather than single PCBs, contribute to altered activity is unknown. The focus of this study is to examine whether PCBs interact in an additive, antagonistic, or synergistic manner at the RyR using the Concentration Addition model (CA model). We conducted radioligand binding assays exposing junctional sarcoplasmic reticulum preparations to single, binary and complex mixtures of PCBs. The activity of binary mixtures or complex mixtures were then compared to expected response curves, or effective concentration (EC) values, calculated using the CA model. We show that binary mixtures of RyR activity NDL PCBs display additivity at the RyR1 and that congeners that do not activate the RyR do not interfere with the activity of active congeners. The RyR activity of complex mixtures varied significantly from that expected when applying the CA model. While the CA model did not adequately describe activity of the complex mixtures, reported concentrations of PCB mixtures found in environmental or serum-based mixtures did cause RyR1 activity. This work helps to further understand the impact of neurotoxic PCBs that dominate the Total PCBs found in environmental mixtures and supports the need for further model development to better describe the neurotoxic hazards presented by ongoing exposure or presentation in serum.

4509 Adverse Effects following Time-Course and Dose-Response Oral Exposures in Sprague Dawley Rats to Complex Groundwater Mixture

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Groundwater containing a complex mixture of contaminants was collected from an industrial site for the identification of target organ toxicity. An initial time-course study involved an oral exposure to a single concentration (0.05% v/v) of groundwater compared to control (after 7, 14, 28, and 60-day exposure) in male Sprague Dawley rats (n=10/group). The second study involved a 60-day oral exposure to drinking water (control group), 10% v/v low impact water from an alternate well in samples as mixtures; however, how these mixtures, rather than single PCBs, contribute to altered activity is unknown. The focus of this study is to examine whether PCBs interact in an additive, antagonistic, or synergistic manner at the RyR using the Concentration Addition model (CA model). We conducted radioligand binding assays exposing junctional sarcoplasmic reticulum preparations to single, binary and complex mixtures of PCBs. The activity of binary mixtures or complex mixtures were then compared to expected response curves, or effective concentration (EC) values, calculated using the CA model. We show that binary mixtures of RyR activity NDL PCBs display additivity at the RyR1 and that congeners that do not activate the RyR do not interfere with the activity of active congeners. The RyR activity of complex mixtures varied significantly from that expected when applying the CA model. While the CA model did not adequately describe activity of the complex mixtures, reported concentrations of PCB mixtures found in environmental or serum-based mixtures did cause RyR1 activity. This work helps to further understand the impact of neurotoxic PCBs that dominate the Total PCBs found in environmental mixtures and supports the need for further model development to better describe the neurotoxic hazards presented by ongoing exposure or presentation in serum.

BRAF mutation levels between 0.29% to 21% were recruited as cases (n=25) and dogs that had no detectable BRAF mutation (with limit of detection <0.1%) and that were breed, sex, and age matched to cases were enrolled as controls (n=76). Dogs wore a silicone passive sampler attached to their collars for five consecutive days. The silicone samplers were extracted and analyzed in the laboratory using both targeted and non-targeted GC-MS approaches. Targeted analyses focused on a suite of 115 chemicals including phthalates, organophosphate esters, brominated flame retardants, polycyclic aromatic hydrocarbons, and pesticides. Of the 115 compounds targeted, 39 were detected in greater than 50% of all samples (n=101). Higher levels (2.3X) of BDE-47, BDE-99, anthracene, and benzyl butyl phthalate were measured in silicone samplers worn by cases compared to controls (p<0.05). BDE-28 and Tris(1,3-dichloroisopropyl)phosphate (TDCPP) were also higher (1.5X) in samplers worn by cases, however this difference was not statistically significant (p=0.09). Measurements of exposure to azo dyes are underway. For suspect screening, data were processed using the deconvolution plugin in the Tracefinder software and screened against three mass spectral libraries (NIST 2017, Thermo Hi-Res Library, In-house library). Using this approach, we identified 1,407 chemical features on silicones worn by cases, of which 12 were statistically higher in cases (p<0.01). Furthermore, the trends observed in these data suggest that there is a higher burden of environmental chemical exposures experienced by our cases, both in the number of chemicals detected and the magnitude of exposures. This study is the largest study to date to investigate such a wide breadth of contaminant exposure levels associated with canine BC and is the first to assess a population with subclinical disease. This study is part of a larger project that will include whole exome sequencing of serially sampled cases as a means to identify signatures of disease progression and facilitate investigation of gene-environment interactions. These data will allow for advances in translational medicine that may one day improve the clinical outcomes for human and canine patients with BC or aid in cancer prevention.

4511 Environmental Mixtures and Breast Cancer: Identifying Co-exposure Patterns between Understudied and Breast Cancer–Associated Chemicals Using Chemical Inventory Informatics

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Although evidence linking environmental chemicals to breast cancer is growing, mixtures-based exposure evaluations are lacking. This study aimed to identify environment chemicals in the inventories that co-occur and share properties with chemicals that have an association with breast cancer, highlighting exposure combinations that may alter disease risk. The occurrence of chemicals within chemical use categories was characterized using the Chemical and Products Database. Co-exposure patterns were evaluated for chemicals that have an association with breast cancer (BC), no known association (NBC), and understudied chemicals (UC) identified through query of the Silent Spring Institute’s Mammary Carcinogens Review Database and the US Environmental Protection Agency’s Toxicity Reference Database. UCs were ranked based on structure and physico-chemical similarities and co-occurrence patterns with BCs within environmentally relevant exposure sources. A total of 6,793 chemicals had data available for exposure source occurrence analyses. 50 top-ranking UCs spanning five clusters of co-occurring chemicals were prioritized, based on shared properties with co-occurring BCs, including chemicals used in food production and consumer/personal care products, as well as potential endocrine system modulators. Results highlight important co-exposure conditions that are likely prevalent within our everyday environments that warrant further evaluation for possible breast cancer risk.

4512 Determination of Variability in Monoterpene Levels in Pine Bark Extract Dietary Supplements Using Gas Chromatography-High Resolution Mass Spectrometry

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Pine bark extract (PBE) dietary supplements are widely used for purported antioxidant, anti-inflammatory, and anti-inflammatory properties. α-Pinene, which has been shown to be a multi-site toxicant in rodents following inhalation exposure, makes up approximately 60% of pine bark essential oil. There are no data available on α-Pinene concentrations in commercial PBE dietary supplements. The objective of this work was to determine the α-pine concentration, along with other monoterpenses, β-pinene, 3-carene, and Δ-limonene, in 23 commercially available PBE dietary supplements and compare to a U.S. Pharmacopeia (USP) PBE standard. Supplements were extracted with ethyl acetate containing the internal standard (isopropylphenol) and subjected to gas chromatography-high resolution mass spectrometry. Quantification of analytes was achieved using solvent calibration curves prepared similarly. A subset of samples was also quantified using the standard addition method to demonstrate the accuracy of using a solvent calibration curve for quantification of the analytes. The 3-carene standard used for
quantification had a ≤ 0.05 min retention time shift from the carene isomer detected in PBE dietary supplements suggesting that the isomer present in the supplement may be different from 3-carene. Monoterpenes had varied detection frequency in 23 PBE dietary supplements investigated. D-limonene was detected in all supplements while α-pinene, 3-carene and β-pinene were detected in 22, 18 and 15 supplements, respectively. Analyte concentrations varied widely within and between supplements (detection concentration varied from 50 µg/g supplement to ≤ 0.1 µg/g supplement; mean concentration of ng/g supplement 3980, 624, 46.0 and 99.0, respectively). Monoterpane concentrations normalized to PBE content per label claim (ng/g PBE) in some manufacturer also varied substantially with observed ranges of 1980–5050, 346–690, 29.9–116.6 and 82.2–389 for D-limonene, 3-carene, β-pinene, and α-pinene, respectively. These data demonstrate the variability in monoterpane concentrations in PBE products on the market including those from the same manufacturer.

4513 A Potential Screening Strategy for Neuroactive Potential of Botanicals
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Botanical product use is widespread and growing in efforts to preserve and enhance human health and well-being. However, safety evaluations of botanicals are often inadequate. Because people are intentionally taking these products for their potential benefits, an urgent need exists to define appropriate toxicity evaluations to support safe use of botanicals. A cross-sector collaboration is addressing this need through an international group of scientific experts to develop a comprehensive toolkit for generating reliable toxicological profiles of these complex mixtures. This effort includes finding assays to screen for neuroactive potential (as an indication of neurotoxicity). A battery of assays and models already exist to control) and a remarkable increase in sub-threshold network bursts. Zebrafish can also be multiplexed with cell viability to aid in prioritization of botanicals for neuroactive botanicals were oleander and kava, inducing hyperactive swimming activity in zebrafish. Dendritic spines, spine density (DS), spine head width (DH), spine neck width (DN), and spine neck length (DSN) were quantified. Dechorionated embryonic zebrafish were continuously exposed to 16 botanicals and 81% induced morphological defects. The most negative, demonstrating a lack of concern for genotoxicity. For other endpoints, transgenic rodent assays (OECD 488) were conducted for each SFPP; results were negative, demonstrating a lack of concern for genotoxicity. For other endpoints, previously conducted 90-day studies (OECD 408) were available for each SFPP; the no observed adverse effect levels (NOAELs) from these studies ranged from 785 mg/kg-bw/day to 1,397 mg/kg-bw/day. For the constituent-based assessment, a robust analytical characterization was performed resulting in the identification and quantification of over 95% of the volatile fraction of the smoke flavors; SF-002 and SF-006 had 48 constituents and SF-005 had 49 constituents. Systematic literature searching was conducted for each constituent following a tiered approach, focusing on EFSAs evaluations, other European or global authoritative bodies, genotoxicity databases and primary literature to identify any available genotoxicity data. For constituents that had available testing data, a hazard-based assessment was performed (SF-002=34, SF-005=39, SF-006=38). If no data were identified (SF-002=14, SF-005=10, SF-006=10) an in silico assessment was performed using read-across and/or QSAR modeling. Of the identified constituents, none were determined to pose a genotoxic risk under the conditions of intended use when considered together in a risk-based analysis with the whole mixture data. The exposure assessment involved a combination of consumption data from the European Union Comprehensive Food Consumption Database as well as use levels of the flavors for a variety of different foods. After utilizing EFSAs intake models (i.e., FAIM, DietEx), it was determined that FAIM is not as reliable as DietEx due to overly broad food categories that greatly overestimate exposure. Therefore, the most reliable exposure estimate, derived by DietEx, was 1.2 mg/kg-bw/day, 0.98 mg/kg-bw/day, and 0.96 mg/kg-bw/day for SF-002, SF-005, and SF-006, respectively. MOS estimates were calculated using the NOAEL values from the 90-day studies and exposure estimates from DietEx. MOS values of 640 for SF-002, 1400 for SF-005 and 1400 for SF-006. Based on their MOS these smoke flavoring primary products are of no safety concern at their proposed use levels, consistent with EFSAs MOS conclusion. Therefore, the data presented supports that the overall safety for these SFPPs continues to be demonstrated. This risk assessment also helps to inform risk-benefit considerations, particularly when comparing the robust safety profile of these SFPPs relative to lack of traditional safety data for conventional smoke.

4515 Valorization of Corncob Agricultural Waste Material for the Removal of Methylene Blue Dye from Aqueous Solution
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In recent years, the treatment of industrial wastewater contaminated with dye is of great importance following the need for the water to be reused. Corncob, an agricultural by-product and a kind of solid waste, is frequently disposed of carelessly, resulting in an environmental burden. Corncob was used in this study as an adsorbent in a batch adsorption technique to remove methylene blue (MB) dye from aqueous solutions. This technique was used to assess the effects of pH, adsorbent dosage, adsorbent concentration, contact duration and temperature on dye removal. The result obtained from the time and concentration variations was used to deduce the kinetic and isotherm study. The dye adsorption investigation found that the removal effectiveness of dye employing corncob was substantial at pH 7 with removal percentage and adsorption capacity as 88.8% and 2.142 mg/g respectively, adsorbent dosage was optimum at 0.1 g with 98.79% and 15.35 mg/g, contact duration was optimum at 1 hour with 97.70% and 18.75 mg/g. Experimental data showed a good fit with the Temkin isotherm models. The pseudo second order kinetic model delivered the highest R² values in the kinetic parameters including the Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) changes indicated that the adsorption of MB was feasible, spontaneous and endothermic in the temperature range of 25°C - 45 °C. The results explored that the low-cost, corncob biosorbent could be used for the effective removal of Methylene blue dye from aqueous solution.
Microplastics (MPs) are plastic fragments less than 5 mm in diameter that have been implicated in intestinal toxicity, altered metabolites, and other adverse health outcomes following exposure. In recent years, MPs have increasingly been found in soils where there is an interaction with such species as Salmonella enterica Typhimurium. Microorganisms like Salmonella can colonize MPs to affect the surrounding environment. Current research has not focused on microbe-MPs co-exposures. This study aimed to elucidate the interaction of polyethylene MPs and S. Typhimurium and how this co-exposure alters the cecal microbiome of exposed birds. We focused on polyethylene MPs which is one of the most abundant plastics found in the environment and in food packaging materials. We assessed two common forms of polyethylene: powder and fiber.

For this study, we utilized chicken cecal mesoconcs (n=60) for a 24 h co-exposure. 16S community sequencing results indicate that polyethylene fiber treatment groups with and without S. Typhimurium had greater changes to cecal taxonomic composition following a 24-hour exposure. However, changes in the total metab- lome were primarily driven by the presence of S. Typhimurium. Further experiments will be needed with this in vitro cecal microbiome model to indicate if and how a high concentration of polyethylene microplastics and S. Typhimurium will lead to phenotypes for gastrointestinal disorders. This will give insight into human health implications from consumption of or interaction with chicken contaminated with this co-exposure.

Pesticidal active ingredients are one of the most heavily regulated and toxicologically evaluated types of molecules on the market. Pesticides must be evaluated for mammalian acute to chronic toxicity, carcinogenicity, and reproductive toxicity across rodent and non-rodent species as a surrogate for humans and wild mammals. Additionally, non-mammalian vertebrate organisms (fish, amphibians, and birds), are evaluated for toxicity to support environmental safety assessment. Likewise, genetically modified crops may contain proteins which increase yield or have other attributes but originate from other organisms. These proteins must also be evaluated for potential effects on mammalian vertebrate species. Traditionally, these toxicological evaluations of mammalian and non-target species for the purposes of human health and environmental assessment are performed in vivo. Consequently, the evaluation of new pesticidal active ingredients and new transgenic events are very animal intensive. An estimated 8-9K animals are used in the toxicity evaluation for human health risk assessment is expected to one new pesticidal active ingredient. Further, while the agrochemical sector has embraced new approach methodologies (NAMs) to reduce animal testing, registrants are simultaneously faced with updated test guidelines and new data requirements can further increase the animal testing burden. At Corteva Agriscience, we are commit- ted to tracking our animal use for the purposes of identifying opportunities to replace, reduce, and refine animal testing wherever possible. This poster provides an overview to the approach Corteva Agriscience has used for 1) determining the average number of animals for use in a typical data package and 2) benchmarking our animal use for the testing programs for our five most recent active ingredi- ents and four recent genomics (GMO Crops). Finally, this poster provides our prospective and iterative approach to the evaluation of animal reduction with the growing inclusion of NAMs as integral to regulatory submissions in the future.
The use of zebrafish embryos provides a valuable platform for the prioritization and design for novel compound development.

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Mancozeb, an ethylenebisdithiocarbamate, is a well-known broad-spectrum contact fungicide. In the United States, an estimated 7-8 million pounds of Mancozeb active ingredient is applied on agricultural lands every year. Mancozeb is metabolized to ethylene thiourea (ETU), one of the major metabolites excreted in rat and human urine. ETU concentration has been considered as a well-estab- lished biomarker of Mancozeb occupational, dietary, or environmental exposure in humans. Mancozeb is a dithiocarbamate, and dithiocarbamates have been known to form organometallic complexes by selectively and strongly binding to most transition heavy metals including cadmium, copper, manganese, nickel, etc. In consideration of this, the aim of this study was to evaluate whether Mancozeb exposure causes mobilization and bioaccumulation of essential trace metals, such as copper, iron, manganese, and zinc, in the kidneys of male and female rats. Age-matched male and female Long-Evans rats were exposed to 0, 50, or 100 mg/kg/day of Mancozeb for 28 days via oral gavage. At the end of the exposure, renal cortex was harvested at necropsy and quantified for essential metals using inductively coupled plasma optical emission spectroscopy (ICP-OES). Additionally, formalin-fixed paraffin embedded (FFPE) whole kidneys from the high-dose group (100 mg/kg/day) were processed for renal histological staining of copper deposits and copper-associated proteins as well as immunohistochem- istry. Navally, the effect of Mancozeb on the kidney cortex (4-HNE), kidney (4-methyl-1-(KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL). A dose-dependent significant increase of 469% and 630% was observed in the copper level in the renal cortex of rats treated with 50 and 100 mg/kg/day of Mancozeb, respectively, relative to vehicle control. In female rats, a dose-dependent trend was observed with a significant accumulation (625%) of copper in the 100 mg/kg/day Mancozeb dose group relative to vehicle control. Furthermore, the zinc concentration was significantly reduced (25%) in the renal cortex of both low- and high-dose Mancozeb-treated male rats; whereas, there were no significant differences in zinc levels observed in females or iron and manganese levels in either males or females. In the 100 mg/kg/ day Mancozeb-treated group, the accumulation of copper deposits demonstrated ubiquitous bioaccumulation of brick-red copper granules within the cytoplasm of the proximal and distal convoluted tubules in both males and females, while there was no visual deposition of copper granules within the glomeruli. In addition, a dramatic increase was observed in the cytoplasmic oenin-positive copper-assoc- iated protein level in the renal proximal and convoluted tubules of the kidneys in both males and females. Immunohistochemistry revealed that copper accumu- lation was accompanied by oxidative damage as indicated by increased 4-HNE adduct detection in the brush border of the proximal tubules and cytoplasm of the distal convoluted tubules in both males and females when compared to vehicle controls. These results suggest that exposure to Mancozeb causes copper accumulation in the kidney, leading to potential renal injury via oxidative stress. Further investigation is warranted to elucidate the mechanism of potential renal toxicity of Mancozeb.

Ziram (ZM) and sodium dimethyldithiocarbamate (Na-DMDTC) are fungicides of the dithiocarbamate (DTC) subclass of dithiocarbamate (DTC) pesticides. Both compounds are complexed to a metal, with ZM complexed to zinc (Zn) and Na-DMDTC complexed to sodium. The widespread use of these fungicides is attributed to their good foliar protection, short environmental persistence and presumed low acute toxicity in humans. However, concerns have been raised regarding their neurotoxicity because dithiocarbamates are a group of highly reactive compounds and there has been evidence of numerous toxic effects. Previous work has suggested that the mechanism of action of these compounds involves the production of oxidative stress, metal-induced toxicity, and toxicity attributed to the organic backbone. In the present study, Long-Evans rats were treated orally with 50% (v/v) PEG 400, 100 µM, and 200 µM ZM and Na-DMDTC for 28 days. A dose-dependent increase in superoxide dismutase 2 protein levels was observed in cells treated with 100 µM ZM and 200 µM Na-DMDTC. Both parameters were significantly increased after cells were treated with 100 µM ZM and 140 µM Na-DMDTC for 24 hours. Superoxide dismutase 2 protein levels were significantly increased in cells treated with 100 µM ZM and 140 µM Na-DMDTC for 24 hours. Furthermore, treatment with 100 µM ZM and 140 µM Na-DMDTC for 24 hours resulted in significant decreases in total antioxidant capacity. Current data indicates that oxidative stress contributes to Mancozeb induced mitochondrial toxicity in HT-29 cells.
toxicity profile of mancozeb, this study used a quantitative high throughput protein array with 167 antibodies to reveal new protein phosphorylation sites in PC12 cells treated with various concentrations of mancozeb for 24 hours. The proteomic results showed some protein phosphorylation status was upregulated, such as Dab1 (70% increase), CaMK2 (60% increase), Gab2 (52% increase), PP2A alpha (62% increase), Presenilin 1/2 (60% increase), Synapsin 1 (42% increase), and TrkB (78% increase) in PC12 cells treated with mancozeb (50 µM) for 24 hours. Some protein phosphorylation status was downregulated, such as NMDA Rl (40% decrease). Interestingly, around 90% of the tested protein expressions were increased after 24 hours of 50 µM mancozeb incubation, such as Atx1 (46% increase), NOS2 (62% increase), Parkin 1 (87% increase), Synapsin 1 (92% increase), and alpha synuclein (42% increase). This study provides a clearer picture of mancozeb effect on PC12 cells. The validation experiment will be conducted to confirm the changes triggered by mancozeb in PC12 cells.

Chlorpyrifos is an organophosphate pesticide that has been widely used for decades for both agricultural and household applications; thus, there is potential for human exposure. The Division of Translational Toxicology is conducting short-term toxicity studies to evaluate cardioxicity following oral exposure of Hsd/Sprague Dawley®/SD® (HSD) rats to chlorpyrifos and chlorpyrifos monoethyl. The goal of the study is to integral to putting toxicological findings into context. However, there are limited efficient and sensitive methods in the literature to quanitiate chlorpyrifos. The objective of this work was to develop and validate a simple LC-MS/MS method to quantitate chlorpyrifos in rat plasma and tissues. Matrix calibration standards were prepared by spiking 10 µL of rat plasma with 10 µL of chlorpyrifos in methanol and 10 µL of internal standard solution (chlorpyrifos-d10). Samples were extracted by protein precipitation with 400 µL of acetonitrile and analyzed by LC-MS/MS in positive ion mode. The method was successfully validated in male Sprague Dawley rat plasma over the concentration range 5 to 1000 ng/mL. Calibration curves were linear (r ≥ 0.99), and accuracy, determined as percent relative error (%RE), was ≤ ±9.9% for standards at all levels. The limit of detection was 1.14 ng/mL. Recovery was ≥ 88% at all concentration levels. Intra- and inter-day precision, determined as % relative standard deviation (RSD), was ≤ 6.4% and %RE was ≤ ±5.3% for quality control standards prepared at 10, 50, and 500 ng/mL. The method was evaluated for HSD rat plasma and heart homogenate (ratio of 1:2 heart:water); %RE values were ≤ ±8.7% and %RSD ≤ 8.5%. Analyte stability in extracted plasma was demonstrated for 4 days at ambient and refrigerated temperatures, as well as in plasma and heart homogenate stored at -80°C for up to 60 days (84.3-96.5% of day 0 concentrations). These data demonstrate that the method is suitable for the quantitation of chlorpyrifos in rat matrices. The method can be easily adapted to other species and matrices.

Neonicotinoids are heavily used in agriculture and to combat household pests, which has led to widespread environmental contamination and human exposure. Modeled after nicotine, they have a high affinity for invertebrate nicotinic acetylcholine receptors (nAChRs) and a comparatively low affinity for mammalian ones, and thus are marketed as safe for human and pet exposure. In mammals they cause oxidative stress in numerous tissues, and can still partially activate mammalian nAChRs. Further, relatively little is known about their behavioral effects, particularly those mediated by nAChRs. Activation of nAChRs influences behavioral and cognitive processes, including learning and memory, anxiety, locomotion, and attention, all of which are disrupted by nicotine. This study examined behavioral effects of imidacloprid (IMI) exposure, with the hypothesis that it affects cognitive behaviors. Adult male and female CD1 mice were orally exposed for 28 days to either 0.5 mg IMI/kg body weight/day, 5.7 mg IMI/kg body weight/day, or the vehicle (either 33% DMSO or corn oil with 2% DMSO). During the dosing period mice were tested daily on two individual tasks: rewarded alternation on a T-maze (procedural memory task) and spontaneous alternation on a Y-maze (working memory task). Following 28 days of dosing the hippocampus was collected and analyzed for the expression of the α4β2 nAChR, which strongly influences learning and memory. For both sexes, both IMI dosages impaired performance on the T-maze but not at later time points, and no significant differences were observed on the Y-maze. Additionally, α4β2 was reduced in male IMI groups compared to controls, which likely reduces a neuron’s responsiveness to IMI and, as an unintended consequence, its responsiveness to endogenous ligands. Thus, IMI exposure acutely impacts performance.
P5 4530 Pulmonary Toxicity of Neonicotinoid Pesticides


Pesticides containing neonicotinoids (NNs) are widely used in both agricultural and residential settings. NNs are nicotine analogs developed as potent pesticides based on their higher binding affinity to nicotinic acetylcholine receptors (nAChRs) in insects over mammals rendering them safer to humans compared to organophosphates. However, emerging data is challenging the perceived safety of NN due to increased human disorders and ecological disasters. Based on the epidemiologic link between the increased burden of respiratory disease and exposure to NNs in US agriculture workers (independent of other known risk factors such as smoking), we chose to investigate the pulmonary toxicity of these compounds. Here, we tested the impact of various NNs on the physiologic properties of airway epithelial cells, including barrier function, epithelial anion transport, and mucociliary clearance, the primary defense of lungs against inhaled irritants and pathogens. Based on the physiologic significance of these protective airway functions, as observed in patients suffering from various respiratory illnesses such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and pneumonia, we specifically tested the impact of NN exposure in laboratory models. Primary human bronchial epithelial (HBE) cells expressing wild-type CFTR were grown at an air-liquid interface to obtain fully differentiated monolayers. These cells were exposed to common NNs such as acetamiprid, clothianidin, dinofeturan, imidaclopid, and thiamethoxam at the reported blood concentrations of 100nM to 1000nM in basolateral media. After exposure to NNs for 24 hours, no apparent difference in transepithelial electrical resistance (TEER), an indicator of epithelial barrier integrity, was observed, suggesting no acute effects on cell viability. However, all five NNs at each concentration significantly decreased CFTR-mediated ion transport function by 20–60% of vehicle-exposed controls, possibly due to increased mucus viscosity by 57%, suggesting negative impacts on mucociliary defense. Overall, our data demonstrate that NNs impair physiologic functions of airway epithelium and provide new insights into the increased respiratory disease among agricultural workers exposed to NNs. These findings support further evaluation of pulmonary toxicity of NNs in animal models to accurately quantify the risk to human health and inform improved regulation of these pesticides.

P5 4531 Neural Exposome Converges on Aging Signaling to Drive Post-acute Neurologic and Cognitive Sequelae of COVID-19

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The dramatic heterogeneity of the clinical outcomes of COVID-19, including highly prevalent long-lasting neurocognitive and neurodegenerative deficits in post-acute sequelae of COVID-19 (PASC or long-COVID), highlights the dire need to understand the pathogenic role of noninheritable environmental factors or the neural exposome in mediating virus and host interaction to initiate and propagate chronic neurodegenerative conditions. Our work and others have provided mechanistic insights to link environmental toxicant exposure to long-lasting neurologic sequelae of virus infection. We have recently identified a strong association of the accumulative pulmonary toxicity of NNs in animal models to accurately quantify the risk to human health and inform improved regulation of these pesticides. In this study, the TH metabolism kinetics of six reference compounds were calculated and compared among two species (rat and human) to differentiate rodent-specific and human-relevant mode of action. A tricuretum system, consisting of primary hepatocytes (human and Sprague Dawley rat), endothelial cells and stromal cells, was used to evaluate mediated perturbations in TH (T3, T4) metabolism (T4G, T4S) and species sensitivity. The model’s physiological functionality was assessed using urea, albumin, and cytokine induction over 10 days of culture. Disruption of T4 metabolism in the tricuretum system was assessed using reference compounds (Phenobarbital (PB), Rifampicin (RIF), Pregnenolone 16α-carbolin (PNC) and Potassium 18-β-D-glucoside (PSB153)), at non-toxic concentrations, in two species (Sprague Dawley rat and human). After 7 days doses of reference compounds, cultures were exposed to a physiologically relevant level of thyroxine (0.05 μM rat, 0.1 μM human), incubated for 0, 4, 8, 12 and 24 hours, and the rate of T4 clearance and metabolite concentration (T4G, T4S, T3) were analyzed in media using LC-MS/MS. The rate of T4 depletion was used to compare compound intrinsic clearance in different species. Furthermore, the in vitro clearance data were used to extrapolate in vivo hepatic T4 qualitative metabolism. Results showed that the model exhibits stable urea, albumin, cytokine and cytokine induction over 10 days of culture. The level of T4 glucuronidation (T4G) peaked at 24 hours in both rat and human culture medium, while the level of T4 sulphate (T4S) was three times more than in human. The level of T3 in human was 2-fold greater than rat. T4 sulphation (T4S) was not identified in either medium. The rate of T4 production increased in rodent tricuretums when treated with PCB153 and PCN, which reciprocated with increased T4 clearance over 24 hours. Intrinsic clearance (CLint) of T4 in the rat model after PCB153 and PCN treatment was calculated at 4.2 and 1.9 μL/min/100mg of liver respectively, and in vitro values of 20.2 and 9.1 mL/min/kg, respectively. In comparison, human tricuretums only showed an increase in T4 with PCB153, but T4 clearance exaggeration was seen with both PCB153 and RIF over 24-12 hours. The CLint in vivo after PCB153 and RIF was calculated at 2.3 and 3.4 μL/min/gm, respectively. The selective TH metabolism response with PCN in rodent and not human, and the RIF selective response in human and not rodent, suggest species-specific responses. In conclusion, the mechanism of TH perturbations in response to CAR/PXR activators was studied in primary human and rat in vitro tricuretum systems. The in vitro system showed measurable TH metabolism responses with some relevant species differences. Further evaluation of intercellular T4G, T4S and deiodinase T4 will be useful for a comprehensive TH metabolism pathways assessment. Future studies will incorporate multiple donor groups of validated species (rat, mice, human) to allow better estimation of TH kinetic responses in larger populations, including male to female variances.

P5 4532 In Vitro Hepatic Triciture System to Evaluate Human Relevance of Chemical-Induced Thyroid Toxicity

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Many chemicals that induce liver metabolizing enzyme and transporter activity cause alterations in thyroid hormone (TH) metabolism. Chronic alterations to TH homeostasis may lead to follicular adenomas and carcinomas. The effects of chemical insult (e.g., CAR/PXR activators), there are two major TH metabolism pathways in the liver (i.e., deiodination and conjugation (sulfation and glucuronidation)), but the response type and rate may differ among species. Advancements in alternative methods have provided more species and population-relevant physiologic in vitro systems to better evaluate hepatic TH metabolism. In this study, the TH metabolism kinetics of six reference compounds were calculated and compared among two species (rat and human) to differentiate rodent-specific and human-relevant mode of action. A tricuretum system, consisting of primary hepatocytes (human and Sprague Dawley rat), endothelial cells and stromal cells, was used to evaluate mediated perturbations in TH (T3, T4) metabolism (T4G, T4S) and species sensitivity. The model’s physiological functionality was assessed using urea, albumin, and cytokine induction over 10 days of culture. Disruption of T4 metabolism in the tricuretum system was assessed using reference compounds (Phenobarbital (PB), Rifampicin (RIF), Pregnenolone 16α-carbolin (PNC) and Potassium 18-β-D-glucoside (PSB153)), at non-toxic concentrations, in two species (Sprague Dawley rat and human). After 7 days doses of reference compounds, cultures were exposed to a physiologically relevant level of thyroxine (0.05 μM rat, 0.1 μM human), incubated for 0, 4, 8, 12 and 24 hours, and the rate of T4 clearance and metabolite concentration (T4G, T4S, T3) were analyzed in media using LC-MS/MS. The rate of T4 depletion was used to compare compound intrinsic clearance in different species. Furthermore, the in vitro clearance data were used to extrapolate in vivo hepatic T4 qualitative metabolism. Results showed that the model exhibits stable urea, albumin, cytokine and cytokine induction over 10 days of culture. The level of T4 glucuronidation (T4G) peaked at 24 hours in both rat and human culture medium, while the level of T4 sulphate (T4S) was three times more than in human. The level of T3 in human was 2-fold greater than rat. T4 sulphation (T4S) was not identified in either medium. The rate of T4 production increased in rodent tricuretums when treated with PCB153 and PCN, which reciprocated with increased T4 clearance over 24 hours. Intrinsic clearance (CLint) of T4 in the rat model after PCB153 and PCN treatment was calculated at 4.2 and 1.9 μL/min/100mg of liver respectively, and in vitro values of 20.2 and 9.1 mL/min/kg, respectively. In comparison, human tricuretums only showed an increase in T4 with PCB153, but T4 clearance exaggeration was seen with both PCB153 and RIF over 24-12 hours. The CLint in vivo after PCB153 and RIF was calculated at 2.3 and 3.4 μL/min/gm, respectively. The selective TH metabolism response with PCN in rodent and not human, and the RIF selective response in human and not rodent, suggest species-specific responses. In conclusion, the mechanism of TH perturbations in response to CAR/PXR activators was studied in primary human and rat in vitro tricuretum systems. The in vitro system showed measurable TH metabolism responses with some relevant species differences. Further evaluation of intercellular T4G, T4S and deiodinase T4 will be useful for a comprehensive TH metabolism pathways assessment. Future studies will incorporate multiple donor groups of validated species (rat, mice, human) to allow better estimation of TH kinetic responses in larger populations, including male to female variances.
and x culture media revealed significantly higher secreted levels of Aβ-40 and Aβ-42 in increased action potential frequency with unique burst-like responses. Analysis of and DDE in mouse primary neurons and human induced neurons (hiN) resulted in DDT and DDE dock within the channel pore. Acute and chronic exposures of DDT and DDE significantly higher serum levels of dichlorodiphenyldichloroethylene (DDE), world; however, little is known of its etiology. We have shown that AD patients framework can also be applied to other routes of pesticide exposure. The combination of toxicological and demographic members in township sections with a greater volume of pesticides applied and toxicity and carcinogenicity. Analysis of U.S. Census data for Ventura County found many also exhibited thyroid toxicity as well as developmental and reproductive index typically had evidence of neurotoxicity at low doses in animal studies and 39 pesticides were associated with mixtures of pesticides experienced in real-world scenarios. This study developed a comprehensive framework to assess toxicity-weighted pesticide use by incorporating data on multiple health effects, including harms to specific organ systems, carcinogenicity, impacts on the developing fetus. The framework was applied to a case study of pesticide use in Ventura County, California (USA). Pesticide data for years 2016-2018 was combined with sociodemographic data to assess potential disparities in pesticide exposure and potential health risks. First, a cumulative toxicity index was calculated incorporating multiple toxicity endpoints for individual pesticides, the severity and strength of association for each endpoint, and the reliability of the data sources used. Next, combining the toxicity index for each pesticide with the pounds applied within each square mile section in Ventura County, the total toxicity was weighted and identified pesticides associated with higher potential risk to health. Overall, 60 pesticides were linked to cancer, 78 pesticides were associated with neurotoxicity, 44 pesticides were associated with thyroid toxicity, 74 pesticides were associated with potential endocrine disrupting effects, 85 pesticides were associated with developmental and reproductive toxicity, and 39 pesticides were associated with respiratory toxicity. The ten pesticides with the highest cumulative toxicity index typically had evidence of neurotoxicity at low doses in animals and study and many also exhibited thyroid toxicity as well as developmental and reproductive toxicity and carcinogenicity. Analysis of U.S. Census data for Ventura County found a greater prevalence of thyroid cancer/Latino (IL-TLS) (OR = 2.6 fold); and b) expression of inflammatory mediators (NF-κB, STAT3 and p38 MAPK- response between 1.5-4.0 fold); autophagy-related proteins (ATG12, ATG16, Beclin1 and p47phox; and gp91 phox - 1.5-4.0 fold); and antioxidants (MnSOD, Catalase & Gpx-2.0-4.0 fold), in PCP-challenged AS49 and HepG2 cells. Interestingly, pretreatment with N-acetyl cysteine (NAC, 200 mM) inhibited PCP-induced responses. Additionally, an increase in the interaction of Hsp70 with Beclin1 (>3.0 times) was observed during PCP exposure in our study models. In brief, our experiments reveal that increase in Hsp70 RESTO-modulated autopagy by directly interacting with Beclin1 in PCP-challenged lung and liver epithelial cells. Further studies are in progress to understand the specific role of posttranslational modifications in Hsp70-mediated responses in our study models. Alzheimer’s disease (AD) is the most common neurodegenerative disease in the world; however, little is known of its etiology. We have shown that AD patients had significantly higher serum levels of dichlorodiphenyldichloroethylene (DDE), the primary metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT). Although there has been a steady decline in environmental DDE levels, the persistent metabolite DDE is still ubiquitously detectable, but DDE has long been considered non-neurotoxic. Molecular docking simulations with the cryo-EM structure of the human voltage-gated sodium channel Na,1 indicated that both DDT and DDE dock within the channel pore to mimic intestinal absorption. We found that increased expression of heat shock protein (Hsp) 70 and oxidative stress thereby resulting in altered protein homeostasis. Earlier studies also demonstrate that increased expression of heat shock protein (Hsp) 70 and oxidative stress trigger autophagy which plays a critical role in cellular homeostasis. In this regard, ROS has been shown to be sufficient to initiate the autophagy process in cellular models. In our preliminary studies we observed a significant increase in the expression of Hsp70 and Beclin1 in PCP-challenged human type I alveolar epithelial cells (AS49) and hepatocarcinoma cells (HepG2). We, therefore, hypothesized that Hsp70-Beclin1 interaction regulates ROS-induced autophagy during PCP exposure. In this regard, we cultured and treated AS49 and HepG2 cells with 1 and 10 μM concentrations of PCP for 24hours. Our results showed increased a) cytokine/ chemokine (IL-1β, IL-10, TNFα, IL-6, IL-2, IL-12, IL-18, IFNγ, TGFβ, IL-1α, IL-1β, IL-2, IL-6, IL-10, TNFα) and b) expression of inflammatory mediators (NF-κB, STAT3 and p38 MAPK- range between 1.5-4.0 fold); autophagy-related proteins (ATG12, ATG16, Beclin1 and LC3B -1.5-3.0 fold), NPAP oxidase subunits (p22phox, p47phox, p67phox and gp91 phox - 1.5-4.0 fold); and antioxidants (MnSOD, Catalase & Gpx-2.0-4.0 fold), in PCP-challenged AS49 and HepG2 cells. Interestingly, pretreatment with N-acetyl cysteine (NAC, 200 mM) inhibited PCP-induced responses. Additionally, an increase in the interaction of Hsp70 with Beclin1 (>3.0 times) was observed during PCP exposure in our study models. In brief, our experiments reveal that increase in Hsp70 RESTO-modulated autopagy by directly interacting with Beclin1 in PCP-challenged lung and liver epithelial cells. Further studies are in progress to understand the specific role of posttranslational modifications in Hsp70-mediated responses in our study models. The neurotoxicity of OPs is well established, ranging from acute cholinesterase inhibition to delayed polyneuropathy. Malathion (MAL) and Chlorpyrifos (CPF) are representative of these activities, with CPF shown to induce a central-peripheral neuropathy. CPF has been shown to promote oxidative stress and inflammatory responses in the mouse brain (microglia, astrocyte, and neuronal cells). In vivo, MAL was reported to elevate inflammatory cytokines in rodent brains. The present study compared the effects of MAL (1-10mM) and CPF (1-10μM) on mouse SIM-A9 MC. Cytotoxicity of MAL and CPF was determined using the MTT viability assay. Nitric oxide production was measured using the Griess method, and qPCR was employed to determine variations in
mRNA expression of key inflammatory mediators. CPF was not cytotoxic at any of the tested concentrations, whereas MAL was associated with a 90.60% decrease in viability at 80 μM (IC50 = 70.99 μM). Neither CPF nor MAL was associated with a significant change in NO production compared to LPS-treated MC. Gene expression showed that both CPF and MAL, alone, did not induce a change of mRNA levels for any of the assessed targets (INOS, TNF-α, COX-2, IL-1β, and IL-6). However, in combination with LPS, CPF significantly enhanced the LPS-induced upregulation of INOS, IL-1β, and IL-6, whereas MAL reduced LPS-induced gene expression. These results are consistent with some published reports of differential and opposite activities in zebrafish and rodents with these agents. It also suggests that their defined neurotoxicity, other than cholinergic, may involve diverse mechanisms of action, inclusive of neuroinflammation in the presence of neurodegenerative events.

Epidemiological studies suggest that low dose chronic prenatal and infant exposures to organophosphorus pesticides (OPs) can lead to life-long neurological damage and behavioral disorders. While inhibition of acetylcholinesterase (AChE) is the shared mechanism of acute OP neurotoxicity, OP-induced developmental neurotoxicity requires an independent mechanism and/or in the absence of significant AChE inhibition, implying that OPs affect alternative targets. Moreover, different OPs can cause different adverse outcomes, suggesting that OPs act through various mechanisms. These findings emphasize the importance of comparative studies of OP toxicity. Freshwater planarians are invertebrates that uniquely allow for automation of experiment and repetitive use to improve the ability to parallel invertebrate models with vertebrate systems in parallel to differentiate neurotoxicity from DNT. Effects found only in regenerating planarians would be indicative of DNT, whereas shared effects may represent neurotoxicity. The planarian Dugesia japonica is especially well suited to study OP toxicity. D. japonica has two cholinesterase enzymes with intermediate properties between AChE and butyrylcholinesterase that are sensitive to OP inhibition. D. japonica can bioactivate chlorpyrifos and diazinon into their respective active metabolites at all life stages and has both cholinesterase and paraoxonase activity. Leveraging these unique strengths, the potential differential effects of OPs on the adult and developing brain were investigated by performing a comparative screen of OPs (CPF, Malathion, Diazinon, Chlorpyrifos, Phosalone, Ethion, Profenofos) across 30 concentrations in quarter-log steps. Neurotoxicity was evaluated using a wide range of quantitative morphological and behavioral readouts. AChE activity was measured using an Ellman assay. The toxicological profiles of the 7 OPs differed across the OPs and between adult and regenerating planarians and were not correlated with levels of AChE inhibition. These results recapitulate findings from mammalian studies showing that different OPs cause different adverse outcomes, and that these differences cannot be explained by AChE inhibition alone. Presumably, adverse outcomes differ between adult and developing organisms due to the OPs' effects on multiple targets. Phenotypic profiles identified in this comparative screen can be used as a starting point for future mechanistic studies of OP neurotoxicity in planarians using proteomics or RNA-seq. The unique strength of planarian rapid screening to directly compare future mechanistic studies of OP neurotoxicity in planarians using proteomics or developing organisms due to the OPs' effects on multiple targets. Phenotypic differences between AChE and butyrylcholinesterase that are sensitive to OP inhibition. Malathion, exposed animals were significantly less active in the open field, includes open field motor activity, rotarod/accelerod and grip strength. In male rats, exposed to malathion performed the same as control animals on the accelerod but had a significant decrease in forelimb grip strength (0.86 ± 0.13 kilograms for control and 0.72 ± 0.12 kilograms for malathion exposed females). These neurological differences may be associated with anatomical changes observed at the neuromuscular junction of hindlimb skeletal muscles in malathion exposed mice that are consistent with peripheral neuropathy. Altogether, our results suggest that the effects of repeated low-level exposure to malathion can affect motor function differentially in male and female rats.

While organophosphates are known neurotoxics, they remain in use as flame retardants, additives to lubricants and plasticizers, and are heavily used as pesticides. Acute exposure to organophosphates inhibits cholinesterase activity and can lead to muscle paralysis and in some cases death. There is also compelling evidence that low or repeated exposures, such as those that may occur in the case of working with or around pesticides, are linked to adverse neurobehavioral effects in humans and animals even though they do not severely impact cholinesterase levels (<10%). Malathion is a commonly used insecticide organophosphate in the United States. Repeated exposure to low levels of malathion correlate with subtle behavioral effects. Despite these impacts, there is a paucity of data available as to how these repeated exposure to low levels of malathion impact locomotion and the structure of motor neurons and their innervation of skeletal muscle at neuromuscular junctions. We hypothesized that low-level repeated exposure to malathion results in motor function deficits with anatomical alterations in the motor unit consistent with neurodegeneration. To test this hypothesis, we exposed male and female adult Sprague Dawley rats to 50 mg/kg of malathion via subcutaneous injections once daily for 5 days a week for 4 weeks total. Locomotor behavior was assessed 1 week following exposure and includes open field motor activity, rotarod/accelerod and grip strength. In male rats, malathion exposed animals were significantly less active in the open field, with activity scores for females being 1484 ± 150 s/rod (mean ± standard deviation) while animals exposed to malathion displayed mean activity times of 1253 ± 151 seconds. Male rats exposed to malathion also fell off the accelerated approximately 54 seconds earlier than control animals, with no significant differences in forelimb or hindlimb grip strength. Female rats exposed to malathion were also significantly less active in open field compared with control animals (1101 ± 218 seconds and 1357 ± 122 seconds, respectively). Unlike males, female rats exposed to malathion performed the same as control animals on the accelerod but had a significant decrease in forelimb grip strength (0.86 ± 0.13 kilograms for control and 0.72 ± 0.12 kilograms for malathion exposed females). These neurological differences may be associated with anatomical changes observed at the neuromuscular junction of hindlimb skeletal muscles in malathion exposed mice that are consistent with peripheral neuropathy. Together, our results suggest that the effects of repeated low-level exposure to malathion can affect motor function differentially in male and female rats.
activity. Relative to DFP mice pretreated with PB or saline, MEC pretreatment also significantly reduced reactive astrogliosis, microglia activation and neurodegeneration in a time and region-specific manner as indicated by immunohistochemical analyses of the piriform cortex and hippocampus at 1, 3, and 7 days post-DFP exposure. Collectively, these findings suggested that mecamylamine, a non-selective nAChR antagonist, is an effective prophylactic treatment for protecting against OP nerve agent-induced SE. Supported by the NIH CounterACT Program (U54 grants NS079202 and NS127756).

4542 Trifloxystrobin: Combination of In Vivo and In Vitro Approaches to Address Neurotoxicity Concerns

Trifloxystrobin is an established fungicide widely used in food and feed crops. As a member of the strobilurin fungicide class it acts as disruptor of mitochondrial respiration by targeting mitochondrial complex III in fungi. Even though the available robust in vivo database for trifloxystrobin showed no indication of neurotoxicity there is increasing concern mainly due to the assumed relationship between mitochondrial complex I inhibitors and Parkinson disease and recent in vitro investigations with strobilurins in neuronal cell models. Based on its chemical structure four possible trifloxystrobin stereoisomers can be formed. The technical grade active substance trifloxystrobin consists of >97.5% of the EE isomer, but under the influence of light photo-isomerization occurs resulting in an equilibrium state between EE, EZ, ZZ and ZE isomer. This is reflected in the EU residue definition for the transformation products. Inclusion of non-EE stereoisomers into the triazole mixture results in three stereoisomers and desmethyl-trifloxystrobin as major transformation products. Exposure to the target tissues, in this case the brain, is a pre-requisite for the elicitation of a toxic effect in vivo. Therefore, the goal of this project was to investigate potential brain exposure to trifloxystrobin plus a comparative toxicity evaluation of the major transformation products with a combination of different in vivo and in vitro methods. Studies with radioactively labelled test material show that residues in rat brain 12 hours after exposure are the lowest of all organs (<0.01% of dose). Due to the extensive metabolism, it is assumed that the radioactive signal more accurately reflects uptake and metabolism in vivo.

