NCSOT Annual Meeting Booklet
Mountains to Coast: The State of Toxicity in North Carolina
September 14, 2023

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Welcome Message from NCSOT President, Anika Dzierlenga,

The NCSOT Executive Committee is pleased to welcome you to the 2023 Annual Meeting!

We are thrilled to be joined by Dr. Dori Germolec, SOT President, to help kick off the meeting with a Society update. We also welcome plenary speakers Dr. John Bang (NCCU), Dr. Jamie DeWitt (ECU), and Dr. Frannie Nilsen (NC DEQ) to present about and discuss some of the most critical toxicology concerns facing our state. The afternoon will feature poster and platform presentations from about 70 trainees and researchers, representing a dozen institutions across North Carolina.

One of our biggest pieces of feedback last year was that NCSOT members love to network, especially at the annual meeting. With that in mind, we do hope you join us at The Glass Jug on Highway 55 directly following the program for an informal Happy Hour. All are welcome!

We would like to sincerely thank the Society of Toxicology for their assistance with meeting registration, our sponsors for this year, and the additional exhibitors and panelists in the undergraduate program for lending their time to inform us about local opportunities for career development. Finally, we would like to thank our gracious hosts at RTI for sharing their beautiful campus and facilities. Our Program allows some time to spread out and appreciate some of the natural features in between sessions.

Thank you all for attending this meeting, and for contributing to our wonderful community of toxicologists. Hope you enjoy!

Sincerely,
Anika

NCSOT Executive Committee 2023-2024
### NCSOT September 14, 2023 – RTI International

**Meeting will begin in the Holden Building**

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<td>Check-in Starts</td>
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<tr>
<td>Welcome and Business Meeting</td>
<td>9:00-9:10am</td>
<td>NCSOT Executive Committee</td>
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<tr>
<td>Welcome to RTI</td>
<td>9:10-9:20am</td>
<td>Dr. Keith Levine</td>
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<td>Analytical Sciences Group, RTI</td>
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<tr>
<td>Update from the SOT President</td>
<td>9:20-9:30am</td>
<td>Dr. Dori Germolec, NIEHS</td>
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**Mountains to Coast: The State of Toxicity in North Carolina**

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<tr>
<td>The Effects of Land Use Practices on PM Exposure in Minority Communities near Durham in North Carolina</td>
<td>9:30-10:00am</td>
<td>Dr. John Bang</td>
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<tr>
<td></td>
<td></td>
<td>North Carolina Central University</td>
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<tr>
<td>The Risk is Real: Immunotoxicological Hazards of PFAS Matter</td>
<td>10:00-10:30am</td>
<td>Dr. Jamie DeWitt</td>
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<td></td>
<td></td>
<td>East Carolina University</td>
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<tr>
<td><strong>BREAK</strong></td>
<td>15 minutes</td>
<td>Refreshments, snacks</td>
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<tr>
<td><strong>PFAS in North Carolina’s Natural Environment</strong></td>
<td>10:45-11:15am</td>
<td>Dr. Frannie Nilsen</td>
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<td></td>
<td>NC Dept of Environmental Quality</td>
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<tr>
<td>Panel Discussion</td>
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**Walk to Haynes Building for posters and exhibitors**

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<td>Lunch, Poster Session, Exhibitor Viewing</td>
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<td>Poster Session #1</td>
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**Undergraduate Program**

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<td>*Exhibitor Introductions</td>
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<tr>
<td>Undergraduate Poster Session &amp; Viewing</td>
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**Walk back to Holden Building for the rest of the afternoon**

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<td>Graduate Student Competition</td>
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<td>Morgan Nalesnik</td>
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<td>President’s Award for Research Competition</td>
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<td>Elise Hickman</td>
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<td>Gilberto Padilla Mercado</td>
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<td>Timothy Smyth</td>
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<tr>
<td><strong>BREAK</strong></td>
<td>20 minutes</td>
<td>Refreshments</td>
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<td><strong>Awards</strong></td>
<td>4:05-4:20pm</td>
<td>NCSOT Executive Committee</td>
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<tr>
<td>Closing Remarks</td>
<td>4:20-4:30pm</td>
<td>NCSOT Executive Committee</td>
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<td><strong>Informal Networking Happy Hour</strong></td>
<td>4:30-6:30pm</td>
<td>The Glass Jug</td>
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*denotes location occurs in Breakout Room*
Guest Speakers:

The Effects of Land Use Practices on PM Exposure in Minority Communities near Durham in North Carolina
Dr. John Bang
Professor, Environmental, Earth and Geospatial Sciences, North Carolina Central University
Dr. John Bang is a professor at North Carolina Central University. He studied Biochemistry and Medicine at the University of Illinois with a focus on respiratory illness and brain pathology with one extra year of tropical medicine in Central America. To further understand environmental impacts on human health, he later did a graduate study in environmental engineering at the University of Texas at El Paso with a focus on the behavioral characterization of nanoparticles. His primary research has been focused on understanding the interaction mechanisms between the cells and the environmental factors at the molecular level. PM exposure assessment, toxicological and pathological consequences after exposure, and potential remediation or treatment method development have been a major focus of his research. In particular, he has been following the ambient PM data near the RTP region and its vicinity to further investigate the health disparity issues in minority communities. Currently, Dr. Bang is working on the effects of PM and engineered nanoparticles on neurodegenerative diseases including Alzheimer’s Disease which affect minority populations at least three times more than non-minority groups.

The Risk is Real: Immunotoxicological Hazards of PFAS Matter
Dr. Jamie DeWitt
Professor, Pharmacology and Toxicology, Brody School of Medicine, East Carolina University
Jamie DeWitt is a Professor in the Department of Pharmacology & Toxicology of the Brody School of Medicine at East Carolina University (ECU). She has BS degrees in Environmental Science and Biology from Michigan State University and PhD degrees in Environmental Science and Neural Science from Indiana University-Bloomington. She completed postdoctoral training in ecotoxicology at Indiana University-Bloomington and in immunotoxicology at the US Environmental Protection Agency (US EPA) in partnership with the University of North Carolina at Chapel Hill. She is a diplomate of the American Board of Toxicology and is active within the Society of Toxicology and the Society of Environmental Toxicology and Chemistry. Her laboratory’s research program focuses on functional effects of environmental chemicals on the immune system and its interactions with the nervous system during development and adulthood. A major emphasis of Dr. DeWitt’s current research program is on effects of exposure to per- and polyfluoroalkyl substances (PFAS). She has published numerous primary research articles, commentaries, and review articles, two book chapters, and edited a book on PFAS toxicity and has served as an external reviewer of PFAS documents for US EPA, the US National Toxicology Program, and the US Agency for Toxic Substances and Disease Registry. She also has served and does serve as a plaintiff’s expert witness in cases involving PFAS.

PFAS in North Carolina’s Natural Environment
Dr. Frannie Nilsen
Environmental Toxicologist, Secretary’s Office, North Carolina Department of Environmental Quality
Dr. Frannie Nilsen is the lead Environmental Toxicologist with the North Carolina Department of Environmental Quality (NC DEQ). Dr. Nilsen earned a Bachelor of Science Degree and Master of Science degree, as well as a certificate in Environmental Policy, all from Hawaii Pacific University. She earned her PhD in Marine Biomedicine and Environmental Toxicology from the Medical University of South Carolina while working at the National Institute of Standards and Technology, and closely with the Florida Fish and Wildlife Conservation Commission, and Integrated Missions Support Systems at NASA’s Kennedy Space Center. Currently in her role at NC DEQ,
she is involved with a variety of projects related to PFAS contamination and exposure including military, industrial, and drinking water facilities. She is the technical liaison to the NC Secretaries’ Science Advisory Board and is responsible for coordinating the PFAS review activities to support reference dose development for PFAS specific to industrial facilities in NC that are not being reviewed by the US EPA. Dr. Nilsen is the lead investigator on a large-scale fish collection study in the Cape Fear River that will support the derivation of regulatory values to be used in concert with the work of the Science Advisory Board. She was recently appointed by the Governor of NC to the NC Advisory Committee on Cancer Coordination and Control to serve as an expert on PFAS toxicity. She previously worked at the US EPA as a Toxicologist and Postdoctoral Fellow combining environmental exposures and genetic predisposition to determine children’s health

Exhibitors:
- Research Triangle Institute (RTI) International
- US Environmental Protection Agency (US EPA), Office of Research and Development
- National Institute of Environmental Health Sciences (NIEHS), Office of Science Education and Diversity

Graduate Student Platform Award Speakers:
Aerosolized Vitamin D Attenuates Ozone-Induced Inflammation and Immune Dysfunction
Kevin Schichlein
UNC-Chapel Hill
Ozone is a common respiratory irritant formed through photochemical reactions of nitrogen oxides (NOx) and volatile organic compounds (VOC). Ozone exposure can cause oxidative stress, inflammation, and epithelial injury, which in turn can lead to increased risk of infection, exacerbation or pathogenesis of lung disease, and a number of systemic health effects. As worldwide ambient ozone levels continue to increase, there is a need for prophylactic and therapeutic interventions to mitigate the harmful health effects of ozone. Vitamin D (VitD) has well-established antimicrobial and anti-inflammatory properties and limited systemic effects when inhaled, making it an ideal candidate. Here, we tested a novel intervention using inhaled VitD aerosols to attenuate ozone-induced pathological responses in primary human bronchial epithelial cells. We exposed cells at an air-liquid-interface (n=3-6) to ozone (0.4 ppm for 4 hours) and administered VitD via different routes (apical, basolateral), forms (cholecalciferol, calcitriol), and timings (30-minute or 24-hour pre-treatment, 30-minute post-treatment). Apical treatment was delivered via aerosol produced by a single-well in vitro exposure system with a vibrating mesh nebulizer while basolateral treatment was added in the media. RNA-sequencing and an MSD 30-plex Human Cytokine assay were then conducted. We found that apical 30-minute post-treatment and 24-hour pretreatment with a cholecalciferol aerosol reduced the ozone-induced secretion of the pro-inflammatory cytokine, IL-8. Gene expression of cathelicidin, an antimicrobial peptide, was reduced by ozone and subsequently rescued with 30-minute apical calcitriol aerosol pretreatment. This indicates VitD inhalation may attenuate ozone-induced inflammation and minimize disruption of antimicrobial defenses. Our data suggest inhaled VitD should be further investigated as a prophylactic/therapeutic in ozone exposure. In the future, we aim to identify mechanisms by which inhaled VitD attenuates ozone-induced inflammation and explore the utility of VitD inhalation as a countermeasure for additional inhalation exposures including military burn pit emissions and wildland fire smoke.
The Impact of Sex on Exposure Response to Wood Smoke Particles in the Human Respiratory Proteome