4543 Combinations of Glyphosate and Mancozeb Alter Metal Levels and Disrupt Redox Balance in Mouse Neuro-2a Cells
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Pesticides-associated toxicities have long been studied. Most of the studies are focused on the effects of exposure to one pesticide at a time rather than the exposure to environmentally relevant combinations of these compounds. In this study, different combinations of two extensively used pesticides, glyphosate and mancozeb, were examined. Glyphosate (GL) is a broad-spectrum systemic herbicide. Mancozeb (MZ) is a broad-spectrum non-systemic fungicide. Residues of both GL and MZ are detected in fruits, vegetables, and beans. Both of these pesticides have been linked to neurotoxicity. GL and MZ are known for their metal-chelating potential and their ability to alter intracellular metal levels. Failure to regulate intracellular metal levels can increase the production of reactive oxygen species and lead to oxidative stress. In the current study, alterations in metal levels and disruptions in redox balance are investigated as underlying mechanisms of neurotoxicity associated with GL, MZ and their combinations using mouse neuroblastoma (neuro-2a) cells. Concentrations used were 100µM for GL and 6µM for MZ. Neuro-2a cells were treated with GL for 30 minutes or 24 hours or treated with MZ for 30 minutes or 24 hours. Cells were also exposed to three different combinations of GL and MZ including exposure to mixture of GL + MZ for 24 hours, exposure to MZ for 30 minutes followed by GL for 24 hours or exposure to GL for 30 minutes followed by MZ for 24 hours. Inductively coupled plasma - optical emission spectroscopy (ICP - OES) was used to analyze metal levels in neuro-2a cells. Copper levels were significantly increased compared to controls in MZ 24 hours treatment, MZ 30 minutes followed by GL 24 hours combination treatment and in GL 30 minutes followed by MZ 24 hours combination treatment. Manganese levels significantly increased compared to controls in MZ 24 hours treatment, GL + MZ 24 hours mixture treatment and in GL 30 minutes followed by MZ 24 hours combination treatment. Zinc levels were significantly increased compared to controls in MZ 24 hours treatment, and in all GL and MZ combination treatments. Iron and magnesium levels were not changed in any of the treatment groups. GL + MZ 24 hours mixture showed significantly higher levels of manganese and zinc compared to glyphosate treatment. Combination of MZ 30 minutes followed by GL 24 hours showed significantly higher levels of copper, manganese, and zinc compared to GL treatment. To assess redox balance disruptions in response to MZ, GL and their combinations, reduced glutathione to oxidized glutathione ratio (GSH/GSSG) was evaluated. GSH/GSSG ratio was significantly reduced in MZ treatments and in all of GL and MZ combinations. GL + MZ 24 hours mixture and combination of MZ 30 minutes followed by GL 24 hours showed significantly lower GSH/ GSSG ratio compared to GL treatment. Combination of GL 30 minutes followed by MZ 24 hours showed significantly lower GSH/GSSG ratio compared to GL and MZ treatments. From these results it is concluded that the degree of alterations in metal levels and in redox balance differs from one GL and MZ combination to another. Combinations of GL and MZ result in more metal levels alterations and induce more redox balance disruptions in neuro-2a cells compared to GL. It is also concluded that exposure to GL followed by MZ induces more redox balance disruptions in neuro-2a cells compared to exposure to each compound separately.

4544 Evaluation of Gut Microbiome Perturbation and Neurotoxicity in C57BL/6 Mice Chronically Exposed to Glyphosate
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Glyphosate is currently the most heavily used broad-spectrum herbicide in the world, and glyphosate exposure is widespread through a variety of sources, such as diet and environment. Significant concerns about its toxicity and health effects of glyphosate in human health remain. Certain controversy in plants, glyphosate disrupts the shikimate pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The subsequent deficiency in EPSPS leads to a reduction in aromatic amino acids that are essential for protein synthesis and plant growth. The shikimate pathway exists in plants and microorganisms but not in mammals, which is a major reason that glyphosate is considered a safer herbicide. However, the human body harbors massive microorganisms in the gastrointestinal tract and the gut microbiome plays a key role in human health. Of particular relevance, many gut bacteria actually encode epsps gene. In addition, aromatic amino acids serve as precursors for the synthesis of neurotransmitters, raising potential concerns that perturbation of the gut microbiome and shikimate pathway by glyphosate may alter neurotransmitter homeostasis and contribute to neurotoxicity. To test this hypothesis, we exposed male C57BL/6 Mice to 0, 0.0175, and 1.75 mg/ mL glyphosate in drinking water for 6 months, followed by gut microbiome analysis and neurotoxicity test. Our results show that glyphosate altered the gut microbiome community structure of selected mice. Furthermore, a significant increase in the GSSG/GSH ratio was observed in the gut microbiome of mice exposed to GL. The results demonstrate that glyphosate can alter gut microbial composition.

4545 Low-Dose Oral Insecticide Exposure Induces Gastrointestinal Dysfunction and Alters Dopamine Homeostasis in Young Adult Mice
Pesticide exposure is a well-established risk factor for Parkinson’s disease (PD). PD is a progressive neurodegenerative disease attributable to the death of dopamine (DA) neurons in a region of the midbrain called the substantia nigra pars compacta (SNc). These neurons project from the SNc to a separate region called the striatum, forming the nigrostriatal dopamine pathway. The death of nigrostriatal DA neurons has been linked to neurodegeneration, neuroinflammation, and oxidative stress. In the current study, alterations in metal levels and disruptions in redox balance are investigated as underlying mechanisms of neurotoxicity associated with GL, MZ and their combinations using mouse neuroblastoma (neuro-2a) cells. Concentrations used were 100µM for GL and 6µM for MZ. Neuro-2a cells were treated with GL for 30 minutes or 24 hours or treated with MZ for 30 minutes or 24 hours. Cells were also exposed to three different combinations of GL and MZ including exposure to mixture of GL + MZ for 24 hours, exposure to MZ for 30 minutes followed by GL for 24 hours or exposure to GL for 30 minutes followed by MZ for 24 hours. Inductively coupled plasma - optical emission spectroscopy (ICP - OES) was used to analyze metal levels in neuro-2a cells. Copper levels were significantly increased compared to controls in MZ 24 hours treatment, MZ 30 minutes followed by GL 24 hours combination treatment and in GL 30 minutes followed by MZ 24 hours combination treatment. Manganese levels significantly increased compared to controls in MZ 24 hours treatment, GL + MZ 24 hours mixture treatment and in GL 30 minutes followed by MZ 24 hours combination treatment. Zinc levels were significantly increased compared to controls in MZ 24 hours treatment, and in all GL and MZ combination treatments. Iron and magnesium levels were not changed in any of the treatment groups. GL + MZ 24 hours mixture showed significantly higher levels of manganese and zinc compared to glyphosate treatment. Combination of MZ 30 minutes followed by GL 24 hours showed significantly higher levels of copper, manganese, and zinc compared to GL treatment. To assess redox balance disruptions in response to MZ, GL and their combinations, reduced glutathione to oxidized glutathione ratio (GSH/GSSG) was evaluated. GSH/GSSG ratio was significantly reduced in MZ treatments and in all of GL and MZ combinations. GL + MZ 24 hours mixture and combination of MZ 30 minutes followed by GL 24 hours showed significantly lower GSH/ GSSG ratio compared to GL treatment. Combination of GL 30 minutes followed by MZ 24 hours showed significantly lower GSH/GSSG ratio compared to GL and MZ treatments. From these results it is concluded that the degree of alterations in metal levels and in redox balance differs from one GL and MZ combination to another. Combinations of GL and MZ result in more metal levels alterations and induce more redox balance disruptions in neuro-2a cells compared to GL. It is also concluded that exposure to GL followed by MZ induces more redox balance disruptions in neuro-2a cells compared to exposure to each compound separately.
idea that some incidences of PD may be triggered from within the gut after oral pesticide exposure, perhaps initiating prodromal stages of disease. Pyrethroids, a class of commonly used insecticides, target the nervous system and are known to disrupt DA signaling pathways. Since ingestion is an important route of pyrethroid exposure, we hypothesize that low-dose oral exposure to the pyrethroid deltamethrin induces gut dysfunction and disrupts nigrostriatal DA circuitry in young adulthood. To test this hypothesis, wildtype mice of both sexes (age 10-12 weeks) were orally gavaged once weekly with a low dose of deltamethrin (3 mg/ kg) or vehicle control in a subchronic (3 weeks) or chronic (12 weeks) exposure paradigm. A panel of GI functional assays was performed to determine whether deltamethrin induces constipation. Gene and protein levels of tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicular monoamine oxide 2 (VMAT2), all important for dopamine synthesis and trafficking, were quantified in gut and brain tissues via qPCR and western blot to determine effects of oral deltamethrin exposure on DA signaling pathways. Motor function was assessed using a variety of behavioral assays to assess fine and gross motor function. Our results indicate that oral deltamethrin exposure triggers intestinal behaviors indicative of constipation. These effects were not due to impairments in gut hormone signaling, but we did observe significantly decreased gene expression and increased protein levels important for DA synthesis (TH) and reuptake (DAT) in the proximal colon of deltamethrin-treated groups compared to controls. Intriguingly, there were also decreased levels of dopamine and its metabolite, DOPAC, in the proximal colon of deltamethrin-treated mice compared to controls. As skunks were unaltered in deltamethrin-treated mice of both sexes, yet the nigrostriatal DA pathway was disrupted as evidenced by significant increases in TH, DAT, and VMAT2 gene expression in the midbrain and increased TH protein levels in the striatum. These findings suggest that epidemiological evidence linking pyrethroid exposure to PD may be due to altered dopamine handling in both the gut and brain, rendering the system more vulnerable to PD-like phenotypes.

5447 APOE-Genotype and Sex Alters Effects of Organochlorine Pesticide DDT in Humanized APOE Mice
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The Apolipoprotein e4 (APOE) gene variant is the strongest genetic risk factor for late-onset Alzheimer’s Disease (LOAD). However, it is not entirely predictive of LOAD, and emerging evidence points to environmental factors in the etiology of the disease. Evidence from numerous epidemiological studies has identified associations between pesticides and lower cognitive scores. Dichlorodiphenyltrichloroethane (DDT) is a pesticide used in various regions, and although its use was banned in 1972 in the US, existing DDT dumpsites and DDT’s long half-life contribute to the trace amounts found in the environment and food chain. We have recently reported novel translational evidence indicating DDT affects the amyloid pathway and induces AD pathology in multiple in vitro and in vivo models. We have also previously shown increased serum levels of DDE, the primary metabolite of DDT, in AD patients compared to age-matched controls. Moreover, APOE genotype modified mini-mental state exam scores and contributed an ~4-fold increase in AD risk. In the present study, we sought to investigate whether the APOE4 genotype alters the pathological response to DDT. To assess the effects of DDT exposure in vivo, three-month-old male and female APOE3 (E3) and APOE4 (E4) mice were exposed to 3 mg/kg DDT by oral gavage every 3 days for 5 months. To investigate DDT-induced APOE genotype and sex-specific alterations at the mRNA level, RNA-sequencing was conducted in the hippocampus of mice. Compared between genotypes in female DDT-exposed mice indicated significantly downregulated cellular component pathways in E4 females, including plasma membrane (Padj = 4.14×10−10), neuron projection (Padj = 9.49×10−10), and vesicles (Padj = 3.48×10−10). Similar comparisons made between genotypes of DDT-treated males indicated that E4 males downregulated biological process pathways, including cell-cell signaling (Padj = 1.1×10−10), chemical synaptic transmission (Padj = 7.3×10−10), and learning or memory (Padj = 7.07×10−10). Cellular component pathways including neuron projection (Padj = 9.38×10−10), and presynapse (Padj = 3.46×10−10) were also downregulated in E3 males exposed to DDT. To confirm these findings, synaptic deficits were assessed by measuring protein levels of postsynaptic density protein 95 (PSD95) and synaptophysin, a pre-synaptic protein associated with small synaptic vesicles. Immunoblotting indicted a ~7% decrease in PSD95 in the hippocampus of DDT-exposed E3 males and females, a ~14% decrease in E4 males, and a 16% decrease in DDT-exposed E4 females compared to their respective controls. Immunoblotting for synaptophysin indicated a ~3.5% decrease in E3 DDT-treated males and females, a 6.5% decrease in E4 males and a 12.5% reduction in E4 females compared to their respective controls. Finally, we tested whether DDT exposure also induced mitochondrial dysfunction in the hippocampus. The mitochondrial light chain (snFL) levels, a biomarker for neurodegeneration, by 1.9 and 2.1-fold in male and female APOE4 mice, respectively, compared to controls. snFL levels were 1.4-fold higher in DDT-exposed APOE3 females compared to APOE4 males. snFL levels did not change in DDT-exposed APOE3 mice. Together, these findings suggest that the gut-brain-gene environment interaction that is modified by sex. These data provide a platform to explore possible mechanisms in AD-related neurodegeneration to support previous epidemiological findings. Supported in part by NIH R01ES026567.

5448 Pesticide Exposure Impairs Neuronal Autophagic Flux in a Zebrafish Model: Toxicological Implications for Parkinson’s Disease Risk

Autophagy is a conserved cellular pathway for clearing aggregated proteins and cellular waste via lysosomal degradation. Dysfunctional autophagy has been implicated in the neurodegeneration associated with Parkinson’s Disease (PD). PD is characterized, in part, by aggregation of alpha-synuclein protein, progressive loss of dopaminergic neurons, and neuroinflammation. Pesticides have broadly been associated with an increased risk of developing PD, however, only a few individual pesticides have been identified as specific risk factors. Zebrafish (ZF), Danio rerio, have emerged as a useful animal model to study neurodegenerative processes due to their optical clarity, rapid development, and conserved brain structures with mammals. We have observed that pesticides alter vesicular and protein components involved in autophagy through a 2-phase cell-based screening using SK-N-MC human neuroblastoma cells and exposing them to pesticides commonly used in the Central Valley of California. We hypothesize that pesticides impair autophagic flux, which then confers increased risk of developing PD. Using a stable transgenic ZF line that expresses eGFP-Map1lc3b in post-mitotic neurons under the HuC promoter, we screened the top pesticide hits from our cell-based screen in vivo. By modulating and quantifying the number of autophagosomes after treatment with known autophagy inhibitors (Bafilomycin A1, protease inhibitors), we determined the relative effect of specific pesticides in ZF neurons. For example, acute exposure to ziram (1 µM; dithiocarbamate fungicide) in combination with Bafilomycin A1 treatment in 3 day-post-fertilization ZF larvae resulted in significantly decreased autophagosome puncta counts relative to Bafilomycin A1 treated controls alone, assessed using

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confocal microscopy. As Bafilomycin A1 acts to prevent autophagosome fusion with the lysosome or lysosomal activation, these findings suggest that ziram is acting early in or upstream of the autophagic pathway in a manner that prevents or decreases autophagosome formation. Further investigations are underway to determine precisely how these pesticides alter autophagic flux in vivo and whether they may be acting similarly or may interfere with the pathway through multiple mechanisms of action. These studies seek to identify specific pesticides that impair protein turnover, increasing risk of neurotoxicity, and to assess the mechanisms by which these pesticides may act.

Decision-Making in Adolescent Rats Exposed Repeatedly to Chlorpyrifos as Juveniles


Chlorpyrifos (CPF) is an organophosphorous insecticide that exerts its toxicity through the inhibition of acetylcholinesterase in both insects and non-target species following higher levels of exposure. However, following exposure to levels that do not inhibit acetylcholinesterase, CPF will target the enzyme fatty acid amide hydrolase (FAAH) which is responsible for the degradation of the endocannabinoid anandamide. Any alteration of the endocannabinoid system in the developing brains could disrupt normal development leading to altered function. In fact, exposure of children to low levels of chlorpyrifos (CPF) has been associated with persistent behavioral problems including attention deficit hyperactivity disorder (ADHD) and decrements in cognitive abilities. However, these types of effects have not been recapitulated in clinical animal models. A rendition of the Iowa Gambling Test (IGT), geared towards rodents, has been used to quantify risk-taking behaviors such as those associated with ADHD. The objective of this study was to determine if risk-taking behavior in the rat IGT is altered by repeated exposure to CPF. Daily from 10-16 days, 10-day old male and female rat pups were orally administered either corn oil, CPF (0.25, 0.5, or 0.75 mg/kg), or 0.02 mg/kg PF-04457845 (PF), a specific inhibitor of fatty acid amide hydrolase (FAAH). FAAH is inhibited following CPF exposure so PF was included for comparison to behavior altered by CPF. On days 32-41, rats were tested in the IGT with 12 trials per day (120 total decisions). The experiment consisted of 2 empty arms and 2 arms containing either food (advantageous arm) or quinine-treated food (disadvantageous arm). The decisions of the rats were scored based on food/quinine arm selection and the subsequent decisions. In terms of overall performance, the male rats exposed to all dosages of CPF and to PF performed better than did controls. This was especially evident during the later days of testing. However, this improvement was not observed in females. The basis for this improvement was that upon encountering a quinine-treated food, treated males had a reduced rate of returning to the same arm thereby increasing the chance of a correct selection.

Potential Interaction of Cannabinoids and Insecticides in Causing Dopaminergic Neurotoxicity

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The therapeutic potential of medical cannabis has gained significant interest in recent years. Over 200 million people in the U.S. now have access to medical cannabis through legalized state programs. Yet, cannabis is not being regulated in the recent years. Over 200 million people in the U.S. now have access to medical cannabis through legalized state programs. Yet, cannabis is not being regulated. The therapeutic potential of medical cannabis has gained significant interest in recent years. Over 200 million people in the U.S. now have access to medical cannabis through legalized state programs. Yet, cannabis is not being regulated. The therapeutic potential of medical cannabis has gained significant interest in recent years. Over 200 million people in the U.S. now have access to medical cannabis through legalized state programs. Yet, cannabis is not being regulated.

Brain-Derived Extracellular Vesicle Biogenesis Alteration by Early-Life Exposure to Deltamethrin Induces Impaired Synaptic Plasticity and Deficits in Learning and Memory

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Medical Center Pyrethroids are a class of synthetic insecticide thought to be safer than their organophosphate predecessors. However, with their popularity in both agricultural and general public use in the past three decades there is growing concern of pyrethroid exposure and the incidence of neurodevelopmental disorders. For example, recent studies have linked pyrethroid exposure, within the NOAEL, guidelines set by the EPA, to neurodevelopmental disorders including attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder. During development, the blood brain barrier is vulnerable to the lipophilic properties of Deltamethrin (DM) and is expected to lead to neural circuit dysfunction. A possible key regulator in this dysfunction include extracellular vesicles (EV), which are phospholipid nanovesicles that, based on their origin or size, include apoptotic bodies, micro vesicles, and exosomes. These molecules are involved in communication between cells and regulating the pathophysiology of several diseases. However, their role in DM-induced neurotoxicity is yet to be explored. Using a developmental exposure model, in which pregnant dams were exposed to 3 mg/kg/72 hours, equivalent to 1mg/kg/day, DM or vehicle through pregnancy and lactation, we have found that early-life exposure to DM causes an increase in the average size of EVs compared to the control. Crucially, this increased size of EVs has been associated with enhanced neuroinflammation linked to neurodevelopmental and neurodegenerative disorders. Functionally, we are determining the effect of the Brain Derived Extracellular Vesicles (BDEV) on neuronal function using naïve cortical neurons by exposing neurons to BDEVs from DM or control animals and then recording the effect on synaptic properties using whole-cell patch clamp electrophysiology. We are determining the effect of BDEVs on synaptic properties of naïve cortical neurons. At the organismal level, their perturbations in neuronal function may give rise to corresponding changes in behaviors associated with neuronal function. Overall, these data suggest early-life exposure to DM causes alterations in EV morphology that are associated with changes in hippocampal plasticity and corresponding behaviors. This research was funded by a NIHES T32 training grant T32ES007254 (JD), an NIEHS R01 grant R01ES031823 (FL) and an NIEHS P30 grant P30ES030285 (FL).
Propiconazole is a triazole fungicide extensively used in agriculture which can harm non-target organisms in aquatic environments through runoff. Chronic exposure to environmental pesticides leads to behavioral impairment in vertebrates including teleosts. However, the potential effect of this fungicide on neurobehavioral impairment and release from it in vertebrates has not been fully explored. In this work, we examined the role of melatonin to rescue fungicide-induced neurobehavioral impairment and mitochondrial dysfunction and its connection with changes in dopaminergic activity in the brain of Mystus cavasius. After fish were exposed to water containing propiconazole at 0, 0.1, 5, and 250 μg/L for 3 days, significant increases of DA, 3,4-dihydroxyphenylacetic acid (DOPAC, a DA metabolite), and their ratio (DOPAC/DA) were observed in whole brains at 250 μg/L concentration. When fish were treated with propiconazole at 250 μg/L for 3 days, there was a significant elevation of DA, DOPAC and DOPAC/DA in diencephalon and pituitary, and only DA in the telencephalon, compared with control fish. Besides, it induced a reduction in extracellular serotonin and had an anxiolytic-like effect, supported by a decrease in cortisol production. Increased locomotor activity, anxiety and aggressiveness, decreased gonadosomatic index with few vitellogenic oocytes in ovaries after propiconazole treatment. When fish were treated with melatonin, D1 (SCH-23390) or D2 (Haloperidol) dopamine receptor antagonists and combined of melatonin and D1/D2 receptor antagonist and was observed melanin + D2 receptor antagonist rescued fungicide induced all behavioral changes in fish. These results indicate that propiconazole increases locomotor activity, anxiety and aggressiveness and decreases reproductive activity, which was rescued by combined treatment of melatonin and dopamine receptor antagonist.

Copper as an essential trace element is crucial for various biological processes but has also been utilized as a constituent of various pesticides for decades. Copper (II) octanoate is one such pesticide utilized as an antifungal agent on a variety of crops and is approved for use in the production of certified organic foods. Previous studies have implicated copper as an inducer of oxidative stress and activator of the detoxification pathways to mitigate redox dysregulation and the subsequent induction of Nrf2-mediated antioxidant/mitophagy in BV2 microglia cells. E2 (10 nM) was pretreated for 18 hours prior to Mn (250 μM) exposure for 24 hours, followed by western blotting of fusion-fission proteins Drp-1, Mfn2 and Opal-1, as well as proteins involved in autophagy/mitophagy such as LC3-I/LC3-II, p62, Parkin, and PINK1 in both whole cell lysates and mitochondrial fraction. Subcellular localization of Drp-1 by immuno-cytochemistry was also performed to determine its translocation from the cytosol to mitochondria. Results showed that E2 (10 nM) inhibited Mn (250 μM)-induced translocation of the fusion protein Drp-1 from the cytosol to the mitochondria. E2 also attenuated Mn-induced reduction of fusion proteins Mfn2 and Opal-1 in both whole cell lysates and mitochondria fraction. In addition, E2 alleviated Mn-induced alterations in mitochondrial protein levels of PINK1 and Parkin in mitochondria fraction. E2 also mitigated Mn-impaired autophagic protein levels of LC3-I/LC3-II and p62. Taken together, our findings suggest that E2 induces protective effects against Mn-induced dysregulation in mitochondrial dynamics and autophagy/mitophagy in BV2 microglia. Supported by NIHES R01 ES031262 and R01 ES010563.

Manganese (Mn) is an essential trace metal, however in excess it is a potent neurotoxin. Previous studies have solely focused on the central nervous system (CNS) and have demonstrated that Mn excess prompts cognitive and behavioral deficits in juveniles. Yet the first site of exposure is through the enteric nervous system (ENS) that is comprised of glial and neuronal cells, that may be affected like observed cells in the CNS by excess Mn ingestion. We hypothesize that there will be a correlation between behavioral and cognitive deficits relative to glial inflammation in the ENS. Mice were administered with environmental-relevant levels of Mn, 50 mg/kg MnCl2, daily via drinking water from d21-d90 postnatal and evaluated locomotor function, memory and learning, and glial activation in both the CNS and ENS. Preliminary results from these studies have replicated sex-dependent behavioral in the juvenile mice previously observed while demonstrating no change in memory and learning of Mn treated mice. Neurochemical analysis in the ENS has demonstrated that there is little alteration of biogenic amines with the low dose exposure cohort compared to controls. Initial analysis of the inflammatory biomarkers, GFAP and s100B, shows sign of inflammation in the ENS yet analysis of these tissues is ongoing. These data indicate that exposure to Mn during development leads to inflammatory activation of the cells in the ENS. These findings lead us to question if high exposures to Mn induce a neurotoxic effect on the gut-brain-axis leading to the behavioral modification.

Manganese levels of targeted neuronal populations: dopaminergic, GABAergic, glutamatergic and cholinergic. Exposure to excess Mn leads to deposition in the basal ganglia of the brain, inducing a debilitating disease known as Mn-induced neurotoxicity. Recent studies have indicated that early-life individuals (newborns, infants, and adolescents) environmentally exposed to increased Mn levels display impairments in postural balance, fine and gross motor function, and cognitive deficits. Despite this, the specific neuronal target and mechanism of early-life Mn-induced neurotoxicity is unknown. To address this, we developed neuron-specific knockout mice for Slc30a10, the critical Mn efflux transporter, to selectively alter Mn levels of targeted neuronal populations: dopaminergic, GABAergic, glutamatergic, or cholinergic neurons. Preliminary validation of the neuron-specific Slc30a10 knockout mice display decreased Slc30a10 mRNA levels using qRT-PCR and increased Mn levels by laser ablation ICP-MS in targeted basal ganglia nuclei. We found that dopaminergic- or glutamatergic-, but not GABAergic-or cholinergic-specific Slc30a10 knockouts displayed a hypolocomotor phenotype at PND56 when compared to littermate controls. Further, the dopaminergic-specific Slc30a10 knockout displayed decreased reflexes during PND9-11 neuron motor assays and increased missteps during PND28 beam balance tests. These data suggest that the expression of Slc30a10 in dopaminergic and glutamatergic neurons is required to protect against early-life Mn-induced motor deficits. In sum, our results suggest that dopaminergic and glutamatergic neurons may be the main targets of early-life Mn-induced neurotoxicity.
Elevated levels of the essential metal manganese (Mn) are neurotoxic and induce neurodegenerative disease. Excretion of Mn by the liver and intestines is a major pathway that regulates body and brain Mn. Notably, the relative contribution of hepatic and intestinal Mn excretion to the homeostatic control of body Mn is unknown, although historic Mn radiotracer studies in wild-type rodents have led to two long-standing tenets: (1) the liver is the primary organ that excretes Mn, and the intestines play a minor role; and (2) Mn excretion is not prominent in pre-weaning early-postnatal life. Here, we leveraged the discovery of SLC30A10 as a critical transporter that mediates Mn excretion to define the role of hepatic and intestinal Mn excretion in controlling body Mn homeostasis. Under basal conditions, tissue Mn levels of liver or intestines-specific Slc30a10 knockout mice were comparable to littermates in pre-weaning or later-life. Thus, under physiological conditions, the liver and intestines compensate for loss of Mn excretory function of the other organ. During elevated Mn exposure in pre-weaning or later-life, tissue Mn levels of liver- or intestine-specific Slc30a10 knockouts were both markedly higher than littermates. Thus, during elevated exposure, Mn excretory function of both the liver and intestines is necessary to regulate body Mn homeostasis. Overall, in contrast with well-established tenets, (1) hepatic and intestinal excretion play equally important roles in regulating body Mn homeostasis; and (2) Mn excretion is indispensable in regulating body Mn homeostasis in both early-postnatal and later-life stages.

Manganese (Mn) is an essential element that participates in several biological processes. However, overexposure may result in neurotoxicity and may contribute to the development of neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease. Although several well-supported mechanisms likely explain the acute neurotoxicity of Mn overload, the molecular mechanisms and factors that trigger the onset and disease progression upon chronic exposure remain largely unknown. Previous studies have demonstrated that the dopaminergic system is affected by Mn exposure, leading to behavioral dysfunction. Therefore, in this study, we investigated if chronic exposure to Mn causes a dose- and dose-duration dependent persistent decline in a DAergic-related behavior phenotype in Caenorhabditis elegans (C. elegans). Specifically, we used wild-type N2 worms at the synchronized larval 1 (L1) stage and assessed the impact of exposure duration and duration on chronic Mn-induced behavioral toxicity on the DAergic-dependent basal slowing response (BSR) phenotype (i.e. DAergic dysfunction correlates with a decrease in the (Δ) between the rate of body bends in ‘no-food’ vs. ‘food’ conditions). Moreover, we evaluated reactive oxygen species (ROS) generation in Mn-exposed neuronal and intestinal cells in C. elegans, a model system for the study of the dopaminergic system. We found that Mn increased oxidative stress measured as a decrease in the difference (Δ) between the rate of body bends in ‘no-food’ vs. ‘food’ conditions. Moreover, we evaluated reactive oxygen species (ROS) generation in Mn-exposed neuronal and intestinal cells in C. elegans, a model system for the study of the dopaminergic system. We found that Mn increased oxidative stress measured as a decrease in the difference (Δ) between the rate of body bends in ‘no-food’ vs. ‘food’ conditions.
Manganese (Mn) and iron (Fe) are micronutrients that play important roles in different metabolic and neuronal processes in the central nervous system. However, occupational exposure to these heavy metals has been shown to have detrimental effects on cognition, mood and motor functioning by promoting oxidative stress and synthesis of reactive oxygen species (ROS). The cerebellum is an important structure for motor control but has not been studied in humans with respect to Mn toxicity. Magnetic Resonance Imaging (MRI) allows us to non-invasively measure the accumulation of Mn, using the R1 contrast, as well as of Fe, using the R2* contrast. Equally, magnetic resonance spectroscopy (MRS) allows quantifying concentrations of the neurotransmitters gamma-aminobutyric acid (GABA), glutamate (GLU) and the oxidative stress marker glutathione (GSH). Using a 3T Siemens MRI scanner, we studied the cerebellum of 24 welders and 12 controls. The participants also underwent an evaluation of their motor function using the Unified Parkinson Disease Rating Scale (UPDRS)-Part III, the Finger-Tapping test and the Grooved Pegboard test. The cerebellar data showed that welders have significantly higher Fe (R2*) concentrations in the left dentate nucleus (p=0.020), but no difference in Mn (R1) was observed. Similarly, welders had significantly elevated GSH levels in the cerebellum than controls (p=0.008), with no significant difference observed in GABA or GLU. In addition, welders also scored significantly higher on the UPDRS scale (p<0.001), indicating reduced motor skills. No differences were found for the Finger-Tapping and Grooved Pegboard tests.

Correlation analysis revealed that higher Mn and GSH levels were significantly correlated with increased GABA levels in both WT and REST cKO mice. The results showed that dopaminergic REST cKO mice worsened Mn-induced impairment of locomotor function and motor coordination compared to WT mice, while REST cKO mice did not exhibit significant changes in phenotype or behavior compared to the loxp WT mice. Mn significantly decreased dopamine and serotonin levels in striatum and midbrain of loxp WT mice, and further decreased these neurotransmitter levels in REST cKO mice in the same regions. Moreover, Mn-induced reduction in tyrosine hydroxylase (TH) protein levels was exacerbated in the striatum and substantia nigra of REST cKO mouse brain compared to WT mice by western blotting and immunofluorescence imaging. On the other hand, Mn’s effects on dopamine and serotonin levels in cortex, cerebellum, and hippocampus showed mixed effects, with a significant increase in dopamine levels in the left dentate nucleus and hippocampus, and a decrease in dopamine levels in the left striatum in both WT and REST cKO mice. Interestingly, Mn significantly decreased GABA levels in striatum, cortex, and hippocampus we tested in WT mice. These findings indicate that dopaminergic REST plays a critical role in modulating Mn’s neurotoxicity, at least in part, by dysregulating dopaminergic and serotonergic neurotransmission in the nigrostriatal system. Chronic exposure to welding fumes increases manganese (Mn) deposition in the brain leading to adverse neurobehavioral health effects. Because the signs of metal-induced toxicity are neurological, it is critical to understand how excess metal deposition distributes across the brain. Our group has shown that excess Mn seems to diffuse along white matter tracts to areas in the cortex; however, these depositions are associated with each other is unclear. Therefore, the objective of this study is to use a network science approach termed "relaxometry covariance network" (RCN) analysis to assess the spatial distribution of Mn (as assessed by R1/1 + R1/2) in different brain regions to analyze Mn accumulation in "silos" but also propagate it to other brain areas. Structural T1-w brain MRI scans from 40 welders (MN) and 30 controls (HC) were parcellated into 192 separate brain regions of interest (ROIs) as masks with a fully automated segmentation software FreeSurfer V6.0.0. Brain regions with a significant amount of cerebrospinal fluid (CSF) were excluded since R1 mapping methods cannot measure the long times of CSF reliability. Next, the segmentation masks were overlaid onto the R1 maps to extract the relaxometry values. Left and right brain hemispheres were averaged, leaving 92 R1 values to construct Pearson correlation matrices representative of the correlation between R1 values across brain regions. To evaluate the covariance change (covChange) behavior, the HC Group correlation matrix was subtracted from the MN Group correlation matrix element-wise and added column-wise. From this operation, high positive values represent increased covariance with the rest of the brain and “coupling” (i.e., correlation) with other brain regions. In other words, these brain regions accumulate Mn and propagate it to other brain areas. Negative values represent that for one specific brain region in the MN Group, the covariance is diminished with the rest of the brain (i.e., not correlated with other brain regions), and that specific brain region is a “sil” that only accumulates Mn but does not distribute it. A two-sample t-test was used to make a stronger case to identify brain regions with statistically significant differences in R1 values across brain regions.

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the two groups. The covChange was then plotted against the p-values to obtain a quadrant plot with quadrants representing brain areas that act as silos versus those acting as propagators. The correlation matrices show higher correlation between the basal ganglia (BG) welders compared to controls. This is to be expected as the BG is one of the primary brain areas where excess Mn accumulates due to occupational exposure to welding fumes. Additionally, more correlation between the BG and other brain regions. When comparing the two groups, nine brain regions are identified: thalamus, putamen, brainstem, amygdala, accumbens-area, optic-chiasm, subsections of the corpus callosum, and the transverse frontopolar gyri and sulci. Two-sample t-test results show statistically significant higher R1 values in the putamen, globus pallidus, and accumbens-area. The quadrant plot shows that the globus pallidus is a silo, while the putamen and accumbens-area accumulate and propagate Mn. Other brain areas that accumulate and propagate Mn are the caudate nucleus, hippocampus, cerebral white matter, amygdala and subsections of the corpus callosum. In conclusion, the preliminary results from the relaxometry covariance analysis allow for identifying silos and propagators of excess Mn deposition in the brain. In addition, the results seem to agree with already established literature (i.e., globus pallidus as a Mn silo) and yield information about other brain regions that potentially play an important role in the distribution of Mn and the development of Mn neurotoxicity.

4565 Decreased Newborn Olivodendrocytes and Demyelination in Corpus Callosum Following Lead Exposure

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The corpus callosum (CC) is an olivodendocyte (OL)-enriched brain region, replenished by newborn OLs differentiated from olivodendrocyte progenitor cells (OPCs) in subventricular zone (SVZ). While exposure to lead (Pb) has been clinically linked to multiple sclerosis, a disease characterized by the loss of the myelin producing OLs, the underlying mechanism remains unclear. This study employed ex vivo neurospheres, in vivo lineage tracing, and real-time MRI technologies to investigate whether Pb exposure altered the population of SVZ-derived newborn OLs, resulting in demyelination in CC. In ex vivo experiments, mice received one ip. injection of 27 mg Pb/kg (as Pb-acetate; saline as controls); brain SVZ tissues were dissected 24 hours later for primary culture of neurospheres which were enriched with OPCs. Immunofluorescent staining showed that, following differentiation, neurospheres from Pb-treated mice had significantly fewer newborn myelin basic protein (MBP)-positive OLs (0.7±1.0 OLs per neuron) than those of controls (12.5±1.7; p<0.01). To verify this discovery, adult mice received daily ip-injections of BrdU (50 mg/kg) for 3 d (for the purpose of tracing newborn OLs in CC), followed by daily oral gavage of Pb at 13.5 mg Pb/kg, 5 d/wk, for 4 weeks. Immunohistochemical staining of CC revealed that chronic Pb exposure decreased BrdU(+)/MBP(+) newborn OLs (5.7±1.8 OLs per neurosphere) than those of controls (12.2±1.7; p<0.005). Quantifying the expression of RICTOR (a critical subunit for mTORC2 that regulates endocytosis and autophagy). GRIN2B, SCAMP, and TMEM, were also investigated. The target genes identified are known for their role in neuronal signaling/plasticity, and neuronal function into NDEVs. RNA sequencing results showed that Pb exposure increases amyloid plaques in the walls of cerebral arterioles (angiopathy), and neuronal tau pathology. Together, these data provide strong evidence that chronic Pb exposure induces CAA pathology as well as the mTORC2-mediated signaling that targets genes for tau phosphorylation, amyloid-beta peptide binding, and neuronal function into NDEVs. The expression levels of these genes were evaluated by qRT-PCR and analyzed in relation to associated Pb concentrations. Our study demonstrates the potential of NDEVs to be used as biomarkers of environmental exposures and AD risk.

4567 Analysis of Neuron-Derived Extracellular Vesicle Lead Levels and microRNA Cargos from Human Plasma in an Aging Minority Population at Risk of Cognitive Impairment and Toxic Exposure


Minority populations are at greater risk of developing neurological disorders and cognitive impairment such as those associated with Alzheimer’s disease and Related Dementias (ADRD) compared to the general population in the United States. A myriad of biological, lifestyle, and social determinants have been proposed to underlie such disparity. However, environmental exposure to toxic metals like lead (Pb) that disproportionally affects minority populations has not been adequately assessed for its neurotoxic contribution in cognitive impairment and ADRD onset and progression in the same population. Extracellular Vesicles (EVs) may play a role in the development and diagnosis of dementia-related neurodegenerative disorders. Lacking access to the brain, we enriched extracellular vesicles of neuronal origin (neuron-derived extracellular vesicles or NDEVs) from plasma to serve as a surrogate of the brain milieu and measure their Pb and microRNA (miRNA) cargo content. In this study, we examine NDEVs from participants at higher risk of mental health illness due to metabolic and cardiovascular disorders (Northern Manhattan Study of Metabolism and Mind or NOMEM), to identify associations with measures of cognition. The 64 minority participants had an average age of 64 years, with equal numbers of men and women and were compared to 32 non-Hispanic white cohorts. We captured NDEVs using neuron-specific immunoprecipitation from total plasma EVs isolated by size-exclusion chromatography from 0.5 mL of plasma and conducted metal analysis using cold-acid digestion for quantification of Pb by ICP-MS/MS. Preliminary analysis showed Pb to be reliably detected in the range between 0.11-0.35 ng/g in NDEVs samples with average NDEV concentration of 9.56±08 particles/mL. We used linear regression to determine associations of Pb concentrations with cognitive scores. Additionally, we explored mechanistic involvement of the lead-exposed potential of Pb in NDEVs. We hypothesized that Pb may influence cognition in aging populations by targeting the biogenesis of NDEVs as well as the miRNA sorting that target genes for tau phosphorylation, amyloid-beta peptide binding, and neuronal function into NDEVs. RNA sequencing results obtained on NDEVs from this population revealed that several miRNAs were differentially expressed in minority groups compared to non-Hispanic whites. Among the target genes of these miRNAs, several previously implicated in Alzheimer’s Disease are: HMG1A2, HMGB1, and CASPR3/IL6, GSK3B, and CDK4/CDK6/CDKN1A. Other target genes identified are known for their role in neuronal signaling/plasticity, and endocytosis and autophagy. GRIN2B, SCAMP, and TMEM, were also investigated. The expression levels of these genes were evaluated by qRT-PCR and analyzed in relation to associated Pb concentrations. Our study demonstrates the potential of NDEVs to be used as biomarkers of environmental exposures and ADRD risk.

Lead (Pb) is an environmental risk factor being suspected of contributing to Alzheimer’s disease (AD) and related dementia. Our previous study has suggested that Pb exposure increases amyloid plaques in the walls of cerebral arterioles and capillary, an early event leading to cerebral amyloid angiopathy (CAA) that is observed in approximately 5%-9% of AD patients. However, it remained unclear whether and how Pb exposure induced CAA pathology. This study was designed to investigate how chronic exposure to Pb induced CAA in APP/PS1 AD transgenic mice. Pb treatments at 0.2% drinking water for 2 months displayed pathological evidence characteristically for significant induction of CAA in the cortical blood vessels of APP/PS1 mice (3 mice with NaAc and 4 with PbAc). Along with the CAA formation, Pb exposure also significantly induced vascular TGF-β signal by immunohistochemical (IHC) analyses, cortical demyelination by both IHC and 3D rosette dual-echo UTE MRI technique, and cognitive deficits by neurobehavioral testing in these mice. Furthermore, Pb exposure also caused the disruption of perivascular drainage and increased the binding affinity of Ab to brain vasculature. Interestingly, by addition of an inhibitor against TGF-β signal, Pb-induced CAA pathogenic outcomes including CAA pathology were markedly attenuated. Taken together, these data provide strong evidence that chronic Pb exposure induces CAA and TGF-β signal is critically involved in the pathogenic processes leading to CAA pathology in this AD mouse model. Supported in part by NIN/NIEHS R01ES027078 and NIA R21AG067923.
Copper chaperone for superoxide dismutase (CCS), also named superoxide dismutase 4 (SOD4), is a copper (Cu) chaperone protein with a primary function to deliver Cu to SOD1 for cellular defense against oxidative damage. CCS also participates in regulation of cellular Cu levels by its influence on the expression of Cu binding proteins, e.g., MT-1, MT-2, ATOX1, COX17, and ATP7A. CCS has been found to be expressed in high amounts in the choroid plexus, a brain tissue that constitutes a barrier between the blood and cerebrospinal fluid (CSF) in brain ventricles and is known to play a vital role in maintaining Cu homeostasis in the central nervous system. Previous data from this lab has established that exposure to toxic metal lead (Pb) causes substantial accumulation of Pb in the choroid plexus. This study was designed to test the hypothesis that Pb accumulation in choroid plexus following chronic exposure interfered with CCS expression in two major cell types of choroid plexus (i.e., choroidal epithelia and endothelia), which may contribute to altered Cu brain homeostasis. CD-1 mice received oral gavage at the dose of 13.5 mg Pb/kg body weight as Pb acetate (saline for control mice), once daily for 28 days. Atomic absorption spectroscopy (AAS) analysis revealed that blood lead levels (BLLs) were 0.1 ± 0.2 (SD) µg/dL and 3.5 ± 0.8 (SD) µg/dL in control and Pb-exposed animals, respectively (n=4, p<0.01). Immunohistochemistry was performed with rabbit anti-SOD4 antibody and, rat anti-CD31 antibody, followed by fluorophore-conjugated secondary antibody to visualize the impact of Pb exposure on CCS expression in brain tissues by confocal microscopic imaging analysis. The data showed that Pb groups had a significantly increased CCS expression in the choroidal epithelial cells by 92.9% (p<0.01), but with no changes in the choroidal endothelial cells, in comparison to controls. Interestingly, CCS was found to express abundantly in the subventricular zone (SVZ) at levels equivalent to that in the choroid plexus. Pb exposure appeared to increase CCS expression in SVZ by 20%, although this increase did not meet statistical significance (p>0.05). These results prove the presence of CCS in both choroidal epithelial and endothelial cells in the blood-CSF barrier in the choroid plexus. Exposure to environmental Pb causes an increased expression of CCS specifically in the epithelial cells that are the structural basis of the blood-CSF barrier and appears to increase defense against Pb toxicity and possible interference with Cu regulation by the blood-CSF barrier. Further research to explore the mechanisms whereby Pb altered the CCS with resulting Cu dyshomeostasis in the CSF is in progress. Supported by NIH/NIEHS R01ES027078.

Expression of Copper Chaperone for Superoxide Dismutase (CCS) in the Blood-CSF Barrier and Impact of In Vivo Lead Exposure in Mice

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The potential for developing Parkinson’s disease (PD)-like neurological dysfunction following occupational exposure to welding fumes (WF) is an emerging concern. Manganese (Mn) in welding consumables is suspected to cause the neurological deficits seen in welders. Indeed, we have shown that Mn-containing WF causes dopaminergic neurotoxicity in rats by provoking neuroinflammation, and reducing PD-related (PARK) proteins in the striatum and midbrain, areas typically affected in PD. Recent studies show that chronic exposure to low doses of Mn causes fine motor and cognitive impairment. Functional magnetic resonance imaging studies of welders reveals Mn accumulation and altered metabolites in cortical and thalamic regions, which correlated with reduced performance in fine motor, working memory, and executive function tasks. Such subclinical motor and non-motor dysfunction often precede the onset of Parkinson’s disease, and are predictive of dopaminergic neurodegeneration. These findings suggest that the neurological underpinnings in PD, manganism, and WF-mediated PD-like manifestation encompasses much more than degeneration of the nigrostriatal dopaminergic pathway and involves brain areas associated with sensorimotor, fine motor, and cognitive tasks, such as the cortex, thalamus, and cerebellum. Here, we examined the effects of WF on the frontal cortex (FCT, including pre-frontal cortex), parietal cortex (PCT, including motor cortex), and thalamus (THL, including subthalamic nucleus) of rats to determine if it instigates neurochemical and synaptic changes that are predictive of sensorimotor and cognitive impairment. Sprague-Dawley rats (male, 3 months old) were exposed by whole-body inhalation to fumes (4 - 6 mg/m³; 3 hours/day × 4 days/week × 5 weeks; for a total of 20 days) generated by gas metal arc-stainless steel (GMA-SS / WF), humanely euthanized at 1, 7, 28 or 112 days-post exposure, and brain areas collected. WF upregulated (1.4 to 1.7-fold) the mRNA transcripts for interferon-gamma (Ifng), inducible nitric oxide synthase (Nos2), matrix metalloproteinase-9 (Mmp9), and dimethylarginine dimethylaminohydrolase-1 (Dma1), while reduced dystrobrevin-18 member A2 (Sib18a2 / Vmat2) in FCT in day 1, suggestive of neuroinflammation and altered monoamine neurotransmitter signaling. Small decrements (10 - 20 %) in norepinephrine (NE) and serotonin (5-HT) were detected in the FCT at 7, 28, and 112 days. Reduced (22 - 30 %) NE, 5-HT, and dopamine (DA) were also seen in THL at 28 days and remained persistently lower at 112 days. Synaptophysin 1 protein increased (43 %), while ubiquitin C-terminal hydrolase L1 (UCHL1 / PARK5) protein levels decreased (44 %) in the FCT at 28 days. The FCT, primarily the prefrontal cortex, is known to coordinate and regulate cognitive tasks, including working memory, decision-making, attention, and learning. NE, DA, and 5-HT principally modulate the pre-FCT, whereas DA and 5HT are involved in sensorimotor function, and they may contribute to dopaminergic neurodegeneration. As dysregulation of corticothalamic region, linked to subcortical and non-motor symptoms, often precedes clinical motor signs, it may provide early insight into the neurodegenerative process. More research is necessary to identify biomarker signatures linked to sensorimotor and cognitive impairment that can aid early detection, intervention, and prevention of motor dysfunction associated with welding and Mn exposure.