Morgan Nalesnik
UNC-Chapel Hill

Exposure to air pollution causes adverse health effects and is estimated to be responsible for over 7 million premature deaths per year. Humans are exposed to a wide variety of air pollutants, three of particular interest include: wood smoke particles (WSP), ozone (O3), and endotoxin (LPS). Controlled human exposure studies revealed that subjects exposed to O3, WSP, or LPS can be categorized into two groups: Responders and Non-Responders (R and NR). The increase, or lack thereof, in sputum percent neutrophils (PMN) between pre- and post-exposure evaluations of each subject defines inflammatory R and NR status, respectively. While the observed neutrophil inflammatory phenotype is consistent across three known inhaled exposures (WSP, O3, LPS), there is a gap in knowledge around other potential biomarkers of R/NR status, as well as the modifiers and mechanisms that underlie the R/NR status. To study mechanistic pathways underlying the R/NR response, it is critical to utilize unbiased analytical approaches to evaluate the pre- and post-exposure respiratory response. To do this, we collected induced sputum from 27 human subjects pre- and post-exposure to 500 ug/m3 WSP for two hours. Using proteomic analysis to study these sputum samples, we found that 1854 unique proteins were differentially expressed between pre- and post-WSP exposure. A total of 350 proteins were significantly (p<0.05) altered and Ingenuity Pathway Analysis IPA predicted inhibition of upstream regulators that affected pathways of cell movement of neutrophils and leukocyte activation. When the subjects were further stratified by R and NR status, 72 and 231 proteins were down-regulated by R and NR subjects respectively. These initial results suggest that human exposure to WSP induces differential protein expression in the central airways that affect pathways of immune cell movement and immune cell activation and that WSP exposure affects R/NR subjects differently.

The Adsorption of House Dust Mite Allergens to Carbon Nanotubes Intensify Allergic Lung Disease in Mice

Ryan Bartone
NC State University

Inhaled particulate air pollution is a major factor that exacerbates allergic asthma in humans. With the increase in nanoscience, nanomaterial, and nanotechnology research in North Carolina, exposure to nanoparticles (NPs) are more prevalent. We previously reported that the co-exposure of mice to house dust mite (HDM) allergens and multi-walled carbon nanotubes (MWCNTs) intensified lung inflammation in mice in vivo and amplified cytokine production by alveolar macrophages in vitro. NPs, including MWCNTs, avidly bind biomolecules to form protein coronas that can modify NP immunotoxicity. Therefore, we hypothesized that exacerbation of innate and adaptive responses from co-exposures of MWCNTs and HDM in mice in vivo is due to the formation of an allergen corona. Coronas were prepared in a cell-free system by co-incubating MWCNTs (NC7000, Nanocyl Inc.) with HDM extract (Greer Laboratories, Inc.), followed by sequential rinsing and centrifugation of the MWCNTs to remove free HDM proteins. Male and female C57BL/6J mice were exposed to a vehicle solution (PBS), pristine MWCNTs, or corona-MWCNTs 6 times over a period of 21 days via oropharyngeal aspiration. Approximately 1% of proteins in HDM extract adsorbed to the MWCNTs to form a stable allergen corona and these HDM proteins included allergens associated with asthma pathogenesis such as Der p 1 and 2. Corona-MWCNTs exacerbated transcription of pro-inflammatory and pro-fibrotic mediator genes including IL-6, CCL11, Arg1, and Col1A1 to a significantly greater extent compared to pristine MWCNTs. Bronchoalveolar lavage fluid demonstrated increased populations of eosinophils, neutrophils, and lymphocytes in mice exposed to the corona-MWCNTs as compared to pristine MWCNTs. Conclusions: Exacerbation of HDM extract-induced allergic lung inflammation in mice by MWCNTs is due, at least in part, to the formations of HDM-allergen coronas. Overall, this study provides new insight to the mechanisms through which inhaled NPs exacerbate allergic asthma.
President’s Award for Research Competition (PARC) Postdoctoral Award

Speakers:
Chemical and Non-Chemical Stressors in a Perinatal Cohort through Wristband and Self Report Data: Novel Links between Increased Chemical Burden and Economic and Racial Stress

Elise Hickman
UNC-Chapel Hill

Bridging the gap between environmental exposure assessments and social biology is imperative in understanding a person’s complete landscape of external stress. This is particularly critical for socially vulnerable populations, such as women who have recently given birth, young children, and historically marginalized communities. Although both external chemical insults and stress factors have been individually assessed and associated with adverse health outcomes, the combined exposure to these stressors has not been thoroughly investigated, despite evidence of co-exposure having interactive effects. This study set out to address this research gap by integrating proximal environmental chemical exposure data collected using silicone wristbands and self-report social stressor data within the Brain and Early Experience (BEE) perinatal cohort, with the goal of understanding co-exposure to environmental chemicals and social stress. Silicone wristbands were worn for one week by mothers throughout central North Carolina who were 6 months postpartum (n = 97). Exposure to 110 different environmental chemicals across eight chemical classes was quantified on silicone wristbands using gas chromatography mass spectrometry. Social stress was evaluated using eight established self-report questionnaires (e.g., Brief Symptom Inventory, Perceived Stress Scale). The chemical exposure landscape and associations between chemical exposure, demographic characteristics, and social stress were characterized through individual variable analyses, cluster and data reduction, and compiled scoring approaches to comprehensively evaluate chemical and social burdens. We found that chemical exposures contain co-occurring patterns largely based on chemical class, with phthalates representing the chemical class with highest exposure and polychlorinated biphenyls the lowest. Integrating social stressor profiling with chemical exposure data identified one particularly vulnerable subset of participants, with high chemical exposure burden coinciding with high experiences of racism and economic stress. Chemicals contributing to this high burden included organophosphates such as isodecyl diphenyl phosphate and triphenyl phosphate, both commonly used as plasticizers and flame retardants. Current efforts are focused on understanding sources of differential chemical exposure between groups, such as the built environment. This is the first study to integrate chemical exposure via wristband and social stressor data in a perinatal cohort. Our data demonstrate co-occurring chemical and social stress, warranting further investigation to better understand how these combined stressors may contribute to disparities in maternal and child health.

Informatics for Toxicokinetics with invivoPKfit and CvTdb

Gilberto Padilla Mercado
ORISE US EPA

Chemical concentration versus time (CvT) data is commonly used to characterize the toxicokinetic (TK) properties of chemical substances. However, obtaining in vivo CvT data requires scientific labor and use of animal subjects. To reduce or eliminate new animal testing, it remains important to maximize reuse of existing CvT data to formulate and evaluate computational TK models. As part of this effort, the Center for Computational Toxicology and Exposure at the US Environmental Protection Agency (US EPA) developed a public repository of toxicokinetic data named the Concentration vs Time Database (CvTdb). CvTdb currently contains manually curated CvT data for hundreds of chemicals. CvTdb continuously updates and expands as more data are extracted by our team of curators and collaborators, from the literature and publicly available resources. Data curation is an important step for efficient and effective testing of computational TK models and to identify trends in CvT data. One example of this is assessing replicability: approximately 89% of replicate observations in CvTdb data from experiments with oral or intravenous routes of administration are within two-fold of the mean concentration. Using CvTdb, we take an informatics approach to characterizing chemical TK. We developed 7
invivoPKfit, an R package to automate the estimation of TK parameters (for 1- and 2-compartment models) from CvT data for hundreds of chemicals, integrating data across all studies available for each chemical, and quantifying uncertainty and variability in TK parameter estimates. invivoPKfit is intended to be sufficiently flexible to allow researchers to derive TK statistics from new chemical data and customized TK models. The development of both CvTdb and invivoPKfit 1) facilitates the reuse of existing CvT data, 2) provides a transparent and auditable workflow for standardized estimation of TK statistics, and 3) informs quantitative evaluation of new approach methodologies, such as TK models used for in vivo-in vitro extrapolation.