Aberration of Corticothalamic Brain Regions in Rats Exposed to Welding Fumes


Variable Activity of Lead (Pb) on Inflammatory Cytokine Gene Expression in SIM-A9 Murine Microglia (MC) Stimulated with Endotoxin

A. Abdelnaser, M. H. Yousef, and H. A. El-Fawal. American University in Cairo, New Cairo, Egypt.

Despite regulatory restrictions, Pb continues to pose a risk to human health, particularly the nervous system (NS). Pb has been shown to induce oxidative stress, impair neural signaling, and cause death of the host neuronal populations. Given its environmental ubiquity, Pb poisoning accounted for 50% of the deaths due to exposure to chemicals in 2019 and 30% of idiopathic cognitive impairment, according to the WHO. While MC play a protective role in the CNS, their levels affect neuronal circuits involved in cognitive processing. In conjunction, increased DRD2 expression is linked to poor cognitive performance. Together, our findings support the notion that a complex interplay of overlapping neural circuits, primarily involving nigrostriatal, cortical, thalamic, and cerebellar tracts are critical for eliciting key motor and non-motor symptoms in PD, and perhaps Manganese, as well as welding-mediated PD-like manifestation. As dysregulation of corticothalamic region, linked to subcortical and non-motor symptoms, often precedes clinical motor signs, it may provide early insight into the neurodegenerative process. More research is necessary to identify biomarker signatures linked to sensorimotor and cognitive impairment that can aid early detection, intervention, and prevention of motor dysfunction associated with welding and Mn exposure.

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expression of genes involved in synapse function and neurotransmitter transpor-
tation. Metagenomic shotgun sequencing data showed that the dysbiosis in in-
fecal microbiome was observed 1 week prior to the onset of learning and memory
deficits. Most notably, over the time course, Cd exposure produced a sustained
decrease of the succinate and acetate-producing A. muciniphila - a producer of the
short chain fatty acid (SCFA) acetate and succinate (a precursor of butyrate), as well
as a sustained increase of a commensal human gut microbe associated with
cognitive deficits and is known for its role in secondary bile acid synthesis.
At the terminal time point, targeted metabolomics showed a decrease of the
neuroprotective short-chain fatty acids acetate and butyrate, as well as an
increase in secondary bile acids (taurine-conjugated (T-) omega muricholic acid,
T-deoxycholic acid, and T- urso-deoxycholic acid), at distinct bio-compartments of
the Cd-exposed mice. Furthermore, butyrate and succinate supplementation rescued
the Cd-induced cytotoxicity in primary cultured adult neural stem cells.
Together, our findings showed that Cd exposure induced gut dysbiosis before the
onset of cognitive deficits in mice, whereas restoring the neuroprotective SFCA
rescued the Cd-induced cytotoxicity in primary cultured adult neural stem cells.
In this study, we examined the effects of developmental exposure to cadmium (Cd)
alone and in a mixture with the model PAHs benzo-a-pyrene (BAP) or fluoranthene
(FA) (0-1.0µM) (Study 1), or binary CD-PAH mixtures during the first five days post-
fermentation (R2, QSM) signal and iron concentrations in a phantom and comparing
them to healthy control subjects. A cylindrical phantom containing nine 15 mL vials of
different concentrations (0.5, 1.0, 2.0, 3.5, 5.0, 7.5, 10.0, 25.0, and 50.0 mM) of iron chloride (FeCl3) diluted with a 1% agar solution was
constructed and imaged on a 3T Siemens Prisma MRI scanner. Quantitative
R2* relaxation maps (Mapit, Siemens Healthcare) were acquired using a multi-echo
gradient-echo (mGRE) sequence. QSM maps were generated using the
open-source software QSMxT using the magnitude and phase images from the
same R2* scan. For the in vivo study, twenty stainless-steel welders (19 M, 1 F, age
range = 45±11 yrs) and 13 controls (8 M, 5 F, age range = 42±12 yrs) were recruited
from a local truck trailer manufacturer and university, respectively. R2* and QSM
images were acquired with the same MRI sequences as in the phantom study.
ROI's were drawn bilaterally on the globus pallidus (GP) and substantia nigra (SN)
to obtain the mean signal intensity. The average mean values were then used for
statistical analysis. In the phantom study, both methods show a strong positive
linear relationship between QSM and R2* signal and increasing Fe concentra-
tions. However, QSM and R2* values were acquired with the same MRI sequences as in the phantom study. The relationship
between QSM and R2* values was strong in the GP for the welder group (R2 = 0.88,
p < 0.001) and moderate in the control group (R2 = 0.75, p = 0.0034). The correlation
between the two contrasts in the SN was not as strong in both the welder group
(R2 = 0.32, p = 0.17) and the control group (R2 = 0.69, p = 0.009). However, it remained
statistically significant. To determine if t-tests revealed statistically significant differences
between welders and controls for QSM values in the SN (p = 0.015). In contrast, R2* values
were statistically different in both the GP (p = 0.004) and SN (p < 0.0001). Overall,
both imaging sequences show strong linear relationships between R2*, QSM, and
iron concentrations. In vivo results show better agreement in the GP, most likely
due to the lesser uniformity of this brain area which potentially decreases suscept-
iblity to artifacts. The R2* values showed statistically significant differences
between the welder and control groups in both brain regions, while the QSM values
only showed a statistically significant difference in the SN. In this study, QSM is
not superior to the gold standard of R2* for measuring brain iron levels in toxicol-
ogy studies. This study was supported by the RO1 E032478 and the International
Manganeese Institute.

### 4574 Neurobehavioral Toxicity of Developmental Cadmium and PAH Mixtures in Zebrasfish

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Developmental exposure to a group of combustion products known as polycyclic aromatic hydrocarbons (PAHs), or to heavy metals, have been shown to cause
perturbation of gene expression and neurobehavioral effects in multiple species. However, interactions between these compounds have not been assessed, despite the fact that air and water pollution frequently contain both classes of contaminants. In two companion studies, we examined the effects of developmental exposure to cadmium (Cd)
alone and in a mixture with the model PAHs benzo-a-pyrene (BAP) or fluoranthene
(FA) (0-1.0µM) (Study 1), or binary CD-PAH mixtures during the first five days post-
fermentation (dpf). In Study 1, single exposures to BaP reduced increased swimming
activity in the dark at 6 dpf. Co-exposure with Cd blocked this effect and caused
a significant BaP-induced hypoactivity to be expressed in the light. At 3 months
of age (adolescence), single exposure to BaP caused locomotor hyperactivity in the
novel tank, but co-exposure with Cd attenuated this effect. In the tap water
trials, BaP generally reduced pre-tap activity, and Cd did not alter this pattern. In
Study 2, in contrast, the effects of FA and Cd did not interact with one another. The
lower concentration of Cd (0.1µM) caused hyperactivity in the dark during the
6 dpf motility test, while 1.0µM FA caused generalized hypoactivity, and each of these
patterns was equally evident across binary mixtures. At 3 months FA generally
reduced the diving response in the novel tank and activity in the tap test without
altering the magnitude of the startle response. Taken together, these data indicate
that Cd exposures can interact with neurotoxic PAHs and influence the expression
of PAH-related deficits, but that these interactions may not be relevant to all PAHs.
PAHs which primarily act as AHR agonists, like BaP, may be particularly sensitive
to these interactions, while PAHs acting as CYP1a inhibitors, like FA, may not. Further
research at later life stages is ongoing. This work emphasizes the need for detailed
risk assessments of mixtures containing contaminants of differing classes, and for
clarity on the mechanisms which allow cross-class toxicant interactions to occur.
Research supported by the Duke University Superfund Research Center (ES010356).

### 4575 Comparison of MRI Methods for Iron Imaging in the Human Brain

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Iron (Fe) is a fundamental element in many normal brain physiological processes
involving neurotransmitter synthesis and metabolism, mitochondrial respiration,
and myelin synthesis as components of certain enzymes. While Fe homeostasis
needs to be maintained, abnormal iron accumulation can be toxic and has
been associated with pathological changes, e.g., neurodegeneration. Fe is also a signifi-
cant constituent of welding fumes and shares similar chemical characteristics with
manganese (Mn); however, Mn is less harmful. By visualizing brain deposition and
metabolism associations with Mn-induced toxicity are inconsistent and scarce. Due to the paramagnetic
properties of Fe, MRI can be used to estimate brain iron non-invasively and has
great potential as a complementary tool to study altered metal-status homeostasis.
Specifically, the effective transverse relaxation rate (R2*) and quantitative suscepti-
bility mapping (QSM) are two MRI contrasts used to quantify Fe concentrations
in the body. However, which method is more specific and sensitive to changes in
Fe concentrations is not yet clear. This study aimed to characterize the quantita-
tive dynamic range of these two methods by assessing the relationship between
R2* and QSM signal and iron concentrations in a phantom and comparing them
in vivo in a cohort of steel welders and healthy controls. A cylindrical phantom
containing nine 15 mL vials of different concentrations (0.5, 1.0, 2.0, 3.5, 5.0, 7.5,
10.0, 25.0, and 50.0 mM) of iron chloride (FeCl3) diluted with a 1% agar solution
was constructed and imaged on a 3T Siemens Prisma MRI scanner. Quantitative
R2* relaxation maps (Mapit, Siemens Healthcare) were acquired using a multi-echo
gradient-echo (mGRE) sequence. QSM maps were generated using the
open-source software QSMxT using the magnitude and phase images from the
same R2* scan. For the in vivo study, twenty stainless-steel welders (19 M, 1 F, age
range = 45±11 yrs) and 13 controls (8 M, 5 F, age range = 42±12 yrs) were recruited
from a local truck trailer manufacturer and university, respectively. R2* and QSM
images were acquired with the same MRI sequences as in the phantom study.
ROI's were drawn bilaterally on the globus pallidus (GP) and substantia nigra (SN)
to obtain the mean signal intensity. The average mean values were then used for
statistical analysis. In the phantom study, both methods show a strong positive
linear relationship between QSM and R2* signal and increasing Fe concentra-
tions. However, QSM and R2* values were acquired with the same MRI sequences as in the phantom study.
These findings not only illuminate a valuable therapeutic target but
highlight a possible interaction between the HD protein and glucose transporters.
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more particles in the brain tissues, owing to age related increase in the permeability of blood brain barrier, and show higher neurotoxicity. To achieve this aim, PM sample was collected from 23 indoor/outdoor sites at Taranto (Southern Italy), a region known to be significantly impacted by air pollution from steel manufacturing, with epidemiological studies highlighting neurotoxicity among the residents of this area. Total elemental concentrations of the homogenized PM sample (< 74 µm) was analyzed using X-ray fluorescence (XRF) microscopy at the National Synchrotron Light Source II (NSLS-II), while the speciation of particulate matter was determined using X-ray absorption spectroscopy (XAS). XRF microscopy showed a significantly higher number of metal hotspots in the telencephalon of exposed killifish compared to the unexposed controls. Speciation analysis confirmed the presence of magnetite (Fe₃O₄) and titanium (TiO₂) in the brain tissues, with similar ratios of elements such as titanium and vanadium to that of the PM sample, confirming the deposition of these elements within the brain tissues. The presence of these elements suggests a role in the neurotoxicity and neuroinflammation observed in the brain.

The world’s population is aging at an unprecedented rate, with an estimate for 2030 that 20% of the U.S. population will reach a geriatric age and will live 20-40 more years as geriatrics. This swelled aged population poses a significant and immediate challenge to healthcare infrastructures due to the increased prevalence of age-related diseases and comorbidities. Exacerbating this challenge is the ubiquitous environmental pollution, which threatens the health of people at any age and can also accelerate biological aging. There are critical knowledge gaps regarding how geriatric populations may be especially vulnerable to environmental pollutants and how these chemicals contribute to biological aging, necessitating a greater need for research at the intersection of aging and toxicology. To tackle this two-sided dilemma, we use the metaphor of a “toxic aging coin.” On the heads side, we consider how age (young, middle-aged, geriatric) affects the toxic outcomes of a chemical. On the tails side, we consider how chemicals accelerate biological aging of the geriatric population; i.e., how chemicals contribute to gerontogenic mechanisms. To assess what qualifies chemicals as gerontogens, we measure their effects on the hallmarks of aging. We propose a gerontogenic mechanism progressing from DNA damage to genomic instability to cellular senescence. Genomic instability is considered the strongest etiological factor for aging, though how it contributes to aging remains largely unknown. Heavy metals are likely candidates as gerontogens. We chose to study hexavalent chromium (Cr(VI)) as it has the best described clastogenic mechanism of the heavy metals. Importantly, Cr(VI) is prevalent in industrial applications and waste effluents contributing the majority of Cr(VI) environmental pollution. Its widespread pollution, alongside its well-defined clastogenic effects, makes it ideal for our gerontogenic mechanism. Since Cr(VI) has been shown to induce reactive oxygen species (ROS) in various cell types, there is a significant lack of data regarding the neurotoxic effects of Cr(VI), and there are currently no federal or state regulations protecting against Cr(VI) neurotoxicity. Hence there is a critical need for research into the neurotoxic and gerontogenic effects of Cr(VI). We propose Cr(VI)-induced DNA double strand breaks promote genomic instability, and subsequent cellular senescence in brain cells. We also use mouse and human immortalized neuronal cell lines to assess this gerontogenic mechanism. Both cell lines were isolated from a glioblastoma and only differ in the fact that M059J cells spontaneously mutated to lose DNA-protein kinase (DNA-PK), a key protein for the repair of DNA double strand breaks. M059K cells still maintain functional DNA-PK and are proficient in DNA double strand break repair. Thus, this pair of cell lines is an ideal model to isolate the effects of DNA repair deficiency on the hallmarks of aging. Cells are treated with sodium chromate (0.1-1 µM) for 5 or 10 days. Our results indicate Cr(VI) induces cytotoxicity and growth arrest in both cell lines, with M059J cells more sensitive to Cr(VI). Ongoing work examines the cellular senescence markers p16 and p21 to confirm the induced growth arrest is a result of senescence. Additionally, examining immunofluorescence of HAAX will link the induction DNA double-strand breaks with senescence. This work is supported by R21ES033327 (JWPJr) and R35ES032876 (JWP Jr).

In fish and shellfish. Compelling evidence identifies both dopaminergic (DAergic) and glutamatergic (Gluergic) neurons as targets of MeHg-induced neurotoxicity, commonly associated with oxidative stress damage and mitochondrial dysfunction. In addition to altering mitochondrial membrane potential, MeHg can impair the oxygen transport chain and affect ATP synthesis by reacting with proteins in mitochondria. Despite their significance as measures of MeHg neurotoxicity, the in vivo effects of MeHg on mitochondrial function and their interconnections remain unclear. In this scenario, the present work sought to evaluate molecular and mitochondrial mechanisms of MeHg-induced neurotoxicity in C. elegans. Worms of the N2 strain (Bristol, wild type) were exposed to 0, 0.5, and 5 µM of MeHg during development (48 hours, L1-L4 stage). As reported previously by our laboratory, after MeHg exposure during 48 hours there was no significant change in lifespan, nonetheless, a development delay was observed following MeHg exposure. In addition, at day 1 of the adult stage, DAergic neuronal morphology was altered by MeHg exposure in the BZ555 (egl1 [datp1::GFP]) strain, manifested by large dots in dendraites, concomitant with morphological alterations or interruptions in dendritic extensions. To evaluate reactive oxygen species (ROS) production, 2,7-dichlorofluorescein diacetate (H₂DCFDA, total ROS) and MitoTracker red CMXRos (mitochondrial ROS) were used. MeHg exposure led to increased ROS levels of both markers at day 1 of the adult stage. Further, mitochondrial morphology was evaluated using confocal microscopy in GL390 [acc2-2::gfp] strain worms exposed to MeHg. An increase in the quantity of punctate or circular mitochondria and a decrease in tubular, interconnected filamentous objects at the Sum MtrH treatment were observed. Since mitochondrial dynamics is a crucial mechanism in mitochondrial function and health, we performed an analysis of Dynamin-Related Protein 1 (drp-1) role in MeHg toxicity. Worms of the strain EU2917 (dnap1::GFP) were used for drp-1 evaluation by confocal microscopy. At day 1 of the adult stage, an increase in the fluorescent intensity of drp-1::GFP warrants after the 5 µM MeHg exposure. Taken together, these results show evidence of altered ROS homeostasis and mitochondrial dynamics underlying MeHg toxicity in C. elegans. Supported by NIH/NIEHS R01 ES073311 (ABB, MA).

Methymercury (MeHg) is an environmental pollutant that causes neuropathological alterations in the developing and mature central nervous system. Despite extensive research over the past decades, the molecular mechanisms mediating MeHg neurotoxicity are still not fully clarified. Our study aimed to evaluate the antioxidant defense, and disruption of calcium homeostasis are mechanisms associated with MeHg toxicity. Protein Tyrosine Phosphatase 1B (PTP1B) is a member of the protein tyrosine phosphatases (PTPs) superfamily. PTP1B acts as a direct negative regulator of several receptors and receptor-associated tyrosine kinases. The knock-down of PTP1B is associated with the pathophysiology of several diseases. Recently, PTP1B has attracted attention in the field of neuroscience since PTP1B inhibition emerged as a promising strategy to prevent neurodegenerative and neuroinflammatory processes. Here, we explored PTP1B as a potential therapeutic target against MeHg-induced neurotoxicity in a neuronal cell model. We transfected immortalized neuronal hypothalamic GT1-7 cells with lentivirus containing murine PTP1B shRNA to generate a stable cell line. Then, we exposed these cells to 5 µM MeHg for 0, 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours. This concentration of MeHg will lead to accumulation of 25 µM protein, capturing the cytotoxic threshold reported in mammalian. Our data show that the knock-down of PTP1B exacerbated MeHg-induced protein and gene expression of heme oxygenase-1. Similarly, PTP1B knock-down lead to a gene expression of NAD(P)H quinine dehydrogenase 1 (NQO1) and some inflammatory genes, such as interleukin 6 (IL6) and IL1β. In contrast, PTP1B knock-down inhibited tumor necrosis factor alpha (TNFα) gene expression. Overall, our novel findings show that PTP1B knock-down may mediate protection against MeHg toxicity by inducing antioxidant and anti-inflammatory defenses. Supported by NIEHS R01 ES073311.

In vitro Inhalation Toxicity Assay of Lambda-Cyhalothrin 5% EC Using EpiAirway
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Lambda-Cyhalothrin 5% EC (LC), a crop protection product, may induce damage to the respiratory tract of the exposed spray operator or bystander via the inhalation route. Inhalation toxicity testing is used to identify potential hazards for such products. Conventionally, this was assessed using OECD TGs 403, 412, 413, 433 or 436. NAMs for respiratory toxicity have been accepted by US EPA and highlighted in an OECD Case Study. 1 The EpiAirway™ Model (AIR-100) is used as a part of the JRF strategy to develop alternative tests, in compliance with the principles of the 3Rs. MatTek developed the model to assess inhalation hazards of xenobiotics. The JRF strategy to develop alternative tests, in compliance with the principles of the 3Rs. MatTek developed the model to assess inhalation hazards of xenobiotics.
at 0.1, 0.25, 0.5, 1, 2.5 and 5 g/L in Dulbecco’s Phosphate Buffered Saline (DPBS) alongside standard controls (LDL) negative, vehicle (treated with DPBS), and positive (10 g/L formaldehyde in DPBS) controls to 6 tissues (per dose group). The tissues were incubated at 37°C in a 5% CO2 humidity saturated atmosphere. At 24 hours, LDH was assayed in the media. LC was removed with DPBS from the tissue surface and tissues processed for viability using the MTI viability assay. Vehicle and positive controls were included in the ALL controls for each test procedure reliability. LDH and TEER controls performed correctly. There was a dose dependent increase in cytotoxicity above an LC concentration of 1 g/L. The calculated IC50 and IC10 for LC were 1.59 and 0.97 g/L respectively. TEER was more sensitive showing a similar cytotoxic effect from 0.5 g/L. LDH was the least sensitive with cytotoxicity observed at the 2.5 and 5 g/L only. Slides were developed for histopathology, and similar observations were identified to the biomarkers in test and control tissues. In conclusion, the MatTek EpiAirway™ model can be used to identify hazards for inhalation toxicity for crop protection products. This assay may either be used as an alternative to the in vivo OECD tests or as a screening tool.

**4582 HUMITIPAA: A Bioinspired Robotic System for Predictive Inhalation Toxicology**

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Over the past decade the use of electronic nicotine delivery systems (ENDS) such as electronic cigarettes (ECs), has surged. In addition to health concerns for healthy adults, the use of ENDS among the youth population and those with underlying lung conditions is particularly of concern. Given the exponentially rapid pace and diversity of new vaping products entering the market, there is a pressing need for a platform that allows on-the-fly insight into potential pulmonary toxicity of these products. Thus, we created a novel biologically inspired robotic system - called Human Vaping Mimetic Real-Time Particle Analyzer (HUMITIPAA). HUMITIPAA generates fresh aerosols for any desired EC in a very controlled and user-definable manner and utilizes an optical sensing system to quantitate and analyze sub-micron and microparticles from every puff over the course of a vaping session in real-time while emulating clinically relevant breathing mechanics and vaping topography. We then utilized this platform for two clinically and society-wise relevant applications: to evaluate whether addition of vitamin E acetate (VEA) or menthol flavoring impacts the profile (size distribution and quantity) of inhaled aerosols, and whether this can correlate with clinical outcome. VEA has been strongly linked to outbreak of EC or vaping product use-associated lung injury (EVALI) that can cause severe respiratory illness and lead to death. On the other hand, toxic effects of menthol-flavored ECs are not fully understood. Using HUMITIPAA, we found that addition of as little as 1.25% VEA was sufficient to significantly enhance toxicity profile inhaled from an EC. Interestingly, nicotine levels inversely yet non-significantly correlated with particles generated from ECs. However, supplementation of VEA led to significant increase in vapor particles of ECs containing nicotine. In addition, by emulating obstructive and restrictive lung disorders breathing, we identified a similar trend in ability of VEA to enhance particle quantities in inhaled vapor and later size distribution. But, notably the magnitude of increase in total particle count due to presence of VEA was highest with the restrictive breathing profile, following sequentially with obstructive and normal breathing. We also discovered that addition of menthol flavoring to e-liquid base propylene glycol-vegetable glycerin leads to enhanced particle counts in all tested size fractions, similar to the effect of VEA supplementation. Similarity, menthol vs. non-menthol e-liquid from popular and commercial ECs using inhaled forced expiratory volume in the first second (FEV1) % predicted and FEV1/ forced vital capacity (FVC) independent of age, gender, race, pack-years of smoking, and use of nicotine or cannabis-containing vaping products. We anticipate HUMITIPAA to serve as a novel preclinical technological platform for evaluating pulmonary toxicity potential of emerging ECs.
mask and whether the NHP model was nasal or mouth breathing was performed to determine the concentration of CO 2 within 3 different mask designs. Providing 6 L/min to the mask using the CFD model with a TV of 30 mL, RR of 30 breaths per minute, 100% mouth breathing and 0% nasal breathing produced 2.5% CO 2 within a Philips Respironics® ProfileLite single port mask. The CO 2 decreased to 0.7% using dual ported masks (designated as 15-22 Mk1 and IPM). A similar CO 2 concentration (1.5%) was produced in the Philips Respironics® ProfileLite mask with 100% nasal breathing at the same TV and RR. Hyper-ventilating conditions (TV=15mL TV, RR=70 breaths per minute) and 100% mouth breathing, produced a CO 2 concentration of 2.4%. The results using the 15-22 Mk1 and IPM masks under these conditions gave CO 2 concentrations of 0.5% to 0.7%. Decreasing the airflow to 3 L/min from 6 L/min increased CO 2 within a Philips Respironics® ProfileLite mask to 2.8%; however, there was a marked increase with both dual ported 15-22 Mk1 and IPM masks to 1.4%. Increasing the airflow to 9 L/min from 6 L/min decreased CO 2 in the Philips Respironics® ProfileLite mask to 2.5%, however, there was a marked decrease with both dual ported 15-22 Mk1 and IPM masks to 0.4%. Intentionally raising or lowering the angle of the mask on the 3D printed NHP model by 7.5° did not result in a significant change in CO 2 concentrations from baseline in terms of ventilated CO 2 for the IPM mask. In conclusion, using a dual ported mask significantly reduces CO 2 concentration in the mask. Reduced CO 2 improves safety and the animal experience allowing for improved welfare and study integrity. However, decreasing mask airflow reduces the clearance in the mask and increases the CO 2 concentration, especially in the dual port designs.

4585 Myeloid Heterogeneity Mediates Air Pollution–Induced Acute Exacerbations of Pulmonary Fibrosis
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Epidemiological evidence indicates that exposure to particulate matter is linked to the development of idiopathic pulmonary fibrosis (IPF) and increases the incidence of acute exacerbations of IPF (AE-IPF). In addition to accelerating the rate of lung function decline, exposure to fine particulate matter (PM 2.5) is a risk factor for increased mortality in IPF subjects. Mice were intra-tracheally administered bleomycin and exposed to PM 2.5 for 4 days/day, 4 days/week for 4 weeks (80 day exposures). Bronchoalveolar lavage (BAL) was performed at 4 and 30 days after the last of the 4 day exposures to assess lung toxicity. BAL fluid lactate dehydrogenase (LDH) was measured as a marker of lung cell toxicity, and total recovered BAL cells were counted as an index of lung inflammation. Animal body weights were measured throughout the post-exposure period to assess general health. The TSC aerosols were generated in a closed spray booth and piped into an animal exposure chamber where they were collected as described. The number composition of each was determined by ICP-AES, including the stainless steel wire (PMET731) (99% Fe, 2% Cr), the Ni-based wire (PMET885 (97% Ni)), and the Zn-based wire (PMET540 (99% Zn)). The particles generated regardless of composition were poorly soluble, complex metal oxides that were arranged as chain-like agglomerates and were similar in size distribution with mass median aerodynamic diameters (MMAD) that ranged from 310 - 378 nm as determined by MOUDI. Inhalation of the Ni-based (PMET885) aerosol caused a significant decrease in body weight compared to the air control at all time points assessed post-exposure for 30 days, whereas the Zn-based (PMET540) and Fe-Cr stainless-steel (PMET731) aerosols had no effect on body weight post-exposure. Exposure to the Ni-based (PMET885) aerosol caused a significant increase in lung injury (BAL fluid LDH activity) and inflammation (total BAL cells recovered) at both 4 and 30 days after exposure. Inhalation of the Zn-based (PMET540) aerosol caused a slight but significant increase in BALF LDH and total BAL cells recovered at 4 but not at 30 days compared to air control. Exposure to the Fe-Cr stainless-steel TSC aerosol had no significant effect on lung toxicity post-exposure. Results of this pilot comparison study of different TSC aerosols indicate that varied lung responses (e.g., LDH > 2000 U/L) are likely dependent on the type of consumables used.

4586 Effect of Crystalline Silica and Welding Fume on Lung-Associated Gene Changes in the Rat
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A major source for the occupational diseases that result in significant morbidity and mortality is exposure to dust or fume containing toxic agents, such as silica and/or welding fumes. More than 424,000 workers in the U.S. and close to one million workers worldwide perform welding as part of their work duties. Because key contributors to the development of lung cancer in stainless steel welders. Our study, assessing the pulmonary toxicity outcome to a mixed exposure of welding fume and crystalline silica concluded that the combined exposure caused a greater expression of disease/innoculation markers and functional pathways associated with lung cancer. Taken together, these results suggest a potentially enhanced effect of the silica + welding fume exposure on genes associated with lung tumor and lung cancer development.

4587 Lung Toxicity in Rats after Inhalation of Aerosols Generated during Thermal Spray Coating Using Different Consumable Materials
NIOSH, Morgantown, WV.

Thermal spray coating (TSC) is an emerging industrial process in which molten metal is sprayed at a high velocity onto a surface as a protective coating. Little is known about the physical and chemical properties of the particles generated and the potential health associations associated with exposure to TSC aerosols. A computer controlled TSC generator and inhalation exposure system has been developed to perform animal studies to mimic workplace exposures. Male Sprague-Dawley rats were exposed at a concentration 1 million workers worldwide perform welding as part of their work duties. Because...
conditions appropriate for a specific study objective, including mouse strain, should be selected. In this study, we focused on mucus secretion and compared the responses to CS exposure among three different mouse strains, C57BL/6, A/J, and BALB/c mice. Female mice of each strain were whole-body exposed to filtered air (Air) or diluted mainstream CS from 1R6F Kentucky reference cigarettes for 5 days per week, up to 1 or 5 weeks. Exposure conditions were set based on our preliminary studies as follows. For A/J and BALB/c mice: 550 μg total particulate matter (TPM)/L for 2 or 4 hours. For C57BL/6 mice: 550 μg TPM/L for 2 or 4 hours, or 750 μg TPM/L for 4 hours. Body weights were assessed twice a week as indicators of CS tolerance. A decrease in body weight was observed only in A/J mice exposed to 550 μg TPM/L for 4 hours. In the C57BL/6 and BALB/c groups, body weights increased throughout the exposure period and these two strains were tolerant to CS exposure at the conditions. Mucus secretion and the effects on the airway tract were evaluated after the completion of exposure. The amount of mucus secreted into the bronchoalveolar lavage fluid (BALF) was measured by staining mucus plugs with Alcian blue and dose-dependent mucus secretion was observed in C57BL/6 and A/J, but not in BALB/c mice. Mucus secretion was increased in a dose-dependent manner in C57BL/6 mice after 5-week CS exposure while the index was less affected in A/J and BALB/c mice. Thus, increased mucus secretion was observed as early as 1 week after exposure whereas airway obstruction occurred after a longer duration of CS exposure in C57BL/6 mice. The number of free cells and inflammatory mediators in the BALF were measured. The numbers of neutrophils and secretion of inflammatory cytokines were increased in all three strains after CS exposure for 1 and 5 weeks. In conclusion, these results suggest that C57BL/6 mice are more sensitive to CS exposure than A/J and BALB/c mice. These results indicate the need to evaluate mucus secretion and related respiratory functions by CS exposure.

**4590** Comparison of the Toxicological Effects of Highly Volatile Inhaled Components of Artificial Butter Flavoring Using In Vivo Rodent and in Vitro Human Approaches

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Artificial butter flavoring (ABF) is used in the food and beverage industry, including in the processing and production of microwave popcorn. Highly volatile organic components (diketones) of ABF include 2,3-butanedione (diacetyl, DA), 2,3-pentanedione (PD), 2,3-hexanedione (Hex), and/or acetoin. Inhalation exposure of microwave popcorn plant workers to DA vapors has been associated with the development of small airway fibrosis in the form of obliterative bronchiolitis (OB). DA has been shown to induce alterations in the molecular pathways regulating mitochondrial function in lung epithelial cells. Recently, novel tobacco products such as Heated Tobacco Products (HTPs) have been marketed as harm reduction products, due to lower levels of toxicants in their emissions compared to cigarette smoke. However, the effects of HTP emissions on human airway epithelial cell metabolism and on the molecular mechanisms regulating mitochondrial biogenesis and mitochondrial-specific autophagy (mitophagy) are unclear. Therefore, in this study, human alveolar epithelial cells (AS549) were exposed to cigarette- or HTP emissions in form of liquid extracts for 24 hours. Both types of extracts similarly induced increases in basal, maximal and non-mitochondrial respiratory capacity. Moreover, we observed alterations in the abundance of regulators of mitochondrial biogenesis and mitophagy in response to both extracts. In conclusion, not only respiratory capacity but also key mitochondrial quality control mechanisms are similarly impacted in human alveolar epithelial cells in response to cigarette and HTP emissions. Which chemical compounds mediate these effects remains to be determined.

**4592** Assessing Toxicity of Microplastics on Airway Epithelial Cells Using Air-Liquid Interface Models

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Human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that human are estimated to be exposed to nearly a thousand micro- and nanoplastics (MNPs) per day, with inhalation as one of the main exposure routes. Evidencing this exposure, MNPs have been detected in human lung tissue. Recent studies indicate that...
cells (in mono or co-culture with endothelial (EA.hy926) and macrophage (5 days PMA differentiated THP-1) cells), bronchial BEAS2B cells or differentiated primary bronchial epithelial cells (PBECs; provided by PLUC facility MUMCH) from healthy donors. Successful differentiation of the PBECs was confirmed by an increase of transepithelial resistance (TEER) and an increase of transcript levels of differentiation genes. The different models were subsequently exposed for 24 hours to different concentrations of MNPs (100 μg/ml, 50 mg/ml, and 1 mg/ml) for 24 hours and 48 hours. For the dose-dependent modulations in representative CIPP-related emissions and alterations in toxicity endpoints, which can potentially inform the safe usage of CIPP technology.

Our laboratory has previously established that following multi-walled carbon nanotube (MWCNT) exposure, female mice develop greater M2a-type inflammatory signaling by alveolar macrophages (AMs) than male mice. Despite AMs being widely considered the primary responder to inhaled nanoparticles and responsible for directing subsequent inflammation, the mechanism responsible for this sex disparity in M2a inflammatory signaling remains unclear. Estrogen receptor (ER) signaling, cholesterol efflux, and oxidized derivatives of cholesterol (oxysterols) signaling, have all been reported to influence ER function and the subsequent sex-based outcomes observed in nanoparticle-in-ductated mice exposed to both H2S and IAV. Taken together, these results indicate that the female bias in MWCNT-induced M2a inflammatory signaling is due to estrogen-mediated enhancement of the M2a phenotype, which is achieved through the modulation of cholesterol metabolism and flux. To test this hypothesis, the ER antagonist Fulvestrant was given in vivo. Fulvestrant attenuated MWCNT-induced M2a development (STAT6 activation and Cxcl2, Chi3l3, Gata3, Il13 expression) and subsequent eosinophil recruitment to the airways of female mice. However, while AMs from Fulvestrant-treated males also displayed reduced MWCNT-induced M2a gene expression, there was no effect on eosinophil recruitment to the airways, suggesting an independent mechanism for the low-level inflammation observed in males. In support of the hypothesis that estrogen signaling modulates cholesterol metabolism and flux during type 2 inflammation, AM expression of cholesterol efflux transporter, Abcg1, and oxysterol synthesis enzyme, Ch25h, were only increased over controls in MWCNT-treated females, not MWCNT-males, and were decreased by Fulvestrant treatment. Accordingly, in vitro incubation with estrogen reduced cholesterol levels (indicative of efflux) while increasing M2a polarization in female, but not male, AMs. Lastly, bone marrow-derived macrophages from SR-BI KO mice, which have impaired cholesterol uptake and efflux, had diminished M2a polarization capabilities following both IL-4 and IL-13 treatment. Thus, the importance of cholesterol flux in M2a development. Taken together, these data provide foundational evidence for the contribution of estrogen-mediated regulation of cholesterol metabolism in AM inflammatory function and the subsequent sex-based outcomes observed in nanoparticle-induced respiratory disease. NIEHS R21ES030978-01.
such as IAV. It enhanced severity of inflammation and lung injury. This novel therapeutic strategy for this disease.

Silanes are widely used as reducing and coupling agents with applications in surface modifications. Because of their reactivity and rapid hydrolyzation, occupational exposure to silanes is possible in the production line. In this study, the INSPIRE Initiative (In vitro System to Predict Respiratory toxicity), human bronchial epithelial cells (BEAS-2B) and a reconstituted tissue model (MucAir™) were exposed to triethylsilane (TES) and trimethylsilane (TMS) as vapors, to predict the ability of these chemicals to cause portal-of-entry effects on the human respiratory tract. Three concentrations were tested for each silane in BEAS-2B cell line (TES: 1, 50, and 150 ppm, and TMS: 1, 25, and 85 ppm) and MucAir™ tissues (TES: 75, 150, and 300 ppm, and TMS: 25, 100, and 300 ppm). All exposures were performed for 30 minutes at the air-liquid interface (ALI) using a VITROCCELL™ 6/4 system and appropriate negative (sodium chloride, incubator control, or nitrogen gas) and positive (nitrogen dioxide) controls were used. Endpoints were assessed 19-24 hours (BEAS-2B and MucAir™) or seven days (MucAir™) after exposure and included cell viability (PrestoBlue™ assay), cytotoxicity (lactate dehydrogenase assay), and secretion of inflammatory markers (electrochemiluminescence immunoassay). For MucAir™ tissues, histology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance (TEER)), and cell beating frequency (CBF) and average active area (AAA) (SAVA system) were also included. In BEAS-2B cells, a dose-dependent response was observed for all endpoints for both silanes. 19-24 hours after exposure of MucAir™ tissues, the results showed decreased cell viability, TEER, and AAA, and an increase in cytotoxicity, inflammatory response, and CBF for all concentrations of both silanes. Seven days after exposure, a further decrease in cell viability and AAA was observed and inflammatory response and CBF remained elevated indicating that silane exposure to silanes was substantial. Interestingly, barrier integrity was restored back to pre-exposure values. The results from both in vitro systems indicate that TMS is more toxic than TES, which is expected based on chemical properties and existing data. Studies are underway to assess additional test chemicals and compare ALI exposure setups with a 3D model to determine the usefulness of in vitro systems in assessing the impact of chemicals on the human respiratory tract and informing regulatory decision-making.

Exposure to pulmonary emmissions to maximize the use of solid surface composite (SSC) materials with power tools has been associated with adverse health effects in humans and laboratory animals. Previous in vitro and in vivo investigations of SSC toxicity have been limited by particle delivery methods that do not fully recapitulate the workplace environment. To represent a real-world particle exposure more accurately, our group constructed a chamber for simultaneous particle generation, characterization, and animal exposure. In order to determine lung deposition and clearance, 6-week-old male C57BL/6 mice were exposed to SSC particles for 4 hours (n = 9) or filtered air control in the exposure chamber. The mice were sacrificed at seven days post-exposure and lungs were divided into two subsets. In one subset, (n = 6), whole lungs were collected and analyzed for aluminum content using inductively coupled plasma atomic emission spectroscopy. In the other subset, (n = 3), in the right lobe was neutral buffered formalin for histopathology, while the left lobe was snap frozen and kept at -80ºC for later molecular analyses. The exposure apparatus was successful in generating inhaled SSC particles, and for this reason that the mechanism of silane exposure to silanes is possible in the production line. In this study, the INSPIRE Initiative (In vitro System to Predict Respiratory toxicity), human bronchial epithelial cells (BEAS-2B) and a reconstituted tissue model (MucAir™) were exposed to triethylsilane (TES) and trimethylsilane (TMS) as vapors, to predict the ability of these chemicals to cause portal-of-entry effects on the human respiratory tract. Three concentrations were tested for each silane in BEAS-2B cell line (TES: 1, 50, and 150 ppm, and TMS: 1, 25, and 85 ppm) and MucAir™ tissues (TES: 75, 150, and 300 ppm, and TMS: 25, 100, and 300 ppm). All exposures were performed for 30 minutes at the air-liquid interface (ALI) using a VITROCCELL™ 6/4 system and appropriate negative (sodium chloride, incubator control, or nitrogen gas) and positive (nitrogen dioxide) controls were used. Endpoints were assessed 19-24 hours (BEAS-2B and MucAir™) or seven days (MucAir™) after exposure and included cell viability (PrestoBlue™ assay), cytotoxicity (lactate dehydrogenase assay), and secretion of inflammatory markers (electrochemiluminescence immunoassay). For MucAir™ tissues, histology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance (TEER)), and cell beating frequency (CBF) and average active area (AAA) (SAVA system) were also included. In BEAS-2B cells, a dose-dependent response was observed for all endpoints for both silanes. 19-24 hours after exposure of MucAir™ tissues, the results showed decreased cell viability, TEER, and AAA, and an increase in cytotoxicity, inflammatory response, and CBF for all concentrations of both silanes. Seven days after exposure, a further decrease in cell viability and AAA was observed and inflammatory response and CBF remained elevated indicating that silane exposure to silanes was substantial. Interestingly, barrier integrity was restored back to pre-exposure values. The results from both in vitro systems indicate that TMS is more toxic than TES, which is expected based on chemical properties and existing data. Studies are underway to assess additional test chemicals and compare ALI exposure setups with a 3D model to determine the usefulness of in vitro systems in assessing the impact of chemicals on the human respiratory tract and informing regulatory decision-making.

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4603: Acet-1 Inhibition Limits Pulmonary Fibrosis

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The enzyme Acet-1/Soa1 catalyzes the conversion of cholesterol to cholesterol esters, a critical step leading to intracellular lipid droplet formation. In previous studies of acute lung injury, we have observed large, lipid-laden cells, which correlate with an increase in pulmonary acellular matrix (PAM) and mature, pro-fibrotic interstitial macrophages (IM). This macrophage phenotype was reduced with the administration of the Acet-1 inhibitor K-604 in vivo. As persistent activation of macrophages is known to contribute to fibrotic processes, we hypothesized that limiting cholesterol esterification through Acet-1 inhibition would reduce the persistence of activated macrophages and limit the progression of pulmonary fibrosis. To induce fibrotic changes in the lung, male and female wild-type mice (C57BL6/J, n = 6-7/group) received an intraperitoneal (IP) injection of bleomycin (IPB, 0.1 U/200 µl) every 3 days from d0-15, with corresponding saline control group (IP, 200 µl). Intratracheal saline or K-604 (10 mg/kg) was administered every 3 days from d10-15. IPB and saline were instilled into the lungs of mice to establish a model of lung injury and fibrosis. Pulmonary macrophages were collected from the lungs by bronchoalveolar lavage (BAL) and analyzed by flow cytometry and intracellular staining. The effects of K-604 were compared with those of controls, with K-604 treatment significantly decreasing the percentage of mature, pro-fibrotic IM (n=7 donors) and Cch-induced MLC phosphorylation (0.550±0.109 of control, p<0.005, n=4 donors). DCPIB attenuated TGF-β1 (0.706±0.061 of control, p<0.005, n=6 donors; 0.687±0.025 of histamine, 0.654±0.037 of histamine, p<0.005, n=8 donors) and Cch-induced MLC phosphorylation (0.706±0.061 of control, p<0.005, n=4 donors). SiRNA-mediated knockdown of LRRC8 reduced histamine-induced MLC phosphorylation (0.600±0.053 of control, p<0.01, n=7 donors) and Cch-induced MLC phosphorylation (0.550±0.109 of control, p<0.005, n=8 donors). These findings were unassociated with agonist-induced cytosolic Ca²⁺. However, DCPIB pretreatment attenuated histamine-induced MLC phosphorylation (0.600±0.053 of control, p<0.01, n=7 donors) and Cch-induced MLC phosphorylation (0.550±0.109 of control, p<0.005, n=8 donors). SiRNA-mediated knockdown of LRRC8 reduced histamine-induced MLC phosphorylation (0.600±0.053 of control, p<0.01, n=7 donors) and Cch-induced MLC phosphorylation (0.550±0.109 of control, p<0.005, n=8 donors). These findings were unassociated with agonist-induced cytosolic Ca²⁺. However, DCPIB treatment significantly attenuated agonist-induced MLC phosphorylation, a key mediator in excitation-contraction (EC) coupling (0.733±0.121 of Cch, p<0.05, n=6 donors; 0.678±0.025 of histamine, p<0.05, n=4 donors). DCPIB attenuated TGF-β1 (0.706±0.061 of control, p<0.005, n=6 donors) and Cch-induced MLC phosphorylation (0.706±0.061 of control, p<0.005, n=8 donors). SiRNA-mediated knockdown of LRRC8 reduced histamine-induced MLC phosphorylation (0.600±0.053 of control, p<0.01, n=4 donors). These findings were unassociated with agonist-induced cytosolic Ca²⁺. However, DCPIB concentration-dependently attenuated Cch-induced Akt (0.639±0.053 of control, p<0.01, n=4 donors) and MYPT1 phosphorylation (0.999±0.002 of control, p<0.01, n=4 donors). Our findings suggest inhibition of LRRC8 activity attenuated the contractile and migratory responses in HASMCs, complementing reductions in agonist-induced MLC phosphorylation. This introduces a novel mechanistic link between LRRC8 activity and asthma progression through altering the function of HASMCs and EC coupling. Supported by NIH P01 HL114471(RAP), UL1 TR003017, and 2T32ES007148-36.

4604: Macrophage-Specific ARNT Signaling Mediates Acrrolein-Associated Acute Lung Injury

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Acrrolein is a potent immunomodulatory respiratory toxicant that promotes acute lung injury (ALI), however, the mechanisms remain to be fully characterized and no treatments currently exist. Pulmonary macrophages are essential for initiating and resolving lung inflammation and are thought to be key drivers of ALI. Additionally, the aryl hydrocarbon receptor nuclear translocator (ARNT) is a crucial modulator of immune homeostasis via obligate cooperation with aryl hydrocarbon receptor (AhR), hypoxia inducible factor (HIF), and NF-kB transcription factors. Accordingly, macrophage specific ARNT signaling could dictate the intensity and duration of the immune response by altering cytokine production and immune cell recruitment, and therefore severity of ALI, after acute acrrolein exposure. Interestingly, ARNT is alternatively spliced into isoform 1 and isoform 3 to promote pro-or anti-inflammatory responses, respectively, and presents a potential target for therapies to treat acrrolein-associated ALI. To investigate the impact of the ARNT isoforms on the initiation of the inflammatory response after acrrolein exposure, 16-week-old male and female transgenic mice that overexpress Arnt-a (murine homolog of human ARNT) were dosed intratracheally with acrrolein (Acr), hypoxia inducible factor (HIF), and NF-kB transcription factors. Accordingly, macrophage specific ARNT signaling could dictate the intensity and duration of the immune response by altering cytokine production and immune cell recruitment, and therefore severity of ALI, after acute acrrolein exposure. Interestingly, ARNT is alternatively spliced into isoform 1 and isoform 3 to promote pro- or anti-inflammatory responses, respectively, and presents a potential target for therapies to treat acrrolein-associated ALI. To investigate the impact of the ARNT isoforms on the initiation of the inflammatory response after acrrolein exposure, 16-week-old male and female transgenic mice that overexpress Arnt-a (murine homolog of human ARNT) were dosed intratracheally with 2 mg/kg acrrolein and sacrificed 24 hours later. Transcriptomic changes attributed to sex, genotype, and treatment were assessed in isolated BAL macrophages, and the inflammatory response was further characterized using differential BAL cell counts, lung histology, and BioPlex cytokine arrays. Transcriptomic analysis revealed sex- and genotype-specific differences in NF-kB and HIF target gene expression after acrrolein exposure, which was further confirmed by alterations in pro- and anti-inflammatory cytokines, chemokactrant proteins, and overall immune cell landscape. Markers of ALI were then measured in the lungs of age-matched animals 5 days post-exposure to assess ARNT isoform specific effects on the inflammatory response. Interestingly, BAL cell counts in female Arnt-a Tg mice reveal persistent neutrophil and lymphocyte numbers that correspond with higher ALI scores relative to non-carrier (NC) controls. Neutrophil and lymphocyte populations were not observed in male Arnt-a Tg or NC mice on day 5, however, Arnt-a Tg males had lower ALI scores relative to NC. Taken together, these results support...
With the legalization of cannabis occurring throughout the world, cannabis use is increasing. Numerous products are now available, including concentrates and distillates for use in vaporizers which are becoming an increasingly popular method of cannabis use for consumers. This includes cannabis vape cartridges that utilize e-cigarette technology to heat the cannabis distillates to form aerosols that are inhaled into the lungs. Cannabis distillates are a purified form of cannabis that typically contain specific compounds from the cannabis plant, particularly the cannabinoid tetrahydrocannabinol (THC), which is the psychoactive component. Despite their growing popularity, the pulmonary effects of cannabis distillates that are inhaled are completely unknown. Therefore, the purpose of this study was to address this gap in knowledge by creating a standardized mouse model for vaporized THC distillate exposure. The objective of this study was to determine whether it is chemically or radiologically induced. This work investigated the in vitro effects of well characterized (<10 μm) depleted (DU), natural (NatU), and highly enriched uranium oxide (UO₂) particles in immortalized (A549) and primary human lung cells (HBTEC and HSAEC). Evaluating cytotoxic effects, biomarker changes, and uranium speciation at various levels of exposure provided an integrated approach to distinguish the radiological and chemical toxicity of internal uranium exposure. We investigated viability, reactive oxygen species, and DNA breakage. Results show that despite the varying isotopic content of UO₂ exposed A549 cells responded similarly with increasing cell death as concentrations of material increased. IC (50)’s were determined with the viability data for each isotopic ratio for A549 cells and primary human lung epithelial cells. Data indicate that the chemical toxicity of the metal exposure may be a stronger effect than the radiological toxicity for UO₂ particulates.
Airway hyperresponsiveness (AHR) and mucus hypersecretion are hallmarks of airway diseases. Exposure to common household allergens and heavy metals acts as stimulator of immune inflammatory response contributing to the severity or existence of chronic airway diseases. Heavy metals like cadmium (Cd) are associated with increased AHR and can contribute to the pathology of existing diseases or infections. Cd exposure can be through cigarette smoke extract (CSE), diesel exhaust or any other occupational and environmental sources. CSE induces mucus production in airway epithelial cells contributing towards dysregulation of air surface liquid homeostasis. Chloride efflux channel plays an important role in maintaining air liquid homeostasis in the airways. In this study, we demonstrate cadmium mediated regulation of ANO1 which is critical for mucus production in airway diseases. Six-to-eight-week-old male CD-1 mice were exposed to Cd extract (CE) through intranasal administration. Sensitization and challenge were achieved with saline, CE alone or CE and Cd together for 5 days. AHR was assessed 24 hours after the last allergen challenge. Lung histology and BAL analysis was performed after 48 hours after the last allergen exposure. ANO1 expression and mucus production were estimated in lung whole cell lysate and airways. Saline exposed mice were used as controls. CE and Cd co-exposure during sensitization and challenge demonstrated increased AHR in mice. ANO1 expression was highly upregulated in mice exposed to CE or Cd alone. Compared to single allergen exposure CE and Cd co-exposure demonstrated significantly upregulated ANO1 expression. Mucus production was analyzed expression pattern of MUC5AC in lung homogenates and tissue sections. Increased MUC5AC intensity was observed in co-exposed mice. In conclusion, regulating ion channel/transporters to alter associated physiological functions is a suitable candidate for controlling the severe outcomes of allergic sensitization. Our results demonstrate that low-dose cadmium induced ANO1 expression may act as an additional mechanism of action exacerbating the airway diseases and accelerate adverse effects of household allergens.

Concentrations of carbon dioxide (CO2) have conventionally been considered a marker of indoor ventilation adequacy rather than a toxic hazard. In the U.S., the current permissible limit for CO2 is 0.05% (500 ppm) set by the Occupational Safety and Health Administration (OSHA). Concentrations of 1,000–2,500 ppm are frequently found in conference rooms, schools, and other poorly ventilated areas. However, in laboratory experiments at or below these levels, acute inhalation exposures to CO2 have produced impaired cognitive performance in humans. For instance, human subjects exposed to CO2, at 600, 1,000, and 2,500 ppm for 2 hours each in a chamber study showed significant declines in components of decision-making performance on the Strategic Management Simulation (SMS) test. Previously in rodent studies it has been suggested that CO2 exposure can lead to inflammation and mucus production. Human, non-immortalized cells were used to assess the role of CO2 in phosphorylating leukocytes. We proposed that in humans CO2 exposure results in vascular inflammation and PMN activation, which is associated with impaired cognitive function. In our blinded, randomized, cross-over design, healthy young volunteers (n=12) breathed filtered air containing CO2 either 600 ppm (control) or 2,500 ppm, for two 2.5-hour intervals one week apart in a controlled exposure facility while completing the decision-making SMS test. Each subject breathed CO2 before exposure and for 4 hours after the end of exposure. PMNs were isolated by differential centrifugation and examined for baseline oxygen consumption (OCR), maximal mitochondrial OCR, glycolytic capacity, and other markers related to PMN activation. Statistical significance (p<0.05) was assessed by paired t-test. Our results showed that CO2 exposure at 2,500 ppm produced significantly decreased scores across all 5 domains of the SMS test (i.e., task management, focused activity, information management, crisis responsiveness, and basic strategy). Only one of the 12 subjects scored higher on all 5 domains for the CO2 exposure at 2,500 ppm compared to the CO2 exposure at 600 ppm. In all subjects, CO2 exposure at 2,500 ppm significantly increased the baseline OCR of PMNs from 13 ± 2.9 pmol/min to 21 ± 3.3 pmol/min (p<0.001) and decreased the maximal mitochondrial OCR of PMNs by 20% (p<0.05). Glycolytic capacity in the PMNs did not significantly change from 37 ± 3.3 mEq/mg to 39 ± 3.2 mEq/mg (p>0.05). In summary, CO2 inhalation exposure increased PMN baseline oxygen consumption in the absence of external stimulation and separately from mitochondrial activity. SMS results showed a strong association between CO2 inhalation and cognitive performance. A likely explanation for these data is increased activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which will be further elucidated by this study’s planned assessment of NLR family pyrin domain containing 3 (NLRP3) inflammasome activity, oxidative burst, and oxygen consumption in the setting of phorbol myristate acetate (PMA). We concluded that these human data are supportive of a vascular inflammatory model as a mechanism of CO2-mediated cognitive dysfunction and add to the urgency of further investigation on the potential toxicity of this ubiquitous gas. Funding was provided by NIEHS grants P30ES005022, R21ES033777-01A1, and T32ES007148.

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IgG controls despite reduced BAL IL-17A concentration. Lung histology also did not show any difference in number of fibrotic bronchial lesions nor inflammatory airway aggregates between DA-exposed groups. BAL neutrophilia remained persistently elevated in DA-exposed rats treated with oltil-17A versus the control. The proportion of CD4+ and CD4+CD25+ T cell populations did not differ significantly between groups while the proportion of CD103+ regulatory T cells increased in IL-17A versus IgG controls (16-HBE). INF-γ, IFN-γ and IL-1β, following DA exposure and oltil-17A treatment. IL-17A neutralization failed to attenuate flavoring related lung disease progression. In contrast, BAL neutrophilia persisted with amplified pro-inflammatory signaling and lung a-SMA expression increased with IL-17A neutralization post-DA exposure. Our results suggest alternate pathways responsible for flavorings related lung disease progression with IL-17A neutralization. Future studies evaluating the lung’s innate immune system may elucidate the differential responses seen in pathogenesis between chemical inhalation exposure and other forms of BO development.

4613 House Dust Mite Allergen and Th2 Cytokine-Mediated Epithelial Barrier Dysfunction Attenuation by Rev-erb Agonists in 16-HBE Cells

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House dust mite (HDM) and Th2 cytokines (IL-4 and IL-13) drive allergic lung diseases such as asthma and allergic rhinitis resulting in altered epithelial barrier function and dysregulated immune response. Prior reports demonstrate the intricate role of circadian clock molecules that regulate epithelial barrier function and immune response. Rev-erb is also known as Nuclear Receptor Subfamily 1 Group D Member 1 (NR1D1), a key circadian molecule that regulates physiological processes such as inflammation, metabolism, and immune response. There are no studies currently available to demonstrate whether activation of Rev-erbs using specific synthetic ligands (Rev-erb agonists) can protect against HDM and Th2 cytokine-mediated epithelial barrier dysfunction in human bronchial epithelial cells (16-HBE). In this study, we utilized the xCELLigence real-time cell analysis-based electric impedance measurement to monitor HDM and Th2 cytokine-mediated changes in transepithelial electrical resistance (TEER) and markers of epithelial barrier function tight junction protein (TJ: Zonula occludens 1) and adherence junction complexes (AJs: E-cadherin and β-catenin) by immunostaining using confocal microscopy in 16-HBE cells. Additionally, we determine the effect of pre-treatment with different Rev-erb agonists (GSK4112, SR9009, and SR9011)/ antagonists (e.g., SR2827) with or without HDM and Th2 cytokines to evaluate their epithelial barrier function (TEER), TJ and AJ proteins at 24 hours post-treatment by confocal microscopy. HDM and Th2 cytokines caused a significant change in the TEER at 24 and 48 hours post-treatment in 16-HBE cells. However, pre-treatment with different Rev-erb agonists for 4 hours along with HDM or Th2 cytokines showed a differential response in attenuation of epithelial barrier dysfunction including augmented expression of AJs and TJ proteins confirmed by confocal microscopy. We for the first-time report that Rev-erb agonists that activate Rev-erbs inhibit HDM and Th2 cytokine-mediated epithelial barrier dysfunction. The exact molecular mechanism through which Rev-erb agonist mediates protective attenuation effects is through a specific clock target or indirectly via other independent mechanisms still needs to be investigated and that may have implications for the treatment of allergic lung diseases. This study is supported by NIH R01 HL142543.

4614 Chronic House Dust Mite Exposure Shows Time-of-Day Difference in Immune-Inflammatory Response and Circadian Clock Gene Expression in the Lung

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Asthma is a chronic inflammatory lung disease that shows a time-of-day effect characterized by increased eosinophilic infiltration associated with Th2 cytokine release and mucus production. Recent studies support that altered rhythms of immune cells and inflammatory mediators contribute to dysregulated innate/adaptive immune response during chronic lung diseases. However, whether a time-of-day response to allergen exposure leads to the difference in immune-inflammatory response and circadian clock function in the lungs remains unclear. We hypothesize that chronic exposure to house dust mite (HDM) allergen shows time-of-day effects in innate and adaptive immune response and altered circadian clock function in the lungs. NCI C57BL/6Ncr (Wild-type) mice were administered with PBS or HDM (30 µg) (intranasally; 5 days/week for 5 weeks, with 2 days interval) at specific time-of-day at ZT0 (6:00 am) or ZT12 (6:00 pm). Lung immunohistochemistry of myeloid cells was performed using flow cytometry. Gene expression of Th2 cytokines and circadian genes were determined by qRT-PCR. Total and HDM-specific immunoglobulins (IgG and IgE) in serum were measured by ELISA. Chronic HDM exposure at ZT12 caused a significant increase in resident eosinophils (EOS: Siglec F+, CCR3-), inflammatory EOS (Siglec F+, CCR3+), Gr1+ EOS, neutrophils, and dendritic cells in the lung compared to ZT0. Alveolar macrophages (AV-MACs) were significantly reduced in airway lumen of the ZT12 HDM exposed group compared to ZT0, and interstitial MACs did not show any time-of-day difference but were increased in the HDM exposed group at ZT0 and ZT12. Gene expression analysis of Th2 cytokines (Il4, Il5, and Il13) was augmented at ZT12 compared to ZT0 in the HDM exposed group. Except for total IgG, total IgE and HDM-specific IgE were higher in HDM exposed group at ZT12 compared to control group. IL-4 receptor (Il4r) expression was decreased at ZT12 compared to ZT0. HDM exposure showed altered expression of core clock genes Nr1d1, Nr1d2, Per1, Per2 and Dbp in the lungs at ZT12 compared to ZT0. HDM exposure at ZT12 showed strong time-of-day effects on the status of immune-inflammatory cellular influx, Th2 cytokine expression, and humoral response associated with circadian clock disruption in the lungs. This study demonstrates that time-of-day allergen exposure affects circadian gene expression leading to an exaggerated immune-inflammatory response in the lung. Our study demonstrates the link between the circadian clock and time-of-day variation in asthmatic lung phenotype. This study is supported by NIH R01 HL142543.

4615 Sex Differences in the Gut Microbiome in a Mouse Model of Allergic Asthma

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Asthma is a chronic inflammatory disease of the airway that compromises lung function and affects more than 300 million patients worldwide. Disparities in sensitivity between men and women to the initiation and exacerbation of asthma phenotypes have been identified, however, it remains unclear whether mediators of such sex differences in such microbiome communities found throughout the body are necessary for initiating immune responses and they contribute to the development of chronic inflammation. In this study, we used a mouse model of house dust mite (HDM) challenge and identified sex differences in the composition of the gut microbiota. The aim was to discover the extent to which the gut microbiota is affected by the induction of allergic asthma in mice challenged with HDM. For this, fecal pellets of male and female C57BL/6 mice intranasally exposed to 25 µg of HDM in 50µL of phosphate buffered saline (PBS) or 50µL PBS (control) daily for 5 weeks (n=5/group) were collected before starting treatment and at the end of week 5. DNA from fecal pellets was extracted using the ZymoBIOMICS®-96 MagBead DNA Kit and analyzed with the ZymoBIOMICS® Service to determine the 16S microbiome: Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA). After treatment, males in both groups showed a significant decrease in the median absolute abundance of bacterial DNA with similar variabilities. Females treated with HDM also displayed a significant decrease in the median absolute abundance with large variability, while females treated with PBS had a very minimal decrease in the median absolute abundance when compared to pre-exposure. On average, males had a higher relative abundance of bacteria from the phylum Firmicutes than females at basal levels with relative ratios of Firmicutes:Bacteroidetes equaling 2.24 and 1.64, respectively. Both sexes saw F:B increases in response to both PBS and HDM exposure suggesting a microbial dysbiosis associated with the treatments. We conclude that the induction of the allergic asthma phenotype with HDM contributes to sex-specific changes in the gut microbiome of C57BL/6 mice.

4616 Alterations in Mitochondrial Respiration in Human Airway Epithelial Cell Cultures as a Function of Time at Air-Liquid Interface


The biochemical pathways involved in the utilization of energy are fundamental determinants that govern every aspect of the interaction of the cell with its environment and are increasingly recognized as having pivotal regulatory roles in the cellular response and adaptation to environmental exposures. Morphologically and functionally, fully differentiated human airway epithelial cells (HAEC) cultured at an air-liquid interface (ALI) on a semi-permeable membrane support represent the most relevant in vitro model of the human airway. The dependence of HAEC on glycolysis and mitochondrial respiration during differentiation at ALI has not been examined. In this study, we used extracellular flux analyses ( Seahorse) to obtain a high temporal resolution metabolic profile of HAEC cultures as a function of time at ALI. Bronchiolar biopsies were obtained from healthy adult volunteers using an IRB-approved protocol. HAEC cultures at days 0, 7, 14, 21 and 28 of ALI were assayed using the Seahorse mitochondrial stress test, in which the cellular oxygen consumption rate (OCR) was measured continuously at each time point following sequential addition of complex 4 inhibitor oligomycin, the protonophore FCCP, and the Complex I and II inhibitors rotenone and antimycin A. The results showed a significant and progressive decrease in baseline ATP production and spare ATP production capacity in HAEC during differentiation days 0-14, stabilizing thereafter, consistent with the notion that HAEC differentiation is accompanied by a decrease in energy utilization. Cinnamaldehyde, an electrophilic phenylpropionanid compound commonly used as a flavorant in foods and electronic cigarettes, was previously reported to suppress mitochondrial respiration in HAEC-ALI. Differentiation at ALI may potentiate the inhibitory effect of cinnamaldehyde on HAEC-ALI. These results

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reveal alterations in the metabolic profile of HAEC as a function of time at ALI, which may be associated with the movement of metabolic quinones in differ-
etiating cells. These findings have implications for the assessment of the risk of adverse effects induced by exposure to ambient air pollutants in health and disease. Funded by NIHES 1R25ES031870-01. This abstract of a proposed presenta-
tion does not necessarily reflect EPA policy.

4617 Sex-Dependent Effects of Early-Life Heavy Metal Exposures on Airway Function

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Developmental exposure to environmental stressors can adversely impact airway anatomy and lung function. Our previous work using murine embryonic lungs showed that cadmium (Cd) and arsene (As) at levels relevant to human exposures via drinking water inhibited arterial branching morphogenesis, indicating that Cd and As may alter the formation of the airway system in fetal development, and the effects were greatly potenti-
ated by a combination of low-level Cd and As. We aim to determine whether early developmental Cd and As effects are linked to postnatal lung function alterations and susceptiblility to pediatric airway disease. Female C57/B16 mice were provided with low levels of Cd and As (250 pg/birth each) in drinking water and purified ALN-935 diet ad libitum for 10 weeks prior to timed breeding and continued until 3 weeks postpartum, thus exposing the pups indirectly during pregnancy and lactation. We measured respiratory mechanics in 3-week-old pre-weaning pups (age and body weight matched between groups) by using the forced oscillation technique (FlexiVent). Airway mechanics were assessed in response to increased dose of inhaled methacholine (a bronchoconstrictor). We found that in response to inhaled methacholine, exposed female pups (n=10) showed increased overall respiratory resistance (R, P < 0.05) compared to non-exposed female pups (n=8). In particular, Newtonian resistance (Rn) which primarily reflects the resistance of the large conducting airway showed a 50-70% increase by Cd and As (P < 0.001) at the baseline and with methacholine, along with increased airway hyper-
activity (P < 0.01) in female pups. Tissue damping (G) which reflects the tissue resistance and small peripheral airway resistance was not changed at baseline but showed increased responsiveness to inhaled methacholine in exposed female pups (n=10). Elastance (E) and tissue elastance (H) were not altered. These results, in line with our prior findings of Cd and As induced inhibition of branch-
ing morphogenesis, indicated that early-life Cd and As adversely impact the large conducting airway at an anatomical level in females causing changes in baseline resistance. On the contrary, in male pups, Cd and As did not cause any differen-
tes in respiratory mechanics (n=10-11 per group). Analyses of high-resolution

4618 Aerosolization and Exposure of a Vaping Mixture to Lung Cell Models for Toxicity Analysis and Degradoant Identification

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Since its inception in 2007, vaping devices have been marketed as a “safe alterna-
tive” to traditional cigarettes. As a result, vaping has become highly popularized among habitual smokers seeking smoking cessation alternatives and first-time tobacco users. Vaping products such as e-cigarettes are battery-powered portable devices comprised of a cartridge which houses the e- liquid, atomizer, and a mouthpiece. E-liquids contain a mixture of an active ingredient (i.e., nicotine or cannabis), water, diluents, and flavoring agents (i.e., terpenes and aldehydes). Although, e-cigarettes are associated with health notices from the CDC surrounding e-cigarette use in adults and youth, e-liquid variations are continuously produced and sold in the market. Due to factors such as e-liquid variability, the testing and regulation of vaping products has proved difficult and thus there is uncertainty surrounding e-cigarette toxicity. Furthermore, toxicological investigations of e-liquid toxicity require the application of mixture toxicity methods to determine which ingredients are more toxic. The purpose of this study is to investigate the resulting cytotoxicity and production of reactive oxygen species (ROS) after exposure to aerosolized vaping mixtures containing a diluent (propyl citrate) and flavoring agent (linoleal). Nuclear magnetic resonance (NMR) spectroscopy was used for the detection of degradant production of aerosolized binary mixtures. The mixture was prepared at two ratios of 97.3 : v/v and 80.20 : v/v (TEC: linoleal) tested against two lung cell models, BEAS-2B and A549. Cells are human epithelial cells representative of the bronchial and alveolar regions of the respiratory tract allowing for analysis of differential responses. Two aerosolization methods were used and compared in this study; a jet nebulizer and vaping device were used for non-heated and heated aerosol generation. Cell viability was measured via MTS and produced potency values. Results showed that toxicity increased after exposure to each mixture containing increased amounts of linoleal. Also, toxicity was higher in cells exposed to the heated aerosol mixtures as compared to the non-heated aerosol mixtures. Oxidative stress was determined to be the molecular initiating event. Exposure to heated aerosolized mixtures resulted in increased levels of H2O2 indicating cellular stress. NMR detected degradants in the heated aerosol mixtures, namely acetaldehyde and ethyl acetate. Additional toxicological investigations of aerosolized vaping mixtures are underway and will be used to adequately define the mechanistic pathways perturbed after exposure to vaping aerosols.

4619 Cancer Potency of Fibrous Balangeriote: Utilizing the Quantitative Structure-Activity Relationship (QSAR) Modeling to Predict Toxicity for a Mixed Mineral Dust


The mineral balangeriote has been identified in an asbestos form from the Balangerio chrysotile mine (Italy). Seven cases of mesothelioma were identified among the mine workers; this level was significantly higher than would be predicted using the average potency of chrysotile asbestos. The sample of balangeriote was obtained from the mine and tested in the laboratory. The lengths and widths of particles from a sample of balangeriote were measured by TEM, and the distribu-
tions of fiber dimensions were determined. Statistical analysis and QSAR modelling were applied to assess toxicological potential of balangeriote in comparison to other mineral occurrences known to cause asbestos-related diseases. Balangeriote fibers are characterized as asbestiform, with geometric mean length of 10 μm, width of 0.54 μm, aspect ratio of 19, and specific surface area of 13.8 (1/μm). The distributions of fiber size follow a fractal dimensions law with D=0.98 for length and D=2.4 for width. Modelled nano-sized mineral fibers in the laboratory. Proximity analysis shows asbestiform anthophyllite as the mineral with dimensions and chemical composition closest to balangeriote. Modeling estimates the average poten-

ty of balangeriote as 0.04% (95 % CI 0.0058, 0.16) for mesothelioma and 1.3% (95 % CI 0.75, 1.77) for lung cancer. Monte Carlo simulation was used to reconstruct the risk level for the workers and explain the mesothelioma mortality rate with good precision.

4620 Quantitative Ex Vivo to In Vivo Extrapolation for Respiratory Toxicity of Inhalable Substances


Methods for the prediction of acute respiratory toxicity in vivo can strongly contrib-
ute to a faster and more effective development of new inhalational drugs and new chemicals as well as to animal welfare. Due to the complex biological and physio-
 logical processes and interactions in the lung as well as differences in the exposure protocols (concentration, exposure time, repeated exposure) and dosimetry, the development of predictive models allowing an in-vivo-to-in-vivo extrapolation remains challenging, however. To assess effects on whole organ level, the model of the isolated Perfused Rat Lung (IPL) providing a fully intact organ system with cellular, structural and functional integrity including pulmonary barrier function and circulation is an interesting tool due to the proximity to the in-vivo situation. The aim of the present works was to apply isolated Perfused Rat Lungs (IPLs) to esti-
mate respiratory toxicity of Nafamostat mesylate prior to a regulatory subacute tox-
icity study. For this purpose, lungs from rats were ventilated and perfused with a physiologic buffer solution. The IPLs were exposed to rat respirable Nafamostat mesylate aerosols in a dose escalation scheme with a single explant using an Aerogen Pro mesh nebulizer. Regional dose deposited for IPLs was calculated with the MPPD (Multiple Path Particle Dosimetry) model using the underlying breathing parameters and aerosol characteristics. Measurement of tidal volume allowed for the on-line assessment of the viability of the lungs. In addition, precision-cut-lung-
slices of the IPLs were prepared and analyzed for viability by confocal micros-
 copy, LIVE/DEAD. The resulting effect level data obtained in- vitro were compared to the corresponding data from a 28-day subacute in vivo investigation study in rats. For high Concentrations of Nafamostat mesylate aerosols a dose-de-
pendent decrease in lung function was observed in IPLs. A No Observed Adverse

Effect Level (NOAEL) and a Lowest Observed Adverse Effect Level (LOAEL) as dose deposited per body weight could be derived. In line with these findings, signif-

cant cell death was observed in particular along the airways but also to a smaller extent in the lung parenchyma for the maximum exposure dose. Comparison to the in-vivo data showed both, a good qualitative agreement regarding the site of the adverse effects and still more important a good quantitative agreement with the data obtained via an In-Vitro to In-Vivo extrapolative approach.
Humans spend 70-90% of their time indoors, however, there is a significant lack of knowledge regarding exposure to microplastic particles and fibers (MPs) within the indoor environment. Fibers make up more than 90% of household settled dust worldwide and have been found in indoor air. Studies have identified MPs larger than 50 micrometers in indoor dust, but these are unlikely to be respirable and little information is available regarding smaller airborne particles. It is evident that humans are inhaling these particles as they have been found in diseased and healthy human lung tissues. We have developed methods to identify plastic particles in settled household dust larger than one micrometer in diameter by distinguishing plastic from cellulosic, proteinaceous, and inorganic materials using two different stains, Nile Red for plastics, and Trypan Blue for cellulosic materials. Proteinaceous and inorganic materials remain unstained. Household settled dust is collected onto silicon nitride nanomembranes where in situ analysis of particles can be characterized. The innovative use of nanomembranes allows for particle capture and multiple analyses to take place on the same substrate. Particle analysis via colorimetric staining and imaging is followed by polymer identification via Raman spectroscopy and subsequently characterized via scanning electron microscopy for size and surface morphology. Using this innovative approach, microplastic particles larger than one micrometer in diameter have been identified in all settled dust samples.

**4622 Effect of Palmitoylation Inhibitor, 2-Bromopalmitate, on Cadmium-Related Changes to Cellular Metabolomes in the Human Lung Fibroblast Cell (HLF)**

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Cadmium (Cd) is a toxic, naturally occurring environmental metal found in foods. Humans do not have an efficient mechanism for Cd removal; thus, Cd burden in humans increases with age. Increasing evidence from cell and mouse studies suggests that Cd exposure to Cd at low environmental levels has an impact on cellular metabolism and on cell growth and proliferation signaling. In our previous mouse study with Cd exposure, we found that zDHHC11, a member of protein S-palmitoyltransferases, plays a role in regulating central metabolism for cell growth and proliferation. This finding led us to hypothesize that inhibition of palmitoylation of proteins potentiates Cd-caused metabolic disruption in lung cells. To test this hypothesis, we examined the effects of inhibiting protein palmitoylation on lung cell metabolism. Human lung fibroblasts treated with 2 μM Cd for 24 hours with or without 20 μM 2-bromopalmitate (2-BP, palmitoylation inhibitor) were analyzed via LCMS-based, untargeted, high-resolution metabolomics (HRM). HRM data was analyzed by pathway ANOVA using xmsPANDA and limma packages in R. A total of 8186 metabolic features were detected. Of these metabolic features, 188 were significantly altered by both Cd plus 2-BP treatment compared with treatment by Cd or 2-BP alone (P<0.05). Pathway enrichment analysis using Mummichog shows that these metabolic features are associated with purine and pyrimidine nucleotide metabolism and with lipoteichoic acid metabolism, suggesting that inhibition of protein palmitoylation potentiates Cd-altered lung cell physiology such as cell growth and proliferation. Therefore, protein palmitoylation could be a potential target mechanism to ameliorate environmental Cd-caused disruption of lung metabolism and subsequent pathologic outcomes.

**4623 IL4i1 Promotes Antioxidant Gene Expression and Inhibits Human Airway Smooth Muscle Cell Shortening**


Mucosal-associated invariant T (MAIT) cells are present in the airways, and MAIT cell deficiency has been implicated in asthma. These lung-resident innate-like T lymphocytes express abundant IL-4-induced-1 (IL4i1) gene, an L-amino acid oxidase, and the secreted IL4i1 has been shown to suppress airway inflammation and airway hyperresponsiveness in animal models of allergen-induced asthma. Here we find that IL4i1 inhibits histamine-induced human airway smooth muscle (HASM) shortening, the end effector of acute airway narrowing in asthma. IL4i1-mediated functional alterations in HASM cells were associated with increases in mRNA expression of anti-oxidant genes NFE2L2, NQO1, HMOX1, and TXNRD1. These data suggest IL4i1 limits oxidative stress by promoting transcription of anti-oxidant pathways, and potentially confers protection against ferroptosis. These findings support that IL4i1 reduces airway hyperresponsiveness by acting on HASM cells and indicate a mechanism for IL4i1 activity, which may present new targets for therapeutics to treat asthma. Funding: P01HL114471.
Primary cells from the different species were expanded in monolayer culture and seeded onto microporous membrane inserts to reconstruct 3D organotypic tissue models. Tissues were characterized for polarity of epithelial cells (histology), epithelial cell markers (IHC), barrier integrity (transepithelial electrical resistance, TEER measurement), and functionality in inhalation toxicological studies by testing 3 well-characterized chemical toxicants (CT). Polyethylene glycol was used as the positive control or another concentration of 1T0 (100 μL) was applied to the apical surface, and tissue inserts were sealed with insert caps (MilliCell-MTK-CAP, MatTek Life Sciences) for 4 hours to mimic in vivo rat exposure experiments. After 4 hours, dosed tissues were washed and allowed to recover for 24 hours at 37°C. Analysis of the 3D tissues from the different species revealed: well polarized epithelium with a physiological TEER value of >300 Ω*cm², cilia formation on the apical surfaces, and mucin production mimicking the airway microenvironment. Acute exposure to CT for 4 hours showed varying levels of tissue viability and membrane integrity by MT and TEER assays, respectively. While the effective dose concentration that reduces tissue viability by 50% (ED-50) for vinyl acetate and chloroacetaldehyde is comparable (<2 mg/tissue) for all species, the TEER value for toluene showed differences: human >20 mg, primates, 16.2±1.7 mg, and rat 13.8±0.1 mg. Based on the MTT viability and TEER values, the test chemicals were ranked ordered from high to minimal toxicity: chloroacetaldehyde > vinyl acetate > toluene > propylene glycol and vehicle control. While the human and primate airway models showed comparable MTT values, the rat airway tissue was more sensitive to the higher concentration of toluene. Although more chemicals need to be tested, the multispecies 3D airway tissue models will be vital translational tools to predict airway inhalation toxicity and to bridge the in vitro in vivo knowledge gap to reliably predict human responses, while providing an alternative approach to animal experimentation.

**4627 Novel Fully Primarily Human Airway Epithelium-Alveolar Macrophages In Vitro Co-cultures Models to Study Host Pathogen Interactions**

B. Boda, C. Bertinetti, O. Vebeke, G. Gunasingam, S. Huang, and S. Constant, Epithelix, Plan-les-Ouates, Switzerland.

Being the first line of defense of the organism against airborne pathogens like bacteria and viruses, the respiratory epithelium acts as a physical barrier as well as an efficiency mucociliary escalator. Furthermore, the airway epithelium is also a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections. Many animal models have been used to study lung infections, but the relevance and predictability of animal models are still questionable and research is established a new co-culture model using well-characterized, standardized human airway epithelium such as Mucari™, SmallAir™ and human lung macrophages (CD45+,HLA-DR+, CD206, CD11b+ and CD14) for studying bacterial and viral infections. The alveolar macrophages were not only able to adhere to the epithelial cells but also functional: The macrophages were capable of phagocytosis, evaluated using phthalo® Red 5 (Cornea Biophotonic Biosystems). Moreover, two monocytes models respond to pro-inflammatory stimuli such as LPS, TNF-α and Poly(I:C) with an increased IL-8 secretion. Upon bacterial infection with methicillin-susceptible Staphylococcus aureus strain (MSSA), compared to Mucari™ monocytes, Mucari™-macrophages showed stronger immune response in the context of bacterial burden. With 1.5Lg10 CFU and 0.03 mg/cm², IL-8 and β-defensin-2 secretions. Interestingly, greater difference was observed for Streptococcus pneumonia (Sp19F). The presence of macrophages led to a decrease of 3.5Lg10 CFU after 24 hours of culture (N=12) versus Mucari™ alone. These novel in vitro models might find applications in understanding the role of immune-epithelial cell interactions in infection diseases and inhalation toxicity assessment.

**4628 Development of Fully Primarily Human 3D Alveolar Model (AlveoAir)**

C. Ferreira, B. Boda, S. Huang, and S. Constant, Epithelix, Plan-les-Ouates, Switzerland.

Chronic obstructive pulmonary disease (COPD) and lower respiratory infections are leading causes of death worldwide. This highlights the necessity for new and more efficient treatments. In order to develop novel drugs and assess lower respiratory toxicity of xenobiotics, robust and relevant in vitro alveolar models would be very helpful. We herein describe the characterization and functionality of a full primary human epithelial-endothelial 3D alveolar model, AlveoAir™. To characterize AlveoAir™, long-term parameters were measured: Biomarkers for ATIs, ATIIs and tight junctions (CAV-1, HTII-280 and ZO-1 respectively, Immunofluorescence); Morphology and lamellar bodies’ presence (Histology & TEM); existence of a maintained alveolar epithelial barrier (TEER) and PCR secretion (ELISA). Using these techniques, the evolution of the culture was monitored for several weeks. Functionality of AlveoAir™ was evaluated by exposure to pro-inflammatory cytokines (IL-1β, LPS, IFN-γ, TNF-α, and IL-6) and changes in barrier integrity (cilia formation on the apical surfaces, actin production mimicking the airway microenvironment). Acute exposure to CT for 4 hours showed varying levels of tissue viability and membrane integrity by MTT and TEER assays, respectively. While the effective dose concentration that reduces tissue viability by 50% (ED-50) for vinyl acetate and chloroacetaldehyde is comparable (<2 mg/tissue) for all species, the TEER value for toluene showed differences: human >20 mg, primates, 16.2±1.7 mg, and rat 13.8±0.1 mg. Based on the MTT viability and TEER values, the test chemicals were ranked ordered from high to minimal toxicity: chloroacetaldehyde > vinyl acetate > toluene > propylene glycol and vehicle control. While the human and primate airway models showed comparable MTT values, the rat airway tissue was more sensitive to the higher concentration of toluene. Although more chemicals need to be tested, the multispecies 3D airway tissue models will be vital translational tools to predict airway inhalation toxicity and to bridge the in vitro in vivo knowledge gap to reliably predict human responses, while providing an alternative approach to animal experimentation.

**4629 The Impact of Age on Ozone Responses in Rodents**

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Ambient Ozone (O₃) is a criteria air pollutant, which causes increased morbidity and mortality, in part, through increased lung inflammation. As ambient O₃ levels continue to rise, mitigating these adverse health effects will require identifying individuals who exhibit different susceptibility to ozone-induced inflammation. Understanding the physiological and genetic factors that are responsible for ozone exposure studies have demonstrated increased O₃ induced inflammation in aged rodents. However, the mechanisms driving these aged responses are poorly understood. The goal was to determine how aging impacts the genetic response to O₃-induced lung injury and inflammation. To address this, we exposed C57BL/6 male mice to O₃ candidate) was assessed on AlveoAir™. The tissues were exposed to 0.003, 0.005, 0.03, 0.3 or 3 μg/cm² of flagellin apically 2 h/day, for 5 days. TEER, LDH, pro-inflammatory cytokines (IL-6 & IL-8) and a panel of genes were evaluated. The flagellin-based formulation had no effect on TEER and cytotoxicity for all tested conditions. However, flagellin did induce a dose-dependent increase of IL-8 starting at 0.03 mg/cm² and upregulate the expression of genes coding for CCL4, TNF, IL-1β, CFX3, or CCL20, with a plateau obtained at 0.03 mg/cm². Altogether, flagellin was well tolerated by alveolar epithelia. Apical exposure induced biomarkers upregulation, demonstrating flagellin’s immunomodulatory potential on alveoli. Finally, co-culture model between AlveoAir™ and primary alveolar macrophages has been developed. Upon bacterial infection with Streptococcus pneumonia (Sp19F) compared to AlveoAir™, AlveoAir™-macrophages showed stronger immune response with reduction up to 3.5Log10 CFU after 24 hours of culture. This novel in vitro model, AlveoAir™, represents a relevant and reliable tool for inhalation toxicity assessment of drugs. It is also highly useful for understanding the cellular and molecular mechanisms of respiratory diseases such as COPD, viral and bacterial infections.
Ozone is a ubiquitous urban air pollutant that causes airway inflammation and hyperresponsiveness in both healthy and susceptible populations. Inflammatory macrophages play a key role in ozone-induced lung injury by regulating the initiation and resolution phases of the inflammatory response. These distinct activities are mediated by macrophage subpopulations broadly classified as M1/proinflammatory and M2/proresolution. Proper control of the inflammatory response requires a balance between M1 and M2 activity. The transcriptional coactivator PPARG coactivator-1 β (PGC-1β) modulates the activity of transcription factors involved in macrophage polarization promoting oxidative phosphorylation, mitochondrial respiration, and cellular metabolic pathways associated with M2 activity. We hypothesized that PGC-1β signaling attenuates ozone-induced lung injury by promoting oxidative phosphorylation, M2 activation and the resolution of inflammation. To test this, we utilized a conditional Cre-lox mouse model in which PGC-1β is specifically knocked out in macrophages and analyzed the effects of this genetic modification on ozone-induced levels of BAL phospholipids, sRAGE, and fibrinogen. We found that the loss of PGC-1β in CX3CR1+ macrophages (PGC-1β KO) was protective against the ozone-induced increases in these markers. In these studies, we analyzed the effects of loss of PGC-1β on pulmonary function and lung disease following chronic ozone exposure. Wild type (WT) and SP-D-/- mice were exposed to air or ozone (1.5 ppm, 3 hours), twice a week, for 3.5 weeks. Bronchoalveolar lavage (BAL) fluid and alveolar macrophages were collected 24 hours later and analyzed for markers of inflammation, emphysema, and disease. Pulmonary mechanics was measured using a Scireq Flexivent. Expression of both WT and SP-D-/- mice to chronic ozone resulted in histological changes in the lung; these included increased in peribronchial infiltration of mononuclear cells and foamy macrophages, significantly greater numbers of foamy macrophages were noted in lungs of SP-D-/- mice when compared to WT controls. SP-D-/- mice exposed to ozone also exhibited increased bronchiolar distention and bronchial epithelial thickening. When compared to air-exposed controls, BAL cell number, protein and IgM levels increased in WT mice after chronic ozone exposure. Loss of SP-D exacerbated the increases in BAL cell number and protein, with no major effect on IgM levels. Greater increases in ozone-induced levels of BAL phospholipids, sRAGE, and fibrinogen were also observed. This was associated with impaired lung function; thus, significant increases in elastance and hysteresis were observed in SP-D-/- mice, but not in WT mice. After exposure to ozone, numbers of alveolar macrophages recovered from the lungs increased in SP-D-/- mice, but not in WT mice. These were activated, as reflected by increases in mRNA expression of Tnfa, Il-1β, Il-3, Ccl5, Ccl7, Ccl22 and Mmp12. This was linked to increased NFκB activity. Expression of M2 anti-inflammatory genes, Tgfβ3, Mmp12 and Mmp28 were also upregulated in SP-D-/- mice after ozone. These data suggest that SP-D plays an important role in protecting against chronic ozone induced pulmonary injury and inflammation. Our data provide new mechanistic pathways into lung pathology following chronic exposure to ozone.

Ozone exposure is known to cause lung injury and increased immune cell recruitment into the lung airspaces. Prior studies have shown that female mice exhibit exaggerated inflammatory responses to ozone exposure as compared to male mice, however, the potential mechanisms for this sex-specific susceptibility of male and female mice to ozone exposure remain unknown. Cells of the hematopoietic as well as the non-hematopoietic lineage respond to ozone exposure via altered morphology, functioning, and survival. Here, we hypothesized that the inherent sex-specific differences within the hematopoietic lineage cells in females versus males account for their variable sex-specific responses to ozone exposure. To test this, we generated cross-sex bone marrow chimeras. Briefly, 10-11-week old C57BL/6 mice were lethally irradiated and then reconstituted within 12 hours post-irradiation with bone marrow (BM) cells obtained from donor mice of either the same or the opposite sex via tail vein injections. The chimeric mice were housed in a sterile environment for 8 weeks post-irradiation and bone marrow transplantation. At 8 weeks, the chimeric mice were subjected to repetitive exposure to either filtered air (FA) or ozone (800 ppb; 4 hours/day for 14 consecutive weekdays) and were necropsied within 12-16 hours post-last FA/ozone exposure. Bronchoalveolar lavage fluid (BALF) and lung tissues were analyzed for immune cell infiltration, levels of inflammatory biomarkers, and histopathological alterations. As expected, ozone-exposed females that received BM cells from female donors exhibited increased immune cell recruitment compared to ozone-exposed males that received BM cells from male donors. Interestingly, ozone-exposed males that received BM cells from female donors exhibited increased immune cell recruitment compared to the ozone-exposed males that received BM cells from male donors; whereas ozone-exposed females that received BM cells from male donors exhibited significantly reduced immune cell recruitment as compared to ozone-exposed females that received BM cells from female donors. Macrophages were found to be the principal cell type contributing to increased immune cell infiltration following ozone exposure, followed by neutrophils. BALF protein and double stranded DNA (dsDNA), both markers of lung injury, were not significantly different among the four ozone-exposed chimeras. Taken together, these data indicate that the cells of the hematopoietic lineage determine the exaggerated responsiveness of female mice to ozone exposure. In support of this, the current study suggests that understanding underlying mechanisms from this study will help us better understand the underlying mechanisms that contribute towards the differential sex-specific susceptibility of mice to ozone exposure.
Ozone-induced acute lung injury and inflammation following one or two days of exposure are not evident if ozone exposure continues for 3 or more days. While a one-to-two-day exposure can induce neutrophilic inflammation, lung protein leakage, and airway irritation, real-life exposures to ozone are more likely to occur in an episodic fashion. Chronic exposure to ozone has been associated with cardiopulmonary disease. The goal of this study was to understand the effects of episodic ozone on lungs responses compared to an acute exposure. Subsequently, an episodic exposure paradigm was established where, over the course of 2 weeks, rats were exposed to ozone on days 1, 2, 4, 9, and 10. Male Long-Evans rats were exposed to either air or ozone (0.4, 0.8 ppm) for 4 hours/day utilizing the aforementioned paradigm or for 2 consecutive days to model an acute exposure. Due to the adaptation response commonly observed with acute ozone exposure, we hypothesized that some phenotypic markers of lung injury would be blunted at 2 weeks while certain molecular alterations would either emerge or persist. Pneth, an index of airflow limitation, was significantly increased on the first and second day of ozone exposure. This response was suppressed over the course of the 2-week study. Rats exposed to only 2 days of ozone displayed concentration dependent increases in bronchoalveolar lavage fluid (BALF) albumin and protein, whereas this response was not evident in the 2-week exposure group. BALF neutrophils, on the other hand, were significantly increased at both the 2-day and 2-week timepoints in a concentration-dependent manner. After 2 days of ozone, genes associated with angiogenesis (Ace, Cdhs, Fti1) and cell metabolism (Ldhb and Pdk1) were altered but did not persist to 2 weeks. Despite the blunted effect in Pneth and protein leakage, differences in genes involved with glucocorticoid signaling and inflammation (Il1r1, Il2r, Il13ra2, Flt1, Tsd22d3, Cxcl2, Cxcl10) and inflammation (Flt1, Cxcl2, Arg1) were observed in ozone exposed rats. Interestingly, male mice had significantly higher expression of the negative regulation of both cell proliferation and inflammation, was significantly reduced at both 2 days and 2 weeks of 0.8 ppm ozone only. Reductions in Dusp1 (which promotes the negative regulation of both cell proliferation and inflammation, was significant) and other genes associated with the negative regulation of both cell proliferation and inflammation, was significantly reduced at both 2 days and 2 weeks of 0.8 ppm ozone only. Reductions in Dusp1 were observed at both 2 days and 2 weeks of ozone exposure only. Reductions in Dusp1 and other genes associated with the negative regulation of both cell proliferation and inflammation, was significantly reduced at both 2 days and 2 weeks of ozone exposure only.

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ChemR23 Axis Regulates Ozone-Induced Lung Inflammation and Injury
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Ozone (O3) is a criteria air pollutant shown to increase morbidity and mortality from chronic lung diseases. O3 induces lung injury and inflammation through the promotion of oxidative stress leading to cell death, as well as inducing the production of pro-inflammatory lipid mediators such as prostaglandins and leukotrienes. Studies have identified a novel class of lipid mediators, termed specialized pro-resolving mediators (SPMs) that resolve inflammation following injury by binding to their respective G protein-coupled receptor (GPCR). ChemR23 is one of the GPCRs that binds to its ligands, Chemerin and Resolin E1 (RvE1). Chemerin is an adipokine that acts as a chemoactivator by activating the migration of inflammatory cells and inducing a pro-inflammatory response. RvE1, an SPM, metabolized from the n-3 polyunsaturated fatty acid (PUFA) Eicosapentaenoic Acid (EPA), also binds to the ChemR23 receptor facilitating the resolution of inflammation and tissue homeostasis. We have previously shown that O3 exposure downregulates select SPMs in the lung and the SPM receptor ChemR23. From this, we hypothesized that the ChemR23 receptor binding to its ligand, RvE1, leads to protection of the lung from O3-induced pulmonary inflammation and injury. To test our hypothesis, male C57BL/6J (WT) and ChemR23 deficient (ChemR23-/-) mice were exposed to either filtered air (FA) or 1 part per million O3 for 3 hours. Mice were euthanized at 24 hours post O3 exposure and bronchoalveolar lavage (BAL) fluid and lung tissue were collected to assess inflammation/injury. Following 3-hour O3 exposure, ChemR23-/- mice revealed increased pulmonary inflammation and injury seen in higher levels of BAL protein and cellular elements, respectively, at the 24 hour time point. Furthermore, the ChemR23-/- mice revealed higher protein production of pro-inflammatory cytokines and chemokines including IL-6, KC, and MCP-1 24 hours post O3 exposure. Both WT and ChemR23-/- mice had increased Chemerin levels in their BAL fluid following O3, however ChemR23-/- mice had significantly more Chemerin in the airspace compared to WT controls. However, ChemR23-/- mice exposed to O3 had a significant decrease in RvE1 BAL when compared to WT controls. Taken together, our results indicate that acute O3 exposure leads to greater pulmonary inflammatory response in ChemR23-/- mice associated with increased Chemerin and decreased RvE1. Future studies will investigate how RvE1 plays a role in the ChemR23/Chemerin pro-inflammatory axis and if increasing levels of O3 exposure through EPA diet leads to enhanced pulmonary production of RvE1 and protects the lung from O3-induced injury and inflammation.

Metabolic and Multi-tissue Transcriptomic Analysis Reveals Complex Phenotypic Interactions of Eight-Week Social Isolation and Acute Ozone Exposure in Rats
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Socially isolated individuals and communities often face psychosocial stressors and are also likely to be exposed to high levels of air pollutants. Social isolation (SI) has been considered a risk factor for neuropsychiatric conditions and is also linked to increased metabolic disorders. The SI model in rats is a well-established model of depressive- and anxiety-like behavior. Based on our research demonstrating the mechanism by which air pollutants and psychosocial stressors through the neuroendocrine system might exacerbate chronic immune and metabolic alterations, we hypothesized that rats under long-term SI will have exaggerated metabolic phenotype through dysregulated neuroendocrine activity and that acute ozone induces a SI-dependent metabolic response in SI relative to pair-housed rats. Male, 4-week-old Wistar-Kyoto rats were either 1 pair-housed (2/cage) with environmental enrichment and frequent handling (no stress (NS) control group), or 2 socially isolated (SI) by single-housing, with no environmental enrichment provided and avoiding frequent handling for 8 weeks. Animals were then exposed to filtered air or ozone (0.8 ppm) as a challenge stress for 4 hours followed immediately by necropsy. The body weight gain of NS and SI were similar during the course of the study, indicating similar food consumption. However, in air-exposed SI animals, circulating cholesterol assessed using clinical assays was increased along with small increases in circulating cytokines, and this effect of SI was exacerbated by acute ozone exposure. Serum metabolomic analysis revealed only a modest effect on circulating metabolites of air-exposed SI relative to NS animals, except for higher levels of several circulating sphingomyelins in SI rats. These increases in sphingomyelins were associated with the absence of higher levels of cholesterol when assessed through metabolomics, suggesting that these sphingomyelins are likely bound to cholesterol and thus some cholesterol signal may be lost during mass spectrometry ionization processes and could cause remarkable changes in circulating metabolites - including higher levels of a variety of free fatty acids, glycerols, branched chain amino acids, and ketone metabolites - typical of an acute stress-mediated metabolic response we have previously demonstrated in rats and humans. These ozone effects also included increases in sphingomyelins in both NS and SI groups. Because sphingomyelins are widely distributed in all cell membranes and enriched in myelinated neurons, and because liver is the master regulator of metabolic processes, we examined transcriptional changes in the stress responsive region of the brain (hypothalamus) and the liver to determine their potential contribution to circulating sphingomyelins in SI animals. There were no significant transcriptional changes attributable to SI except in the hypothalamus in the levels of air-exposed ozone exposure, on the other hand, caused 940 hypothalamic genes to be changed in NS and 1192 in SI, and 2398 liver genes in NS and 3055 genes in SI, with no significant interaction between SI and ozone in either tissue. Thus, these data indicate which other complex mechanisms responsible for exacerbation of systemic metabolic and inflammation phenotypes together with increases in circulating sphingomyelins in SI by acute ozone exposure. These subtle effects of subchronic SI modifying the metabolic response to acute ozone exposure indicate the importance of understanding how prior stressors modify the subsequent stress responses. This abstract does not reflect the US EPA policy.