**Human Monocyte-Derived Macrophages Demonstrate Polarization-State Dependent Responses to Urban Particulate Matter**

Timothy Smyth  
**UNC-Chapel Hill**

Particulate matter (PM) exposure has been linked to increased morbidity and mortality in human respiratory infections. Macrophages, a major innate immune cell, exist on a spectrum between the pro-inflammatory M1 and pro-resolution M2 polarization state and demonstrate elevated pro-inflammatory cytokine expression, reduced phagocytosis, and reduced pathogen killing following PM exposure. We hypothesized that exposure to PM collected from ambient air in an urban environment would induce polarization-state dependent effects on macrophages and that M2 macrophages would demonstrate a previously undescribed phenotype following reprogramming using M1-polarizing stimuli. Monocyte derived macrophages were generated from the blood of healthy human donors (in compliance with the UNC IRB) and left unpolarized (M0) or polarized into pro-resolution M2 cells (20 ng/mL IL-4) for 24 hours. Following polarization, macrophages were exposed to M1-polarizing conditions (20 ng/mL LPS and IFN-γ) with or without 25 μg/cm² PM for 24 hours. Following these exposures, macrophages were assessed for phagocytic ability, pro-inflammatory cytokine secretion, and gene expression profiles. Surprisingly, only M1-polarizing conditions induced changes in phagocytic function while PM had a limited effect on macrophages of any polarization state. In contrast, cytokine secretion profiles varied widely between polarization states with and without PM co-exposure, with reprogrammed M2 macrophages demonstrated the greatest cytokine secretion both at baseline and following co-exposure to PM. Finally, major differences in global gene expression patterns between polarization states with and without PM co-exposure were detected through RNA-seq. In particular, reprogrammed M2 macrophages demonstrated reduced expression of regulatory pathways of inflammation, with increased activation of pro-inflammatory pathways following PM co-exposure. Together, these data suggest macrophages demonstrate polarization-state dependent responses to airborne particulate matter. These data further suggest that reprogrammed M2 macrophages transition to a unique, highly inflammatory population of macrophages which may contribute to the poor health outcomes observed in humans following PM exposure during respiratory infections.
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Ajayi, Tolulope
NC A&T
Investigating the Anti-Proliferative Effects of *Cnidoscolus aconitifolius* Leaf Extracts on Breast Cancer

*Cnidoscolus aconitifolius* (Pax) I.M. Johnst (Euphorbiaceae), is used in the traditional medical practice of Africa, Asia, and some parts of North America for the treatment of several diseases. Its anticancer uses have recently been reported. The present study evaluated the antiproliferative and cytotoxic activity of the extract of the leaves of *C. aconitifolius* against the 1806 breast cancer cell line. The dried leaves of *C. aconitifolius* were macerated in a 70:30 mixture of ethanol-water. The evaluation of the antiproliferative and cytotoxic activity of the extract on the 1806 breast cancer cell lines was performed using the MTT assay. The extract of the leaves of *C. aconitifolius* significantly inhibited the proliferation of the 1806 breast cancer cell line at 250 μg/mL concentration without apparent cell damage. However, at concentrations above 250 μg/mL, the extracts showed weak cytotoxicity. This study concluded that *C. aconitifolius* leaf extract indeed showed a significant antiproliferative effect against 1806 breast cancer cell lines. However, the extract appeared to cause some cell damage at a higher concentration.

Alewel, Devin
ORISE & US EPA
Differential Transcriptomic Alterations in Nasal Versus Lung Tissue of Acrolein-Exposed Rats

Acrolein, a volatile aldehyde frequently encountered through wildfire and cigarette smoke, is associated with COPD and asthma. Toxicology studies have parsed together acrolein’s molecular mechanisms of toxicity; however, few studies have provided an unbiased global transcriptional characterization of the sex-specific airway response. Thus, a comparative assessment of upper versus lower airway outcomes is needed for improved respiratory toxicity evaluation. Due to the likelihood for nasal but not lung deposition based upon chemical hydrophilicity and rodent breathing mode, we performed a comparative assessment of upper versus lower airway injury. Here, male and female Wistar-Kyoto rats were exposed nose-only to air (0ppm) or acrolein (~3.16ppm) for 4hr. In the first cohort of rats, male and female nasal and bronchoalveolar lavage injury/inflammation markers were analyzed, and in a second cohort of males, nasal epithelial and left lung tissues underwent mRNA sequencing. We identified sex- and site- specific injury/inflammation and cytokine responses, where only male nasal lavage indicated neutrophilic end eosinophilic inflammation, as well as increased IL-6, TNF-α, IL-1β, and IFN-γ. Comparative mRNA assessment revealed acrolein-induced pro-inflammatory signaling, organelle stress, and changes in glucocorticoid signaling that was unique to the nose (452-DEGs). In the lung (95-DEGs), small acrolein responses indicated xenobiotic metabolism. In male rats, expression patterns of glutathione (GSH) of aldo-keto reductase (AKR) aldehyde metabolizing enzymes indicated nasal acrolein GSH conjugation and lung AKR activity, suggesting site-specific interactions of inhaled acrolein metabolism. These results demonstrate site- and region-specific differences in acrolein airway penetration, respiratory aldehyde metabolism, and oxidative stress, which might have implications for upper airway and systemic disease susceptibility associated with acrolein inhalation. Further investigations using rodents to explore health implications of acrolein inhalation in humans should consider species and sex differences in airway deposition and metabolism. This abstract does not reflect US EPA policy.

Arechavala, Rebecca
NIEHS
Evaluating the use of Physiological Monitoring in Rodent Inhalation Toxicity Studies

Clinical monitoring of physiological measures, such as heart rate, blood pressure and body temperature are regular endpoints in human health care appointments. In traditional toxicity rodent studies conducted for hazard assessment, physiological measures have not been. Instead, terminal endpoints such as body weight, organ weights, and clinical and anatomic pathology endpoints are most often utilized. Furthermore, inhalation toxicity studies include inherently stressful exposure paradigms requiring animals to be housed in alternative housing chambers. Little is known regarding how inhalation exposure housing conditions impact toxicity outcomes, but we hypothesize that physiological measurements can provide insight to understanding this research question.
We are evaluating if these health markers can provide earlier or additional information on general stress or adverse health effects as well as provide insight on functional changes not otherwise captured in traditional toxicological endpoints. In this study, male and female Sprague Dawley rats are surgically implanted with radio-telemetry devices which collected data on intra-arterial blood pressure, electrocardiogram (ECG), heart rate variability, activity, and body temperature. After a recovery period, animals are housed in whole-body inhalation chambers for exposure to the known respiratory toxicant, 2-ethyltoluene (2-ET) for 6 hours per day, 5 days per week, for 4 weeks at concentrations of 0 ppm, 1.5 ppm, or 150 ppm 2-ET. The selected doses represent a high dose to induce known respiratory toxicity, and a low dose not expected to induce nasal lesions. In addition to traditional terminal toxicity endpoints, physiological endpoints collected daily will be assessed to provide information on the function of the cardiac system, autonomic nervous systems, and systemic markers of stress and health. As this study is on-going, our results will include statistical analysis of changes to physiological measures to validate the use of physiological monitoring as a tool for better understanding adverse health outcomes in rodent toxicity studies.

Avenbuan, Oyemwenosa
UNC-CH
Manganese Alters Trophoblast Invasion and Migration in SVneo Cells In Vitro: Links to Preeclampsia

Preeclampsia (PE) is a pregnancy-related disorder that impacts up to 8% of pregnant women worldwide. Clinically, PE is diagnosed through emergence of de novo hypertension and proteinuria development during the second half of pregnancy. This disorder impacts multiple maternal organs and end-organ damage which include (but are not limited to) the cardiovascular, renal, and hepatic systems. Importantly, PE can be life-threatening to both the mother and fetus. The suspected etiology of PE is related to superficial invasion of trophoblast cells into the myometrium, which supporting evidence shows is related to exposure to toxic agents, including metals. While research has demonstrated the damaging effects of exposure to toxic metals, few studies have investigated the potential for essential elements to mitigate PE's effects. In epidemiological studies, low levels of manganese (Mn), a ubiquitous and essential trace metal, has been shown to reduce the risk of PE, yet few toxicological studies assess the molecular mechanisms underlying this relationship. This study aimed to evaluate the impact of exposure to low levels of Mn, on the migration and invasion of trophoblast cells. SVneo trophoblast cells were exposed to four low and environmentally-relevant concentrations of Mn, ranging from 0.18 – 5 μM, and were assessed for the effects on migration and invasion using the scratch-wound assay. The results showed that 0.91 and 2.8 μM Mn trended toward (p>0.05) increased migration and invasion in SVneo cells, respectively. This study has shown that Mn at low concentrations may influence in the invasion and migration of trophoblast cells. These results would support a solution-oriented follow-up supplement for women at high risk for PE. Given the high prevalence of PE worldwide, solution-oriented research is of great importance for public health promotion.

Bailey, Aleah
UNC-CH
Evaluating the Effects of Enriched versus Depleted Housing on Systemic Immunity and Allostatic Load in Mice

Wildfires are an increasing public health concern. Wildfire smoke (WS), which contains large amounts of fine particulate matter less than 2.5 μm in diameter (PM2.5) and other toxic chemicals, is strongly associated with increased inflammation and altered immune function. Social factors, such as low SES, poor housing conditions, and limited access to healthcare, also contribute to altered immunity, inequitable wildfire health outcomes and allostatic load (AL), physiological dysregulation due to chronic stress. AL forces the body to adapt to stressors by altering ideal, homeostatic ranges to maintain abated organ function. However, there is a lack of research on the synergistic effects of AL and WS exposure. To address this knowledge gap, a cohort of C57/BL6 mice (n=63) were housed in enriched (EH) or depleted (DH) conditions for 20 weeks prior to exposure to filtered air or 0.2mg/m3 flaming eucalyptus smoke for 1 hour. DH conditions serve as a psychosocial stress model and are synonymous to built environments devoid of healthy physical and social factors. Collected serum was analyzed for immune mediators associated with WS exposure and AL biomarkers that were reduced to an allostatic load index (ALI) using the rat cumulative allostatic measure (rCALM). Results indicate housing conditions and WS exposure significantly altered cytokine expression, primarily in female mice. We observed housing-dependent
changes in MCP-1, IL-17, and IL-2 only in female mice. WS exposure increased IL-2 in DH females and IL-27 in EH males. At baseline, female mice had higher levels of corticosterone, HbA1c, and fibrinogen than males. Post-exposure, female DH mice had higher HbA1c and corticosterone, and female EH mice had higher epinephrine. Overall, female mice had higher ALI scores than males. Together, these findings indicate that housing conditions and WS exposure alter immune and stress mediators in a manner that increases AL, which may increase WS health effects.

Barbo, Nadia
ORISE & US EPA

Effects of Larval Age and Previous Testing on Larval Zebrafish Behavior

Zebrafish (Danio rerio) are often used to investigate the effects of chemical exposure during development. Brain development in the zebrafish larval stage is rapid, so it is common for larvae to be assessed at various developmental stages, such as 4, 5, and 6 days post fertilization (dpf). Chemical screening and assay development can benefit from repeated behavioral assays on the same individuals over consecutive days because such experiments allow for a better understanding of exposure over time. With multiple timepoints being assessed using the same fish, it is important to understand: (1) how larval age affects locomotor behavior and (2) how previous behavior testing impacts later behavior profiles. To accomplish this, we concurrently reared two 96-well plates of zebrafish embryos/larvae at 26°C (one individual per well, experiment repeated 3x) and conducted a light-dark transition test on larvae at both 4 and 5 dpf, or only 5 dpf. When comparing behavior at 4 to 5 dpf (n=180), 4 dpf fish moved significantly less, especially in the light phase (~90%). When assessing whether prior testing affected behavior, there was a significant but small (9%) decrease in locomotion in the dark phase among individuals who experienced previous testing (n=279-281). This decreased movement in previously tested fish, although statistically significant, is within normal behavior variability and is unlikely to impact experimental outcomes when using a smaller number of individuals per treatment group. Our results demonstrate that larval age can affect zebrafish locomotor behavior and should be considered when comparing light and dark responses across different studies. We also found that, at 5 dpf, there is no practical difference observed in locomotion between larvae who had undergone previous behavior testing and those who had not. This abstract does not reflect the official policy of the US EPA.

Bhattacharya, Shamik
NCSU

Model Averaging Toolbox for Climate Change Projections: Methodology and Implementation

Climate change modeling is a challenging task that necessitates the integration of various climate models and observations, making predictions more accurate and reliable. To solve this issue, this research proposal presents a novel toolbox for averaging climate models to generate an ensemble of predictions. The Bayesian Model Averaging (BMA) methodology and other approaches are implemented in the toolbox to address the model averaging problem and increase the accuracy of climate change predictions. In the proposed model averaging toolbox, techniques such as BMA (as will be used in the 5th National Climate Assessment), the Sanderson approach (as was used in the 4th National Climate Assessment), and the AIC (Akaike Information Criterion) methods were used to estimate model weights, compute model averages, and compare the multi-model ensemble generated predictions to observed data. The toolbox allows for the discovery of optimal sets of model weights that can be used for post-processing. This toolbox will, in particular, feature a model independence estimation, a vital statistic that has gained prominence in recent model averaging methods. Using the obtained model weights, researchers will be able to assess the level of skill and independence for each climate model. Existing model weighting approaches generally rely on predefined criteria to assess model skill and independence, limiting forecast flexibility and accuracy. The suggested toolbox, on the other hand, gives users a wide range of alternatives for the model weighting problem, allowing them to effectively address diverse climate change investigations.
Bomstein, Zachary
UNC-G

The Effect of DEHP on Non-Malignant Colonocyte

Bis(2-ethylhexyl) Phthalate (DEHP) is a plasticizer thought to be an important component of the human exposome, with human exposure estimated to be around 8.2 μg/kg/ body weight/day. Though usage of DEHP has declined considerably, its ability to persist in environments such as landfills, sewage plants, water systems, and airborne particulates makes human exposure to it a continued threat. In fact, biomonitoring estimates derived from the National Health and Nutrition Examination Survey (NHANES) found 95% of participants had DEHP metabolites in their urine. In vitro and in vivo evidence point to DEHP exposure deleteriously affecting reproductive health, particularly when exposure occurs 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 non-malignant colonic epithelia using the Young Adult Mouse Colonocyte (YAMC) cell line. 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. To determine the manner in which DEHP reduced cell count, we assessed its influence on cell proliferation and apoptosis. Seventy-two-hour DEHP exposures of 10 μM, 100 μM, 1 mM and 10 mM resulted in statistically significant decreases in cellular proliferation. 12-hour DEHP exposure at concentrations of 100 μM and 1 mM led to a statistically significant increase in apoptosis. To understand the pathways involved with our proliferation and apoptosis data we used Real-Time Quantitative Polymerase Chain Reaction (RT-PCR) to measure the expression of genes involved in these processes. 24-hour DEHP exposures at concentrations of 100 μM and 1 mM led to statistically significant changes in cellular proliferation. 12-hour DEHP exposure at concentrations of 100 μM and 1 mM led to a statistically significant increase in apoptosis. To understand the pathways involved with our proliferation and apoptosis data we used Real-Time Quantitative Polymerase Chain Reaction (RT-PCR) to measure the expression of genes involved in these processes. 24-hour DEHP exposures at concentrations of 100 μM and 1 mM led to statistically significant changes in cellular proliferation. 12-hour DEHP exposure at concentrations of 100 μM and 1 mM led to a statistically significant increase in apoptosis. Furthermore, 24-hour DEHP exposures of 10 μM, 100 μM and 1 mM led to statistically significant changes in the expression of genes associated with oxidative stress (Nrf2, HO-1, GCLC, and CHOP). 24-hour DEHP exposure at concentrations of 100 μM and 1 mM significantly increased Reactive Oxygen Species (ROS) production compared to vehicle control. Collectively, our findings illustrate that the growth-inhibitory effect of DEHP on colonic epithelial cells is a product of apoptosis and inhibition of cellular proliferation. Additionally, we demonstrate that the short-term toxicity conferred by DEHP exposure in colonocytes may be a product of oxidative stress.

Britton, Katy
ORAU & US EPA

Using Zebrafish to Screen the Developmental Toxicity of a PFAS Chemical Library

Per- and polyfluoroalkyl substances (PFAS) are compounds found in many consumer and industrial products. While there is evidence that some PFAS, notably perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), cause developmental toxicity in mammals, the vast majority of PFAS have not been studied regarding their developmental toxicity potential. Our goal was to use zebrafish data to fill this knowledge gap and paint a clearer picture of the overall pattern that many of these PFAS have on development in vertebrates. To assess whether PFAS cause developmental effects in vertebrates, we used a larval zebrafish, medium-throughput assay to conduct a dose-response study with 182 unique chemicals in the US EPA PFAS chemical library. Embryos were collected at 0 days post fertilization (dpf) and exposed to either dimethyl sulfoxide (vehicle, 0.4% v/v), or one of the PFAS (n=6 embryos/ chemical/ concentration; ≤ 100 μM, 8 concentrations per chemical). At 6 dpf, two independent observers, blinded to the treatment conditions, graded developmental landmarks (endpoints) for each larva (e.g., mortality, hatching, swim bladder inflation, edema, abnormal spine/tail, and cranial-facial abnormalities). Of the 182 PFAS tested, 53 (29%) produced developmental toxicity commonly seen as mortality, edema, or uninflated swim bladders. Interestingly, while PFOS was developmentally toxic to the zebrafish embryos with a benchmark concentration (BMC) of 7.5 μM, perfluorooctanesulfonamide (PFOSA) was the most potent with a BMC of 0.26 μM, followed by ((perfluorooctyl)ethyl)phosphonic acid (BMC=0.58 μM), N-methylperfluorooctane sulfonamide (BMC = 0.70 μM) and perfluorohexane sulfonamide (BMC =1.45 μM). Therefore, both mammals and zebrafish have exhibited toxicity after developmental exposure to PFOS, but the developmental toxicity profile for these other sulfonamide-containing PFAS in mammals is largely unexplored.
These observed effects in zebrafish may further inform the toxicity profile of these chemicals in mammals. This abstract does not reflect USEPA policy.

**Brocke, Stephanie**  
**UNC-CH**  
**Seasonal Air Pollution Samples from Xinxiang, China Induce Disparate Toxicological Outcomes in a Human Airway Cell Model**  
Fine particulate matter (PM2.5) is a major component of ambient air pollution and is a criteria pollutant regulated by the US Environmental Protection Agency. Previous *in vitro* and *in vivo* studies have linked exposure to single sources of PM2.5—such as diesel engines, cigarettes, and lab-generated woodsmoke—to increased risk of respiratory viral infections. Additionally, epidemiological evidence suggests that higher ambient PM2.5 corresponds with increased hospitalization rates for influenza infections and others. However, it is known that ambient air pollution is a complex mixture of chemicals from many contributing sources and varies geographically, seasonally, and temporally. Throughout 2021 and 2022 we collected samples of PM2.5 from a single location in Xinxiang, China. We hypothesized that treating human airway epithelial cells with PM2.5 from disparate times of year followed by influenza infection would yield different toxicological outcomes and infection responses due to variance in the chemical makeup of the PM. We exposed primary human nasal epithelial cells grown at air-liquid interface to Xinxiang PM2.5 samples from different seasons (22 μg/cm²) for 2h, followed by infection with influenza A at an MOI of 0.001. To analyze how PM exposure affected response to infection, we collected basolateral supernatants at 24 and 48 h post infection and measured levels of 21 chemokines and cytokines related to viral infection response. Additionally, chemical analyses of the PM2.5 samples were performed to measure levels of metals, non-metals, and carbonaceous components. The findings of this study begin to highlight how the chemical composition of real-world air pollution can impact its toxicological effects in the airway.