Loss of G-Protein-Coupled Receptor ALX/FPR2 Inhibits Inflammatory and Resolution following Acute Ozone Exposure
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Ozone (O3) is a toxic air pollutant that exacerbates pulmonary diseases by activating the innate immune response, inhibiting the resolution of inflammation, and causing chronic lung injury. Based on our research demonstrating the SPM maresin 1 was increased in the lungs of FPR2-/- mice after O3 exposure. However, the biological significance of ALX/FPR2 signaling is currently unknown in O3-induced pulmonary inflammation and/or resolution. Therefore, we hypothesized that ALX/FPR2 deficiency exacerbates inflammation and inhibits resolution of inflammation following O3 exposure. To test this hypothesis, we exposed male ALX/FPR2 wild type (FPR2+) or ALX/FPR2 knockout (FPR2-/-) mice to filtered air (FA) or 1 ppm O3 for 3 hours and then collected bronchoalveolar lavage (BAL) 6, 24, or 48 hour after exposure to assess the initiation, peak, and resolution phases of lung inflammation respectively. At the 6-hour time point, FPR2+ mice had significantly less pulmonary neutrophil infiltration and production of cytokine TNF-a, and chemokine MCP-1 and MIP-2 compared to mice exposed to O3, while FPR2-/- mice had increased Chemerin and decreased RvE1. Then 24 hours following O3 exposure, pulmonary neutrophilia and BAL cytokines and chemokines were equivalent between FPR2+ and FPR2-/- mice but the SPMS maresin 1 was increased in the lungs of FPR2+ mice after O3 exposure. Lastly at 48 hours after exposure, FPR2+ mice had increased BAL protein (a marker of lung injury) and macrophage infiltration after O3 exposure compared to FPR2-/- mice. Taken together these data indicate that ALX/FPR2 may contribute to the balance of initiating the O3-induced pulmonary inflammatory response along with activating the resolution phase of inflammation.

The Role of CD163 and HO-1 Signaling in Ozone-Induced Lung Antioxidant Responses
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Ozone (O3) is a criteria air pollutant that has been linked to adverse pulmonary and cardiovascular outcomes when present in elevated concentrations. The mechanism by which acute O3 exposure causes these adverse outcomes are currently unknown. Previous studies have shown that acute O3 exposure increases pulmonary oxidative stress and inflammation. These effects can be partly attributed to the presence of reactive oxygen species (ROS) and depletion of antioxidant responses. CD163 is a scavenger receptor expressed by macrophages responsible for the activation of the heme oxygenase 1 (HO-1) pathway which is an essential antioxidant response element known to protect the lung from oxidative damage. However, it is unknown if CD163 reduces O3-induced oxidative stress responses in the lung. Therefore, we hypothesize that O3-induced oxidative stress responses in the lung. To test this hypothesis, we exposed C57BL6 (WT) and CD163 knockout (CD163-) female mice to either filtered air (FA) or 1 ppm O3 for 3 hours. This 3-hour exposure is equivalent to acute ambient increases in ground level O3. Mice were necropsied at 6 and 12 hours following exposure to collect bronchoalveolar lavage (BAL), lung tissue, and blood for serum. Lung tissue was used to measure mRNA levels of antioxidant response genes via real time PCR including glutamate-cysteine ligase catalytic subunit (GCLC), NAD(P)H quinone dehydrogenase 1 (NQO1), nuclear factor erythroid 2-related factor 2 (Nrf2), and HO-1. Six hours following O3 exposure, HO-1 gene expression in the lung was increased in WT mice but not in CD163-/- mice. Twelve hours following O3 exposure, WT mice maintained a
A significant increase in cardiorespiratory morbidity and mortality is associated with acute episodes of air pollution increase. Ozone is the most reactive gaseous component of air pollution and is a critical air pollutant regulated by the Environmental Protection Agency (EPA). NLRX1 (Nod like receptor X1) is a recently discovered NOD-like receptor, implicated in multitude of cellular responses including mitochondrial injury, reactive oxygen species formation, inflammation, and cell death. However, its effect in mediating environmental toxicant induced lung insult is yet to be studied. We hypothesized that ozone induced lung injury is mediated by the NLRX1. C57Bl/6J (Nlrx1+/+) and NLRX1 knockout (Nlrx1-/-) mice were exposed to filtered air (control) or 1 ppm ozone for 3 hours. We utilized 6-12-week-old male and female mice in these studies. The animals were euthanized 24 hours post exposure and changes in lung lavage cellularity, cell death, alveolar barrier damage, lung inflammation and oxidative capacity were measured. Ozone exposure induced a significantly greater increase in neutrophils in bronchoalveolar lavage fluid of Nlrx1-/- animals compared to the Nlrx1+/+ animals. Moreover, Nlrx1-/- mice displayed a significant induction of inflammation (KC, TNF-α, IL-1β) both in terms of gene expression on the lung tissue and protein secretion in the bronchoalveolar lavage fluid. We also observed a decrease in Nrf2 activity in Nlrx1+/+ mice compared to Nlrx1+/+ mice. Electron paramagnetic resonance studies demonstrated significantly greater induction of free radicals in the lung tissue, bronchoalveolar lavage and serum of Nlrx1-/- mice compared to Nlrx1+/+ mice. Mitochondrial function studies using sea horse assay confirmed greater mitochondrial dysfunction (proton leak, glycolytic capacity and decrease in ATP production) in Nlrx1-/- mice compared to Nlrx1+/+ mice. In summary these studies confirm the role of NLRX1 in mediating ozone induced lung inflammation and cellular damage. Funding: NIH R01 ES031253 (SH).

Gestational Ozone Exposure Augments the Amniotic Environment with Osteopontin-Driven Placental Adaptation

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Urban air pollution impacts millions worldwide influencing an estimated 4.2 million premature deaths a year. A highly reactive component of air pollution, ozone (O3) is associated with adverse vascular, respiratory, and neuronal health outcomes as well as developmental defects. While many of these outcomes are exacerbated, the effects of ozone inhalation during critical windows of gestational development remain unclear. The epidemiology shows an increased risk for pre-eclampsia, gestational diabetes, and lower fetal birth weight at term, the symptoms of which have been recapitulated in modeled ozone exposures. Yet, little is known about the mechanisms by which the placenta and the enclosed amniotic environment are augmented during different periods of gestation. In the current study, we hypothesized that an acute rat maternal ozone exposure will elicit a more robust impact on the amniotic environment compared to sham exposure. Placental tissues were sectioned, prepared for immunofluorescence microscopy, and stained for OPN as well as associated markers. OPN levels increased two-fold across gestational windows post exposure (GD10 to GD20). While the GD10 time point generally excludes the relevance of OPN on implantation, OPN can play an anti-inflammatory role by reducing MMP-2 expression and limiting matrix remodel- ing. MMP-2 staining affirmed a corresponding reduction with GD10 and not GD20 O3 exposure, while staining of TIMPs 1 and 2 showed no response at either time point. Together, these findings suggest an enduring suppression of MMP-2 that is independent of acute TIMP inhibition. Increased OPN expression has also been linked with both angiogenesis and vessel wall thickening. PECAM staining (platelet and endothelial cell adhesion molecule) was correspondingly increased selectively with GD10 O3 exposure, biased to the decidual later attached to the uterine wall. However, it could not be distinguished whether this was due to hypervascularization or a thickening of the vessels, the latter which is to occur with advancing gestational age. Uterine artery resistance at term, however, significantly increased with GD10 O3 exposure, supporting the later conclusion. In all, results from these studies demonstrate a gestational-window dependent influence on the amniotic environment bathing the fetus with a prominent and lasting increase in placental OPN as an anti-inflammatory response to O3 exposure that is also associated with vascular changes that may predispose for preeclampsia.
Differences regulate airway hyperresponsiveness to ozone-induced asthma. Male and female C57Bl/6 mice were untreated or sensitized with DRA, O3 or DRA-O3 combined to examine the effects of these two hits in regulating airway inflammation. Total number of cells within the airways were highest in the DRA only group for both male and female groups. H&E staining of lung sections showed increased inflammation in DRA and DRA-O3 groups compared to untreated and O3 only groups. Taken together, these data suggest sex differences regulate airway hyperresponsiveness to ozone-induced asthma.

Ozone is a highly reactive molecule derived from sunlight-induced reactions with volatile organic chemicals and oxides of nitrogen. Ozone reacts rapidly within the lung upon inhalation and is known to cause widespread systemic health effects, including cardiovascular impairment, immune cell influx into the lung, along with acute respiratory symptoms. Inhalation exposure to ozone induces blood-brain barrier permeability and neuronal inflammation, or reactive gliosis. In this study, dietary supplementation with different fatty acid diets was analyzed as a potential intervention for ozone-induced outcomes. Supplementation of standard chow with saturated and polyunsaturated fats (PUFAs) was conducted in order to determine how diet impacts the effects of ozone inhalation. As ozone is highly reactive with alkenes, we hypothesized that PUFAs in the lung would buffer most of the oxidative reactions and limit the systemic (neural) implications of exposure compared to saturated fats. Consequently, mice can be on diets supplemented with 6% oil enriched with the respective fatty acid species for 3 weeks before being exposed to 1ppm ozone for 4 hours. Diet types included coconut oil (saturated fat) soybean oil, (PUFA, Omega-6), flaxseed oil (PUFA, Omega-3) and a standard grain chow. 24h after ozone exposure, brain tissue was collected for lipidomics, metabolomics, and gene expression assays. Primary mouse brain endothelial cells were assayed for barrier integrity evaluation using electric cell-substrate impedance sensing (ECIS). Cells were treated with serum from the mice in the diet study. Cells treated with the supplemented diet serum demonstrated higher resistance between cells, indicating tighter junctions. In contrast, cells treated with standard chow serum had the lowest resistance levels. Gene expression data demonstrated higher levels of Claudin 5 within the brain with ozone exposure. This suggests a protective compensatory mechanism in response to a toxic interaction at the blood-brain barrier. Claudin 5 is a transmembrane protein that plays an important role in barrier integrity, expression of which appears to be upregulated in response to ozone. Lipidomic data analysis revealed both dietary and exposure-based changes in the lipid species and metabolites. Ozone exposure increased the expression of phosphatidylcholine, platelet activating factor, and phosphatidylethanolamine species decreased with ozone exposure and a saturated fat diet. In platelet activating factor demonstrated the most significant changes in the soybean oil group (omega 6), showing decreased expression with ozone exposure. The omega-3 PUFAsupplemented group (flaxseed oil) showed only modest changes in both lipid species and metabolites, suggesting a protective effect against ozone. This is in contrast to coconut oil, which exhibited more extensive changes in brain lipids and metabolites in response to ozone.

Inhalation exposure to air pollutants such as ozone (O3) is associated with respiratory disease in humans. In mice, repeated O3 exposure induces airway inflammation and remodeling in both the airways and parenchyma (fibrosis). However, typically high ozone exposures are required to induce airway inflammation and remodeling in classical inbred mouse strains such as C57BL/6J. To overcome these limitations, we utilized the Collaborative Cross (CC), a genetically diverse multi-parental mouse population, to identify strains with enhanced susceptibility to O3. In a previous study of 12 CC strains exposed to O3 for 9 days 0.8 ppm O3 the CC002 strain that was highly susceptible to O3-induced eosophinophilic airway inflammation. Intriguingly, histopathological analyses of O3-exposed CC002 lungs revealed the presence of fibrotic lesions adjacent bronchoalveolar duct junctions; while this phenotype has been observed previously in other rodent models, it has never been observed on this short time scale. Here, we tested whether CC002 mice also exhibit a heightened response to acute O3. At 1ppm C57BL/6J mice. After a single 4-hour exposure to 0.8 ppm O3 CC002 mice exhibited earlier and more prominent epithelial alarmin responses (lung IL-6, IL-33, and IL-1bala expression 2 hours post exposure) and greater epithelial cell injury (# of Ki67+ cells 20 hours post exposure) compared to C57BL/6J mice. These CC002 mice also exhibited a large drop in body temperature (10 deg C) and rapid, shallow breathing. CC002 mice also maintained body temperature (3 deg C drop) and exhibited shortened changes in breathing patterns. In conclusion, CC002 exhibits a unique susceptibility to O3 exposure that is associated with altered dosimetry and leads to fibrosis on very shortened time scale compared to other rodent models. The difference in dosimetry increases the relevance of CC002 as a model for O3 exposure induced pulmonary disease in humans. In the future, we aim to identify the genetic basis of CC002’s susceptibility.

The Impacts of Ozone Inhalation on the Brain Lipidome and Blood-Brain Barrier Integrity as Modulated by Dietary Fatty Acids

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Lung injury caused by inhalation of ozone is thought to be due, in part, to a failure to resolve acute inflammation. Macrophage effectorcytosis is known to play a role in inflammation resolution; thus, identifying mechanisms regulating this response is key to limiting ozone toxicity. Regulatory T (Treg) cells are a subset of CD4+ T cells that have been reported to stimulate macrophage effectorcytosis. Herein, we assessed the effect of ozone on T lymphocyte populations accumulating in the lung. Lung cells were collected by bronchoalveolar lavage with gentle massage 24 and 72 hours after exposure of mice to air or ozone (0.8 ppm, 2 hours). Immunostaining of lung cells with anti-CD3 antibody revealed that the total number of CD8+ T cells was increased at 72 hours post-ozone exposure relative to air controls. Macrophage efferocytosis was increased by a greater than 2-fold. Ozone exposure induced a unique susceptibility to CD45+CD4+CD3- cells detected at this time. Notably, this included a significant increase in CD25+ Treg cell subsets, and a subpopulation of these cells expressed the T cell activation marker CD69. In contrast, ozone had no effect on natural killer (NK) cell, B cell, or CD8+ and NK T cells at either post-exposure time point. Single cell RNA-sequencing identified a clearly defined cluster of cells exhibiting high expression of the Treg marker gene Il2ra. In this cluster, there were 42 differentially expressed genes (fold change > 1.5) and FDR-corrected p-value < 0.05) at 72 hours post exposure; no genes were differentially expressed at 24 hours. Pathway analysis of the differentially expressed genes using Ingenuity Pathway Analysis.
software identified yes-associated protein 1 (YAP-1), a transcriptional regulator that promotes Treg differentiation, as a significantly enriched upstream regulator. Similarly, Elongation Initiation Factor 2 (EIF2) signaling was also a significantly enriched canonical pathway and was predicted to be upregulated (z-score = 3.87), a response consistent with increased protein synthesis, which is a hallmark of lymphocyte activation. Collectively, these results indicate that activated Tregs accumulate in the lung during the later resolution phase of inflammation following acute injury induced by ozone. We speculate that these cells are important in promoting macrophage efferocytosis, resolution of inflammation, and limiting lung injury caused by ozone. This work was supported by NIH ES032473, ES004738, ES005022 and ES033698.

4654 Effects of Prolonged Wildland Fire Smoke Inhalation on Murine Respiratory Structure and Function
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Rising global temperatures and prolonged droughts world-wide are increasing the size and severity of wildland fires, which are in turn increasing the occupational risk of smoke inhalation for wildland fire fighters (WLFs). While epidemiological evidence shows varied evidence of cross-shift or seasonal declines in lung function, the long-term impacts of wildland fire smoke (WFS) inhalation remain unclear. To uncover the biological underpinnings of WFS exposure, we performed a preclinical assessment of long term WFS inhalation in a hypercholesterolemia mouse model (ApoE-/-). A custom-built smoke generation apparatus was used to produce consistent smoldering Douglas fir smoke (DFS, Missoula, MT). Daily particulate matter (PM) concentration was measured gravimetrically, carbon monoxide (CO) was monitored continuously (Enerac 700, Enerac, NY), and the particle size distribution was measured using an engine exhaust particle sizer (EEPS 3090, TSI, MN). Male, 8-week-old ApoE-/- mice were exposed for 2 hours/day, 5 days/week, for 8 or 16 weeks (9 ppm CO2) exposure to room air as control (N=6) or to forest smoke (3 ppm CO2) 6 mice to FA or O3 (2 ppm x 3h) and performed BAL 12 and 24 hours post-exposure. We observed overall increased numbers of BAL neutrophils in the MerTK null mice at 12 and 24 hours post-exposure. Consistent with clodronate experiments, MerTK null mice demonstrated persistence of O3-induced neutrophilic inflammation. In addition, O3-exposed MerTK null mice when compared to controls exhibited increased oxidative stress as measured by a protein carbonyl assay. Overall, this data support that tissue-resident AMØ promote resolution of O3-induced inflammation via MerTK-mediated efferocytosis. These data suggest distinct roles for macrophage subsets in the resolution of air pollution-mediated lung injury.

4656 Dietary Modulation of Pulmonary Lipids Influencing Toxicological Responses to Ozone Inhalation in Mice
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Lipid and protein composition of the epithelial lining fluid of the lung facilitate interactions with xenobiotic agents and the degree of inflammatory reactions within the alveolar space. As a source of oxidative damage, the inhalation of ozone is a known driver of inflammation in the lung that leads to systemic release of biological mediators into the circulatory system effecting vascular tone. The current study examines how dietary supplementation of saturated and polyunsaturated fatty acids (SFA, PUFA) can impact the lung, as well as how these changes can modulate the biological effects of ozone inhalation on the lungs. The experimental design utilized female C57BL/6 mice as a model, with animals receiving diets containing 6% soybean, flaxseed, or coconut oil. Animals were then exposed to either 1ppm ozone or filtered air for 4 hours. 24 hours post exposure, animals were evaluated for pulmonary inflammation via bronchoalveolar lavage (BAL), and lungs were prepped for Matrix-assisted laser desorption/ionization (MALDI) imaging. This study highlights how dietary supplementation of fatty acids can have large effects on organ systems beyond the digestive tract and provides clues for potential dietary intervention strategies to limit risks to human health due to air pollution induced pulmonary damage.

4657 When You Give a Tissue-Resident Macrophage an Apoptotic Cell
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Acute ozone (O3) exposure exacerbates respiratory diseases through induction of lung inflammation and is a burden to global public health. A critical cell type regulating O3-induced inflammation is alveolar macrophages (AMØ). AMØ functions are dependent on their cellular origin (i.e., tissue-resident or recruited monocyte-derived). Our prior work demonstrated that AMØ are not recruited after acute O3 exposure, while tissue-resident AMØ expand during O3-induced inflammation. However, the specific functional role of tissue-resident AMØ in O3-induced lung injury has yet to be defined. We hypothesized that tissue-resident AMØ promote resolution of O3-induced lung inflammation via their role in efferocytosis, the removal of apoptotic cells. To test this hypothesis, AMØ were depleted from the lungs of male C57BL/6 mice by oropharyngeal aspiration of clodronate liposomes (50µL of 5mg/mL) or PBS vehicle control. AMØ depletion was confirmed by whole lung flow cytometry assessment. These mice (AMØ sufficient or depleted) were then exposed to FA or O3 (2 ppm x 3h) and airspace inflammation and cytokine production were measured following a harvest 12 and 24 hours post-exposure. To assess for the requirement of tissue-resident AMØ in efferocytosis following acute O3 exposure, AMØ were depleted with clodronate versus vehicle and then apoptotic and Calcein AM labeled T cells, jurkats, were instilled via oropharyngeal aspiration for 90 min. Bronchoalveolar lavage (BAL) was collected and processed for flow cytometry to define Calcein AM positive cells as a measure of the clearance of apoptotic cells. We observed that clodronate ablation of AMØ caused persistence of airspace neutrophils and of pro-inflammatory cytokine production after acute O3 exposure. In addition, clodronate ablation of tissue-resident AMØ decreased in vivo efferocytotic capacity as defined by an increase in lavagable Calcein AM positive cells. Given that AMØ highly express Mer tyrosine kinase (MerTK) and this receptor regulates efferocytosis, with exogenous MerTK delivery (1pmol) or sufficient MerTK expression (MerTK null) mice to FA or O3, and performed BAL 12 and 24 hours post-exposure. We observed overall increased numbers of BAL neutrophils in the MerTK null mice at 12 and 24 hours post-exposure. Consistent with clodronate experiments, MerTK null mice demonstrated persistence of O3-induced neutrophilic inflammation. In addition, O3-exposed MerTK null mice when compared to controls exhibited increased oxidative stress as measured by a protein carbonyl assay. Overall, this data support that tissue-resident AMØ promote resolution of O3-induced inflammation via MerTK-mediated efferocytosis. These data suggest distinct roles for macrophage subsets in the resolution of air pollution-mediated lung injury.

4658 Activation of the Integrated Stress Response in Lung Macrophages after Exposure of Mice to Ozone
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Ozone is a pulmonary irritant known to oxidize biomolecules in the respiratory tract, including phospholipids, which cause stress in lung macrophages. This can lead to proinflammatory and anti-inflammatory mediators that exacerbate lung injury. In response to stress, cells upregulate the Integrated Stress Response (ISR), a protective pathway that functions to promote the resolution of inflammation; aberrations in the ISR have been implicated in chronic inflammation and exacerbation of tissue injury. Central to activation of the ISR is phosphorylation
of eIF2α via intracellular kinases, this initiates signaling pathways that promote cell survival. Oxidized phospholipids have been identified as a trigger of the ISR. In these studies, we analyzed the effects of acute ozone exposure on the accumulation of oxidized phospholipids in lung macrophages and determined if this was associated with activation of the ISR. Female C57BL/6 mice (12 wk) were exposed to filtered air or ozone (0.8 ppm, 3 hours). At 24 hours and 72 hours post-exposure, lung macrophages were collected, dye-labeled with anti-Scnn1b, and cultured with glutathione to reduce lung cell damage and analyzed for oxidized phospholipids and for ISR activation. Following ozone inhalation, we observed an accumulation of oxidized phospholipids in lung macrophages, as determined by immunofluorescence staining. Western blot analysis of macrophages revealed that this was associated with ISR activation, characterized by significant increases in phosphorylated eIF2α protein in lung macrophages, a response most prominent 72-hour post ozone exposure. Analysis of isolated lung macrophages by RNAseq showed significant alterations in known ISR-regulated genes at this time; these included downregulation of Atf4, Atf5, Tnb3, Asns, and Txnip, and upregulation of Ikbk, Ihd2, and Mdm2. This was accompanied by activation of proliferation pathways in macrophages as determined by Ingenuity Pathway Analysis. These results demonstrate that acute ozone exposure induces an accumulation of oxidized phospholipids in lung macrophages, which may contribute to activation of the ISR and restoration of homeostasis. As such, the ISR could be a potential target to promote the resolution of inflammation and mitigate the effects of ozone-induced toxicity. Supported by NIH ES032473, ES004758, ES033639, ES005022, and GM198804.

4655 Ultrafine Particle and Ozone Co-exposure Predisposes to Greater Microbiome Dysbiosis after Acute Lung Injury

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Exposure to ultra-fine particulate matter and ozone are associated with significant cardiorespiratory morbidity and mortality. Microbiome plays a significant role in modulation of the immune system. Acute Lung Injury (ALI) is characterized by severe lung inflammation causing alveolar damage and poor lung compliance. Limited information exists on the adverse effect of environmental exposure on microbial dysbiosis in the lung and gut in the context of regenerating lung. We hypothesized that co-exposure to carbon black (CB) and ozone (O2) will significantly alter the microbiota in lung and gut microbiome after acute exposure. C57Bl/6J male and female mice were exposed to air, or a mixture of carbon black (1 mg/m3 + ozone (250 ppb)) for 3 hours/day, 5 days/week for three weeks. At the end of exposure, animals were treated intra-tracheally with bleomycin (3U/kg) or PBS and euthanized on 7th and 14th day post bleomycin treatment. We performed 16S sequencing on lung and colon contents, serum short chain fatty acid contents were measured using mass spectrometry and inflammation was assessed using real-time PCR and ELISA. Real-time monitoring and characterization of the aerosols demonstrated generation and exposure to stable aerosols at the desired concentrations. We observed significant shift in microbial abundance in bleomycin treated group. Following bleomycin exposure, (number of altered OTUs, alpha and beta diversity) was significantly altered in case of pre-exposure to carbon+ozone aerosols. Additionally, digital PCR based analysis of the absolute bacterial load demonstrated a sex-based response in the animals post co-exposure. The females demonstrated significantly reduced bacterial load post-bleomycin treatment and exposure. Real-time PCR analysis for mRNA abundance of free fatty acid receptor FFR2 and FRR3 demonstrated significant induction after 7days time point. The short chain fatty acid analyses in the serum demonstrated significant reduction in acetate, propionate and butyrate in males compared to females after 7- and 14-days post-bleomycin treatment. In summary, we demonstrate significant alteration of lung-gut microbiome dysbiosis and systemic short chain fatty acid levels in ultrafine particle and ozone co-exposed and bleomycin injured lungs.

4656 Cardiopulmonary Effects of the Combined Exposure to Urban Particulate Matter and Ozone and Analyses of Substances P and Cytokines


The number of people living and working in urban areas has been steadily increasing over the last several decades as populations have grown towards cities, and the number of megacities continues to grow. As the number of people in these environments increases, their activities likely contribute to higher levels of various forms of air pollution, thereby increasing the risk of adverse effects from exposure to air pollution. From a health perspective, two important components of urban air pollution are particulate matter (PM) and ozone gas. Urban PM is a complex mixture of particles and consists of organic and inorganic substances generated from a variety of sources including natural activities, industrial processes, and traffic-related emissions. Ozone (O3) is an important gaseous pollutant that is formed from numerous activities, including combustion. In this study, we simulated an urban environment in a chamber with airborne urban PM and ozone and exposed a rodent model in order to investigate the potential adverse health effects. Brown Norway rats were exposed for 3 hours in single whole-body inhalation exposure chambers to filtered air, National Institute of Standards and Technology (NIST) Urban PM standard reference material 1648a collected in the St. Louis, MO area (0, 10, or 20 mg/m3), O3 (0, 1.0, or 2.0 ppm), or a combination of PM and ozone. Exposed rats were evaluated the next day for cardiac and lung function, and changes in levels of Substance P and inflammatory cytokines in the blood and...
bronchoalveolar lavage fluid (BALF). Results from a small animal echocardiography system used to measure changes in cardiac and major artery function revealed that animals exposed to 20 mg/m³ PM demonstrated significant cardiac effects including increased pulmonary artery acceleration time and pulmonary artery ejection time/pulmonary artery acceleration time compared with animals exposed to filtered air. Further, cardiac output was significantly lower for animals exposed to 20 mg/m³ PM. Conversely, animals exposed to either of the two O₃ concentrations and the co-exposures to O₃ and PM showed no significant effects on cardiac function. Airway hyper-responsiveness was measured by FlexiVent. To measure the mechanical function of the lungs, animals were exposed to increasing doses of inhaled methacholine and various parameters including respiratory system resistance (Rrs), respiratory system elastance (Ers), central airway response (Rn), tissue damping (G) and tissue elastance (H) were measured. When compared to animals exposed to filtered air alone, there were statistically significant differences in Ers, Rn and G in animals exposed to combined PM and O₃ (10 mg/m³ PM + 1 ppm O₃ and 20mg/m³ PM + 1 ppm O₃); Rrs in animals exposed to 1 ppm O₃; and H in animals exposed to 20 mg/m³ PM in combination with 1 or 2 ppm O₃. Plasma and BAL fluids were analyzed by immunoassay for the neuropeptide Substance P and inflammatory cytokines (IFN-gamma, IL-4, IL-5, IL-6, IL-10, IL-13, TNF-alpha, and KC-GRO).

Overall, changes in lung function were found in rats exposed to combined exposure of PM and ozone while changes cardiac parameters were observed in the animals exposed only to PM. Under the conditions of this study, it appears that the PM is the major contributor to the observed pulmonary changes.
neurogenesis. In contrast to what is observed in SCZ, however, male mice exposed to Cyp1a1 deficiency had a reduced, albeit non-significant, ventricle area and perimeter, compared to control males. In contrast, Cyp1a1-exposed females showed significantly increased ventricle area and perimeter in the more caudal section of brain. Myelination was examined in the largest white matter tract of brain, the corpus callosum which was split into three subregions: the truncus, the medial external capsule (MEC) and lateral external capsule (LEC). This was done to account for the large area of the brain which the corpus callosum spans, and the large variety of other brain regions to which it interconnects. No significant differences in myelination, measured either as myelin basic protein positive counts or in staining intensity, were found in relation to Cu exposure, in either sex. Neurogenesis was assessed by number of parvalbumin (PV) positive (PV+) fast spiking, GABA-ergic neurons, which impact the brain’s excitatory-inhibitory balance, an imbalance of which is seen in SCZ, in two regions: the A32 of the frontal cortex, and the Dorsal Subiculum (DS), both regions dysregulated in SCZ. In the A32, the PV+ neurons examined were basket cells, while in the DS, the PV+ neurons were chandelier cells. In the A32, male mice tended toward having fewer PV+ neurons following Cu exposure, while there was no change in females. In the DS, females in general had more PV+ neurons than males. There was also a trend toward both females and males having fewer PV+ neurons following Cu exposure in the more rostral areas of the DS. Overall, these data indicate a pressing need to further elucidate the effects of different components of AP on neurodevelopmental disorders, including SCZ, as many of these disorders do not have a clearly defined etiology. Due to the increasing evidence that developmental exposure to AP may be involved in these disorders, increased understanding is of importance to public health. Supported by NIH Grants R01 ES032260 and R35 ES031689.

4663 Single-Cell RNA Sequencing Analysis in Atherosclerosis Reveals Unique Cell Types and Differential Profiles in Response to Chronic Exposure to Ambient Air Pollution


Air pollution is a major contributor to the development of chronic noncommunicable diseases, including atherosclerotic cardiovascular disease (ASCVD). Atherosclerosis is the primary cause of cardiovascular disease and causes >50% of deaths worldwide attributable to air pollution. While our lab has provided multiple mechanisms linking exposure with atherosclerosis, the cell specific pathways remain unclear. The goal of this study was to identify the transcriptomic landscape and the dynamics of cell population heterogeneity within the atherosclerotic plaque of atherosclerosis prone mice following inhalation of particulate matter. Male ApoE-/- mice were exposed to filtered air (FA) or aerosolized particulate matter (PM) for 5 days a week, 6 hours per day for a total duration of 36 weeks using a Versatile Aerosol Concentration Enrichment System (VACES), a whole-body exposure system, used routinely in our laboratory to accomplish long term exposures to PM2.5, at levels that are 8-10 x ambient levels (70-80 μg/m³). Aortas were carefully dissected, pooled, and cells were processed following the 10X genomics protocol for single-cell RNA sequencing (scRNA-seq). Following sequencing, we analyzed 61,311 cells in total, and kept 25,692 high-quality cells with a mean sequencing depth of 11,900. In the process we retained only 12,823 genes that could be detected. After data normalization, Uniformed Manifold Approximation and Projection (UMAP) and an unsupervised graph-based clustering method (K-nearest neighbor combined with a modularity optimization technique) were used to identify 12 cell clusters for FA and 14 cell clusters for PM-exposed mice. Characterization of subsequent aortic cell population, cell subtypes, and cell lineages was done by functional analyses (gene markers, gene ontology, molecular pathways). We identified 7 cell populations (vascular smooth muscle cells (VSMC), endothelial cells, monocytes/macrophages, mast cells, pericytes, and 4 cell types in PM): 1) FA mouse aortas and 2 additional cell populations in PM mouse aortas. A dendritic cell population and an unannotated cell population were identified upon PM exposure. This unannotated cell population demonstrated increased gene expression for TIMP1, EGR4, TNFRSF11B, suggesting a fibroblast-like cell population. Air pollution air exposure caused macroaphages’ gene expression profile changes from more fibroblast markers (DCN, LUM, and CS) to resolution phase macroaphage markers (H2-Eb1, H2-Ab1, CD74, and H2-Aa). Overlapping cell clustering and cell typing data also allowed the identification of potential cell subtypes. In FA mice, we identified 4 vascular smooth muscle cell subtypes and 2 fibroblast subtypes. In PM mice, we also identified 4 vascular smooth muscle cell subtypes and 3 fibroblast subtypes. Lastly, we used a supervised graph method (Minimum Spanning Tree and Principal Curve) to deduce branching and cell ordering for novel cell lineage pseudotime/pseudospace inference. We observed 2 trajectory lineages in FA mice (monocytes to VSMC and monocytes to EC) and 2 additional ones in PM mice (monocytes to fibroblasts and monocytes to EC and cells/T cells). These trajectory cell lineages hint at potential new paths to cell differentiation and plasticity in response to air pollution exposure. Overall, scRNA-seq technology combined with statistical and functional analyses demonstrated that a previously uncharacterized biological complexity of aortic cells in terms of cell populations, cell subtypes, and cell lineages in response to PM exposure can be unraveled. Our findings have significant implications for renewed understanding of the links between air pollution exposure and ASCVD.
Results of epidemiologic studies independently suggest that either ambient fine particulate matter (PM$_{2.5}$) air pollution exposure or a disturbed circadian rhythm (circadian dysynchrony) are important contributing factors to the rapidly evolving type-2 diabetes (T2D) epidemic. In the present study we investigate whether and how PM$_{2.5}$ exposure increases metabolic injury in circadian dysynchrony. For this, we exposed mice maintained on a regular (12/12 h light/dark) or disrupted (18/6 h light/dark, light-induced circadian dysynchrony, LICD) light cycle to concentrated PM$_{2.5}$ (CAP, 6 h/day, 30 days). Exposures during Zeitgeber times (ZT) 3-9 or 11-17 tested for time-of-day PM$_{2.5}$ sensitivity (chronotoxicity). At the end of the exposure experiments we examined systemic and organ-specific changes in glucose tolerance and insulin sensitivity in mice inhaling CAP or air (control). Mice transgenic for lung-specific overexpression of extracellular superoxide dismutase (ecSOD-Tg) were used to assess the contribution of pulmonary oxidative stress. We found that CAP exposure from ZT3-9 or ZT11-17 increased systemic glucose intolerance and insulin resistance in mice with LICD, but not in mice kept on a regular light cycle. Although changes in glucose homeostasis in CAP-exposed LICD mice were not associated with obesity, they were accompanied by adipose tissue inflammation, impaired insulin signaling in skeletal muscle and liver, as well as systemic and pulmonary oxidative stress. Overexpression of ecSOD prevented the effects of CAP on glucose tolerance and insulin sensitivity that occurred in LICD. Taken together, these data suggest that CAP exacerbates glucose intolerance and insulin resistance in circadian dysynchrony by inducing pulmonary oxidative stress. Our results demonstrate that circadian dysynchrony is a susceptibility state for PM$_{2.5}$ exposure.

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Osteoarthritis (OA) is a complex and multifactorial, with many interconnected cellular pathways influencing disease onset and progression. Epidemiological studies have shown that environmental factors such as environmental pollution may contribute to an increased risk of OA. Recently, investigators have reported an increased prevalence of pulmonary disease in relationship to PM$_{2.5}$ exposure and have made a link to musculoskeletal conditions. While some work has investigated PM in animal models of induced post-traumatic OA or rheumatoid arthritis, there are no studies to date that explore the effect of PM$_{2.5}$ exposure in an age-related, naturally-occurring model of OA. We have demonstrated that chronic exposure to PM$_{2.5}$ impairs OA progression, likely through a cascade of events initiated by compromised systemic inflammation and inflammatory mediators affiliated with systemic inflammation and joint degeneration. Specifically, mRNA expression of interleukin-1beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF), and complement component 3 (C3) protein (serum and synovial fluid) and C3 gene expression (lung, IFP, cartilage, menisci). Significantly, mRNA expression of interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1β antagonist (IL-1β-A), interleukin-6 (IL-6), TNF, and interleukin-1β (IL-1β) was significantly increased in all tissues, including lung and knee joint connective tissue (interleukin-1β antagonist, IL-1β-A) and lung, menisci, synovial fluid, and cartilage.

Further, primary chondrocytes exposed to PM$_{2.5}$ for 72 hours significantly increased ROS production when compared to control primary chondrocytes. Similarly, Annexin V staining corroborated a significant increase apoptotic chondrocytes exposed to PM$_{2.5}$. Cumulatively, this in vitro work provided the foundation primary chondrocytes are undergoing oxidative stress and programmed cell death with PM$_{2.5}$. To evaluate the effect of PM$_{2.5}$ on mRNA expression of key inflammatory mediators associated with joint inflammation and degeneration within lung and joint knee tissue (interleukin-1β antagonist, IL-1β-A) and lung, menisci, synovial fluid, and cartilage.


Epidemiological studies suggest a link between air pollution exposure and neurocognitive dysfunction. Current assessment tools are insufficient to assess changes in brain function including Alzheimer’s disease (AD). However, there is a lack of understanding of potential mechanisms. Accordingly, we aimed to evaluate the effects of chronic exposure to air pollution (particulate matter <2.5µm, or PM$_{2.5}$) on cognitive function and hippocampal dysfunction using a variety of behavioral tasks in mice. We exposed mice to PM$_{2.5}$ at levels that are 8-10x ambient levels (70-80 µg/m$^3$). Wild-type (WT) littermates were exposed to PM$_{2.5}$ at levels that are 8-10x ambient levels (70-80 µg/m$^3$). Wild-type (WT) littermates or transgenic 5xFAD mice were exposed to PM$_{2.5}$ or filtered air for 24 hours. Following polarization, macrophages were exposed to M1-polarizing conditions (20 ng/mL LPS and IFN-γ) with or without 25 µg/cm$^2$ DEP for 24 hours. Following these exposures, macrophages were assessed for phagocytic ability, pro-inflammatory cytokine secretion, gene expression profiles, and bioenergetic properties. Both M0 and M2 macrophages exposed to either M1-polarizing conditions or DEP demonstrated a significant reduction in pro-inflammatory cytokine secretion compared to control primary chondrocytes. Repolarized M2 macrophages (M2->M1) and directly polarized M0->M1 macrophages (M1) demonstrated similar gene expression profiles. DEP exposure induced complex gene expression changes which were similar in both cell types, suggesting DEP exposure induces broad changes in gene expression patterns independent of initial polarization state. M2->M1 macrophages demonstrated a more pro-inflammatory profile characterized by elevated glycolytic and mitochondrial oxidative phosphorylation rates. Co-exposure with DEP induced a shift from glycolysis to oxidative phosphorylation in M1 and M2->M1 macrophages. However, co-exposed M2->M1 macrophages maintained their overall rate of oxidative phosphorylation compared to M1 macrophages. Together, these data support the hypothesis that DEP exposure may be capable of repolarizing to a pro-inflammatory phenotype while retaining some of their original polarization programming. This mixed M1/M2 phenotype is characterized by dysfunctional phagocytosis, intermediate bioenergetic properties, and elevated pro-inflammatory cytokine secretion. DEP co-exposure during the repolarization process demonstrated a dose-dependent increase in cytokine secretion and bioenergetic and pro-inflammatory property changes, and induce a shift from glycolysis to oxidative phosphorylation. In conclusion, our data indicate that particle exposure during the M2->M1 repolarization process may generate a unique, highly inflammatory, highly energetic population of macrophages with diminished anti-microbial functions.


Epidemiological studies suggest a link between air pollution exposure and neurocognitive dysfunction. Current assessment tools are insufficient to assess changes in brain function including Alzheimer’s disease (AD). However, there is a lack of understanding of potential mechanisms. Accordingly, we aimed to evaluate the effects of chronic exposure to air pollution (particulate matter <2.5µm, or PM$_{2.5}$) and its effects on previously implicated pathways in neurocognitive dysfunction. A validated transgenic model of Alzheimer’s Disease (AD; 5xFAD) was tested and exposed to PM$_{2.5}$. Wild-type (WT) littermates were exposed to 5xFAD mice were exposed to PM$_{2.5}$ at levels that are 8-10x ambient levels (70-80 µg/m$^3$). Wild-type (WT) littermates or transgenic 5xFAD mice were exposed to PM$_{2.5}$ or filtered air for 24 hours. Following polarization, macrophages were exposed to M1-polarizing conditions (20 ng/mL LPS and IFN-γ) with or without 25 µg/cm$^2$ DEP for 24 hours. Following these exposures, macrophages were assessed for phagocytic ability, pro-inflammatory cytokine secretion, gene expression profiles, and bioenergetic properties. Both M0 and M2 macrophages exposed to either M1-polarizing conditions or DEP demonstrated a significant reduction in pro-inflammatory cytokine secretion compared to control primary chondrocytes. Repolarized M2 macrophages (M2->M1) and directly polarized M0->M1 macrophages (M1) demonstrated similar gene expression profiles. DEP exposure induced complex gene expression changes which were similar in both cell types, suggesting DEP exposure induces broad changes in gene expression patterns independent of initial polarization state. M2->M1 macrophages demonstrated a more pro-inflammatory profile characterized by elevated glycolytic and mitochondrial oxidative phosphorylation rates. Co-exposure with DEP induced a shift from glycolysis to oxidative phosphorylation in M1 and M2->M1 macrophages. However, co-exposed M2->M1 macrophages maintained their overall rate of oxidative phosphorylation compared to M1 macrophages. Together, these data support the hypothesis that DEP exposure may be capable of repolarizing to a pro-inflammatory phenotype while retaining some of their original polarization programming. This mixed M1/M2 phenotype is characterized by dysfunctional phagocytosis, intermediate bioenergetic properties, and elevated pro-inflammatory cytokine secretion. DEP co-exposure during the repolarization process demonstrated a dose-dependent increase in cytokine secretion and bioenergetic and pro-inflammatory property changes, and induce a shift from glycolysis to oxidative phosphorylation. In conclusion, our data indicate that particle exposure during the M2->M1 repolarization process may generate a unique, highly inflammatory, highly energetic population of macrophages with diminished anti-microbial functions.

FA, PM-exposed wild-type mice along with both exposure groups of transgenic 5xFAD mice were used to investigate the impact of oxidative stress on cognitive function. The leakage volume of dextran in WT PM-exposed mice was significantly higher when compared to WT FA-exposed mice. The presence of amyloid plaques using Thioflavin-S staining (fluorescent signal depicted from Thioflavin-S corroboration of amyloid-beta presence) was tested. Fluorescence quantification of Thioflavin-S in the cerebral cortex of PM- and FA-exposed wild-type mice revealed a significant trend toward deposition as expected. In contrast, 5xFAD mice demonstrated robust plaque deposition, with no significant difference between FA and PM exposure. This study provides new and potentially important mechanisms of cognitive impairment caused by air pollution and may provide new mechanistic understanding of the underlying mechanisms linking air pollution exposure with cognitive decline.

4670 An Improved Machine-Learning Approach MacLEAP for the Recognition and Quantification of Carbon Content in Airway Macrophages
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Long-term exposure to combustion-emitted particulate matter (CE-PM) increases cardiopulmonary morbidity and mortality. Macrophage carbon load (MaCL) is a novel spumut cytology-based method that measures accumulative lung deposition dose of total CE-PM exposure at an individual level. MaCL has been considered a highly effective assessment tool in pulmonary and extra-pulmonary outcomes in epidemiological and occupational exposure settings. Conventional pathological scoring of MaCL is labor-consuming and scorer-dependent, with a limitation on efficiency in large-scale studies. We have previously developed a state-of-the-art method based on the deep convolutional neural network Mask R-CNN: Machine Learning approach for Encrusted Carbon Particles (MacLEAP), which automatically distinguishes (segments) the macrophages from other cell types on the sputum images and quantifies the phagocytosed nano-scale black carbon particles (PM 2.5 - PM 10) from 17 subjects. In the current report, we further optimized and strengthened the artificial intelligence (AI) algorithm with additional 1043 sputum images from 66 individuals of the LoveUckes Smokers Cohort. Our current optimized MacLEAP algorithm successfully recognized 96.3% macrophages from all 1789 sputum images. We found that MacLEAP exhibited greater robustness on challenging images. It is notable that the AI algorithm also recognizes more macrophages (3.5%), mostly cells with broken cytoplasm or irregularly stained nuclei, which were excluded during the visual inspection of images. Excellent correlations were identified for the mean number and median area for carbon particles between manual and AI counting in 66 subjects used for the development of the MacLEAP algorithm. Excellent correlations were also identified for the mean number (R²=0.76) and median area (R²=0.91) of carbon particles between manual and AI counting in 22 subjects used as the external validation of the MacLEAP algorithm. Combined results in 88 subjects were 0.86 and 0.83 for the mean number and median area of black carbon particles between manual and AI counting, respectively. In conclusion, this study established a MacLEAP machine learning model, which provides a high-throughput and scorer-independent approach to quantify MacCL levels from thousands of slide images, making large-scale epidemiological studies involving lung deposition dose of black carbon feasible.
Environmental Protection Agency (EPA)'s annual standard of PM2.5 (0.015 mg/m³). These findings shed light on the exposures and health outcomes of museum staff and will help shape the future of health and safety in this field. The team will investigate other hazards such as mold and bioaerosols in future research. This work was supported by the IUPUI Office of the Vice Chancellor for Research under a Research Support Funds Grant (RSFG). This work was supported by the United States National Institute of Occupational Safety and Health Grant (T03OH008615).