**Brown, Eric**  
**UNC-CH**  
**Prenatal Metals Mixtures are Associated with Differential Methylation of Neurodevelopment-Associated Genes Within the Extremely Low Gestational Newborns (ELGAN) Cohort**  
Placental DNA methylation (DNAm) has been proposed as a primary mechanism underlying associations between prenatal exposure to environmental metals and adverse health outcomes throughout the life course. Numerous epidemiological studies have associated exposure to individual metals to alterations in placental DNAm. Of concern, pregnant mothers are exposed to a complex mixture of environmental stressors which may alter individual relationships. We set out to evaluate the association between fetal exposure to metal mixtures and placental DNAm within a cohort of 216 singletons from the Extremely Low Gestational Age Newborn (ELGAN) cohort. Umbilical cord tissue samples were collected at birth and analyzed for levels of 11 metals (As, Mn, Cd, Pb, Hg, Cu, Sb, Sr, Se, Ba, and Zn). CpG methylation was assessed in the placenta using EPIC arrays. The novel ACllustsCCA approach was used to examine the relationship between the mixture of the 11 different metals measured in the umbilical cord and differentially methylated regions (DMRs) in the placenta. We used canonical variables representing regions and exposure mixtures to test for associations, and computed permutation p-values adjusting for covariates. A total of 29,399 genomic regions of correlated DNAm were identified in placenta. Of these 29,399 genomic regions, 14,383 DMRs (13,383 genes) was associated with prenatal exposure to Cd. Notably, exposure to Cd was associated with a DMR on the X-chromosome that transcribed Methyl-CpG Binding Protein 2 (MECP2), a gene critical to neurodevelopment. These findings highlight the biological mechanisms associated with exposures to complex mixtures and neurodevelopment.

**Brown, Shalyn**  
**NIEHS**  
**Quantifying Xenobiotic Induced Oxidative Stress by Exploiting the Xc-Cystine/Glutamate Exchange System at the Blood-Brain Barrier**  
Inhibition of cystine uptake via the cystine/glutamate antiporter, Xc-, indirectly decreases intracellular levels of glutathione, 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 cystine uptake through the Xc- Cystine/Glutamate antiporter of rat brain endothelium. Ferroptotic inducing chemotherapeutic agent, erastin, and a known Xc- exchange inhibitor, sulfasalazine, were used as investigative tools to affect the Xc- antiporter’s activity. Sulforaphane (SFN) was used as a positive control, a chemical known to upregulate the oxidative stress proteins: nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) enzyme. The utility of the assay was successfully established in rat brain capillaries and subsequently used to screen three xenobiotics – Ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX), Perfluorooctanoic acid (PFOA), and Perfluorooctane sulfonate (PFOS) – to determine their oxidative effects on the Xc- Cystine/Glutamate exchange system. The implications of our findings and the associated data will be discussed. It is important to note that this assay can be easily adapted to a 96-well format for the rapid screening of chemicals (xenobiotics) for their ability to induce oxidative stress in brain capillaries or in human cell lines.

Cao, Kevin
UNC-CH
Long-Term Respiratory Mucosal Immune Response to SARS-CoV-2 mRNA Vaccination Mediated by Exogenous Inhaled Chemical
Despite the incidence of COVID-19-related infections and deaths having drastically decreased since the peak of the pandemic, variants of concern (VOC) are still emerging. As a result, vaccine developers began targeting the respiratory mucosal surface for vaccination which may prevent or reduce initial infection rates. Supporting this initiative, our previous study showed that intramuscular (IM) mRNA vaccination induced intranasal neutralizing antibodies against wild-type SARS-CoV-2 strain. However, the duration of respiratory mucosal antibody presence and activity and whether there are other factors, such as inhaled chemical exposure, that may alter antibody production over time is not well characterized. Inhaled chemicals, e-cigarettes, have been found to alter nasal antibody production with live attenuated influenza infection, thus they are an interest for our study. Here we sought to characterize the duration of intranasal and systemic antibodies after IM mRNA SARS-CoV-2 vaccination in healthy individuals and identify potential modification of duration in e-cigarette users. Blood and nasal epithelial lining fluid (NELF) were collected from healthy human volunteers from March 2021 to March 2022 across key vaccination schedule timepoints (prior to primary dose, 2-11 months post-primary dose, and 2-3 weeks post-booster). Serum and NELF were analyzed via multiplex ELISAs for anti-Spike IgG and IgA as well as neutralization activity against SARS-CoV-2 variants. Our results indicate that mRNA booster vaccination induces virus-specific IgG and IgA antibody production in the nasal cavity and systemically against multiple variants, with no difference in booster brand (Pfizer vs. Moderna). Booster-induced nasal antibodies displayed greater neutralization efficacy compared to systemic antibodies. Interestingly, we observed altered nasal and systemic virus-specific IgA concentrations following booster in e-cigarette users. We are continuing to analyze the data and are comparing antibody longevity in NELF and serum. Overall, these data suggest that mucosal immunity is induced with continued IM mRNA vaccination and response may be mediated in individuals exposed to exogenous inhaled chemicals.

Cobos-Uribe, Catalina
UNC-CH
Wood Smoke Exposure and its Effects on the Respiratory Microbiome and Health
Wildfire-derived smoke is a well-known air pollutant that adversely affects respiratory health. During the recent Canadian wildfires, unhealthy air contaminated with wildfire smoke reached North Carolina. Given its expanding reach and potential risks to large populations, it is important to understand the respiratory health implications of wood smoke exposure, a model for wildfire smoke, to control and mitigate its hazardous effects. This study focuses on the respiratory microbiome and its role as a mediator of the respiratory host response to wood smoke. To achieve this, we collected induced sputum samples from 54 healthy volunteers before and after controlled exposure to 500 μg/m3 of wood smoke for two hours. DNA was extracted from sputum samples and sequenced for the 16S rDNA V3-V4 region. Sequences were analyzed using QIIME2 for alpha- and beta-diversity metrics. Taxonomic identification was carried out using SILVA and eHOMD databases. Advanced analysis and correlations with biological variables, such as lung function, sputum %PMN, and sputum cytokine concentrations, will be executed in R. The top three most abundant phyla were Firmicutes, Proteobacteria, and Bacteroidetes. Streptococcus, Veillonella, and Prevotella were the most abundant genera. No significant alterations in
microbiome structure across the different exposure times were detected, indicating that acute exposure does not introduce or eradicate specific bacteria. Interestingly, there is a difference in alpha diversity based on their respiratory inflammatory response, sputum %PMN. This suggests that the baseline microbiome composition may underpin the inflammatory response and possibly point toward bacteria with protective or detrimental effects on the host. Our ongoing research seeks to pinpoint bacteria that correlate with the host's respiratory response to wood smoke. This comprehensive study will provide valuable insights into the interplay between wood smoke exposure, respiratory microbiota, and host respiratory immune responses, paving the way for protective interventions to safeguard public health.

Combs, Sarah
UNC-CH
Evaluation of Emerging and Legacy PFAS Mixtures and Their Effect on Mammary Epithelial Differentiation and Markers of Lactation In Vitro
Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured, persistent chemicals that have been used in since the 1940's. PFAS are highly stable chemicals known to contaminate air, soil, water, and to bioaccumulate within organisms. PFAS are linked with adverse health effects. “Legacy”, or older PFAS compounds, have started to be replaced with novel “emerging” compounds – whose toxicity is less understood. Six longitudinal cohort studies now report an association between PFAS exposure and decreased breastfeeding duration. In in vitro cell models several novel or legacy PFAS promote proliferation of mammary epithelium, but effects on differentiation to the lactating state have not been examined. Exposing the HC11 mammary epithelial cells to emerging and/or legacy PFAS mixtures decreases cell differentiation, interfering with lactogenic markers. HC11 mouse mammary epithelial cells (MEC) will be cultured and dosed with mixtures of legacy and/or emerging PFAS, to examine the effect on MEC differentiation. HC11 cells will be exposed to PFAS mixtures at different times during differentiation: 1) HC11 cells continually grown in PFAS, 2) dosed with PFAS during differentiation, 3) dosed with PFAS shortly after beginning differentiation. Milk proteins and cell-type specific biomarkers will be measured across time (0, 24, 48, 96 hours post-differentiation) via PCR using primers Sn2, WAP, Pip, CD44, and CD10. Milk protein antibodies will be used to confirm changes in gene expression. PFAS mixtures will be used to examine how North Carolina-specific drinking water contamination of PFAS impacts lactation. This study will help to understand possible PFAS interference on lactation function. We will determine at which stage PFAS may interfere in the development of a differentiated state, and the exposures associated with adverse changes. Known mechanisms involved in lactogenesis will be interrogated once we determine the stage most affected by PFAS.

Conley, Jessica
ORISE & US EPA
Human Brain Organoid Model to Study Developmental Neurotoxicity
Human in vitro models of the developing brain are important for studying neurodevelopment and the potential developmental neurotoxicity (DNT) of environmental and pharmacological compounds. Exposure to these compounds during neurodevelopment can potentially cause adverse effects such as morphological alterations and/or functional changes in the developing brain. Over the past 15 years, in vitro two-dimensional models have been developed as assays to characterize chemicals for potential DNT hazard. Organoids are a three-dimensional (3D) cell culture system that mimic the complex structure and development of the human brain better than their two-dimensional counterparts. In this study, we establish and characterize an induced pluripotent stem cell (iPSC)-derived brain organoid model containing mature neurons and glial cells originally developed at Johns Hopkins University. Immunocytochemistry and high-content imaging of the organoids show a decrease in proliferation and neural progenitor markers and an increase in neuronal, astrocyte, and oligodendrocyte markers during differentiation (weeks 0-10), indicating that during this time the organoids are progressing along a neurodevelopmental ontogeny. Neural organoids were plated on a high-density microelectrode array (hdMEA) to measure the spontaneous electric field potentials produced. Treatment with Picrotoxin (PTX), Tetrodotoxin (TTX) and Nicotine (NIC) resulted in the expected changes in spontaneous electrical activity following an acute one-hour exposure; PTX increased network activity while TTX and NIC decreased network activity. This work demonstrates the importance of 3D organotypic models for neurotoxicological studies, especially as rat primary cortical cultures do not have robust responses to nicotine.
Future studies will develop an exposure protocol relevant to neurodevelopment and assess effects of additional compounds, including neonicotinoid insecticides and per- and polyfluoroalkyl substances (PFAS). This abstract does not represent US EPA policy.

Connors, Ashley
NCSU
Per- And Polyfluoroalkyl Substances (PFAS) Impact Macrophage Function In Vitro
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). Previous studies on the innate immune system indicate that PFAS exposure can influence the numbers of innate immune cells, cellular signaling, and functional endpoints. We are evaluating how macrophages are affected by a 2-day in vitro exposure to ten PFASs: perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), 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-PFAS cytotoxicity studies with human macrophage-like THP-1 cells, exposure to 320 μM PFDA, PFNA, PFOS, and Nafion Byproduct significantly reduced viability. We observed no changes in cell viability at or below exposures to 80 μM PFAS. To test how PFAS modulate phagocytosis, macrophage-like THP-1 cells were exposed to 80 μM PFAS for 48 hours, then challenged with fluorescent heat-killed E. coli. Phagocytic index and number are measured with flow cytometry. Thus far, we have observed that PFOS and PFNA increase the average extent of phagocytosis. Additionally, we are measuring cytokine production by both unstimulated and stimulated THP-1 macrophages. Thus far, we have observed that PFOS and PFNA modulate secreted levels of IL-6.

Understanding how PFASs 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.

Dai, Emily
FDA
Challenges and Solutions in Measuring Commonly used Hepatotoxicity Biomarkers in a Liver-On-A-Chip Platform
Liver-on-a-chip (liver-chip) is a microphysiological system (MPS) designed to better maintain hepatic functions and viability of liver cells cultured under in-vitro conditions. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are clinically used biomarkers for liver injury and are therefore have been proposed hepatotoxicity biomarkers for liver MPS. In our study of acetaminophen hepatotoxicity, we used Emulate® liver-chips, which coculture primary human hepatocytes (PHH) with non-parenchymal cells (NPCs) consisting of sinusoidal endothelium, Kupffer, and stellate cells. We unexpectedly found that commonly used assays for ALT and AST activities did not work in liver-chips, and efforts were undertaken to address this issue. Human PH and NPCs were cultured in the top and bottom channels, respectively, of the Emulate® liver-chips. The perfusing medium, also called effluent, was collected to determine ALT and AST. Cells were lysed using 1% Triton X-100, and then the lysate was stored in cell culture incubator for 0, 24, 48, and 72 h. ALT and AST activities were measured using the method recommended by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC method). In parallel, an enzyme-linked immunosorbent assay (ELISA) was used to determine protein levels of ALT and AST. The activities of ALT and AST of the effluent from intact cells were undetectable (i.e., < 5 U/L). In lysed cells, ATL and AST activity were 20 and 70 U/L, respectively. However, the activity of ALT, but not AST, spontaneously decreased by about 50% every 24 h, suggesting ALT activity is not suitable for measuring cell death in liver-chips. In contrast, protein levels of ALT and AST measured by ELISA were stable over time and readily detectable in the effluent of intact cells. Our findings suggest that ALT and AST protein levels, but not activity, should be used to determine cell death in Emulate liver-chips.
Daly, Fiona  
NCSU  
Use of Integrated Approach to Testing and Assessment (IATA) on Selected Chemicals to Determine Their Developmental Neurotoxic Potential  

Developmental Neurotoxicity (DNT) is the effect of chemicals on the developing nervous system. Most chemicals have not been tested for DNT due to high cost and extended time of in vivo testing. To enhance the assessment of chemicals for DNT, a battery of 16 new approach methodologies (NAMs) made up of human and animal cell models as well as Zebrafish was developed that assess key neurodevelopmental events. The Division of Translational Toxicology at the National Institute of Environmental Health Sciences has used this battery to generate screening level information on 115 chemicals that were nominated by various stakeholders due to DNT concern. Based on preliminary data, two chemicals were selected for this project, Mifepristone (drug) and Pyraclostrobin (fungicide). Both compounds were active in most of the DNT NAMs battery, Mifepristone at concentrations 0.98μM-44.5μM and Pyraclostrobin at concentrations 0.005μM-54μM. The most sensitive assay for both compounds was the network formation assay. About half of the assays had specific effects, meaning that the benchmark concentration (BMC) of the DNT endpoint was at non-cytotoxic concentration. Integrated approaches for testing and assessment (IATA) case studies will be developed to determine if Mifepristone and Pyraclostrobin have DNT potential in humans. IATA allow the combination of a variety of information sources e.g., in vivo, and NAMs approaches to understand hazard for chemical risk assessment. The IATA framework can increase the confidence in transition from animal test data towards more NAM-based approaches. A systematic literature review will be performed using the software, Sysrev. Additionally, physiologically based pharmacokinetic (PBPK) and in vitro to in vivo extrapolation (IVIVE) models will be utilized to view data gathered from the DNT NAMs battery and compared to data found in the literature search. The IATA case studies can help build confidence in NAM based approaches and categorize potentially toxic chemicals more efficiently.

Dameris, Logan  
NCSU  
The Role of Imprinted IncRNA H19 in Cadmium-Induced Steatotic Liver Disease  

The most prevalent chronic liver disease in the United States is steatotic liver disease (SLD) which is characterized by the accumulation of lipids in hepatocytes. The pathogenesis of SLD and its associated risk factors are well-understood; however, its etiologies – genetic or environmental – are not clear. We and others have demonstrated that exposure to environmental stressors including the heavy metal cadmium (Cd) can facilitate SLD. Cd is naturally-occurring throughout the Earth’s crust, but anthropogenic activities have significantly increased its presence in the environment. The World Health Organization (WHO) has denoted Cd as a top ten chemical of major public health concern, given its ubiquity, long biological half-life, and association with numerous adverse effects including liver disease. Notably, we and others have shown that imprinted genes – which are defined by their expression from only one parental allele – are especially sensitive to environmental perturbations; therefore, we propose that aberrant imprinted gene activity links Cd exposure to the onset of SLD. The imprinted gene H19 has previously been implicated in the pathogenesis of SLD. H19 encodes a long non-coding RNA (lncRNA) that activates transcriptional programs underlying hepatic steatosis and fibrosis and regulates liver metabolism and homeostasis. We hypothesize that hepatic IncRNA H19 upregulation facilitates Cd-induced SLD. To test our hypothesis, we leveraged a novel H19 knockout (KO) mouse model and exposed KO or wildtype (WT) mice to 0 ppm or 50 ppm CdCl₂ in their water for 14 weeks. We then performed histological, biochemical, and molecular analyses on liver tissue to determine if H19 ablation can protect against SLD onset after exposure to Cd. The results of our study will reveal the importance of IncRNA H19 in regulating Cd-induced SLD pathogenesis.

Eastman, Zahkura  
RTI International  
Investigating the Impact of Tire Rubble Microplastics Sourced from Recycling Facilities Dosed onto Human Epithelial Lung Carcinoma Cells  

Recycling is a commonly used alternative to divert plastics from accumulating in landfills and polluting the environment. However, the process of preparing plastics for recycling requires mechanical grinding of plastics into smaller fragments known as microplastics. Microplastics from recycled products can be inhaled and are a
source of human health concern for occupation workers in recycling facilities and for children that use recycled tire fields for recreational purposes. In this study, we used microscopy and various in-vitro techniques to characterize tire microplastic particles sourced from recycling facilities and determine their toxicity in human lung cells. Shredded tire rubble were physically and chemically characterized using Fourier-transform infrared spectroscopy and X-ray diffraction. Additionally, these microplastics were incubated at 37 °C in appropriate cell media at 0.1 mg MP / mL media and 0.05 mg MP / mL media for 1 or 7 days. After incubation, A549 human lung carcinoma cells were treated with microplastic media leachate and proliferation was assessed over 72 hours using an IncuCyte live cell imager. In addition, cell viability/metabolism was assessed at 24 hours and 72 hours using a MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. Microscopy results demonstrate differences in microplastic structure, show various elements on the polymer surface, and confirm polymer types of the samples. In vitro cell results demonstrate a statistically significant decrease in cell viability in cells treated with tire rubble (11% decrease using 0.05 mg MP / mL media and 22% decrease using 0.1 mg MP / mL at 24 hours) compared to cells dosed with control only. Interestingly, cell proliferation increased at 24 hours in cells dosed with tire rubble leachate. However, we observed a decrease in proliferation rate in cells dosed with either tire rubble concentration for 120 hours. These results indicate the possibility of a toxic interaction between the physical particle or the chemicals released from this microplastic. To further elucidate the molecular mechanisms that could be affected by tire rubble, we will investigate the generation of reactive oxygen species, changes in the expression of inflammatory markers and metabolomics in lung cellular models.

Falls, Ashlee
UNC-CH
Evaluating Known and Novel PFAS in Exposed Populations with Non-Targeted Liquid Chromatography, Ion Mobility Spectrometry and Mass Spectrometry Measurements
Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals of great concern due to their resistance to breakdown and known accumulation in the environment and human body. PFAS, however, are still widely used in consumer products, firefighter turnout gear, and the aqueous film forming foam (AFF) used to extinguish fires. Due to their utility in firefighter gear and job-related products, firefighters have extensive exposure to PFAS both at ambient conditions and high heat. We performed a non-targeted analysis of 29 firefighter serum samples from Durham, North Carolina to investigate and identify PFAS present in their blood and will compare them to both contaminated and control groups from Pittsboro, North Carolina. The combination of targeted and non-targeted PFAS assessments allowed for an evaluation of both known PFAS and new species. A liquid-liquid extraction was performed on 50 μL of serum, including an acetonitrile protein crash to isolate PFAS prior to reconstitution. Non-targeted analysis was performed using a liquid chromatography - ion mobility spectrometry - collision induced dissociation - mass spectrometry (LC-IMS-CID-MS) platform. Data analysis was performed using a Skyline library with over 100 PFAS for a targeted assessment and by evaluating all features for their mass defects and collision cross section (CCS) vs. m/z trendlines to identify new PFAS. This data will be further used to analyze the relationship between observed lipid changes and the corresponding presence of PFAS.