4672  Utilizing a Human Lung Epithelial Cell Macrophage Co-culture Model to Assess the Effects of Environmental Toxicants In Vitro
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The development of representative in vitro models to investigate the effects of inhalation exposure of the lung environment to particulate matter is an essential step in the inhalation toxicology field. The inhalation of particulate matter from environmental sources such as vehicular sources, energy production, mine dusts, as well as microplastics contributes to increased rates of diverse diseases, such as autoimmune, hypertension, and interstitial lung diseases. While AS49 cells are routinely used as a model for the testing of the effects of toxicants in the lungs, there is insufficiency in the representation of these cells to model the lung environment due to the lack of important immune cell populations and the inability to model the effects of their response and recruitment to sites of exposure. The addition of differentiated THP-1 monocytic cells into macrophages onto AS49 cells in a co-culture model helps to investigate the interactive responses to deposited particulates as an immune-epithelial interface. We developed this model to investigate the toxic effects following particulate exposure in vitro. For the mine dust studies the AS49 and THP-1 macrophages were plated in a 10:1 ratio and cells were exposed for 24-48-hours to soluble arsenic (6/12 μM) and vanadum (60/120 μM), with and without the addition of aluminum silicate powder (20 μg). Aluminum silicate comprises roughly 25% of the mine dust from abandoned mine site waste in the four corners area of the United States, so we sought to determine whether there was a compounding effect of the aluminum silicate in combination with the heavy metals present in the mine dust. Analysis of the co-culture after treatment with the metals +/- silicate was performed by flow cytometry where a CD45 antibody was utilized to distinguish the macrophages from the epithelial cells. Markers for DNA damage and cytotoxicity-phosphorylated γ-H2AX and Live/Dead staining were also included in the panel. The results indicated that within the co-culture there was a differential toxicity response where the epithelial cells showed no change in DNA damage after treatment with arsenic or vanadium, yet the macrophages showed a significant increase in DNA damage following the arsenic treatment. We also observed that the macrophage varies in the four corners area of the United States, this workforce is comprised primarily of migrant, seasonal, and immigrant farmworkers that face many social inequities, leading to challenges in gaining exposure and health information. To begin to address PM exposure and respiratory infection incidence in crop farmworkers in Florida, we recruited mostly Mexican-American farmworkers that worked in fernery harvesting, nursery greenhouses, and strawberry harvesting. Participants (N=30) completed a questionnaire that captured information on demographics, chronic respiratory symptoms, work-related training, and work-related task. Each participant was then fitted with a personal air sampler to assess occupational exposure to respirable PM (< 2.5 μm) during an 8-hour work week which was used to calculate cumulative occupational exposure to PM2.5 for total years worked in agriculture. We then conducted prospective weekly follow-ups of participants during influenza season to determine the incidence of acute respiratory symptoms and viral respiratory infections. Nasal swab biospecimens were collected from symptomatic participants and tested for 20 different respiratory pathogens using a PCR panel array (Biofire). Descriptive statistics were used to demonstrate continuous and categorical variables, meanwhile regression models were used to demonstrate the association of reported respiratory symptoms and PM2.5 exposure. Data from this study show that 2 participants (1800.14 μg/m³ and 17820.89 μg/m³) had PM exposures that exceeded the OSHA permissible exposure limit of 15mg/m³ and strawberry harvesting yield the greatest geometric mean (121.35 μg/m³). A Poisson Regression analysis demonstrated that measured PM2.5 and calculated cumulative PM2.5 values had a positive association with reported symptoms of chronic coughing (OR 4.11 vs OR 3.02) and wheezing (OR 1.51 vs OR 1.40). During the follow-up, for participants reporting acute respiratory symptoms and pathogenic molecular analysis revealed positive results for human rhinovirus (n=2), and SARS-CoV-2 (n=1), whereas 1 individual reported symptoms but tested negative in the panel used. To our knowledge, this is the first study to assess occupational PM2.5 exposure and incidence of respiratory infections in Southeastern US-hired farmworkers which support PM exposures may be significant and associated with adverse respiratory health outcomes in this population.

4674  Assessing Personal Particulate Matter Exposure and Respiratory Virus Infections among Farmworkers in the Southeastern United States
A. Manrique, K. Clarke,1 J. Lednicky1, T. Sabo-Attwood,1 and E. Coker2 1University of Florida, Gainesville, FL, and 2British Columbia Centre for Disease Control, Vancouver, BC, Canada.

Industrialization of the agricultural industry has led to an increased risk of exposure to hazardous pollutants for workers. Among crop farmworkers, occupational studies have identified particulate matter (PM) as an emerging concern. Particulates from agricultural dust may be multi-pollutant mixtures of pesticides, heavy metals, mineral silicas, air pollutants, and microbes and previous reports show that high PM exposures are associated with harvesting tasks. Furthermore, chronic respiratory conditions and mortality attributable to acute respiratory infections are notably elevated among crop farmworkers, yet the association between PM exposure and susceptibility to respiratory infections has not been well explored. In this study, we established an immune-epithelial interface. We developed this model to investigate the interactive responses to deposited particulates as an immune-epithelial interface. We developed this model to investigate the interactive responses to deposited particulates. The addition of differentiated THP-1 monocytic cells into macrophages onto AS49 cells in a co-culture model helps to investigate the interactive responses to deposited particulates as an immune-epithelial interface. We developed this model to investigate the toxic effects following particulate exposure in vitro. For the mine dust studies the AS49 and THP-1 macrophages were plated in a 10:1 ratio and cells were exposed for 24-48-hours to soluble arsenic (6/12 μM) and vanadum (60/120 μM), with and without the addition of aluminum silicate powder (20 μg). Aluminum silicate comprises roughly 25% of the mine dust from abandoned mine site waste in the four corners area of the United States, so we sought to determine whether there was a compounding effect of the aluminum silicate in combination with the heavy metals present in the mine dust. Analysis of the co-culture after treatment with the metals +/- silicate was performed by flow cytometry where a CD45 antibody was utilized to distinguish the macrophages from the epithelial cells. Markers for DNA damage and cytotoxicity-phosphorylated γ-H2AX and Live/Dead staining were also included in the panel. The results indicated that within the co-culture there was a differential toxicity response where the epithelial cells showed no change in DNA damage after treatment with arsenic or vanadium, yet the macrophages showed a significant increase in DNA damage following the arsenic treatment. We also observed that the macrophage varies in the four corners area of the United States, this workforce is comprised primarily of migrant, seasonal, and immigrant farmworkers that face many social inequities, leading to challenges in gaining exposure and health information. To begin to address PM exposure and respiratory infection incidence in crop farmworkers in Florida, we recruited mostly Mexican-American farmworkers that worked in fernery harvesting, nursery greenhouses, and strawberry harvesting. Participants (N=30) completed a questionnaire that captured information on demographics, chronic respiratory symptoms, work-related training, and work-related task. Each participant was then fitted with a personal air sampler to assess occupational exposure to respirable PM (< 2.5 μm) during an 8-hour work week which was used to calculate cumulative occupational exposure to PM2.5 for total years worked in agriculture. We then conducted prospective weekly follow-ups of participants during influenza season to determine the incidence of acute respiratory symptoms and viral respiratory infections. Nasal swab biospecimens were collected from symptomatic participants and tested for 20 different respiratory pathogens using a PCR panel array (Biofire). Descriptive statistics were used to demonstrate continuous and categorical variables, meanwhile regression models were used to demonstrate the association of reported respiratory symptoms and PM2.5 exposure. Data from this study show that 2 participants (1800.14 μg/m³ and 17820.89 μg/m³) had PM exposures that exceeded the OSHA permissible exposure limit of 15mg/m³ and strawberry harvesting yield the greatest geometric mean (121.35 μg/m³). A Poisson Regression analysis demonstrated that measured PM2.5 and calculated cumulative PM2.5 values had a positive association with reported symptoms of chronic coughing (OR 4.11 vs OR 3.02) and wheezing (OR 1.51 vs OR 1.40). During the follow-up, for participants reporting acute respiratory symptoms and pathogenic molecular analysis revealed positive results for human rhinovirus (n=2), and SARS-CoV-2 (n=1), whereas 1 individual reported symptoms but tested negative in the panel used. To our knowledge, this is the first study to assess occupational PM2.5 exposure and incidence of respiratory infections in Southeastern US-hired farmworkers which support PM exposures may be significant and associated with adverse respiratory health outcomes in this population.

4675  The Use of Low-Cost Sensors for Worker Exposure Assessment: Testing the Impact of Various Particulate Matter Compositions on Sensor Performance
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Rising public interest in air quality has led to the rapid development and surge in popularity of low-cost air quality sensors which have expanded the field of air quality surveillance and opened new avenues for research. Prior studies have demonstrated that low-cost Particulate Matter (PM) sensors can be successfully used for environmental sampling, but research on their ability to occupational PM exposures is limited. Today’s low-cost PM sensors have several advantages over the current industry standard devices that would make them optimal for use in occupational settings including their lower cost, smaller size, and ability to stream location, temperature, humidity, and PM concentration data wirelessly to phones or computers via Bluetooth or Wi-Fi. However, previous studies have demonstrated potential limitations to these low-cost PM sensors, such as poor performance during fog, humidity, and temperature extremes, and reliability issues. Furthermore, low-cost PM sensors use light scattering similar to those used in the current industry standard devices, to measure PM concentrations, which may impact PM concentrations measurements due to varying compositions of PM. Therefore, the goal of this study was to test the efficacy of the second
and third generations of a selected low-cost PM sensors device (Airbeam) and investigate how particle composition can impact PM measurements and calibration coefficients. The Airbeams differ from the PDR 1500 in that they sample all PM and use an algorithm to estimate the size/size distribution of PM, whereas the PDR 1500 uses a physical cut cylinder for size selective sampling of PM. To perform the calibrations five Airbeam 2s, three Airbeam 3s, and one PDR 1500 with a pre-weighed filter were placed into an airtight chamber where the devices were run simultaneously with each taking measurements at 1-minute intervals. They were then exposed to one of three common occupational aerosols (combustion engine exhaust, biomass smoke, and construction dust) and allowed to measure PM concentrations in the chamber until the PDR 1500 measured a concentration of 0 µg/m³, after which the filter was removed from the PDR 1500 and weighed again to get the pre and post weight difference. These in-station corrections were then used to generate calibration curves for each Airbeam and to compare the total concentration measured by each device to the actual total concentration sampled, determined from the gravimetric analysis of the filter. Our results showed that there was a strong linear relationship and high R² value between the Airbeams' and the PDR 1500’s measurements below 300 µg/m³. However, plateauing and variability between the Airbeams’ measurements typically began at around 300 µg/m³. Size selective Airbeam measurements (i.e. PM1, PM2.5 and PM10) were observed to be influenced by the device equation and were different when changed to the sensor’s default equation. In conclusion, our current findings demonstrate that Airbeams have the capacity to be successfully used to measure occupational exposures to PM of varying chemical compositions below a certain range, and importantly should be calibrated with advanced instruments prior to use in research.

### Study Overview

Studies in both the US and in developing areas abound show robust associations between acute and chronic air pollution particulate matter (PM) exposure with cardiovascular mortality and morbidity. Ambient ultrafine particles (UFPs) generated via tailpipe emissions induce pro-inflammatory effects in the blood and alter plasma lipoproteins. While almost weightless, UFPs represent 85-90% of PM2.5 and the small size of UFPs contribute to their biochemical and biophysical properties that lead to increased toxicity. The gastrointestinal (GI) tract may be exposed to UFPs as rapid bronchial mucociliary clearance transports the inhaled particles to the oropharynx, followed by swallowing. When UFPs reach the GI tract, they may influence the gut microbiome, which modulates host metabolism, immunity, and inflammatory responses, leading to pathogenesis. We hypothesize that UFP inhalation exposures result in alterations of the fecal microbiome in hyperlipidemic and normolipidemic mouse models. Ambient ultrafine particles (UFPs) generated via tailpipe emissions induce pro-inflammatory effects in the blood and alter plasma lipoproteins.

### Methods

UFPs represent 85-90% of PM2.5 and the small size of UFPs contribute to their biochemical and biophysical properties that lead to increased toxicity. The gastrointestinal (GI) tract may be exposed to UFPs as rapid bronchial mucociliary clearance transports the inhaled particles to the oropharynx, followed by swallowing. When UFPs reach the GI tract, they may influence the gut microbiome, which modulates host metabolism, immunity, and inflammatory responses, leading to pathogenesis. We hypothesize that UFP inhalation exposures result in alterations of the fecal microbiome in hyperlipidemic and normolipidemic mouse models. Ambient ultrafine particles (UFPs) generated via tailpipe emissions induce pro-inflammatory effects in the blood and alter plasma lipoproteins.

### Results

Fecal and dietary interventions were performed using ICP-MS to characterize elements present (n=16) and GC-MS to extract the DNA of fecal pellets. Chemical analysis was performed using ICP-MS to characterize elements present (n=16) and GC-MS to extract the DNA of fecal pellets. Chemical analysis was performed using ICP-MS to characterize elements present (n=16) and GC-MS to extract the DNA of fecal pellets. Chemical analysis was performed using ICP-MS to characterize elements present (n=16) and GC-MS to extract the DNA of fecal pellets.
Female Mice Are Protected against Fine Particulate Matter– Induced Glucose Intolerance in Circadian Dysynchrony but Not Cardiovascular Insulin Resistance

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Epidemiological studies have shown that exposure to elevated levels of ambient fine particulate matter (PM$_{2.5}$) increases the risk of developing cardiovascular disease (CVD) and type 2 diabetes (T2D); however, the underlying mechanisms remain elusive. Results of our previous work in male mice showed that exposure to concentrated PM$_{2.5}$ exacerbates glucose intolerance and insulin resistance in models of diet-induced obesity and circadian dysynchrony. We also found that while PM$_{2.5}$ alone did not affect systemic insulin sensitivity, it induces insulin resistance in the heart and aorta. This study investigated whether PM$_{2.5}$ exposure has similar effects in female mice. Female wild-type (WT) or mice transgenic for lung-specific overexpression of extracellular superoxide dismutase (ecSOD-Tg) kept on a normal light cycle (12 hours light: 12 hours dark) or with light-induced circadian dysynchrony (LICD, 18 hours light: 6 hours dark) were exposed to concentrated ambient PM$_{2.5}$ (CAP) or inhaled HEPA-filtered air for 30 days. Glucose tolerance was tested after 21 days, and tissue-specific insulin sensitivity (1.5 U/kg insulin, i.p., 15 min) was analyzed after 30 days in skeletal muscle (SKM), liver, heart, and aorta by Western blotting. Vascular inflammation was also assessed by Western blotting. Plasma concentration of the nitrite (NO) was determined by Griess assay. Statistical significance (p < 0.05) was determined for glucose tolerance using an unpaired Student’s t-test. Comparison of insulin stimulation in mice exposed to HEPA-filtered-air or CAP was done by Two-way ANOVA with Tukey’s post-hoc test. As previously observed in male mice, CAP exposure decreased the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). Female mice exposed to CAP were protected against circadian dysynchrony (LICD) and PM$_{2.5}$-induced effects on glucose homeostasis in LICD. Yet, female mice seem not to be protected against the cardiovascular toxicity elicited by PM$_{2.5}$-exposure, which is dependent on pulmonary oxidative stress.

Study of Lung Toxicity by PM Exposure in HDM-Induced Asthma Model

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Particulate matter (PM) is associated with the exacerbation and incidence of asthma. However, its pathogenesis is complicated, and there is little evidence on the effect of PM in asthma. We investigated the effect of PM in an asthma model and performed transcriptomic analysis. For asthma model, C57BL/6 mice were intratracheally instilled with three times with house dust mite (HDM). Artificial PM (APM), which was synthesized in a laboratory system, and diesel exhaust particles (DEP) were used as PM. The experiments were designed as follows: Group I and II were intratracheally instilled five times with 1.25, 2.5, or 5 mg/kg of PM before and after asthma induction, respectively. We assessed asthmatic features by determining the cell number and cytokine levels in bronchoalveolar lavage fluid (BALF), immunoglobulin E (IgE) in serum, and histopathological changes. As results, group I showed that exposure to PM dose-dependently inhibited HDM-induced eosinophilic inflammation by decreasing the number of inflammatory cells and Th2 cytokine levels in BALF and IgE production in serum. Also, in histological examination, eosinophilic cell infiltration and mucus cell hyperplasia were significantly decreased by exposure to PM in HDM-induced mice. Interestingly, Group II showed that PM exposure increased more HDM-induced the inflammatory cytokines including Th2 cytokines in the BALF and the increased IgE production in serum. Especially, PM exposure synergistically induced granulomatous inflammation and pulmonary fibrosis in lungs, accompanied by increased PM-pigment alveolar macrophages in the lungs of HDM-exposed mice. In transcriptomic profiling, group I showed that HDM-induced the expression of Rnase 2A and CCL24 genes was statistically down-regulated by PM exposure. Group II showed that the expression of endoplasmic reticulum stress, phospho-p38 mitogen-activated protein kinase, and fibrosis-related genes were higher in HDM pre-instilled and PM-exposed group than in HDM group. These results suggest that PM can trigger different immune responses in asthma depending on the timing of PM exposure.

Toxicity of Ultrafine Particles from Combustion of High Aromatic and Non-aromatic Fuels Using in Vitro ALI Exposure System

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The differences in traffic fuels have been shown to affect the combustion emissions and their toxicity. The importance of aromatic content of the fuel is central considering the particulate matter (PM) emissions. Moreover, the role of ultra-fine particles (UFP) as a traffic emission component has been increasing due to connection with various health effects, and the large surface-area to mass ratio of UFPs, resulting in the efficient transport of particle surface-bound components deep into biological systems. Therefore, studying the toxicity of the UFPs and how different fuels can be used in mitigating the toxic emission and toxicological impact is crucial. In the present study, emissions from a heavy-duty diesel engine and different cars were used to assess the exhaust emission toxicity with a thermophoresis-based in vitro air-liquid interface (ALI) exposure system. Aim of the study was to evaluate the toxicity of UFPs and the potential effect of distinct fuels to the emission toxicity. The results of the present study indicate that the aromatic content of both diesel and gasoline fuels increase the emission cytotoxicity and genotoxicity. Further, the increase in genotoxicity was most likely due to the PM phase of the aerosol, as removing to the particles high-efficiency particulate absorbing (HEPA)-filter, resulted in reduced genotoxicity with the heavy-duty diesel engine exposures. Overall, decreasing the aromatic content of the fuels could be a significant aspect for mitigating the PM emissions from traffic-related combustion emissions.

Examining Differential Behavioral Responses and Gene Expression of Two Zebrafish Strains Exposed to PM$_{2.5}$

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Particulate matter (PM) exposure is a complex mixture of solid and liquid components of combustion and combustion products. PM$_{2.5}$ exposure has associated with various human health conditions, especially those affecting the cardiorespiratory system. Even though PM$_{2.5}$ contains particles small enough to enter the bloodstream, little research has been conducted to determine the effects of PM$_{2.5}$ on other organ systems, such as the reproductive system. Therefore, we aimed to identify the effects of PM$_{2.5}$ exposure on the expression of genes related to reproductive function and oxidative stress in two zebrafish strains. Zebrafish embryos (6 hpf) from two wild-type strains (AB and SD) were exposed to 12.5, 25, 50, 100, and 200 μg/mL whole particle and soluble fractions of fine particulate standard reference material (n=33/group). Health, morphology, and a behavioral endpoint (the photomotor response) were measured prior to storage of larvae for gene expression analysis of oxidative stress and reproductive function-related genes. All exposures have been completed and morphological assessments were concentration-dependent, but not strain-dependent. Significant differences in behavioral responses were observed between PM$_{2.5}$ concentrations and between zebrafish strains. For example, at the lowest exposure concentration of 12.5 μg/mL, swim distance differed significantly between AB and SD strains (P=0.020, two-way ANOVA Holm-Sidak). RT-qPCR is underway to compare gene expression levels in the two strains following PM$_{2.5}$ exposure, including genes related to oxidative stress (superoxide dismutase, catalase) and reproductive function (vitellogenin, estrogen receptors, and thyroid hormone receptors). Overall, this study is exploring differences in morphological, behavioral, and molecular responses based on the concentration of exposure and zebrafish strain. This information will aid in comparing studies between research groups in the expanding field of utilizing zebrafish to study the effects of particulate matter exposures.

The Effects of Fluorene Exposure in Conjunction with High-Fat Diet on Toxicant-Associated Fatty Liver Disease

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Fluorene belongs to the class of polycyclic aromatic hydrocarbons (PAHs) and is a major component of particulate matter (PM$_{2.5}$). Previous studies have demonstrated positive associations between PAH mixtures and risk for fatty liver disease (FLD). However, the impact of fluorene in the context of toxicant-associated FLD (TAFLD) remains to be elucidated. The current study aims to investigate the effects of fluorene exposure in TAFLD and identify mechanisms contributing to hepatic steatosis and metabolic disruption. Male C57BL/6 mice were fed a feed control diet (CD) or high fat diet (HFD,42%) for 10 weeks; each diet group was then administered corn oil (vehicle control) or fluorene (50 mg/kg/day, daily gavage, 4 weeks). Mice were then euthanized; plasma and tissues were collected for downstream analysis. Results were analyzed using 2-WAY ANOVA for two factors: “diet” and “fluorene”.

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followed by Tukey’s post hoc test; p<0.05. HFD-fed mice showed hepatic steatosis (H&E staining of liver sections), while fluorene exposure resulted in increased liver inflammatory infiltration. Consistently, assessment of hepatic gene expression demonstrated fluorene upregulation of mRNA levels for the inflammatory/fibrotic marker (Fgf21) in HFD-fed mice. In terms of lipid metabolism, HFD upregulated hepatic expression of genes involved in lipid uptake (Cpt1), and decreased expression of genes for de novo lipogenesis (FASN) and lipid binding (FABP1). Fluorene decreased FASN and increased FABP1 mRNA levels in CD-fed mice, implicating fluorene-mediated disruption of lipid metabolism irrespective of HFD. Additionally, fluorene altered hepatic expression of genes involved in glucose and glycogen metabolism (increased gluconeogenic Pck1, decreased Gck expression) in CD-fed mice although this group exhibited lower fasting glucose levels, further implicating fluorene-mediated disruption of glucose metabolism. HFD feeding also decreased mRNA levels of genes involved in hepatic recovery/function (Fgf21, Hnf4a) while fluorene further decreased Fgf21, indicating exacerbation of HFD-induced liver damage with exposure. Fluorene did not impact HFD-mediated lowering of hepatic glutathione levels. Lastly, assessment of hepatic xenobiotic receptor activation demonstrated fluorene-mediated induction of target genes for the AhR (Cyp1a2), CAR (Cyp2b10) and PXR (Cyp3a11). The current findings suggest that fluorene is a metabolism-disrupting chemical that can contribute to TAFLD, partially through hepatic receptor activation.

4684 Cardiorespiratory Response to Respiratory Gunshot Residue and Impact of RAGE Signaling In Vivo
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Gunshot residue (GSR) is a complex mixture comprised of organic and inorganic compounds released after shooting a firearm and includes components with known environmental and human health effects such as Pb, Ba, and black carbon (BC). This study presents a new approach to gaseous health effects since particles less than 2.5 microns in aerodynamic diameter, PM_{2.5}, can penetrate the respiratory tract and potentially enter the bloodstream. Currently, there is very limited research investigating the effects of GSR particle size and composition on cardiopulmonary health; however, the high concentrations of toxic compounds in GSR and epidemiology studies indicate the need for further research. One potential mechanism of action involved in GSR-related responses is the advanced glycation end products (AGEs) and their receptor (RAGE) signaling cascade which plays a role in inflammation and oxidative stress. This study collected PM_{2.5} during a University of Mississippi law enforcement firearms qualification. The GSR PM_{2.5} samples were used to conduct a chemical composition of characteristic GSR marker and the in vivo effects of exposure with a focus on the role of RAGE-mediated responses. Following collection, PM_{2.5} samples were pooled together, concentrated, and resuspended in saline for in vivo exposures or DI water for chemical analysis (elements via ICP-MS). PM_{2.5} samples and controls (vehicle, blank filter) were used to conduct in vivo exposures in male wild-type (C57BL/6) and RAGE knockout (RKO) mice at 0 or 100 µg/mouse (n=7-8/group). Pre- and 24-hr post-exposure physiological parameters were measured using echocardiography to determine cardiac function in the left and right ventricle. Tissues (heart and lung) were collected for histological (H&E, Picric acid-Sirius red, and Verhoeff-Van Gieson) analysis (elements via ICP-MS). PM_{2.5} samples were pooled together, concentrated, and resuspended in saline for in vivo exposures or DI water for chemical analysis (elements via ICP-MS). PM_{2.5} samples and controls (vehicle, blank filter) were used to conduct in vivo exposures in male wild-type (C57BL/6) and RAGE knockout (RKO) mice at 0 or 100 µg/mouse (n=7-8/group). Pre- and 24-hr post-exposure physiological parameters were measured using echocardiography to determine cardiac function in the left and right ventricle. Tissues (heart and lung) were collected for histological (H&E, Picnic acid-Sirius red, and Verhoeff-Van Gieson) analysis (elements via ICP-MS). PM_{2.5} samples were pooled together, concentrated, and resuspended in saline for in vivo exposures or DI water for chemical analysis (elements via ICP-MS). PM_{2.5} samples were pooled together, concentrated, and resuspended in saline for in vivo exposures or DI water for chemical analysis (elements via ICP-MS). PM_{2.5} samples were pooled together, concentrated, and resuspended in saline for in vivo exposures or DI water for chemical analysis (elements via ICP-MS).

4685 Interaction of Aryl Hydrocarbon Receptor (AhR) and Toll-Like Receptor (TLR) Signaling Induced by Exposure to Particulate Matter (PM)
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The aryl hydrocarbon receptor (AhR) plays a critical role as a modulator of the immune response. Exposure to compounds and environmental pollutants interacting with the ligand-activated transcription factor AhR can modify the function of immune cells. Consequently, the AhR may influence inflammatory processes, infectious and common chronic diseases. Recently, it has been found that exposure to particulate matter (PM) collected from ambient air or from wild fires may activate AhR signaling. Here, we examined the effects of particulate matter (PM) on Interleukin (IL)-22 and IL-33 expression in macrophages. IL-22 is critically involved in gut immunity and host defense. In different circumstances IL-22 may contribute to pathological conditions or act as a cancer promoting cytokine secreted by infiltrating immune cells. IL-33 is closely involved in the onset and progression of asthma. The exposure to traffic-related PM and PM collected from wildfire smoke induced the expression of IL-33 and IL-22, respectively, in macrophages. Inhibitor studies and analysis of macrophages derived from AhR and TLR4 knockout mice confirmed that the synergistic induction of IL-22 and upregulation of IL-33 by PM and endotoxin depend on the expression of AhR and TLR. The results suggest that exposure to air pollutants containing PM and endotoxin may contribute to diseases associated with inflammation. TLR agonists such as endotoxin are critical components of PM collected from traffic related sources and wildfires activating AhR and Toll-like receptor (TLR) signaling leading to the upregulation of IL-22 and IL-33. Conclusively, the induction of IL-22 and IL-33 in macrophages exposed to PM may implement signals of the canonical and non-canonical AhR pathway.

4686 Mechanistic Role of the Aryl Hydrocarbon Receptor (AhR) in the Tumor Microenvironment and Development of Breast Cancer
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Air pollution and occupational exposure studies have reported positive associations with the risk of developing breast cancer. Air pollution and ambient particulate matter (PM) contain a complex mixture of compounds, including polycyclic aromatic hydrocarbons (PAHs) and various metals (e.g., iron, nickel, copper), which may induce oxidative stress and inflammation and stimulation of the progression of breast cancer. The aryl hydrocarbon receptor (AhR) is known as a sensor of environmental exposure to pollutants and may act as a critical player in tumor promotion and progression. Here, we investigated the role of AhR in mammary tumorigenesis, using mouse models. We observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM. Following exposures in the wild-type mice, we observed a trend of reduced ejection fraction and protein expression changes, with over 1-fold differences in exposure to PM.
New approach methodologies (NAM) are being widely used to reduce, refine, and replace, animal use in toxicology. This includes in vitro and in silico alternatives to replace traditional in vivo methods for inhalation toxicity. We have recently demonstrated the predictive power of combining in vitro ALI exposure assays with in-vitro-to-in-vivo extrapolation (IVIVE) based on computational airway dosimetry models. Differentiated AE1/E-Cad epithelial cell cultures were exposed acutely to 1,3-dichloropropane (1,3-DCP) vapor at the ALI, acute toxic responses were measured, and in vitro point of departure (POD) was determined. PBPK model of 1,3-DCP airway dosimetry was then utilized to extrapolate the in vitro POD to a rat or human equivalent inhaled concentration (EIC), which served as an estimate of the in vivo POD. Measurement of cytotoxicity using lactate dehydrogenase (LDH) assay and transepithelial electrical conductor measurement for epithelial integrity were used as the endpoints to see the effect of 1,3-DCP. Five different cultures representing different parts of the human respiratory systems were used. MucliAir Nasal cultures were found to be most sensitive to 1,3-DCP. Here, we have expanded the endpoints to better understand the effect of 1,3-DCP and to bring more confidence to our model. The MucliAir Nasal cultures were exposed to 6 sub cytotoxic concentrations of 1,3-DCP vapor: clean air, 6.25 pp, 12.5 pp, 25 pp, 50 pp, 100 pp, and 200 pp for 4 hours and the acute toxicity measured 24 hours after initiation of exposure except for Glutathione (GSH) assay which was done immediately after exposure. The major metabolic pathway of 1,3-DCP in vivo is GSH conjugation to form mercapturic acid. Measurement of depleted GSH was done using the GSH assay immediately after exposure and found that GSH depletion starts at very low concentration of 1,3-DCP. The benchmark dose was calculated and IVIVE determined with the obtained data. We observed that the BMD for GSH was 6.7 which was 20 times lower than LSD and transepithelial conductance measurements. Recent advances in transcriptomics analysis is ongoing to understand the in vitro mode of action of 1,3-DCP in lung organoid cultures. Further histological analysis was also done using H&E staining, which showed that there is change in cellular morphology with increasing concentrations of 1,3-DCP at exposure levels of 100 pp. The clean air group had normal pseudostratified epithelium while the 200-ppm group. The change from a pseudostratified columnar epithelium to pseudostratified cuboidal, cuboidal, and then squamous is indicative of an increase in the severity of the injury. The density of ciliated cells and the cilia length are also dose-related and decreased as the concentration of 1,3-DCP increased. The number of necrotic cells also increased with increasing concentration. To look at the cytotoxicity, media was analyzed using LDH assay and the epithelial integrity was measured using TEER (transepithelial resistance) and observed increased cytotoxicity and decreased resistance over the time. Histopathological comparison of chronic and acute exposure condition indicates that chronic exposure had caused much damage as compared to acute exposure. The use of multiple endpoints for the same chemical has increased the reliability of our system and confidence in the approach we are using for the development of NAMs for inhalation exposures.
compared to BIT (31%, 71% vs 97% BIT). This result suggests that some isothioci
azolone may become unstable during nebulization, affecting their disposition and
human exposure significantly.

4693  Altered Urinary Levels of Oxidative Stress Biomarkers in Pregnant Women Exposed to Oil and Gas Sites in Northeastern British Columbia

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Northeastern British Columbia is a hotspot for oil and gas extraction by hydraul
ic fracturing. Oil and gas sites often release volatile organic compounds (VOCs),
which have been shown to increase oxidative stress, disrupt antioxidant enzyme
activity, and increase the incidence of adverse pregnancy outcomes. This study
aimed to 1) measure biomarkers of oxidative stress and antioxidant activity in urine
samples of a cohort of pregnant women living in Northeastern British Columbia; 2
analyze the associations between indoor air VOCs, density and proximity of oil
and gas wells and biomarker levels. Up to 7 urine samples were collected for eighty-five
pregnant women living in Northeastern BC. Passive air samplers were also installed
in participants’ residences to measure VOCs in indoor air. Density and proxim
ity metrics of oil and gas wells were determined in 2.5 km, 5 km, and 10 km radii
around the participants’ residences. Colorimetric and fluorescent assay kits were
used to measure concentrations of catalase (CAT), superoxide dismutase (SOD),
glutathione S-transferase (GST), total antioxidant capacity (TAC), 6-hydroxymet
holation sulfite, malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG),
and 8-isoprostane (8-IP). Associations between biomarker concentrations and exposure
dimensions were determined using multiple linear regression models with covariables
adjusting for participant age, gestational week, smoking status, sample collection
time, creatine content, and melatonin levels for some biomarkers. Biomarker levels
were also compared between Indigenous and non-Indigenous participants. Urinary biomarker levels were statistically different between Indigenous and non-
Indigenous participants. Increased density and proximity of oil and gas wells was
associated with a significant increase in CAT at all buffer radii. SOD, 6-hydroxymet
holation sulfite, 8-OHdG, and 8-IP all decreased with elevated density and proximity
to oil and gas wells. These trends tended to increase in strength as the buffer radii
became smaller. 6-hydroxymelatonin sulfite and 8-OHdG also had strong negative
associations with hexanal levels. No significant associations were found between
TAC, GST, or MDA and our exposure metrics. These results may suggest that
chronic exposure to oil and gas activity could impair protective oxidative stress
pathways in pregnant women. More research is needed to elucidate the underlying
mechanisms associated with changes in oxidative stress and to what degree these
changes could affect birth outcomes.

4694  Quantification of Sulforaphane Metabolites in Human Breast Milk for Protection against Infant Respiratory Toxicity

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Respiratory syncytial virus (RSV) is a significant cause of infant hospitalization,
increasing long-term risk for chronic respiratory disease. Environmental exposures
to a variety of pollutants have been implicated in enhancing the severity of RSV
infection necessitating intervention strategies. Sulforaphane (SFN), a phytochem
ical derived from cruciferous vegetables, is a known dietary activator of the NRF2
antioxidant response pathway that has been shown to mitigate RSV severity in
clinical derived from cruciferous vegetables, is a known dietary activator of the NRF2
pathways associated with changes in oxidative stress and to what degree these
changes could affect birth outcomes.

4695  Serum Metabolic and Tissue Transcriptomic Profiling Reveal Acrolein Inhalation Variably Impacts Multi-organ Stress Responses in Male and Female Rats


Acrolein is a well-studied significant constituent of anthropogenic and wildfire
smoke emissions and thus, an environmental risk factor contributing to adverse
health outcomes. Acrolein is on EPA’s priority list of hazardous air pollutants
and is a sensory irritant associated with adverse cardiopulmonary health effects;
however, the ability for acrolein to induce systemic metabolic derangement has
not been systematically investigated. Further, sex-specific stress responses are not
examined in prior acrolein exposure studies, which in terms of metabolic stress is
crucial considering the prevalence of metabolic disease often differs between
sexes. In this study, male and female rats underwent acrolein nose-only inhalation in incremental concentrations (0, 0.1, 0.316, 1 ppm) for 30 min for head-out plethysmography assessment, followed by a 3.5 hr exposure at 3.16 ppm (n=8/group). We performed serum metabolic profiling in males and females, identifying 887 known circulating biomolecules. Males showed
extensive alterations in circulating metabolites relating to lipolysis, muscle protein
turnover, and mitochondrial respiration cycle shifts, whereas these markers in
females were not changed by acrolein exposure. In males, acrolein exposure induced a release of long-chain polysaturated fatty acids and phospholipids, including
glycerols and sphingomyelins, into circulation, which ultimately altered circulating
levels of fatty acids and cholesterol changes in TCA cycle intermediates.

4696  Association of Ambient Volatile Organic Compounds with Circulating Endothelial Cell–Derived Microparticles


Volatile organic compounds (VOCs) are ubiquitous environmental pollutants
abundant in indoor and outdoor air. Increased levels of VOCs are also present at
various Superfund and other hazardous waste sites. Our studies in experi
mental animals showed that VOCs induce endothelial toxicity. In humans, VOC exposure is associated with depletion of peripheral blood endothelial progenitor
cells (EPCs), indicating compromised endothelial repair capacity. However, little is
known about the association of ambient VOC exposure with endothelial activation/
iny. We applied flow cytometry to quantitate circulating endothelial micropartic
les (MPs), activated endothelial MPs, EPC MPs, lung MPs, lung endothelial MPs,
and activated endothelial lung MPs in 413 non-smokers (urinary cotinine level <40
ng/mg creatinine) with low-to-high CVD risk. Urinary metabolites of VOCs were
measured by liquid chromatography mass spectrometry (LC/MS/MS). Generalized
linear models were used to assess the association of MPs with VOC metabolites.
Models were adjusted for age, sex, race, BMI, diabetes, hypertension, daily ozo
ej, and daily PM2.5 levels. Our data show that all the circulating MPs were positively
associated with the metabolites of acrylamide, 1,3-butadiene, crotonaldehyde,
NN-dimethylformamide and styrene. Percentage change in MPs per 2-fold increase
exposure to acrolein was confirmed through transcriptomic analysis of liver, adipose, and muscle tissue, supporting a similar pattern across all three organ systems. Metabolic stress responses observed in serum metabolic profiling. In conclusion, acrolein inhalation orchestrated a multi-organ metabolic stress response in males, impacting multiple homeostatic metabolic pathways. Basal sex differences in relative metabolite quantity were pervasive throughout all metabolic super-pathways, suggesting a further need to understand how basal metabolism between males and females may impact the stress response, as well as suscept
ibility to metabolic diseases from exposure to irritant air pollutants. Importantly,
male rats showing an increased susceptibility to respiratory irritation induced
metabolic effects relative to female rats may have implications for sex-specific risk
of metabolic disease from acrolein and other volatile irritant pollutants. Does not
reflect US EPA policy.
was positively associated with endothelial MPs, activated endothelial MPs, and EPC MPs (20-25% change per 2-fold increase in the metabolic Environmental Risk Scores (cumulative effect of all the VOCs) was also significantly increased for all the MPs. Stratification of blood endothelial MP data by sex showed that acrylamide and 1,3-butadiene exposure display greater increase in men, whereas styrene/ethylbenzene exposure showed greater increase in women. Association of acrylamide metabolite was also higher with MPs in older (>55 years) than younger (<55 years) participants. Collectively, our data suggest that low-level ambient VOC exposure is associated with increased endothelial cell-derived MP formation, which reflects endothelial injury, and could potentially increase cardiovascular disease risk.

**4697 Role of Transfected Human Aldo-Keto Reductases on HPRT Gene Mutagenicity in V79-4 Cells Exposed to Nitroarenes**


1-Nitropropane (1-NP), 1,8-dinitropropane (1,8-DNP), and 3-nitrobenzantrone (3-nitro-7H-benzo(de)anthracen-7-one, 3-NBA) are nitroarenes that are air pollutants found in diesel exhaust and ranked as either group 2A probable or group 2B possible human carcinogens by the International Agency for Research on Cancer. The metabolic activation of these compounds by nitroreduction contributes to the mutagenicity of these compounds. Specifically, the hydroxylamine intermediate can be O-acetylated to result in a good leaving group for the formation of tautomeric nitrenium and carbenium ions that bind guanine of DNA. Aldo-keto reductases (AKR) 1C1-1C3 catalyze the aerobic nitroreduction of 1-NP, 1,8-DNP, and 3-NBA, and NADPH:quinone oxidoreductase 1 (NQO1) is a non-metabolizing enzyme that reduces the nitroreduction of 3-NBA. The extent to which these enzymes contribute to the mutagenicity of these nitroarenes is unknown. We investigated the mutagenicity of these compounds at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene locus in V79-4 Chinese hamster fibroblasts using transfection in response to transfection AKR1C1-1C3. Transfection of AKR1C1, AKR1C2, or AKR1C3 plasmid at 2 µg/mL did not cause mutagenicity in the absence of nitroarene exposure. 1-NP at 1, 5, or 10 µM in the absence of AKR1C3 transfection did not increase mutagenicity relative to vehicle (0.1% DMSO). In the presence of AKR1C1, AKR1C2, or AKR1C3 transfection at 1 or 2 µg/mL, 1-NP at 5 or 10 µM increased mutagenicity relative to the untransfected controls, showing mutagenicity was dependent on AKR1C3 transfection. 1,8-DNP or 3-NBA at 5 or 10 µM caused mutation in the absence of AKR1C1, AKR1C2, or AKR1C3 transfection; however, 1 or 2 µg/mL of AKR1C1, AKR1C2, or AKR1C3 plasmid increased this mutagenicity. Mutagenicity for 3-NBA in the absence of AKR1C1 could be attributable to NQO1 present in V79-4 cells; however, the enzymes involved in 1,8-DNP nitroreduction in the absence of transfected AKR1C3 are unknown. Our study shows that transfection of human AKRs is sufficient to cause mutation by nitroarenes at the HPRT gene locus.

**4698 Evaluating the Accuracy of Spatial Interpolation Models for Estimating Residential Ambient PM$_{2.5}$ Exposure Concentrations in Kampala, Uganda**


Fine particulate matter (PM$_{2.5}$) is an ambient air pollutant estimated to cause over 4 million deaths globally. Ambient PM$_{2.5}$ levels are especially high in rapidly growing urban cities. However, little air pollution epidemiology research has been conducted in African cities. One barrier to conducting such research in Africa is a lack of air pollution monitoring to estimate exposures. The aim of this study was to determine the accuracy of spatial interpolation methods for estimating residential ambient PM$_{2.5}$ levels in Kampala, Uganda. This was done by setting up low-cost air quality sensors, combined with spatial interpolation methods, are sufficient to facilitate PM$_{2.5}$ exposure assessment in under-resourced cities like Kampala.

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**4699 Self-Reported Health Outcomes and Fine Particulate Matter at Western Montana Residences during the 2022 Wildfire Season**

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Throughout recent decades wildfires have destroyed parts of the Western United States. Increases in temperature and droughts caused by climate change are correlated with a surge in the number of wildfires recorded as well as wildfire duration. These natural disasters are damaging not only the ecosystems they burn but the public health of the residential areas nearby (primarily from smoke). Previous studies have shown the implications of wildfires on public health; however, few have been done for the entire duration of a wildfire season. This project investigated the Bitterroot Valley of Montana during the entirety of the 2022 Montana wildfire season (July-October). At the beginning of the study, 20 participants were mailed two PurpleAir (PAII-SD, PurpleAir, Inc, USA) air pollution sensors and a blood pressure monitor. The sensors were installed inside and outside of the homes to measure fine particulate matter (PM$_{2.5}$) two-minute intervals starting in July and were removed in November. Throughout the duration of wildfire season, participants submitted weekly electronic surveys to record their health outcomes and behaviors/activities. Health outcomes included blood pressure and self-reported health symptoms (e.g., headache, shortness of breath). The mean age of the 20 participants was 48 years (range: 29 to 88) and 17 participants (85%) reported sex of female. In preliminary analyses, median daily outdoor PM$_{2.5}$ at the households was 6.6 µg/m$^3$ (25th percentile [P25]=4.5, 75th percentile [P75]=11.2) during the entire study period and 41.1 µg/m$^3$ (P25=28.0, P75=68.9) during a 2-week period in September impacted by wildfire smoke. Systolic blood pressure was higher during PM$_{2.5}$ (P25=161.0 mmHg, standard deviation = 16.0) compared to the rest of the study (mean = 112.6 mmHg, standard deviation = 14.1). We also observed higher diastolic blood pressure during the wildfire-impacted period (mean = 77.2 mmHg, standard deviation = 10.0) compared to the rest of the study (mean = 74.8 mmHg, standard deviation = 8.4). In a wildfire-impacted period, there were significantly more high blood pressure compared to the rest of the summer among 20 adult participants. This finding indicates a potential association between wildfire smoke and subclinical cardiovascular health. In future analyses, we will further assess the associations between wildfire smoke and blood pressure as well as other self-reported health outcomes.

**4700 Gestational Ultrafine Particulate Matter Exposure Alters the Fetal Lung Transcriptional Profile**

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Exposure to ultrafine particulate matter (UFP) during pregnancy significantly increases the risk of adverse developmental outcomes, including long-term impacts on childhood respiratory health. Yet, the molecular mechanisms driving the effects from gestational exposure remain unclear. To improve our understanding of the underlying mechanisms mediating poor childhood respiratory health, we exposed pregnant C57Bl/6n mice to either a low (LD, 100 µg/m$^3$) or high dose (HD, 600 µg/m$^3$) of UFP mixture or filtered air (control) for 4 hours every day throughout an 18-day gestation period. This whole-body exposure setup resulted in an average 24-hour exposure of 25 µg/m$^3$ and 125 µg/m$^3$ for the LD and HD exposure groups, respectively. Following the exposure on gestation day 18, we collected sex-separated fetal lung tissue and conducted transcriptomic analysis through total RNA-sequencing. There were no significant transcriptomic changes in the fetuses exposed to the HD or LD or high dose of the UFP mixture compared to the control. However, there were 200 and 240 significantly upregulated targets in the fetal lung of the LD and HD exposed female offspring, respectively. Interestingly, 176 of these upregulated targets were shared between both exposure groups. In line with this large overlap, the upregulated targets from both groups were significantly associated with signaling pathways involved in lipid metabolism (farnesoid X receptor/ retinoid X receptor activation, LD: p=1.3 x 10^{-4}, HD: p=7.94 x 10^{-4}, inflammatory responses (acute phase response signaling, LD: p=2.0 x 10^{-8}, HD: p=6.31 x 10^{-10}), and coagulation activation (coagulation system signaling, LD: p=4.0 x 10^{-7}, HD: p=2.0 x 10^{-9}) among others. As these pathways have all been implicated in the development of adverse respiratory diseases, these data suggest these pathways may be an important mechanistic link between gestational UFP exposure and early life respiratory disease. Collectively, these data suggest the lungs of female offspring may be more susceptible to adverse impacts from gestational UFP exposure. These results align with our prior findings showing altered placenta physiology, shortened fetal length, and more severe lung pathology following a secondary challenge to respiratory syncytial virus (RSV) in female offspring after LD gestational UFP exposure. In addition, despite the LD group residing within the safe 24-hour limits set for particulate matter 2.5 (PM$_{2.5}$), the large overlap in the altered transcriptional response and enriched pathways between UFP LD and HD exposure groups suggest safe limits for PM$_{2.5}$ may not reflect safe levels of UFP exposure. This study provides a critical advancement towards understanding the mechanistic links between gestational UFP exposure and early life respiratory disease. Furthermore, this work can improve our ability to assess the health risks of UFP exposure and determine proper regulations that can ultimately improve pediatric respiratory health.
Chronic respiratory disease is among the most representative environmental diseases, and ambient particles are the major risk factors. Herein, we collected particles from the air of a port city in Korea using a high-volume sampler and investigated the toxicity and the mechanism in mice and mice alveolar macrophage cells. Compared with ambient particles used in our study previously, these particles contained more arsenic, and the hydrodynamic diameter was approximately 2577.8 nm. When instilled intratracheally in mice for 13 weeks, the particles were persistent in the lungs of the mice and induced the formation of multicellular nucleated macrophages and monolayers of alveolar macrophage cells in the murine lung. Meanwhile, among mediators measured in this study, we found that only pulmonary levels of CXCL-1 and IL-1β increased with the treatment of ambient particles. In addition, these particles formed autophagosomes together with increased production of free radicals in alveolar macrophage cells, causing structural disturbance of the cist-Golgi matrix and increased expression of nuclear ribosomal RNA. Considering the crucial role of the Golgi network in the biosynthetic output from the ER, we concluded that ambient particles might cause chronic respiratory disease by disturbing protein synthesis.

**Pharmacokinetic and Meta-analysis of Tissue Distribution of Nanomaterials in Tumor-Bearing Mice**

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Nanoparticles (NPs) have been extensively studied both as a diagnostic agent to detect and a therapeutic agent to treat cancer. However, only a small number of NP-based drug formulations have been successfully translated to clinical usage, partly due to low targeted delivery efficiency (DE) to the tumor and a lack of quantitative tools that accurately predict the distribution of NPs to tumors and major organs. In the present study, the NP tumor DE and biodistribution to major healthy organs in tumor-bearing mice were calculated from data in relevant articles published between 2005-2021. Four pharmacokinetic metrics, including standard area under the curve (AUC) - based method of percentage of the injected dose (%ID), the maximum DE based on the peak concentration in the targeted tissue or tumor (Cmax), the AUC ratio between targeted tissue or tumor versus blood (e.g., AUCtumor/AUCblood), and the relative distribution index over time (RDI-OT) were assessed for total protein, total cell count, differential cell count, lactate dehydrogenase (LDH), and hydroxide (OH-) leakage from cultured cells. In the present study, the pulmonary toxicity of Er2O3 NPs was assessed by examining tissues and bronchoalveolar lavage fluid (BALF) from rats after nasal instillation. Following 14 days of exposure, the animals exhibited irregular respiration and discolored lungs but no mortality, resulting in an LC50 of > 5 mg/L. An acute inhalation study was conducted in rats at the dose of 5 mg/L in accordance with the upper end of the dose range for GHS classification criteria. After a single 4-hr nose-only inhalation exposure and following a 14-day observation period, the animals exhibited irregular respiration and discolored lungs but no mortality, resulting in an LC50 of > 5 mg/L. Ultimately, no mortalities were observed after oral or inhalation exposure of NiO nanoparticles at doses that align with the GHS classification criteria for acute toxicity. These acute toxicity results are consistent with results previously obtained with the traditional non-nanoscale micron particles, which also did not result in mortality at these doses and are not classified for acute oral or inhalation toxicity. Our bioaccessibility analyses and OECD acute toxicity studies suggest that potential differential acute toxicity classifications for NiO nanoparticles would not be warranted.