Farrell, Matthew
NCSU
Relation of PFAS Chemical Structure to Developmental Toxicity in Zebrafish Larvae
Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants used in manufacturing and numerous commercial products. Some PFAS have been connected to harmful health effects, but to this point the number of different PFAS chemicals being produced and released into the environment has greatly outpaced the scientific community's ability to study them. This has emphasized the importance of establishing which structural characteristics of PFAS are responsible for toxic effects, so that models can be established to predict harmful chemicals. This study seeks to relate carbon chain lengths, functional acid groups, and ether linkages of PFAS with acute toxicity. Zebrafish embryos were aqueously exposed to seventeen individual PFAS over the course of five days. Increased carbon chain length and sulfonic acid functional groups were found to positively correlate with mortality and several symptoms of developmental toxicity, including spinal malformation, swim bladder non inflation, and yolk sac / pericardial edema. Inclusion of ether linkages showed negative
correlation with these same phenotypes. This study reinforces the links between key PFAS chemical characteristics and toxicity by examining a wide range of structurally similar PFAS under identical conditions.

**Fiamingo, Michelle**  
**UNC-CH**  
**Depleted Housing Elicits Cardiovascular Dysfunction After a Single Wildfire Smoke Exposure in ApoE(-/-) Mice**

The American Heart Association released a scientific statement describing the importance of housing on cardiovascular health and emphasized the impact of the broader physical and social environment. Thus, housing is recognized as a major social determinant of cardiovascular health, however the impact of housing on subsequent environmental exposures remains understudied, specifically for populations that have pre-existing cardiovascular morbidities. This study sought to examine the effects of enriched (EH) versus depleted (DH) housing on the cardiovascular response to a single 0.4mg/m³ flaming eucalyptus wildfire smoke exposure in male and female apolipoprotein E knockout mice, which develop atherosclerosis spontaneously. Mice were kept in either EH or DH for 18 weeks, which is sufficiently long enough to develop fatty streaks and mild atherosclerotic lesions. High-frequency echocardiography was conducted 1-wk before exposures, 24-hrs post-exposure, and 1-wk post-exposure. There were significant sex differences in the cardiovascular response to both housing and wildfire smoke (WS). Here, male DH mice exposed to WS exhibited a decrease in ejection fraction (EF) and stroke volume (SV), and an increase in isovolumic relaxation time (IVRT) after the exposure, with decreases in fractional shortening (FS) from DH before exposures. These changes imply that DH elicits left ventricular diastolic dysfunction in mice following a WS exposure. Females, on the other hand, exhibited hemodynamic changes, with increases in the myocardial performance index (MPI) 24-hours after the exposure, and increases in the ratio between pulmonary acceleration time (PAT) and pulmonary ejection time (PET) and IVRT 1-wk post-exposure. This is indicating a global decline in left ventricular functioning and decreased right ventricular systolic pressure. Hence, these results indicate that depleted living conditions worsen the cardiovascular response to a single WS exposure and implicate the need to evaluate non-chemical stressors as a modifier of the toxicological response. (This abstract does not reflect US EPA policy).

**Fletcher, Brenda**  
**RTI International**  
**Simultaneous Analysis of 1H, 1H, 2H, 2H - Perfluorooctanesulfonic Acid (6:2 FTS), 2-Chloro-2,3,3,3,-Tetrafluoropropanoic Acid (CTPFA), and Nonadecafluorodecanoic Acid (PFDA) in Rat Plasma by UPLC-MS/MS**

Per- and polyfluoroalkyl substances (PFAS) are a large yet diverse class of compounds that are widely used for their stain-resistant, waterproofing, and friction-reducing properties. Through their manufacture, use, and disposal, these compounds have become anthropogenic contaminants in air, soil, and drinking water. These chemicals tend to be bioaccumulative and persistent in the environment, and several have been detected in human blood. Some PFAS compounds are associated with chronic effects such as immunotoxicity and potentially cancer. However, for many of the PFAS compounds, the available data on the potential *in vivo* toxic effects from acute and chronic exposure are limited. The objective of this work was to develop a method to simultaneously quantitate 1H, 1H, 2H, 2H – perfluorooctanesulfonic acid (6:2 FTS), 2-chloro-2,3,3,3,-tetrafluoropropanoic acid (CTPFA), and nonadecafluorodecanoic acid (PFDA) in rat plasma in support of toxicology studies. Samples were extracted by fortifying 50 µL of rat plasma with 10 µL of methanol and 10 µL of internal standard solution (6:2 FTS-13C2D4 in methanol). Samples were extracted by protein precipitation with 300 µL of methanol and freezing the extracts to separate the phospholipids. The extracts were analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in negative ion mode for simultaneous analysis of 6:2 FTS, CTPFA and PFDA. The method was assessed over the concentration range ~5-1000 ng/mL for each analyte in male Sprague Dawley rat plasma. Matrix standard curves were linear (r ≥ 0.99), and the percent relative error (%RE) values were ≤ 15% for standards at all levels. The lower limits of quantitation in male plasma were 5.12 ng/mL for 6:2 FTS, 5.00 ng/mL for CTPFA and 5.08 ng/mL for PFDA; the limits of detection were 1.43, 1.72, and 0.999 ng/mL, respectively. Samples found to be outside of the calibration range can be diluted up to 1:100 for 6:2 FTS and up to 1:500 for CTFPA and PFDA. These data demonstrate
that the method is suitable for simultaneous analysis of 6:2 FTS, CTPFA and PFDA in rodent plasma in support of toxicology studies of PFAS compounds.

**Freedman, Anastasia**
**UNC-CH**

**Using Genomic Signatures to Elucidate Individual and Mixture Effects of Inorganic Arsenic and Manganese in Placental Trophoblasts**

Prenatal exposure to the toxic metal inorganic arsenic (iAs) is associated with adverse pregnancy and fetal growth outcomes. These adverse outcomes have been tied to physiological disruptions in the placenta. While iAs is known to co-occur in the environment with other metals such as manganese (Mn), there is a gap in the knowledge of the effects of metal-mixtures on the placenta. To address this, we exposed placental trophoblast cells to iAs, Mn, and an iAs-Mn mixture, at three low-level concentrations and evaluated subsequent transcriptome-wide gene expression. We hypothesized that co-exposure to an iAs-Mn mixture would result in a synergistic/enhanced transcriptomic effect compared to either metal alone. We hypothesized that genes involved in inflammatory or immune-related pathways would be differentially expressed in relation to the mixture compared to single-metals. In this study, iAs exposure alone had a stronger genomic response than Mn exposure, with two-fold the number of genes displaying differential expression. When analyzing genes that displayed differential expression across all concentrations of study, the iAs-Mn mixture resulted in the greatest number of differentially expressed genes (DEGs). Our results highlight that iAs exposure alone influences the expression of toll-like receptor-initiated response pathways, while Mn exposure alone influences the expression of NAD biosynthesis pathways. In contrast, exposure to the iAs-Mn mixtures resulted in altered expression of inflammatory and immune response-related pathways, including the NRF2-mediated oxidative stress response pathway. Our findings provide novel mechanistic insights into metals-induced placental disorders and suggest that in utero exposure to metal mixtures may influence placental gene expression.

**Grant, Kyla**
**UNC-G**

**Uptake of Nanoplastics by Human Aortic Endothelial Cells and Potential Uptake Pathways**

Society has become increasingly dependent on the versatility of plastic, leading to widespread use in products worldwide. Microplastics and Nanoplastics (M-NPLs) from these products have made their way into the human body through consumption of food and beverages, ultimately infiltrating blood and tissues. Given the ever increasing number of plastic products it is important to explore their uptake into vascular cells. Their potential pathways are key to understanding their effects on cells. To investigate this, Human Aortic Endothelial Cells (HAEC) were cultured and treated with various concentrations of M-NPLs (80nm). The cells were then collected and analyzed under flow cytometry to determine where there were interactions between the cells and M-NPLs. Cell lines were also treated with various endocytic inhibitors in conjunction with M-NPLs and analyzed under flow cytometry to identify potential endocytic routes. Our results indicated that M-NPLs interact with cell membranes and become endocytosed primarily through micropinocytosis. Endocytosis of these particles allows them to interact with cellular structures, potentially leading to unforeseen consequences. Knowing endocytic pathways may allow preventative actions against the ever-growing threat of M-NPLs. As these particles continue to infiltrate tissues, information on how they interact with our cell lines will become crucial.

**Green, Emily**
**Duke University**

**Integrating Air Sampling and In Vivo Exposure Assays to Investigate Health Impacts of Hog CAFOs in North Carolina Communities**

North Carolina counties, Sampson and Duplin, host the largest population of hogs in the US. Population health data illustrates residents living in proximity to high-density hog concentrated animal feeding operations (CAFOs) have higher incidence of disease such as cardiovascular, gastrointestinal, lung, and kidney disease. Hog CAFOs commonly store waste in large lagoons and spray it onto fields as fertilizer, releasing aerosolized particles such as particulate matter, antibiotic-resistant bacteria, volatile organic chemicals, and harmful gases into surrounding communities, including residents’ homes. However, a causal mechanistic relationship between environmental exposure to hog CAFOs and decreased community member health is not defined, especially regarding kidney
disease. Therefore, we developed a method to capture aerosolized chemicals and microbes near CAFOs for use in in vivo zebrafish exposure assays. Novel air sampling devices, InnovaPrep Bobcat and AirPrep Cub Sampler, are being utilized to capture aerosolized chemicals and microbes (>0.01um) around community spaces and resident homes in high-density CAFOs areas. The captured particles will be eluted in solution using a buffer determined not to cause larval zebrafish toxicity. Half of the eluent will be used to amplify 16S rRNA for bacterial species identification and the other half will be utilized in larval zebrafish exposure assays. Embryos will be exposed to a 1:4 dilution of eluent in Danieau's media at 6 hours-post-fertilization (hpf) until assay endpoint. Survival and deformities will be recorded every 24 hours and Agilent Seahorse BioAnalyzer (XFe96) will measure mitochondrial respiration rates in exposed larvae at 32hpf. A zebrafish transgenic line (Tg[fabp10a:NL-D3]) will assess tubular reuptake deficiency as a measure of kidney function in exposed larvae at 96hpf. This method for integrating air sampling devices with in vivo exposure assays will establish associations between hog CAFOs exposure and negative health outcomes as well as be easily adaptable for similar environmental exposure studies.