**Evaluation of Pulmonary Toxicity to Inhaled Erbium Oxide Nanoparticles in Golden Syrian Hamsters**

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Erbium oxide nanoparticles (Er2O3 NPs) are used in various products from simple paper-based products to highly technical devices. The usage of these nanoparticles has increased over the years leading to an elevated risk of accidental occupational exposure. The Er2O3 NPs used in this study were characterized by dynamic light scattering (223 ±1 nm), TEM (20 ±1 nm), and zeta potential (4.4 ±1 mV). In the present study, the pulmonary toxicity of Er2O3 NPs was assessed by examining tissues and bronchoalveolar lavage fluid (BALF) from rats after 4 hours of exposure. Tissues and BALF were evaluated for bioaccessibility, both nanoparticle samples agglomerated in vitro to a similar extent and released similar amounts of nickel ion, suggesting a similar toxicity of nanoparticles within this size range. These bioaccessibility results were followed with OECD guideline acute toxicity studies evaluating the smaller of the two nanoparticles (20 nm). An acute oral toxicity study (OECD 425) was conducted in rats at doses that align with the Globally Harmonized System (GHS) of classification criteria for acute toxicity (175 - 5000 mg/kg). After a single exposure via oral gavage and following a 14-day observation period, the animals were active and healthy with no gross abnormalities or mortality, resulting in an LD50 of > 5000 mg/kg. An acute inhalation toxicity study (OECD 403) was conducted in rats at the limit dose of 5 mg/L, in accordance with the upper end of the dose range for GHS classification criteria. After a single 4-hr nose-only inhalation exposure and following a 14-day observation period, the animals exhibited irregular respiration and discolored lungs but no mortality, resulting in an LC50 of > 5 mg/L. Ultimately, no mortalities were observed after oral or inhalation exposure of NiO nanoparticles at doses that align with the GHS classification criteria for acute toxicity. These acute toxicity results are consistent with results previously obtained with the traditional non-nanoscale micron particles, which also did not result in mortality at these doses and are not classified for acute oral or inhalation toxicity. Our bioaccessibility analyses and OECD acute toxicity studies suggest that potential differential acute toxicity classifications for NiO nanoparticles would not be warranted.

**Investigation of the Bioaccessibility and Acute Toxicity of Nickel Oxide Nanoparticles**


Nanomaterials continue to be an emerging issue due to their unique and often beneficial characteristics with growing uses in various industries. However, there is currently unknown and even conflicting information regarding potential adverse effects and toxicity of nanoparticles. Our work presents a collection of data focused on evaluating the acute toxicity of nickel oxide (NiO) nanoparticles. An initial literature review was conducted on the health effects of NiO nanoparticles in which we noted that many short-term studies reported adverse effects (e.g., inflammation, cytotoxicity, reactive oxygen species, etc.), yet mortality was not reported. Additionally, the short-term studies identified and evaluated were not guideline studies and did not establish appropriate toxicological dose descriptors (e.g., LC50, LD50, etc.) that could prove useful in the acute toxicity classification of NiO nanoparticle in the future. To obtain a preliminary idea of the potential acute toxicity of NiO nanoparticles through the oral and inhalation routes of exposure, in vivo bioaccessibility analyses were conducted to measure the release of nickel ions in various simulated biological fluids, where increased nickel ion release suggests increased likelihood of toxicity. Three NiO particle samples were evaluated for bioaccessibility: two in the nanoparticle size range (20 nm and 80 nm) and one in the traditional non-nanoscale micron particle size range (15 μm). These samples were evaluated in simulated gastrointestinal fluids for 2 hours and simulated lung fluids (lysosomal and interstitial) for 24 and 72 hours. The overall results showed that NiO nanoparticles released less nickel ions than traditional NiO micron particles, with the most significant differences observed in gastric and lysosomal fluids. These results suggest that NiO nanoparticles are not expected to pose the same acute toxicity as the traditional non-nanoscale micron particles of different sizes were evaluated for bioaccessibility, both nanoparticle samples agglomerated in vitro to a similar extent and released similar amounts of nickel ion, suggesting a similar toxicity of nanoparticles within this size range. These bioaccessibility results were followed with OECD guideline acute toxicity studies evaluating the smaller of the two nanoparticles (20 nm). An acute oral toxicity study (OECD 425) was conducted in rats at doses that align with the Globally Harmonized System (GHS) of classification criteria for acute toxicity (175 - 5000 mg/kg). After a single exposure via oral gavage and following a 14-day observation period, the animals were active and healthy with no gross abnormalities or mortality, resulting in an LD50 of > 5000 mg/kg. An acute inhalation toxicity study (OECD 403) was conducted in rats at the limit dose of 5 mg/L, in accordance with the upper end of the dose range for GHS classification criteria. After a single 4-hr nose-only inhalation exposure and following a 14-day observation period, the animals exhibited irregular respiration and discolored lungs but no mortality, resulting in an LC50 of > 5 mg/L. Ultimately, no mortalities were observed after oral or inhalation exposure of NiO nanoparticles at doses that align with the GHS classification criteria for acute toxicity. These acute toxicity results are consistent with results previously obtained with the traditional non-nanoscale micron particles, which also did not result in mortality at these doses and are not classified for acute oral or inhalation toxicity. Our bioaccessibility analyses and OECD acute toxicity studies suggest that potential differential acute toxicity classifications for NiO nanoparticles would not be warranted.
of the tissue in the control groups, Group-1 (<1) and Group-2 (<1). The inhalation exposure of Er₂O₃ NPs to Golden Syrian Hamsters caused no significant differences in signs of inflammation and cytotoxicity in the lungs when compared to the controls.

### 4705 Toxicity of Silicon Dioxide Nanoparticles on Rat Pleural Mesothelial Cells and the Lungs of Golden Syrian Hamsters

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Silicon Dioxide Nanoparticles (SiO₂ NPs) are widely used in the occupational, chemical, and cosmetics industries. Such particles are pulmonary toxicants, however, the mechanism of toxicity is uncertain. In the current study, toxicity was assessed using an in vivo and in vitro model. The mechanism of SiO₂ NPs induces cell toxicity was studied in rat pleural mesothelial cells (RPMCs). The protein levels were significantly increased (77% and 44% respectively) in cultures treated for 60 days with 10 and 100 μg/ml SiO₂ NPs. For the in vivo study, Golden Syrian Hamsters were exposed to inhalation of SiO₂ NPs in a whole-body exposure chamber. Experimental animals were divided into 4 groups: Group 1: room air control, Group 2: aerosolized water vehicle, Group 3: 6mg/m³ and Group 4: 12mg/m³ exposed for 4 hours/day for 8 days. For the in vitro model, cell toxicity of SiO₂ NPs was investigated using microscopy, MTT assay, Apoptotic, Necrotic, & Healthy Cells Quantification Kit and measurement of LDH. Measurements for dynamic light scattering of SiO₂ NPs in the BALF was removed, and lungs inflated and fixed with formalin. Tissue sections were cut, stained with hematoxylin and eosin or by the TUNEL Assay. The BALFs were assessed for cell number, differential leukocyte counts, total protein, alkaline phosphatase, and LDH. Measurements for dynamic light scattering of SiO₂ NPs in the BALF of Group 4 was significantly increased (600%) when compared to the BALF of Group 4 animals' total protein and alkaline phosphatase control. Results from the in vivo experiment indicate that exposure of RPMCs to SiO₂ NPs at 12mg/m³ in Golden Syrian Hamsters led to altered tissue morphology, cytotoxicity, and an inflammatory reaction when compared to the controls.

### 4707 Toxicological Assessment of ZnO Nanoforms to Substantiate Grouping

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Various zinc oxide nanoparticles were grouped to fulfill information requirements of the European Chemicals Regulation, REACH. The grouping was to be substantiated by new experimental data on selected nanoforms within this group [1]. The toxicological study included subchronic inhalation toxicity, reproductive and developmental toxicity, and genotoxicity. 28 Nanoforms of ZnO were characterized: (i) their distribution and aspect ratio (by TEM, according to the NanoDefine Method) [2]; (ii) protein and aerodynamic diameter (by the small rotating drum method, EN 171994-4); (iii) dissolution rate in lysosomal simulant (by continuous flow system); according to [2, 3]. Two nanoforms were selected having the same size and shape, but different surface characteristics: uncoated and hydrophobic coated. Soluble zinc sulfate monohydrate and micron-sized ZnO particles were tested as reference substances at equimolar Zn concentrations. Rats were exposed up to 10 mg/m³ ZnO for 90 days according to OECD test guideline (TG) no. 413 with, in addition, reproducibility and developmental toxicity screening tests (TG no. 421), including developmental neurotoxicity on PND 22. Recovery groups of rats were included to check for any reversibility, progression, or delay of the toxic effect. A comet assay (TG no. 489) was performed after 14 days inhalation exposure. The target tissues analyzed in the comet assay were the nasal epithelium and the lung (site of contact) as well as the liver and the bone marrow. Zinc oxide nanoparticles caused local effects at high exposure, i.e. in any of the tested substances with the exception of some increased neutrophil blood counts in male animals exposed to 10 mg/m³ coated nano ZnO. There were no indications for impairment of fertility, developmental toxicity, or developmental neurotoxicity. After the recovery period, all parameters in laveage fluid returned to the control level in all animals, irrespective of the exposed test and reference substance. With regards to histological findings in the respiratory tract, all changes reduced significantly in incidence and severity. The Comet assay confirmed the absence of genotoxic effects in all of the examined tissues, after exposure to both nano ZnO forms or to the high concentration of micron-sized ZnO or ZnSO₄ monohydrate. Both ZnO nanoform, micron-sized ZnO particles of Zn were included into 4 levels in a dose response-relationship. No genotoxic effects did not cause systemic, reproductive, developmental, or genotoxicity. The ZnO nanoforms were comparable and may be grouped together. No pronounced difference was found between nanoforms micron-sized ZnO. This grouping approach helps to minimize the number of animal studies to be performed. References: [1] J. G., et al. “Rationale and decision rules behind the ECETOC NanoApp to support registration of sets of similar nanoforms within REACH.” Nanotoxicology 15.2 (2021): 145-166. https://doi.org/10.1080/17435390.2020.1842933 [2] K. J., et al. “Predicting dissolution and transformation of inhaled nanoparticles in the lung using abiotic flow cells: The case of barium sulfate. Scientific reports, 10(1), 1-15. https://doi.org/10.1038/s41598-019-56872-z [3] K. J., et al. “Variation in dissolution behavior among different nanoforms and its implication for grouping approaches in inhalation toxicity.” Nanotoxicology. 23, 100341. https://doi.org/10.1016/j.nantox.2021.100341.

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The exploitation of nanomaterials (NMs) in products such as food, cosmetics, electronics, textiles, and medicines is increasing. The hazards of NMs therefore need to be thoroughly assessed to ensure the safe and responsible exploitation of nanotechnology. This is challenging given the rapid growth of the nanotechnology industry and the production of a huge diversity of NMs with varied physico-chemical properties. NM hazard assessments often focus on whether an inflammatory response is stimulated, with a reliance placed on using rodents. Thus, alternative models are needed to support the implementation of the 3Rs principles in nanotoxicology. Early life stages of transgenic zebrafish have been used to develop models for the future development of biomarker panels that may be representative for different categories of nanomaterials or conditions, and the left lung was preserved for pathology analysis. Serum was collected for analysis. Lavage was performed on the right lungs to characterize lung injury and inflammation. Early life stages of transgenic zebrafish can be used to screen for NM toxicity via assessment of neutrophil responses. C. Tucker3, a test model for screening NM toxicity via assessment of neutrophil responses, is more ethical, quicker, cheaper and potentially more predictive. The aim of this study was to investigate the suitability of using transgenic zebrafish larvae as a test model for screening NM toxicity via assessment of neutrophil responses. We undertook a series of experiments to investigate the suitability of transgenic zebrafish for NM toxicity screening. Zebrafish larvae were exposed to TiO2 or ZnO NMs at concentrations ranging from 11-71, depending on the dose and the particle exposure. The number of significantly different between control and exposed animals at postnatal months 3, 6, and 12. Plasma samples were collected from offspring rats at 3, 6, 9 and 12 months of age and analyzed for markers of kidney injury and function. Kidneys were collected from offspring rats at 9 months of age, and tissue was sectioned for histopathological analysis. Plasma neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) concentrations were measured using enzyme-linked immunosorbent assays. No differences in NGAL or CysC levels were observed between TiO2 NP-exposed and control groups at 3, 6, 9 and 12 months. No differences in plasma concentrations of urea nitrogen, creatinine, albumin, phosphorus, sodium, and potassium were observed between control and exposed groups at 3, 6, 9 or 12 months. Plasma concentrations of urea nitrogen (17.7 ± 4.34 mg/dL vs. 13.25 ± 2.22 mg/dL, p<0.01), creatinine (0.375 ± 0.05 mg/dL vs. 0.3 ± 0.08 mg/dL, p=0.07) and albumin (4.525 ± 0.68 g/dL vs. 3.875 ± 0.59 g/dL, p=0.20) trended higher in exposed compared to control progeny at 6 months, respectively, although the results did not reach statistical significance. Overall, exposure to TiO2 NPs during late gestation did not affect kidney function or induce kidney injury in adult rat progeny. Subsequent studies will further assess indicators of interstitial fibrosis in progeny kidneys.
were ingested by Sprague Dawley rats. Proteomics assessment revealed more than 400 hundred proteins in the liver that may be affected. These proteins are involved in such processes as catalysis of fatty acids by CoA, homocysteine metabolism, beta oxidation and the condensation of carbamyl phosphate in the urea cycle, among others. Further analysis of the protein associations by DAVID bioinformatics tool showed gene ontology (GO) categories including fundamental biological processes, cellular components (CC) and molecular function (MF). GO categories including325 biological processes, 140 molecular functions and 70 cellular components appear to be affected from the ingestion of TiO2. Quantitative analysis of specific mRNA transcripts indicated CMLB, GSTM1 and SDS were differentially expressed.

**4712 Oral Toxicological Study of Titanium Dioxide Nanoparticles with a Crystallite Diameter of 6 nm in Rats**


Titanium dioxide (TiO2) is generally considered to have a low impact on the human body; however, the safety of TiO2 containing nanosized particles (NPs) has attracted attention. We found that the toxicity of silver NPs markedly varied depending on their particle size, as silver NPs with a diameter of 10 nm exhibited fatal toxicity in mice, unlike those with diameters of 60 nm and 100 nm. Therefore, the toxicological effects of the smallest available TiO2 NPs with a crystallite size of 6 nm were examined by repeated oral administration at 10, 100, and 1,000 mg/kg bw/day for 28 days and at 100, 300, and 1,000 mg/kg/bw/day for 90 days. In both the 28- and 90-day studies, no toxicity was observed in any group, and no treatment-related adverse effects were observed in body weight, urinalysis, hematology, serum biochemistry, and organ weights. Histopathological examination with hematoxylin and eosin staining revealed TiO2 particles as depositions of yellowish-brown materials. The particles observed in the gastrointestinal lumen were also found in the nasopharynx, as well as in the epithelium and stromal tissue in the 28-day study. In addition, they were observed in the Peyer’s patches in the ileum, cervical lymph nodes, mediastinal lymph nodes, bronchus-associated lymphoid tissue, and trachea in the 90-day study. Notably, no adverse biological responses, such as inflammation or tissue injury, were observed around the deposits. The determination of titanium concentration in the liver, kidney, and spleen of rats treated with TiO2 NPs were hardly absorbed and accumulated in these tissues. Immunohistochemical analysis of colonic crypts showed no extension of the proliferative cell zone or abnormalities of colonic crypts, and induction of DNA strand breaks and chromosomal aberrations.

**4713 Pulmonary Effects after Exposure to Cadmium Sulfide Nanoparticles: Comparison of In Vitro and In Vivo Changes in Proteome**


The manufacturing of quantum dots using cadmium sulfide (Cds) nanoparticles (NPs) has been expanding. Cds quantum dots are semiconductors with a direct intermediate bandgap and excellent thermal stability and thus have shown strong potential for wide scale use in solar cells, light emitting diodes, and specialty lasers. Industrial scale manufacturing of Cds quantum dots requires large batch of NPs. To evaluate pulmonary toxicity associated with exposure to Cds NPs and to compare changes in proteome, we performed in vitro (acute) and in vivo inhalation (acute and sub-acute) studies. Primary particle size by TEM was 10.7 nm (SD=3.1 nm). In vitro studies utilized human monocytes (THP1 cells) exposed to either 12.5 or 25.0 µg/mL. Outcomes were evaluated 12 h postexposure. In vivo studies employed a nose-only inhalation exposure system and C58B16, female mice. Cds nanoaerosols had geometric mobility diameter of 41 nm (geometric SD=1.8) and exposures targeted a concentration of 3.5 mg/m3 for 4 h (acute) or 2 wk (sub-acute). Mice were necropsied immediately (0 wk) or 3 wk postexposure. Studies were controlled using either untreated cells (in vitro) or animals exposed nose-only to filtered air (in vivo). THP1 cells and lungs were processed for LC-MS/MS analysis (Orbitrap). Label-free quantitative proteomics and DAVID ontology analysis of data were performed. Bronchoalveolar lavage (BAL) fluid and lung tissues were evaluated for inflammation. Neutrophils in BAL fluid after acute inhalation exposure increased to 30% within 24 h. Immediately after sub-acute exposure, neutrophil infiltration into the lungs was 41% - this neutrophilic inflammation persisted through 3 wk postexposure (29%). These increases corresponded to elevated concentrations of inflammatory cytokines at these time points. Histopathology of lung tissue confirmed the presence of inflammation. There was evidence of lipid peroxidation in both lung tissue and serum of exposed mice (TBARS assay) after sub-acute exposure. Proteomics analyses of THP1 cells did not show significant changes in protein expression as opposed to acute and sub-acute in vivo exposures which showed significant and distinct exposure group separation from controls as well as between 0 wk and 3 wk post-sub-acute exposure. Out of ~2500 proteins quantified, 824 were shown to be significantly differently expressed in sub-acute exposed compared to controls. Functional annotation and biological processes of the following biological processes were shown to be the most significantly affected by Cds NP exposure: cell adhesion, extracellular matrix organization, positive regulation of cell-substrate adhesion, regulation of cell shape, immune system and metabolic processes, positive regulation of peptidyl-tyrosine phosphorylation, and cellular responses to interferon-β. The highest number of differently expressed proteins were in observed in the extracellular exosomes and plasma membrane. The results from in vitro studies were not predictive of in vivo results. Funded by NIH U01 ES027252.

**4714 Immunomodulatory Effects of Subacute Inhalation Exposure to Copper Oxide Nanoparticles in House Dust Mite–Induced Asthma and Allergen Immunotherapy Mouse Models**


Inhalation exposure to copper oxide nanoparticles (CuO NPs) results in pulmonary inflammation. However, immunomodulatory consequences after CuO NP inhalation exposure have been less explored. We investigated the effect of CuO NP aerosol inhalation on immune responses and asthma pathology in a novel model of allergic asthma in mice (A/J). Mice were sensitized and challenged to house dust mite (HDM) aerosols to induce allergic asthma (A/J-t) as well as allergic asthma in A/J-t mice treated with A/J-t mice treated with CpG-loaded nanoparticles (CpG NPs) by subcutaneous (s.c.) injection while being exposed to CuO NP aerosols (4 h/day, 10 days) starting on the 1st immunization day. Mice were sensitized twice by s.c. injection of HDM alone and were challenged 10 times with HDM extract by intranasal instillation. The asthmatic mouse model followed the same timeline, except that they received no immunizations. Mice were euthanized 24 h after the last HDM challenge; macrophages, neutrophils, lymphocytes, eosinophils and bronchoalveolar lavage (BAL) fluid were counted. Non-lavaged lung tissues were homogenized and selected immune cell populations were analyzed by flow cytometry (T1, T2, T17, and Tc17, cells). Lung histopathology using H&E and PAS staining was also performed along with measurements of cytokine/chemokine levels in BAL fluid, HDM-specific immunoglobulin levels (IgE, IgG1, IgG2) in serum, and pulmonary mechanics after methacholine challenge. Mice that were exposed only to CuO NP showed significant increases in neutrophils and T1-like Treg cells (T-bet+FoxP3+), but no increases in T1 cells were observed compared to sham mice. Inhalation exposure to CuO NPs followed by HDM exposure (asthmatic model) resulted in higher T1 responses and lower T2 responses; with elevations in T1 cells and reductions in IL-4, IL-2, and T2-cell (GATA-3 FoxP3+) cells, compared to non-CuO NP aerosol-exposed asthmatic mice. There was no immunomodulatory effect of CuO NP aerosol inhalation in mice treated with A/J-t mice treated with HDM exposure to induce asthmatic conditions. Inhalation exposure to CuO NPs prior to HDM sensitization caused increased T1 immune responses and increased levels of T cells in the lung homogenates, suggesting skewing of normal immune balance. The effect of CuO NP inhalation exposure on A/J-t treated asthmatic mice did not result in significant differences in T1 or T2 immune responses. This work was funded by NIH U01ES027252 and P30 ES05565.

**4715 Preliminary Report on a Two-Year, Four-Week Interval Intermittent Whole Body Inhalation Study of the Multiwalled Carbon Nanotube (MWNT-7) in Male Mice**

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In a whole-body inhalation toxicity study on gaseous test substance, the lungs are exposed to a same concentration throughout the study. Therefore, "area under the curve" (AUC) of the lung concentration-time curve is rectangular in shape. In contrast, in conventional inhalation studies, the pulmonary concentration of the test particles is biopersistent; the concentration of the aerosol of the particle is constant throughout the inhalation period, and the lung burden or the amount of the particle deposited in the lung gradually increases over time, from zero at the beginning to a maximum amount at the end of the study. If the chronic lung toxicity is induced by the deposited particles, the particulate matter affects small effects at the beginning and larger effects towards the end of the study. In other words, particulate matter study has a triangle-shaped AUC of the lung tissue concentration-time curve which is half of the AUC of gaseous test substances. We started a project to seek the experimental conditions to make the lung burden constant during the two-year period.
period by boosting it at the beginning of the study followed by intermittent maintenance exposure to shape a rectangular AUC. As a first step, we performed a 4-week intermittent exposure 2 year inhalation study without initial boost, mimicking the increment of lung burden of conventional inhalation study by referring a rat study by Kasai et al., 2016. Male C57BL/6 mice were exposed to Mitsui MWNT-7 aerosol generated by a Taquann system (J. Toxicol. Sci. 2013) using 53 micrometer mesh filters 6 hours per day, once per every 4 weeks, for a total of 26 times for 2 years. Mass concentrations were 2.7 ± 0.1 mg/m³ for the low concentration group (group L) and 5.2 ± 0.2 mg/m³ for the high concentration group (group H). MMAD was ca. 500 nm. No difference in mortality was observed between the groups. Grossly, the lungs were grayish-white to gray and “voluminous” along with an increase in weight in a dose-dependent manner, i.e. control, 165.5±8.8 mg, group L, 336.2±25.2 mg, and group H, 369.4±25.5 mg. The lung burden of MWNT-7 at 2 years was 61.1±2.2 microgram/lung in group L and 91.6±21.5 microgram/lung in group H. Histologically, chronic granulomatous foreign body responses against MWNT-7 and fibrosis in a form of respiratory bronchiolitis was observed along with a proliferation of terminal bronchial epithelium and proliferation of type II cells of the alveolar ducts. Severe and pleural inflammation was also observed. Nodular lesions, diagnoses as adenocarcinoma, were identified in two cases of group L. Adenocarcinoma cells were positive for TTF-1 and negative for CC10, indicating type II alveolar epithelial origin. Further details will be presented.

Health and Labour Sciences Research Grant, Japan.

Effects of Subacute Inhalation Exposure to Multiwalled Carbon Nanotubes in B6C3F1/N Mice and Sprague Dawley Rats

Multicored carbon nanotubes (MWCNT) are a subset of carbon nanotubes ranging from 10nm to 200nm long. MWCNT are applied in construction, engineering, and electronic applications, and have several physicochemical properties as well as a high aspect ratio. Materials with high aspect ratios can be easily inhaled, and this quality has been a concern in the safety of inhaled carbon nanotubes. To date, MWCNT studies have utilized intratracheal or intrapharyngeal aspiration routes of administration, and studies using inhaled MWCNT have focused on chronic rather than subacute effects on pulmonary health. The goal of this study was to elucidate the subacute effects of inhaled MWCNT in mice and rats, and their ability to clear MWCNTs from the lung. It is hypothesized that subacute inhalation of MWCNT results in increased lung inflammation, total cell counts in bronchoalveolar lavage (BAL), retention of MWCNT in alveolar macrophages, and increased inflammatory cytokine expression.

Mice were therefore assigned into one of four treatment groups for MWCNT exposure control (0.06, 0.2, and 0.6mg/m³; n=5 per group). Animals were whole-body exposed to assigned doses of L-MWCNT-1020 via a particle attachment chamber using a single jet disperser/aerosolizer. Exposures were conducted for 6 hours/day, 5 days/wk for 5 weeks total, followed by a 1 week or 4 week recovery period. Animals were then anesthetized via an intraperitoneal injection of Beuthanasia and tracheotomized. Bronchoalveolar lavage (BAL) was harvested, and right lung lobes were collected for downstream RNA and protein extraction and analysis via RT-qPCR and ELISA respectively. BAL samples were cytospun onto slides for neutrophil counts and cell differentials. Left lung lobes were inflated and fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned onto slides for H&E staining. BAL from mice in the 1-week recovery group conveyed significantly higher number of total cells when exposed to 0.6mg/m³ MWCNT compared to those exposed to lower concentrations. Neutrophil counts from BAL of mice in the 0.2 and 0.6mg/m³ MWCNT exposure groups were predictive of the outcome observed. In both models, the 5-week recovery period groups exhibited an attenuation of these effects. For both mice and rats, the highest dose treatment group exhibited significantly higher levels of neutrophil counts compared to control after 4 week recovery. MWCNT were found to be retained in BAL and lung tissue of both 1 week and 5 week recovery groups for both species. In conclusion, acute inhalation is observed 1 week-post-exposure but resolves at the 5-week recovery time point. However, the continued retention of MWCNT inclinations in lung macrophages without the presence of overt inflammation or injury raises concerns about the potential long-term effects of MWCNT inhalation exposure. Funding: National Toxicology Program in MWCNT Research, NIEHS U01 ES020127.

Characterization and Toxicity Assessment of Aerosolized Particles Generated during Cutting of Carbon Nanotubes—Embedded Concrete
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Carbon nanotube (CNT) as a reinforcement enhances durability and performance of concrete composites. Exposure to some forms of CNT is known to cause adverse pathological outcomes. To understand potential toxicity arising from use of CNT-enabled concrete composites as it goes through various occupational life cycle stages, we evaluated 1) the physicochemical characteristics and toxicity of the used carbon nanotubes, 2) potential wear induced during concrete manip-ulation (e.g., cutting), is altered by CNT incorporation. Physical dimensional profiling indicated the as-produced CNT had a geometric mean length of 0.72 µm and 32 nm in diameter. Pulmonary injury and inflammation exerted by the CNT was assessed by exposing C57BL/6J mice by oropharyngeal aspiration to a bolus dose of 4.4 x 10⁴ ug in 5µl over 4 days and 28 days. The toxicity of the as-produced CNT was similar to an agglomerated CNT studied in our previous research, lacking the more severe toxicity associated with longer length and diameter CNT. Mechanism-based screening of the initiating events in vitro in differentiated THP-1 macrophages were predictive of the in vivo outcome observed. For evaluating the second aim, three types of concrete blocks, 0% (reference), low%, and high% CNT, were used in a custom designed enclosure housing an apparatus for the cutting of a block with an automated computer-controlled process. The highest particle number concentration (163,821 particles/cm³) was measured for the reference cylinder, while others showed similar concentrations (131,689 particles/cm³ and 140,954 particles/cm³ for the low% and high% blocks, respectively). There was no shift in the size distribution of the released aerosols from addition(s) of CNT. The released particulate was predominantly respirable particulate consisting of quartz, feldspar, etc. and often appeared to be agglomerated materials that included both paste and aggregate minerals with an aerodynamic diameter ~5µm. No free CNT were observed by electron microscopy from low% and high% CNT. The released aerosols were not significantly different from that of a raw material.

4716 Effects of Subacute Inhalation Exposure to Multiwalled Carbon Nanotubes in B6C3F1/N Mice and Sprague Dawley Rats

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The inhalation toxicity of CNTs is not clearly known due to relatively few related studies reported. An acute inhalation study and short-term inhalation study (5 days) were therefore conducted using Sprague-Dawley rats. In the acute inhalation study, the rats were grouped and exposed to a fresh air control or to low (0.238 ± 0.197), moderate (1.935 ± 0.159), or high (24.66 ± 6.336 mg/m³) CNT concentrations for 6 hours and then sacrificed at 14 days. For the short-term inhalation study, the rats were grouped and exposed to a fresh air control or low (0.593 ± 0.019), moderate (2.487 ± 0.213), or high (10.345 ± 0.541 mg/m³) CNT concentrations for 6 h/day for 5 days and sacrificed at 1, 3, and 21 days post-exposure. No mortality was observed in the acute inhalation study. Thus, the CNT LC50 was higher than 25 mg/m³. No significant organ weight changes were noted during the 5 days short-term inhalation study or during the post-exposure period. No significant effects of toxicological importance were observed in the hematological, blood biochemical, and coagulation tests. In addition, the bronchoalveolar lavage (BAL) fluid cell differ- ential counts of BAL and BMF from the rats exposed to CNTs in the acute study showed no significant changes. The histopathological examination also found no CNT-exposure-relevant histopathological lesions. Thus, neither acute nor 5 days inhalation exposure to CNTs induced any noticeable toxicological responses.

4717 Characterization and Toxicity Assessment of Aerosolized Particles Generated during Cutting of Carbon Nanotubes—Embedded Concrete

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Atherosclerosis is known to be the main cause of cardiovascular disease and is regulated by pro-inflammatory molecules such as macrophage chemoattractant protein-1 (MCP-1), interleukin 1 beta (IL-1 beta), interleukin 6 (IL-6). A new class of nanoparticles, Carbon Nanodots (CNDs), have been expressed as potential candidates for biomaging, biosensing, and drug delivery. The liver is known to be the main metabolic target that is involved in metabolizing abnormal lipid metabo-lism and complex inflammatory disease. However, the effect of CNDs on the liver has not been investigated. In this study, the impact of CNDs on TNF-α-mediated expressions of pro-inflammatory genes in mouse liver tissues was examined. C57BL/6 mice were treated with TNF-α (25mg/kg bw), CNDs (2.5 mg/kg CNDs), both TNF-a and CNDs, or neither to serve as the control. Spleen tissue was collected and homogenized, and the isolated RNA was used to make cDNA for the expression of various genes associated with the inflammatory response using real-time PCR. Our results showed that TNF-α increased the expression of pro-inflammatory genes, including TNF-a, IL-1β, and IL-6, and decreased the expression of anti-inflammatory genes, including glutathione s-transferase and heme oxygenase-1. CNDs treatment improved the expression of these genes in the liver of TNF-α-treated mice. This study would contribute to a better understanding of the role of CNDs on inflammation in vivo.

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Cardiovascular disease affects many people around the world, and atherosclerosis is one of the main causes of cardiovascular disease. Atherosclerosis is the hardening of the blood vessels and is strongly regulated by various pro-inflammatory molecules such as macrophage chemoattractant protein-1 (MCP-1), interleukin 1 beta (IL-1 beta), interleukin 6 (IL-6). The liver is closely related to the metabolism of abnormal lipids and complex inflammatory disease and is the organ we have been studying. There is a new class of nanoparticles, called Carbon Nanodots (CNDs), which have been noted as potential candidates for bioimaging, biosensing, and drug delivery. However, there is not much research on the effects of CNDs on inflammatory disease and the liver. In this study, we have investigated the impact of CNDs on TNF alpha mediated expressions of pro-inflammatory genes in mice in both of the previously mentioned tissues. C57BL/6 mice tissues have been treated with either TNF alpha (25µl/kg bw), CNDs (2.5 mg/kg CNDs), both TNF alpha and CNDs, or neither to serve as the control. The real-time PCR performed shows that the TNF alpha increased the expression of MCP-1, IL-1 beta, and IL-6 beta in the liver tissues studied. Other experimental data are still in progress. This study will gain a better understanding of the actions of the CND on TNF alpha-induced inflammation in vivo.

Microplastics (MPs), with a diameter less than 5 mm, have become widespread contaminants in the environment, where human consumption is inevitable. While the health impact of MP inhalation uptake is still under investigation, previous work has demonstrated that the consumption of polystyrene (PS) beads by mice promotes adiposity and indices of insulin resistance. To develop an understanding for the basis of these outcomes, we supplied male C57BL/6 mice at 13 weeks of age with normal water or that containing polystyrene beads (5 µm or 0.5 µm; 1 µg/ml) for 14 weeks and assessed adipose immune cell infiltration, adipokine levels, as well as metabolic and inflammatory markers in the plasma and liver. We observed a significant increase in adipose tissue macrophage abundance in those mice drinking PS-containing water compared to mice drinking normal water. This was accompanied in the same group by increases in the expression of adipo C122 and Ins2 and in plasma by increased leptin levels. The livers of PS-exposed mice demonstrated increases of cholesterol and protein CysS3S, but no changes in triglycerides and a decrease in the levels of carnitine palmitoyltransferase-2 (Cpt2). In additional mechanistic studies we tested if delphinidin, a plant- and berry-derived anthocyanin with antioxidant and anti-inflammatory properties, could alleviate PS-effects. Thus, groups of PS-exposed mice received either intraperitoneal injections of DMSO or delphinidin (20mg/kg/3x per week). After 4wk we assessed weight gain, and body composition. We observed that mice receiving consuming PS beads and receiving the delphinidin injections gained significantly less weight and had a smaller percentage of body fat, compared with those mice receiving consuming PS beads and receiving DMSO injections. These results suggest that ingestion of PS beads promotes metabolic disturbances likely through mechanisms involving oxidative stress and inflammation, which could be alleviated by supplementation with delphinidin.

Two-dimensional (2D) nanomaterials are a large class of engineered nanoparticles with a multitude of applications in electronics, biosensors, and more. Increased demand for these materials, including graphene, nanoclay, transition metal dichalcogenides (TMDs), such as WS2 and MoS2, and hexagonal boron nitride (hBN), has elevated the potential for occupational respiratory exposure, notably during manufacturing. The goal of the current study was to conduct a comparative toxicity study of representative 2D materials for different categories listed above using high throughput in vitro screening assays. The five materials were thoroughly characterized for size, density, surface area, hydrodynamic diameter, and more. A battery of toxicity assays was performed using human bronchial epithelial cells (BEAS-2B) and human THP-1 monocytic cells in doses ranging from 1-100 µg/ml. Cytotoxicity and cell proliferation were assessed using WST-1 and Alamar blue for each cell type. Significant reduction in cell viability was found to occur with graphene at doses ≥ 12.5 µg/ml. Nanoclay and hBN had significant changes at doses ≥ 25 µg/ml, while little to no changes were seen, even at the highest doses (100 µg/ml). Inflammasome activation was assessed in THP-1 cells. IL-1β was found to be significantly increased at an average of 3.6 (6.25 µg/ml) and 4.8 (25 µg/ml) times the control level following nanoclay exposure, and a significant 1.8-fold change occurred following hBN (6.25 µg/ml) exposure. A 4-fold change in Caspase-1 also resulted from exposure to 25 µg/ml nanoclay. In
BEAS-2B cells, there was a trend for cell cycle arrest in G0/G1 following the 25 µg/ml exposure to nano-CuO. These initial findings suggest that the TMD category is relatively less toxic than the other classes of 2D materials and nanoclays may be of greater toxicological concern overall. Future work will further screen these materials for genotoxicity, oxidative stress, and inflammatory effects, as well as conduct statistical analyses of the relationship of material properties to these outcomes.

**Development of Toxicity Evaluation Method for Nanomaterials Using Activation of THP-1 Cell as an Index**


**Nano-Ceramics of Engineered Nanoparticles with Various Sizes for Skin Sensitization Studies**

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Despite the expanding number of applications for engineered nanoparticles (ENPs), human health concerns associated with ingested nanoparticles are poorly understood. In this study we utilized 3D human small intestinal (SMI) tissue model to develop a physiologically relevant test system to assess nanotoxicity of ingestible nanomaterials. For dose response experiments, we tested ENPs of various sizes: TiO2 (5 nm, 900 µg/ml), ZnO (15 nm, 900 µg/ml), and SiO2 (300 nm, 900 µg/ml). The cytotoxicity of THP-1 cells was determined by MTS assay and alamarBlue. The cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue. The cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue. The cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue. The cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue. The cytotoxicity of Nano-CuO in cells was determined by MTS assay and alamarBlue.
Exosomes have emerged as key signaling mediators of cell-cell communication in a wide variety of biological processes. When cells secrete exosomes, various biologically active factors such as RNA, protein, lipid, and metabolites are loaded; thus, exosomes can serve as carriers of complex intercellular information. The effects of nanoparticles (NPs) exposure on the pulmonary system are well established; however, the underlying mechanisms of how inhaled NPs affect the other organs need further investigation. Although some studies on the direct effects of NPs on systemic distribution suggest that NPs may be translocated directly from the alveoli into the microvascular bed, it is unclear that how NPs and biological mediators from the lung reach to other organs to induce extrapulmonary toxic effects. We hypothesized that the exosomes released from human lung cells as responses to NPs exposure play a key role in cell-cell communication for extrapulmonary toxicity of NPs. Here, we characterize by lung epithelial cells after exposure to NPs and examined the roles of isolated exosomes in NP-induced extrapulmonary toxicity. In this study, We characterized the exosomes released by human alveolar epithelial cells (A549) following exposure to copper oxide (CuO) NPs and evaluated the cytotoxicity (AlamarBlue assay and LDH assay) and oxidative stress (intracellular ROS) of A549 cells (for pulmonary responses) and human umbilical vein endothelial cells (HUVEC, for extrapulmonary effects) as responses to CuO NPs exposure. Exosomes were isolated from the exposure medium following NP exposure using ExoQuick-TC. To characterize exosomes, we measured the expression of exosome membrane marker proteins (e.g., CD63 and Flotillin) by Western blot assay, observed morphology using transmission electron microscopy, and measured the size and concentration through Nanoparticle Tracking Analysis (NTA). Then, we profiled miRNA signatures in the isolated exosomes using NanoString. In addition, cellular responses of HUVEC in the presence of exosomes released from NP-exposed A549 cells were assessed to examine the effects of exosomes in the extrapulmonary toxicity of NPs. CuO NPs induced the dose-dependent cytotoxicity effects and intracellular reactive oxygen species (ROS) in A549 cells and ROS-induced cytotoxic effects were found in the experiment with N-acetyl cysteine as a ROS scavenger. Furthermore, the size and concentrations of exosomes released from NP-exposed A549 were different compared to those of untreated cells. Isolated exosomes released from NP-exposed A549 cells induced cytotoxicity in fresh A549 cells and HUVECs. Differentially expressed exosomal miRNAs in NP-treated cells were associated with cellular toxicity, which further emphasizes the potential role of exosomes in NP-induced toxicity. Our results suggest that exosomes have a potential as key mediators for carrying cytotoxic-related factors to surrounding cells or target organs in NP exposure, which can induce extrapulmonary toxic effects.

Gold nanoparticles (AuNPs) are promising nanomaterials for biomedical applications that range from sensing to diagnostic imaging, to drug delivery and therapeutics. However, the insufficient understanding of NP interactions with biological systems has proven to be a barrier to the clinical translation of nanotechnology, impacting the development of the whole area. New and more advanced mechanism-based approaches, including OMICS and systems biology (toxicology), are emerging to provide quantitative information on how biological networks are perturbed by nanomaterials in living systems. MicroRNAs (miRNAs), short non-coding RNAs that mediate gene expression repression, have emerged as key regulators of biological pathways. Herein, we report the first study focused on the in vitro long-term cellular responses of a low B16 cell line exposed to various sized and surfaces coatings in the global miRNA expression profile of primary human dermal fibroblasts after chronic and acute (non-chronic) exposure. Our results show that the exposure condition and surface chemistry of the AuNPs do have a significant impact on the modulation of miRNA levels. At the systems-level perspective, the biological response for a long-term low dose is distinctly different: the non-chronic exposure led to significant perturbations in miRNA networks enriched for target genes involved in cell proliferation pathways, with cells under this exposure condition potentially suppressing proliferative signaling pathways, possibly in an attempt to restore cell homeostasis disrupted after the treatment with AuNPs, while changes in miRNA co-expression networks enriched for target genes related to activation of proliferative and suppression of apoptotic pathways were observed in cells chronically exposed to one specific type of AuNP. In this case, miRNA dysregulation might be contributing to enforce a new cell phenotype during stress. Collectively, our data suggest that miRNAs play critical roles in the cellular responses to the stress provoked by AuNP stimuli. The mechanistic understanding of nanomaterial-induced molecular changes and perturbation in biological pathways provides comprehensive response profiles of nanomaterial exposures, enabling the development of safe-by-design nanomaterials as well as opening new avenues for further nanotechnology applications.

In the present study, silver nanoparticles (Ag-NPs) were synthesized using Azadirachta indica extract and evaluated for their in vitro antioxidant activity and cytotoxicity efficacy against MCF-7 and HeLa cells. The silver nanoparti- cles (Ag-NPs) were formed within 40 min and after preliminary confirmation by UV-visible spectroscopy (peak observed at 375 nm), and were characterized using a transmission electron microscope (TEM), and dynamic light scattering (DLS). The TEM images showed the spherical shape of the biosynthesized Ag-NPs with particle sizes in the range of 10 to 60 nm, and compositional analysis was carried out. The cytotoxicity and antioxidant activity of various concentrations of biosynthesized silver nanoparticles, Azadirachta indica extract, and a standard ranging from 0.125 to 10 mg/mL were evaluated. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of the biosynthesized Ag-NPs and aqueous leaf extract increased in a dose-dependent manner, with average IC50 values of the biosynthesized Ag-NPs, aqueous leaf extract, and ascorbic acid (standard) of 0.70 ± 0.07, 1.63 ± 0.09, and 0.25 ± 0.09 mg/mL, respectively. Furthermore, higher cytotoxicity was exhibited in both the MCF-7 and HeLa cell lines in a dose-dependent manner. The average IC50 values of the biosynthesized Ag-NPs, aqueous leaf extract, and cisplatin (standard) were 0.90 ± 0.07, 1.75 ± 0.01, and 0.56 ± 0.08 mg/mL, respectively, with MCF-7 cell lines and 0.85 ± 0.01, 1.76 ± 0.08, 0.45 ± 0.10 mg/mL, respectively, with HeLa cell lines. Hence, this study resulted in an efficient green reductant for producing silver nanoparticles that possess cytotoxicity and antioxidant activity against MCF-7 and HeLa cells.

Silver nanoparticles (AgNP) are widely consumed nanomaterials that have been increasingly incorporated in food-related and hygiene products, which thereby could lead to their intentional or accidental ingestion. Increased oral exposure to AgNP may result in extensive exposure of intestinal cells, which may result in local adverse effects and intestinal inflammation. Hence, it becomes imperative to thoroughly evaluate the potential pro-inflammatory effects of AgNP, especially at the intestinal level. This study aimed to evaluate the possible pro-inflammatory effects of polypyrrolidine (PVP)-AgNP of two different sizes (5 and 50 nm) in intestinal epithelial C2BBe1 cells. The size-dependent effects of PVP-AgNP on cellular viability were firstly evaluated by flow cytometry. It was observed a trend in the occurrence of early and late apoptotic events for the 50 nm PVP-AgNP. The size-dependent pro-inflammatory effects of PVP-AgNP were also evaluated through a western blot analysis of inducible nitric oxide synthase and iкBα, and also through the quantification of reactive oxygen species (measured by DCFH-DA) and reactive nitrogen species (measured by the Gness reagent). The results revealed the activation of an inflammatory response after the exposure of C2BBe1 cells to the larger PVP-AgNP (50 nm), which was mainly characterized by an increase of expression levels of inducible nitric oxide synthase and a decrease of expression levels of iкBα levels. This response was followed by an increase in the levels of reactive nitrogen species, although there was not observed a significant increase in the amount of reactive oxygen species. This study indicates that PVP-AgNP may be deleterious to intestinal cells, through the activation of several steps of the inflammatory process. This work received financial support from FCT/MCTES (Fundaçao para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for the national funds received through the projects PT17/EEổi/00019/2014, IREN, POCI-01-0145-FEDER-000030, FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/00056/2020 and UIDP/00056/2020. AS and MF thank FCT for the PhD grant SFRH/BD/150656/2020 and the contract 2020.04126.CEECIND/CP1596/CT0006, respectively. MF also thanks to LAQV/REQUIMTE her contract LA/P/0008/2020. SFRH/BD/150656/2020 and the contract 2020.04126.CEECIND/CP1596/CT0006, respectively. MF also thanks to LAQV/REQUIMTE her contract LA/P/0008/2020.

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Micro-nanoplastics (MNPs) generated by aging and fragmentation of plastics over time or by municipal incineration of plastic waste are environmental contaminants of increasing concern in recent years. MNP-induced disruption of organismal physiology and behaviors has been widely reported in aquatic organisms. More recently, it has been postulated that exposure to MnPs is a serious threat to human health. Ingestion is considered one of the most common routes of MNP exposure in humans, as MNPs can ascend the food chain by trophic transfer or be released from plastic food packaging and have been found in a wide variety of foods and drinks. The genotoxicity of MNPs, while gaining increasing attention, remains underdetermined. This study is focused on investigating the potential genotoxicity of MNPs in small intestinal epithelium using a high-throughput microarray analysis of MNPs, including primary carboxylated polystyrene (PS) spheres with sizes of 25 and 1000 nm (PS25C and PS1K), and environmentally relevant secondary polystyrene (PE) MNPs generated by incineration of pristine PE pellets (PEi, PEpm). We used the in vitro compound-genotoxicity assay (iCCG) to measure MNPs’ genotoxicity in this study. iCCG utilizes human airway organoids derived from airway epithelial cells to assess the genotoxic effects of test substances. The in vitro compound-genotoxicity assay (iCCG) is a high-throughput method for the detection of DNA damage in cells. This method is particularly useful for testing the genotoxicity of small particulate matter, such as microplastics, due to its high sensitivity and specificity. By using this assay, we were able to measure the genotoxic potential of MNPs in a linear and dose-dependent manner. The results of this study indicate that MNPs can cause DNA damage in human airway organoids, suggesting that exposure to MNPs may pose a significant threat to human health. Further research is needed to determine the extent to which MNPs are ingested and the potential long-term health effects of MNP exposure.
of primary and environmentally secondary MNPs in an integrated in vitro ingestion platform including simulated GIT digestion and three small intestinal segments. These findings suggest that ingestion exposures to high concentrations of MNPs could have serious genotoxic consequences in the small intestinal epithelium. Supported by SP300505022 for H.Z. and P.D.

4738 Evaluation of Glucose Uptake Activity and Cytotoxicity of Diterpenes and Triterpenes Isolated from Lamiaceae Plant Species
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The prevalence of diabetes mellitus (DM), considered as one of the most common metabolic disorders, has dramatically increased and resulted in higher rates of morbidity and mortality around the world in the past decade. It is well known that insulin resistance in target tissues and a deficiency in insulin secretion from pancreatic β-cells are the main characteristics of type 2 diabetes. The aim of this study was to evaluate the glucose-uptake ability of compounds isolated from three selected plant species namely Salvia africana-lutea, Leonotis oymifolia, and Plectranthus madagascarensis. The glucose-uptake ability of each compound was then evaluated in mammalian cells using 2-deoxyglucose-6-phosphate. The cytotoxicity of each compound was established via the MTT assay. Chromatographic purification of the three plant species yielded sixteen pure terpenoids namely 19-acetoxy-12-methoxyxarnocin (1), 3β-acetoxy-7α-methoxyxarnosanol (2), 19-acetoxy-7α-methoxyxarnosanol (3), 19-acetoxy-12-methoxyxarnosanol (4), climpereolides A (5) and B (6), oleandroic, and usorolic acids (7, 8), β-amyrin (9), camosanol (10), 6β,7α-dihydroxyxarnoleanone (11), 7α-acetoxy-6β-hydroxyxarnoleanone (12), horminone (13), and coleun U quinone (14), Leonurin (15) and 20-acetoxy-9α,13-dihydroxy-15(16)-epoxylabd-14-en-6β-lactone (16).

The results suggested that several compounds demonstrated a marked increase in glucose uptake, while two of the compounds exhibited signs of cytotoxicity. It may therefore be suggested that these compounds be considered as potential candidates for novel plant-derived alternative therapies in the treatment of type 2 diabetes.

4739 Investigation of Blue Cohosh Extract Compositions and Human Hepatocellular Effects

Botanical ingredients found in dietary supplements are widely used throughout the world as ‘natural’ remedies and food additives. Blue cohosh (Caulophyllum thalictroides) is purported to reduce the pain of menstrual cramps and induce labor, yet limited information is available to verify the quality of these supplements (i.e., constituent composition) or understand their potential human health effects (e.g., interactions with other botanicals or drugs). Recent studies indicate that ingestion of specific alkaloids in blue cohosh preparations can produce birth defects and neonatal heart failure, and contain saponins, which may be responsible for uterine-stimulating effects. Previous studies in our labs have established patient-derived human liver cells to study botanical-induced toxicity and drug interaction potential. In this study, we applied NMR, LC-MS, and cell-based assays to better characterize the adaptive effects of blue cohosh on hepatocellular pathways governing metabolism and transport (e.g., pregnancy X receptor (PXR), constitutive androstane receptor (CAR)). Study data revealed 3 out of 9 commercially available blue cohosh products contained constituent compositions that reflected possible contamination or adulteration. Furthermore, morphology, cytotoxicity, and gene expression assays in cultures of primary human hepatocytes revealed suppression of ABCB11, CYP1A2, CYP2B6, CYP3A4, and HMGC52 consistent both with selective effects and cellular stress responses at higher exposure levels. These data revealed commercially available botanical supplements may not reflect authentic constituent compositions and may have potential to suppress CYP3A4A metabolism that could contribute to observed in vivo effects.

4740 Platycodon D Prevents Endothelial Dysfunction through eNOS Phosphorylation and NO Synthesis

Endothelial dysfunction is characterized by an inflammatory response due to decreased thrombomodulin and adhesion molecules. The inhibition of NO production induces the inflammatory adhesion molecules, including ICAM-1 and VCAM-1. Platycodon D (PCD), a saponins from the root of Platycodon grandiflorum, have been reported to various physiological activities such as anti-cancer, anti-oxidant and anti-obsity. Despite the various physiological activities of PCD, studies on the molecular mechanisms for the protection of endothelial dysfunction is unclear. In this study, we investigated the protective effect of PCD on endothelial dysfunction via eNOS phosphorylation and NO production. PCD suppressed TNF-α induced the monocyte adhesion and the expression of ICAM-1 and VCAM-1. Treated with PCD was increased NO production via eNOS phosphorylation in human endothelial cells. In addition, PCD increased intracellular Ca2+ concentration and phosphorylation of eNOS (Ser1177) and cGMP production in HUVECs and SKBr3 (Her-2 over-expressed cell line). Compound D (AMPK inhibitor) and KN-62 (CaMKII inhibitor) were inhibited eNOS phosphorylation and NO production induced by PCD. Taken together, PCD promote NO synthesis and eNOS phosphorylation through the Ca2+/CaMKII and CaMKKβ/AMPK signaling pathways, consequently PCD attenuated TNF-induced the monocyte adhesion and the expression of inflammatory cytokines. These results suggest that PCD can exert to prevent endothelial dysfunction and improve cardiovascular health.

4741 The Acute Toxicity of Water Hemlock (Cicuta douglasii) in a Goat Model
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Water hemlock (Cicuta douglasii) is one of the most toxic plants in North America. It has been reported to cause numerous poisonings in livestock species, as well as humans. Humans eat water hemlock because they mistake the tuber of water hemlock to be wild carrots or wild parsnips. Clinical signs in humans poisoned by water hemlock include nausea, sweating, salivation, vomiting, severe seizures and death. The toxins in water hemlock are C17 polyacetylenes, with cicutoxin being the most common and a potent inhibitor of Na+/Ca2+ exchange due to their action as noncompetitive gamma aminobutyric acid (GABA) receptor antagonists in the central nervous system. Little information is known regarding the amount of tuber required for death to occur. Therefore, the objective of this study was to determine a lethal dose of water hemlock in a goat model. Tubers and above ground parts were dosed separately to goats via oral gavage of freeze-dried ground plant material. All goats dosed with tubers at 2.0, 0.5 and 0.25 g/kg developed severe seizures and were dead/euthanized by 30-120 min after dosing, with a clear dose-dependent response as to the severity and time of onset of the seizures. None of the goats dosed with tubers at 0.1 g/kg developed any clinical signs of poisoning. Similarily, none of the goats dosed with above ground parts of the plant at 2.0 g/kg showed any clinical signs. When goats were dosed with above ground parts at 10.0 g/kg, one goat developed seizures 2 hours post dosing and was euthanized. Two other goats showed minor signs of poisoning, while the fourth goat exhibited no adverse effects. The results from this study confirm anecdotal observations that the above ground parts of water hemlock are much less toxic than tubers. The results from this study indicate that cicutoxin is the most toxic of the C17 polyacetylenes, as the concentration of cicutoxin is much greater in the tubers compared to the above ground parts of the plant. This results also suggest that the NOEL for tubers in goats is 0.1 g/kg. This would correspond to 1-2 small fresh tubers.

4742 Modulation of B[a]P-Induced DNA Damage via Diallyl Trisulfide (Garlic Organosulfide) in Breast Epithelial (MCF-10A) Cells

Invasive and in situ breast cancer incidences are positively correlated with higher urbanization in women. Benzo[a]pyrene (B[a]P) is the most characterized polycyclic aromatic hydrocarbon, and well-known endocrine disrupter formed during incomplete combustion of organic materials. Reactive metabolites of B[a]P produce mutagenesis via DNA adducts and strand breaks leading to cancer. Long-term cumulative airborne B[a]P exposure is significantly associated with the risk of breast cancer. Our lab and others have previously reported that diallyl trisulfide (DATS), a garlic organosulfide compound (OSC), affects chemoprevention/ chemotherapy, apoptosis, and cell cycle arrest in DNA damaged normal breast epithelial, breast cancer cells, as well as other cancers. The objective of this study is to determine the effectiveness of DATS in preventing B[a]P induced initiation of cancer. In this study, we evaluated the ability of DATS, in blocking B[a]P induced ROS formation and DNA damage in breast epithelial (MCF-10A) cells. In order to assess the toxic effect of B[a]P and/or DATS on MCF-10A cells over time, we measured: the number of live cells using the WST-1 cell viability assay; cell proliferation via the Bromodeoxyuridine (BrdU) cell proliferation assay; aqueous peroxides (AOX) via the reactive oxygen species (ROS) formation assay; and more specifically, confirmed ROS formation using DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) dye for 4′,6-diamidino-2-phenylindole (DAPI) staining. In the assay using the 8-hydroxyguanosine (8-OHdG) DNA damage quantitation assay. Cell viability of MCF-10A cells exposed to B[a]P and/or DATS was measured over 24-72 hours. Our results indicate in a dose-and time-dependent manner, B[a]P (10 nM - 4 μM) significantly increased while DATS alone (12.5 - 200 μM) significantly decreased MCF-10A cell viability when compared to the control. To assess cell proliferation, BrdU, a pyrimidine analog incorporated in place of thymidine into newly synthesized DNA of proliferating cells, was measured over a 12-24 hour period. 1 μM B[a]P significantly increased cell proliferation, while DATS alone (40, 60, and 80 μM) had minor but significant increase on MCF-10A cell proliferation when compared
Delta-9-tetrahydrocannabinol (THC), one of the main psychoactive compounds in marijuana, has been shown to act as a potent anti-inflammatory agent. While the role of THC in attenuating T cell functions has been well characterized, the effects of THC on myeloid cells are less understood. In this study, we investigated the effects of THC on bone marrow cell proliferation and differentiation. Inasmuch as, macrophage colony-stimulating factor (M-CSF)-induced myeloid cell proliferation and differentiation is a major factor in diseases such as atherosclerosis, arthritis, and neuroinflammation, we studied the effects of THC on M-CSF-induced proliferation and differentiation of bone marrow (BM) progenitor cells, monocytes, and macrophages. The addition of M-CSF to bone marrow cell cultures resulted in the differentiation of large numbers of CD45+CD11b+F4/80+ macrophages and these cells were further increased in the presence of lipopolysaccharide (LPS). Importantly, we found that the addition of THC resulted in a significant and dose-dependent decrease in the differentiation of these macrophages. Interestingly, the BM cultures treated with M-CSF showed an increase in Ly6G+ granulocytic cells in the presence of THC. Further, RNASeq analysis of these cultures following treatment with THC showed significant difference in the gene expression profiles when compared to vehicle treated controls. Specifically, on day 2 following Thc treatment, there was a significant upregulation in mRNA transcripts involved in the retinoic acid synthesis pathway and heat shock proteins while on day 5, the THc group showed a granulocytic phenotype while the vehicle-treated group displayed a mature macrophage genotype. miRNAs targeting mRNA transcripts that were downregulated as seen from RNaseq data. FPA analysis listed mir-150-3p, mir-486-5p, mir-2137 targeting mRNA transcripts involved in myeloid cell differentiation and proliferation. Together, these data demonstrate that THC suppresses myeloid cell differentiation and proliferation through the regulation of miRNA expression. Supported by NIH grants R01ES030144, P01AT003961, P20GM103641, and R01AI23947, R01AI160896.

Poisoning by Paraquat, a widely used herbicide and well-known oxidative stress inducer due to cytotoxicity and reactive oxygen species (ROS), is a major medical problem. The very high case fatality of Paraquat is due to inherent toxicity and lack of effective treatments. Annona muricata, also known as soursop, graviola and guanabana, is a fruit tree member of the Annonaceae family with a long history of traditional medicinal use. A. muricata contains chemicals such as acetogenins, alkaloids, flavonoids, vitamins, phenolic and lipophilic compounds responsible for the biological activity, including anticancer, antconvulsant, anti-arithmetic, antiparasitic, antimarial, antilucifer, antidiarrheea, antioxidant, antihypertensive, hepatoprotective and antidiabetic. However, there are few studies that evaluate the biological properties of A. muricata previously described in vivo models. Caenorhabditis elegans is a free-living nematode with 40% of its genes with a human ortholog. It is used to evaluate possible toxic effects in vivo of contaminants and other substances with the possibility of being extrapolated to higher eukaryotes. The objective of this work was to evaluate the effect of aqueous extracts of A. muricata on protection against oxidative stress and lipid accumulation in Caenorhabditis elegans induced by Paraquat and sucrose, respectively, in addition to other endpoints such as reproduction and growth. For this, the wild strains of C. elegans (N2) and two mutagenic protoprotective effects Sod-3, Gpx-4, Ggt-4 were used, which were exposed for 72 hours to different concentrations of aqueous extracts of A. muricata and later they were exposed to Paraquat, to evaluate the protection against oxidative stress; or sucrose, to value the antiobesogenic activity. For this last test, quick red O staining (q-or) was used to color the lipid deposits inside the nematodes' cells. The results indicate that the aqueous extracts of A. muricata do not significantly alter the growth and reproduction of C. elegans, however, they manage to reduce the expression of oxidative stress regulator genes in concentrations higher than 100 mg/L. In addition, a slight decrease in red coloration of nematodes exposed to concentrations higher than 100 mg/L of A. muricata extracts, indicates a possible antiobesogenic activity. Future studies would clarify the metabolites involved in the antiobesogenic and antioxidant activities of A. muricata to promote treatments of natural origin.

Obesity induces the accumulation of ectopic lipids and desensitizes insulin signal- ing in skeletal muscle and adipose tissue, resulting in systemic insulin resistance and the occurrence of type 2 diabetes mellitus (DM2). The exact mechanisms are still unknown, but the evidence involves the generation of reactive oxygen species (ROS), oxidative stress and pro-inflammatory state. The use of functional foods such as dietary supplements, bioactive components, nutraceuticals and botanicals have become an alternative approach to prevent and alleviate the complications of hyperglycemia in patients with DM2, to mitigate inflammation and maintain an oxidant-antioxidant balance. This study used the biological model Caenorhabditis elegans to evaluate the anti-obesogenic effect of Bixa orellana seeds. Clean, dried and powdered Bixa orellana seeds were obtained from a local market and were used to promote treatments of obesity and diabetes. The results indicate that the aqueous extracts of B. orellana do not significantly alter the growth and reproduction of C. elegans, however, they manage to reduce the expression of oxidative stress regulator genes in concentrations higher than 100 mg/L. In addition, a slight decrease in red coloration of nematodes exposed to concentrations higher than 100 mg/L of A. muricata extracts, indicates a possible antiobesogenic activity. Future studies would clarify the metabolites involved in the antiobesogenic and antioxidant activities of A. muricata to promote treatments of natural origin.
As a helpful assistant, I don't have the ability to read images or natural text. If you have any questions or need assistance with a specific topic, feel free to ask!
Urolithin A is a polyphenol derived from the multi-step metabolism of dietary ellagitannins found in nuts, seeds, and other plants. Urolithin A is known to reduce oxidative stress, promote mitophagy, and improve arsenic-induced gut barrier dysfunction. Most adults harbor a microbiota capable of urolithin A production; however, the bacteria and enzymes that metabolize its dietary precursor (urolithin C) are unknown. We discovered a putative urolithin C metabolism operon found in three Enteroxoc scler species. Using liquid chromatography-mass spectrometry, we identified E. asperagelma, E. bolete, and E. citrinum as urolithin A producers in a panel of six validated Enteroroc scler species. RNA-sequencing of producers revealed a three-gene cluster that was highly upregulated in urolithin C-treated bacteria. The three-gene cluster was not present in the genomes of non-producers. Each gene of the three-gene cluster was expressed to a similar level and was found to be transcribed from the same mRNA due to an operon. Growth experiments showed that urolithin C, but not urolithin A, delayed growth in Enteroroc scler species, suggesting that metabolism is a detoxification mechanism. Future experiments using CRISPR-based recombining approaches will be used to probe the physiological role of urolithin A production in gut bacteria.

There have been various medications proposed to treat COVID-19 patients, one of which is Lianhua Qingwen (LHQW), a traditional Chinese herbal medicine. In this study, compounds were used to test the viability between gene and protein targets of LHQW and that of COVID-19, in order to analyze the potential mechanism of action of LHQW. Four compounds in LHQW, including quercetin, luteolin, wogonin, and kaempferol, showed the highest relevance to the gene targets of COVID-19. With gene correlation studies, several potential gene targets of LHQW were identified, including IL-6, IL-4, and TNF in the pathway of inflammatory cytokines. Additionally, protein target screening of quercetin and luteolin identified Thyroid hormone receptor alpha (THRA) as a strong candidate, which is associated with the immune response. As COVID-19 patients tend to have high expression of inflammatory cytokines, potential anti-inflammatory effects of LHQW are essential for the treatment of COVID-19 symptoms. Furthermore, through molecular docking, LHQW phytochemicals were shown to interact with cell proteins ACE2, ADAM17, TLR4, and Galectin-3, which potentially ameliorate COVID-19 symptoms. LHQW was also shown to directly inhibit COVID-19 NSP5 and S proteins, which suggests its treatment as COVID-specific. Through this research, it was concluded that Lianhua Qingwen is effective in treating COVID-19 symptoms, through a synergistic effect of enhancing anti-inflammation and targeting COVID-specific proteins.

Visceral Leishmaniasis (VL) affects millions of people in rural, tropical environments and with a 95% fatality rate VL is one of the top ten neglected tropical diseases. VL is caused by two protozoan parasites, *Leishmania donovani* (LD) and *Leishmania infantantium* (LI), which causes hepatosplenomegaly and when left untreated leads to death. The transmission of the *Leishmania* parasite occurs via the bite of an infected vector; the Phlebotomine sandfly. Leishmania parasites have a biphasic life cycle including an extracellular promastigote found in the sandfly and an intracellular amastigote that replicates within human macrophages. Although there has been progress in treatment over the past decade, the most common anti-leishmanial drug, liposomal amphotericin B (AMB), has poor selective toxicity, requires intravenous infusion and cold storage and is limited in countries where VL is endemic. Moreover, with no effective vaccine against Leishmania, there is an urgent need to identify more cost-effective, less cytotoxic compounds for VL patients. Our research focuses on the use of naturally occurring compounds to inhibit growth of the extracellular promastigote and intracellular amastigote as a novel, alternative therapy against VL. Sulforaphane (SFN), which is a compound found in broccoli and other cruciferous vegetables, inhibits promastigote growth of LI and LD with IC50 concentrations of 16 and 23 μM, respectively. We hypothesize that reactive oxygen species (ROS) generated by SFN may lead to mitochondrial membrane changes that promote promastigote cell death. Within macrophages, autophagy is inhibited early on during Leishmania infections to promote parasite survival. Conversely, the nuclear factor erythroid 2-related factor 2 (NRF2) pathway is activated early during infections and increases the expression of heme oxygenase (HO-1) to limit host inflammation. We found that 10 μM SFN leads to significantly less toxicity than AMB in human macrophages and significantly reduces amastigote formation. Our data indicate that SFN alters the expression and localization of the selective autophagy receptor (P62/SQSTM1) possibly to decrease parasite survival within autophagosomes. Our data indicate that SFN may be a promising lead compound to further investigate novel intracellular mechanisms of actions against amastigote proliferation in VL patients.

The world production of chemicals has increased from 1 million tons in 1930 to several hundred million tons today, which poses a potential risk to public health, especially in the case of their irrational use. According to estimates by the World Health Organization, worldwide more than 25% of non-communicable diseases, including the acute poisons, are determined by the action of chemical substances. The statistics data indicates that 2 million lives and 53 million disability-adjusted life years were lost due to these kinds of poisons. Also, the number of cases of acute unintentional poisoning ranges between 3.5-5.5 million cases, among these, 3 million cases are severe, leading to 20,000 deaths annually. The aim of this paper is the hygienic estimation of the risks for the public health associated with chemical substances in the Republic of Moldova. Data was used from a statistical report on state surveillance and control of public health in the district, as well as the data from a register of persons with acute non-professional exogenous poisonings of chemical etiology. Over the last few years, the Republic of Moldova is facing unfavorable conditions regarding the quality of the environment, which is becoming more and more precarious due to the excessive involvement of the human factor. Excessive chemical substances, thus conditions risks for human health, are most frequently generating acute chemical poisonings. A total of 13,082 chemical poisonings were registered during 2016-2021, including 243 deaths (1.8%). Among the children, 4,807 (36.7% of the total chemical poisonings) cases were registered, of which 22 children died (9% of total deaths). Drug poisoning is the most common type of chemical poisoning both nationally and worldwide. Thus, 4,963 persons were poisoned following the irrational administration of drugs in different dosages and forms. Alcohol abuse is the second leading cause of chemical poisonings. Excessive alcohol consumption affected 3,137 persons. The poisonings generated by the carbon monoxide and other gases and vapors, as well as other kinds of smoke and harmful substances, constituted 1,888 affected. In the same way, during the analyzed period, 655 poisonings were caused by pesticides, 58 cases of poisonings by nitrates, 760 poisonings caused by liquid chemicals into the human body (acidic, basic solutions, solvents), etc. Another concern of the public health and the community is acute poisoning with paint vapors, which is not the most common type of chemical poisoning both nationally and worldwide. It is important to raise public awareness of the importance of chemical safety, proper handling of chemicals to reduce and prevent their impact, including the prevention of poisoning among the population caused by free access, improper storage, repackaging of products in containers that can be confused with water, food, non-compliance with instructions on the label, and ignoring the use of personal protective equipment.

Bromodomain and extraterminal (BET) proteins have important impact on cellular proliferation and differentiation through epigenetic process to regulate gene transcription, and therefore BET inhibitors (BETI) represent promising treatment options for patients with cancer, including cancers associated with deregulated stem cell renewal and oncogenes such as MYC. However, in both clinical trials and rat 4-day in vivo studies, thrombocytopenia was observed with BETI treatment, although the adverse effect was reversible. In previous studies, the pathway of BETI affected genes that are regulated by GATA1 and associated with erythropoiesis and thrombopoiesis was investigated. The blood samples from the patients treated with BMS-986158 (clinical trial-NCT02419417) and bone marrow collected from rats treated with BMS-961658 for 4 days showed downregulation of NFE2 and P4F4 transcriptional expression. The current study is to further explore the mechanism and clinical pharmacology of BMS-986158 induced thrombocytopenia in the rat, which could bridge potential translational relationships between pre-clinical changes in the rat and clinical changes in patients treated with BETI's. Rat bone marrow cells were collected after 4-day treatment of BMS-986158 for megakaryocyte (MK) evaluation using Colony-Forming Unit assay for CFU-MK colonies and flow cytometry of MKs (CD61+ cells). Rat blood samples were also collected in the same study to evaluate the gene profile regulated by GATA1 and associated with thrombopoiesis. The results demonstrated that there was dose-dependent reduction of megakaryocytes in the bone marrow and platelets in the blood...
NAD+ homeostasis in SHY5Y cells mediated by increased and HT116 cells can induce decreased energy production and disruption of cellular viability and ATP production was found in SHY5Y cells; when co-exposed to CM exposed with CM from colorectal cancer lines for 24h, a significantly decreased cell viability, NAD+ and ATP production in both differentiation and undifferentiating SHY5Y cells. About 70% of colorectal cancer survivors who undergo chemotherapy often have complaints, such as fatigue, depression and cognitive decline. Even though the underlying mechanisms remain elusive, these complaints are highly related to neurotoxicity and disruption of the normal neuronal functions. Anti-neoplastic therapy can induce (cancer) cell senescence, with concomitant senescence-associated secreted secretory phenotype (SASP) known to secrete several inflammatory proteases and chemokines. These cytokines can by-pass the blood-brain-barrier and induce inflammation by activating CD38. CD38 uses NAD+ as its substrate and has been identified as the major regulator of cellular NAD+ levels. NAD+ decline was also reported to be related to ageing and neurodegenerative diseases. This study investigated if the metabolic pathway can lead to neurotoxicity and may provide a biological explanation towards neuropathy and fatigue syndrome observed in cancer survivors. Colorectal cancer lines HTC116 and HT29 were exposed with three senescence inducers hydrogen peroxide, 5-fluorouracil and dexamethasone for 24h and continued culturing with growth media for 7 days. The cellular senescent phenotype was validated by increased beta-galactosidase activity, and significantly increased mRNA levels of p16, p21, IL-6, TNF-a, IL-1β and MMP9. Conditioned medium (CM) from the senescent HT29 and HTC116 cells was used to treat human micro-glial cells (HMC3 cell line) and neuronal cells (SHY5Y cell line) in a medium (CM) from the senescent HT29 and HTC116 cells was used to treat human micro-glial cells (HMC3 cell line) and neuronal cells (SHY5Y cell line) in a mono- and a co-culture. Neuronal cells were also exposed to CM from microglial cells (CM-HMC3) treated with CM for 24h. Exposure of the human neuronal cells to CM-HMC3 for 2h and 24h, respectively, significantly decreased cell viability, NAD+ and ATP production in both differentiation and undifferentiating SHY5Y cells. Significantly higher IL-6, TNF-a, IL-1β, TGF-α levels, M1 phenotype marker CD40 and a biological explanation towards neuropathy and fatigue syndrome observed in cancer survivors. Colorectal cancer lines HTC116 and HT29 were exposed with three senescence inducers hydrogen peroxide, 5-fluorouracil and dexamethasone for 24h and continued culturing with growth media for 7 days. The cellular senescent phenotype was validated by increased beta-galactosidase activity, and significantly increased mRNA levels of p16, p21, IL-6, TNF-a, IL-1β and MMP9. Conditioned medium (CM) from the senescent HT29 and HTC116 cells was used to treat human micro-glial cells (HMC3 cell line) and neuronal cells (SHY5Y cell line) in a mono- and a co-culture. Neuronal cells were also exposed to CM from microglial cells (CM-HMC3) treated with CM for 24h. Exposure of the human neuronal cells to CM-HMC3 for 2h and 24h, respectively, significantly decreased cell viability, NAD+ and ATP production in both differentiation and undifferentiating SHY5Y cells. In a co-culture system, when exposed with CM from colorectal cancer lines for 24h, a significantly decreased cell viability and ATP production was found in SHY5Y cells; when exposed to CM with 50 nM CD38 inhibitor 78c, ATP production, mRNA levels on kynurenine pathway, inflammation markers, NAD+ status, and mitochondrial stress test by seahorse assay were preformed after exposure with CM in both differentiation and undifferentiating SHY5Y cells. Significantly higher IL-6, TNF-a, IL-1β, TGF-α levels, M1 phenotype marker CD40 mRNA levels, and increased CD38 activity was found in microglial cells treated with CM for 24h. Exposure of the human neuronal cells to CM-HMC3 for 2h and 24h, respectively, significantly decreased cell viability, NAD+ and ATP production in both differentiation and undifferentiating SHY5Y cells. In a co-culture system, when exposed with CM from colorectal cancer lines for 24h, a significantly decreased cell viability and ATP production was found in SHY5Y cells; when exposed to CM with 50 nM CD38 inhibitor 78c for 24h, the cell viability and ATP production was restored in SHY5Y cells. Our data suggest that SASP released by senescent HT29 and HTC116 cells can induce decreased energy production and disruption of cellular homeostasis in SHY5Y cells mediated by increased CD38 activity.

Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the United States. While hepatocytes are the primary cell type affected by APAP toxicity, it is now evident that resident and infiltrating immune cells are involved in the recovery phase after a moderate APAP overdose. Previous work has indicated that female mice have lower susceptibility to a moderate APAP overdose (300mg/kg) compared to male mice, but their response to a severe APAP overdose (600mg/kg) and the role of infiltrating immune cells at this dose has not been investigated. Our objective was to determine if there is a significant difference in liver injury recovery, and immune cell recruitment in female mice administered either 300 mg/kg (A300) or 600 mg/kg APAP (A600) during the APAP hepatotoxicity time course. We found that liver injury was significantly exacerbated with a substantial delay in recovery of liver glutathione in A600 compared to A300. Correspondingly, while there was no detectable mitochondrial JNK in the female A300 mice, there was sustained mitochondrial JNK in the A600 mice at 12h post APAP. Similar to previous reports in male mice, A600 female mice had delayed liver regeneration and a sustained induction of p21 indicative of cell cycle arrest. Interestingly, there was a delay in Ccr2+ macrophage recruitment to the liver at A600, which are critical for successful liver recovery after a moderate overdose. Hepatic neutrophil infiltration was much greater and sustained at A600, highlighting a potentially altered role for neutrophils at the higher dose. This was possibly due to the greater expression of the chemokine, Cxcl14 at A600, which has been implicated in neutrophil recruitment. Collectively, we find that though female mice may have lower susceptibility to liver injury after a moderate APAP overdose, the response after a severe overdose is similar to that of male mice. Interestingly, significant differences in the innate immune response are evident at severe overdose especially with regards to neutrophil infiltration, suggesting a potentially altered role for these immune cells after a severe APAP overdose. Future work targeting Cxcl14 and neutrophils at the A600 dose will be completed to examine how they impact liver injury and recovery during APAP hepatotoxicity.


4775 Delayed Liver Regeneration and Enhanced Neutrophil Recruitment after Severe Acetaminophen Overdose in Female Mice S. Smith, D. Umbaugh, B. Baum, A. Ramachandran, and H. Jaeschke. University of Kansas Medical Center, Kansas City, KS.


The pregnane X receptor (PXR) is a xenobiotic receptor with a well-established role in regulating drug metabolism and clearance. Recent research has shown that PXR is involved in other cellular processes, including cell proliferation, apoptosis, and energy homeostasis. It is important to identify compounds that may modulate PXR activity to prevent drug-drug interactions, distinguish chemicals which could potentially generate toxicity, and identify compounds for further development towards therapeutic use. The National Center for Advancing Translational Sciences (NCATS) Pharmacologically Active Chemical Toolbox (NPACT) library, which consists of 4,916 unique pharmacologically active synthetic and naturally derived small molecules, was screened in this study to identify PXR antagonists. One hundred and forty compounds were identified as potential PXR antagonists through a primary screen and confirmation study. Based on efficacy, potency, and novelty, 20 compounds, including gamma-secretase modulator 2 (GSM2) and fusidic acid, were selected to further study their effect on the cytochrome P450 (CYP) 3A4 mRNA expression and their PXR antagonistic ability using metabolically competent HepaRG cells. A pharmacological shift study was then performed to confirm the activity of the 20 selected compounds against PXR. Further investigation may provide useful information regarding possible drug-drug interactions involving these compounds, as well as the detection of potential therapeutic effects or toxic consequences.

4757 Nucleoside Transporters

4758 Identification of Chemicals That Inhibit Pregnane X Receptor Activity

4759 Identification of Chemicals That Inhibit Pregnane X Receptor Activity

4760 Developing an Inhibition Screening Protocol for Equilibrative Nucleoside Transporters

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Dopamine Receptor D2 (DRD2) TaqA Gene Polymorphism and Acute Risperidone-Induced Changes in Body Weight, Plasma Glucose, and Lipid Profile

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The transient blockade of the dopamine receptor D2 (DRD2) may explain the low incidence of extrapyramidal side effects (EPS) seen with atypical antipsychotics such as risperidone. There are indications that the interaction of this class of antipsychotics with the DRD2 is related to their metabolic side effects. We therefore examined the relationship between TaqA polymorphism of the DRD2 gene and risperidone-induced metabolic changes. We recruited 153 newly diagnosed patients with psychotic disorders (71 males and 82 females) from the Federal Neuropsychiatric Hospital, Yaba, Lagos, Nigeria. Body weight, fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TChol), low density lipoprotein cholesterol (LDLChol) and high-density lipoprotein cholesterol (HDLChol) were all determined at the end of the 6-week administration of risperidone (2 mg twice daily). DNA was also extracted from peripheral blood and genotyping was carried out using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The relationship between the mean changes in the metabolic indices and the DRD2 TaqA genotype were statistically determined. The frequencies of the A1A1, A1A2 and A2A2 were 0.229, 0.412 and 0.360 respectively. The population however was not in Hardy-Weinberg equilibrium (x2 = 4.023, p<0.01). The mean weight change and the mean changes in FBG, TG, TChol, LDLChol were significantly (p<0.05) higher among participants with A1A1 genotype followed by the heterozygous (A1A2) participants and lowest among those homozygous for the A2 allele. However, there was no significant difference in the mean change in HDLChol across all genotype groups. The DRD2 TaqA1 allele is associated with higher risperidone-induced weight gain and metabolic changes among Nigerians.

Determination of Efficacy, Cytotoxicity, and Mutagenicity of Bexarotene Analogs for Potential Cancer and Alzheimer’s Treatments


Bexarotene is a retinoid that was approved by the FDA in 1999 as a treatment for Cutaneous T-Cell Lymphoma (CTCL). It is typically designated for those patients who have not responded favorably to other therapies. Bexarotene has also shown to be effective in treating Alzheimer’s, lung cancer and breast cancer. It is relatively well tolerated in use but, unfortunately, it has also been shown to have numerous adverse effects. For our purposes, we were most concerned about the increase of lipids and other fats in the bloodstream. This causes the patient’s cholesterol levels to increase, sometimes to dangerously high levels. Because Bexarotene selectively binds with and activates the retinoid X receptors (RXRα, RXRβ, RXRγ) in the body, we believed that different analogs of Bexarotene can induce RXR with varying levels of efficacy. The purpose of this research was to test different Bexarotene analogs for efficacy, cytotoxicity, and mutagenicity. Each analog was prepared in a 1/100 dilution of Dimethylsulfoxide (DMSO). A sample of healthy Saccharomyces cerevisiae was then added and the new compound was centrifuged. The supernatant was removed and the remaining pellet was resuspended in a solution of YPD and potassium phosphate buffer. This new compound was then added to DMSO (negative control), ethidium bromide (positive control) and each analog. After incubating for 24 hours, various doses were plated on three different types of selective media to act as a readout for cytotoxicity and mutagenicity. The plates were allowed to incubate for another 72 hours and then the numbers of colonies present on each plate were counted. Efficacy was measured by utilizing a yeast 2-hybrid (Y2H) assay while cytotoxicity and mutagenicity were analyzed by a D7 yeast-based assay. Of the 26 analogs tested to date, 19 were found not to be toxic or mutagenic. Further analysis occurred in silico with the cytotoxicity and mutagenicity results to those generated via the EPA Comptox Chemicals Dashboard. The predicted cytotoxicity and mutagenicity results did not correlate with our results. Additional studies will be needed to further narrow down the promising analogs and determine which has the least effect on patient cholesterol levels.

Consequences of PBDE Exposure in Preterm Breast Milk: A Link to Abnormal miR-22-3p and miR-30b-5p Levels

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Preterm birth is a complex condition with potential links to environmental exposures such as polybrominated diphenyl ethers (PBDEs). PBDEs are persistent flame retardants that maintain high serum levels in North American women despite an EPA ban in 2004. Maternal exposure to PBDE-47 has been linked to endocrine disruption, impaired maternal-fetal tolerance, and incidence of preterm birth. Maternal serum levels of PBDE-47 are also correlated with infant exposure during pregnancy as PBDE-47 crosses the placenta. Prolonged exposure to PBDE-47 is possible through breastfeeding as the high lipophilicity of PBDE-47 enables sequestration in maternal fat tissue. Both PBDE-47 exposure and preterm status are associated with neurodevelopmental delay, the combined effect of both conditions creates a concerning threat to vulnerable preterm infant health. This presents a threefold insult to preterm infant neurodevelopment through preterm status, in utero exposure, and postnatal PBDE-47 exposure through breast milk. PBDEs migrate from breast milk to the mammary gland which may alter the breast milk profile. Breast milk naturally contains a dynamic population of extracellular vesicles (EVs) that transport miRNA to the developing infant. Breast milk EV cargo are highly stable with high bioavailability to the infant upon ingestion. While studies have shown that PBDE-47 may alter the levels of placental miRNA few have assessed effects on breast milk EV-bound miRNA. The present study compared breast milk of mothers who delivered preterm (<37 weeks gestation) and full term (at 40 weeks gestation) milk for PBDE-47 concentration and quantity of protective miRNA. Breast milk PBDE-47 concentrations were quantified using an indirect ELISA. This novel immunoassay employs a competitive binding reaction between magnetic beads conjugated to an anti-PBDE-47 antibody against an enzyme labelled PBDE-47 conjugate. Results showed a significantly higher level of PBDE-47 in breast milk of mothers who delivered preterm versus those who delivered full-term. To evaluate changes to the EV profile, EVs were isolated from preterm and term breast milk samples via differential ultracentrifugation and characterized by Zetaview nanoparticle tracking analysis. EVs were between 90-250 nm in diameter. Total EV-RNA was isolated and quantified via droplet digital PCR using the Taqman Advanced miRNA assay. Preterm milk was deficient in neuroprotective miR-22-3p and anti-inflammatory miR-30b-5p. These results indicate a potential triple insult to infant neurodevelopment from preterm status, direct PBDE-47 exposure during breast feeding, and reduction in protective breast milk miRNA. This study may have clinical implications such as use of the ELISA to facilitate faster, cheaper, more efficient screening for PBDE-47 in vulnerable populations. Increased awareness of the risks could further inform the decision to breastfeeding or supplement with donor milk. Additional studies are also warranted to address the social determinants of health in relation to early-life PBDE-47 exposure.

Identification and Quantitation of Microplastics Exposure in Human Placenta

M. García1, E. Castillo2, E. Barrozo3, M. Suter2, G. Herbert3, S. Lucas1, D. Scieska1, E. El Hayek4, J. Gonzalez-Estrella2, A. Konys2, K. Aagaard2, and M. Campen3. 1University of New Mexico, Albuquerque, NM; 2Baylor College of Medicine and Texas Children’s Hospital, Houston, TX; and 3Oklahoma State University, Stillwater, OK.

Global plastic use has exponentially increased over the past century, and microplastic (MP) pollution and ingestion are emerging environmental issues with uncertain impacts on human health. There is a significant knowledge gap in the quantitation of systemic uptake and distribution of ingested or inhaled microplastics (MPs), especially indoors. To better appreciate for potential health effects, MPs ultimately enter the ecosystem and become ingested or ingested by both animals and humans, potentially leading to toxicity and adverse health outcomes. This study focuses on the impacts of MPs and establishes how they accumulate within the placenta during gestation. We obtained frozen, uniformly collected and banked placenta samples from PerBank, Baylor College of Medicine and Texas Children’s Hospital’s perinatal biorepository from 81 subjects. Samples were analyzed for MP accumulation using complementary techniques to identify, isolate, and quantify MPs to aid in the early identification of MP-associated placental health outcomes associated. Placenta samples were weighed, and tissue digestion was performed with 3x the tissue volume using 10% KOH, incubated at 40°C for 72 hours with agitation, and ultracentrifuged for 4 hours at 30,000g. MP accumulation was determined by weight of the resulting pellet formed, normalized to compositional distributions determined by micro-FTIR spectroscopy. Further analysis included using confocal microscopy, which revealed significant translocation of MPs and fibers into the placenta. We also utilized micro-FITIR to establish the number of particles detected and the identification of particle types in the placenta samples. Results showed an average of 2.33 (±0.58) fibers, 16 (±1.73) fragments, and 80.33 (±3.21) particles per sample, and a mass concentration range of 0.14-4 mg/placenta. We identified the highest concentrations to be rayon, polystyrene, and polyethylene (delfin). Complete quantification of plastics concentration in placental tissues is further enabled by dissolution in nonpolar solvents and gas chromatographic assessment. This study shows further evidence that MPs are ubiquitous in human placental samples and provides important information related to size, shape, and composition of contaminant materials. Future research will explore the influence of MPs on gestational health.
Vaccines are paramount to global health as a preventative measure in the ever-evolving fight against infectious disease. Vaccines prime the adaptive immune system to elicit prompt, specific cell- and humoral-mediated responses. Successful vaccine development is contingent on thorough evaluation of both efficacy and safety, particularly in avoiding excessive, immediate immune activation effects after administration (reactogenicity). Highly reactogenic vaccines have induced potent inflammatory responses and severe adverse reactions, including multi-organ failure and death. Accurate prediction of vaccine reactogenicity and mitigation of severe adverse reactions is therefore highly desirable and a critical goal in vaccine safety. Combining pre-clinical in vitro experiments with modeling and simulation to assess the reactogenicity of clinical candidates and dosing regimens will accelerate the vaccine development process and provide additional confidence in clinical safety. Using the MIMIC® PTE module, peripheral blood mononuclear cells from 40 healthy donors were treated with 10 different vaccines of varying reactogenicity and the supernatants analyzed via flow cytometry and a multi-cytokine assay. In parallel, an extensive adverse event dataset for the vaccines of interest was assembled from publicly available clinical trial data. A novel generalizable scoring framework accounting for the frequency and severity of local and systemic adverse events was applied to the clinical data. Then a machine learning approach was employed to translate in vitro assay measurements to vaccine reactogenicity estimates. In human subjects, validation of model performance demonstrated the need to include vaccines representing extremes in the frequency of clinical adverse events and in vitro cytokine responses for optimal model robustness. The current framework was able to accurately recapitulate clinical reactogenicity scores for the original 10 considered treatments and a forward-validation study with a third dataset and out-of-distribution vaccine not used in model development. Evaluation of model performance demonstrated a significant role in the risk of developing adverse events. To our knowledge, this is the first regression-based translational model that predicts the relative likelihood of local and systemic adverse event risk for vaccines at the clinical stage with in vitro data. These results derived from the coupling of the MIMIC® PTE system and a translational model demonstrated the potential utility and application of non-animal alternate methods in vaccine safety.

4767a Evaluating Policy and Health Equity in Carbon Monoxide Poison Prevention

T. Dodd-Buter1, H. Li1, M. L. Beaman1, and M. DerMousessian1

1Azusa Pacific University, Azusa, CA; and 2California State University, San Bernardino, CA.

Despite national and regional efforts for poison prevention, carbon monoxide (CO) poisoning remains a significant public health issue. Evidence exists of CO exposures in vulnerable populations during power outages post-disasters. Immediate impact of education and policy is important due to increasing disaster occurrences. Thus, assessing circumstances of exposures and susceptibility is valuable for evaluating effectiveness, efficiency, and equity (the “3Es”). A logic model provides an illustration of an underlying conceptual framework and the logical connection within and between systems. Based on the assumption that poisoning is an essential consideration for emergency preparedness, a logic model was utilized to evaluate prevention policies and detection of CO exposure. The logic model demonstrated the potential utility and application of non-animal alternate methods in vaccine safety.

4765 Clinical Test Results Present a Solid Foundation for the FDA GRASE Determination of Bemotrizinol: A New US FDA OTC Sunscreen Active Ingredient

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Protection against sunburn, skin damage and the carcinogenic effects of ultraviolet light are the primary health benefits associated with UV filters used in topical sunscreen drug products. Since 1999 US consumers have been desperately waiting for new UV-filters and more modern sunscreens. Currently, a generally recognized as safe and effective (GRASE) determination is being sought by DSM for the inclusion of a new sunscreen active ingredient called Bemotrizinol (BEMT) 6% on FDA’s OTC Sunscreen Monograph. BEMT represents the first new sunscreen active ingredient to be evaluated under FDA’s revised GRASE and new Maximum Use (MUST) testing requirements. As part of these testing requirements, a clinical Phase 1 pilot and Phase 3 pivotal MUST Pharmacokinetic (PK) study and clinical dermal safety testing are required. Results from the completed pilot maximal usage PK study showed that BEMT concentrations rarely exceeded FDA’s indicated threshold (0.5 ng/mL) in plasma and that there was no evidence for BEMT accumulation or steady-state concentrations above threshold. More recent studies evaluating 6% BEMT dermal safety in market image sunscreen formulations and in a dispersion of petrolatum as the only vehicle showed no evidence of dermal or cumulative irritation, sensitization, phototoxicity or photoallergenicity. Furthermore, the analysis of the results from the first recent open-label, randomized, single-blind, 3-arm (MUST) testing conducted at the FDA OTC testing laboratory demonstrated the need to include vaccines representing extremes in the frequency of clinical adverse events and in vitro cytokine responses for optimal model robustness.

4766 Thinking Zinc: An Intervention to Address Environmental Metal Exposure on the Navajo Nation


1University of New Mexico, Albuquerque, NM; and 2Southwest Research and Information Center, Albuquerque, NM.

More than 500 abandoned uranium mines (AUMs) are located on the Navajo Nation and previous studies find an increased risk for chronic diseases related to AUM waste exposure. Experimental models demonstrate that metals such as uranium and arsenic disrupt certain zinc finger motifs and affect protein function: supplement zinc confers protection against the metal effects. Based on this evidence, a community and academic partnership developed an intervention trial called Thinking Zinc. This trial tests the hypothesis that dietary zinc supplementation at the recommended daily allowance will modulate biomarkers of oxidative stress, inflammation and immune dysregulation, and decrease DNA damage in a metal-exposed population. Extensive community engagement and collaboration was utilized to evaluate prevention policies and detection of CO exposure. The logic model was assembled from publicly available clinical trial data. A novel generalizable data. These results derived from the coupling of the MIMIC® PTE system and a translational model demonstrate the potential utility and application of non-animal alternate methods in vaccine safety.

4767 A Predictive Model of Vaccine Reactogenicity Using Data from an In Vitro Human Immune Immunity Assay System

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Vaccines are paramount to global health as a preventative measure in the ever-evolving fight against infectious disease. Vaccines prime the adaptive immune system to elicit prompt, specific cell- and humoral-mediated responses. Successful vaccine development is contingent on thorough evaluation of both efficacy and safety, particularly in avoiding excessive, immediate immune activation effects after administration (reactogenicity). Highly reactogenic vaccines have induced potent inflammatory responses and severe adverse reactions, including multi-organ failure and death. Accurate prediction of vaccine reactogenicity and mitigation of severe adverse reactions is therefore highly desirable and a critical goal in vaccine safety. Combining pre-clinical in vitro experiments with modeling and simulation to assess the reactogenicity of clinical candidates and dosing regimens will accelerate the vaccine development process and provide additional confidence in clinical safety. Using the MIMIC® PTE module, peripheral blood mononuclear cells from 40 healthy donors were treated with 10 different vaccines of varying reactogenicity and the supernatants analyzed via flow cytometry and a multi-cytokine assay. In parallel, an extensive adverse event dataset for the vaccines of interest was assembled from publicly available clinical trial data. A novel generalizable scoring framework accounting for the frequency and severity of local and systemic adverse events was applied to the clinical data. Then a machine learning approach was employed to translate in vitro assay measurements to vaccine reactogenicity estimates. In human subjects, validation of model performance demonstrated the need to include vaccines representing extremes in the frequency of clinical adverse events and in vitro cytokine responses for optimal model robustness. The current framework was able to accurately recapitulate clinical reactogenicity scores for the original 10 considered treatments and a forward-validation study with a third dataset and out-of-distribution vaccine not used in model development. Evaluation of the translational model components suggested the relative levels of IL1B, IL6, IL10, and CCL4 play a significant role in the risk of developing adverse events. To our knowledge, this is the first regression-based translational model that predicts the relative likelihood of local and systemic adverse event risk for vaccines at the clinical stage with in vitro data. These results derived from the coupling of the MIMIC® PTE system and a translational model demonstrate the potential utility and application of non-animal alternate methods in vaccine safety.

4767a Evaluating Policy and Health Equity in Carbon Monoxide Poison Prevention

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The numerals after the author names refer to the abstract number(s). The asterisk after the abstract number indicates that the author is the presenter.

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