Gutkowski, Justin
NCSU
Differentiating Embryonic Stem Cells into Dopaminergic Neurons to Perform Developmental Neurotoxicity Screening
Early in development, animals start off as clusters of embryonic stem cells (ESCs). ESCs are pluripotent, meaning they can differentiate into any other cell type. Researchers can control this differentiation in vitro to obtain cultures of specific cell types. One cell type neurotoxicologists are particularly interested in are dopaminergic (DA) neurons, or brain cells that produce dopamine. Dopamine plays a large role in mood regulation and reward pathways. Dysregulation of dopamine levels and the degeneration of DA neurons is associated with multiple psychological and neurodegenerative disorders. DA neurons are also particularly sensitive to environmental influences, leading some researchers to hypothesize that environmental exposures might contribute to aberrant brain development and the development of these disorders. The objective of this project is to develop a protocol to grow DA neurons in vitro, differentiating them from ESCs. Once this protocol is established, it will be used in a developmental neurotoxicity screen to assess the effects of environmental influences on the development of DA neurons. Differentiation protocols have been designed by other research groups, but these often lack methodological details and have not been applied for toxicology screening. Multiple protocols are currently being tested to develop a reproducible high throughput screening approach. To ensure consistency over several trials, the gene expression of the cells is measured at multiple timepoints using qPCR and immunostaining. The proportion of DA neurons in the culture is also measured at the end of each trial. The development of this DA neuron differentiation protocol will provide greater insight into methodological details for deriving DA neurons from ESCs and help developmental neurotoxicologists design more consistent and repeatable experiments. The results produced by the screens will allow researchers to evaluate the effects of different chemicals on developing DA neurons and assess the likelihood that exposures can impact neural development.

Huber, Erin
RTI International
Common Liquid Application Dosing Conditions Alter Toxicity Testing Endpoints in Air-Liquid Interface Primary Bronchial Epithelial Culture
The use of in vitro systems to model inhalation exposures has focused on using primary cells as they can recapitulate key features of the airway epithelium in vivo. For inhaled chemicals, achieving this goal has relied on using differentiated primary human bronchial epithelial cells (dHBEC) as a representative model of the barrier that separates materials inhaled into the respiratory tract from the underlying lung tissue in vivo. Achieving physiological relevance with these models require cultures be maintained under air-liquid interface (ALI) conditions. Evaluating potential adverse effects typically involves applying an aqueous solution containing the test substance to the surface of ALI cultures, thus abolishing ALI conditions. While practical, the impact of liquid application on ALI culture phenotype and response to experimental exposures is poorly understood, limiting the interpretation of these studies and integrating the data into decision making. To address this knowledge gap, we sought to determine the effects of common liquid application dosing conditions on in vivo physiologically-relevant
endpoints in dpHBEC cultures. Application of 50µL resulted in dpHBECs remaining fully covered at 24-hours, 10
and 30µL lost full coverage after 1-hour. Application of ALI medium or 0.9% saline to dpHBEC-ALI cultures
caused significant transcriptional upregulation of pro-inflammatory cytokines: IL-6, IL-8, and IL-1α, and the
growth factor PGF. The hypoxia-responsive regulating transcription factor HIF-1α and glucose transporter
GLUT3 were upregulated following application of 30 and 50µL of liquid suggesting induction of hypoxia.
Consistent with these findings, application of these volumes led to decreases in dpHBEC-ALI culture barrier
integrity, measured by trans-epithelial electrical resistance. These findings support the need to further
characterize the effects of liquid application on dpHBEC cultures. Doing so will build confidence in the use of
these models to recapitulate the human respiratory tract for chemical testing. [Abstract does not reflect views or
policies of the US EPA].

Hudson, Sarah
UNC-G
Exposure to Microplastics and Nanoplastics Triggers Pro-Inflammatory Gene Expression in
Human Aortic Endothelial Cells
Cardiovascular disease (CVD) is the leading cause of mortality. It remains a major economic and health burden
in the USA and worldwide. In the US alone, CVD was responsible for 1 in every 5 deaths recorded in 2021.
Among the many manifestations of CVD, atherosclerosis and intra-arterial plaque formation are the most
notorious forms, and inflammation-induced aortic endothelial inflammation plays a key role in the occurrence
and development of the disease. Many environmental contaminants such as BPAs and PFAS are well-known
human cardiovascular health hazards. Microplastics and nanoplastics (MNPLs) are emerging environmental
pollutants detected in human tissues, and their adverse effects on cardiovascular health are of increasing
concern. This study explores the impact of MNPLs on the expression of pro-inflammatory cytokines, including
IL-1β, IL-6, and IL-8, in human aortic endothelial cells (HAEC), which is a protective monolayer within the arterial
lumen. HAEC cells were treated with 0.08-sized MNPLs at doses of 20, 120, 240, and 480 µg/mL for 72 hours
of incubation. The inflammatory gene expression was quantified using qRT-PCR. Our results showed a
significant (p < 0.05) increase in the expression of IL-8 and IL1-β at doses of 240 and 480 µg/mL as compared
to the control in a dose-dependent manner. Whereas no significant changes were detected in the expression of
IL-6. Taken together, our results suggest that exposure to MNPLs could induce pro-inflammation in HAEC cells.
The results of this study will enhance our assessment of the health effects of human exposure to the emerging
environmental pollutant MNPLs.

Huff, Katelyn
UNC-CH
Prenatal Over-the-Counter Acetaminophen Use is Associated with Lower Birthweight in ECHO
Cohort
Acetaminophen is among the most commonly used over-the-counter medications during pregnancy. However,
some experimental and epidemiological studies suggest acetaminophen use during pregnancy alters
fetal growth. Our objective was to evaluate the relationship between prenatal acetaminophen use and the
following outcomes: (1) preterm birth, (2) birthweight, (3) small-for-gestational-age (SGA), and (4) large-for-
gestational-age (LGA). The study population consisted of 8,950 mother-infant pairs from 36 pediatric cohorts
participating in the National Institute of Health (NIH) Environment influences on Child Health Outcomes (ECHO)
program. Regression modelling with inverse probability weighting and covariate adjustment was used to examine
the relationship between acetaminophen use during pregnancy with each of the four perinatal health outcomes
of interest. Approximately half of the mothers reported using acetaminophen at some point during their
pregnancy (n=4,449). After adjustment for relevant covariates, on average, prenatal acetaminophen use was
associated with lower birthweight (adjusted beta: -41 g; 95% CI: -59, -22), and lower odds of LGA (adjusted odds
ratio (AOR): 0.74; 95% CI: 0.68, 0.81). Further, of borderline significance, we observed higher odds of SGA
(AOR: 1.10, 95% CI: 0.98, 1.25) and lower odds of preterm birth (AOR: 0.95, 95% CI: 0.88, 1.03) in infants
whose mothers used prenatal acetaminophen. In a large and diverse cohort, prenatal acetaminophen use was
associated with modest reductions in birthweight. Future research should test for dose-response, biological
mechanisms, trimester-specific responses, and factors that influence inter-individual response to exposure.
Jackson, Zaria  
NCCU  
**Determining effectiveness of a catalyst on the depolymerization of polyethylene terephthalate using kinetics**

Kinetics research is essential for expanding the field of polymer recycling as well as restoring sustainable methods for the conversion of waste plastics into valuable chemical resources. The depolymerization of the biobased polyester polyethylene terephthalate (PET) using a zinc-oxide catalyst has shown to be a functional and efficient approach in recycling and reusing PET waste. The catalyst was composed of zinc-oxide and picolinic acid to form a zinc-picolinate. The efficiency and effectiveness of the catalyst was characterized and evaluated by conducting a kinetics study on the depolymerization of PET in order to determine the rate of reaction. These research methods are vital for advancing the economy and decreasing the negative effects of plastic waste on the environment.

Jameson, Laura  
Duke University  
**Investigation of the Impact of 6PPDQ on Metabolic State of Mitochondria in C. elegans**

The presence of tire rubber leachate 6PPD and its metabolite 6PPD quinone (6PPDQ) in roadway water runoff and streams, has resulted in mass toxicity, notably in Coho salmon. There is considerable inter-species variability in susceptibility to 6PPDQ toxicity; therefore, investigation of the mechanisms of action is necessary to accurately model risk and environmental contamination. Some previous literature suggests that 6PPDQ may interfere with mitochondrial function by uncoupling mitochondria. Here, we exposed the model organism *Caenorhabditis elegans* to 6PPDQ in a wide range of concentrations from 0.1 nM to 33μM and measured growth and metabolic state. We observed a slight decrease in volume, but not length, at the highest concentrations. Neurons are highly energetic cells with known susceptibility to energetic and redox challenges, so we measured the ratio of ADP:ATP in dopaminergic neurons with a reporter strain. This ratio can be used as a broad measure of mitochondrial energetic state potentially reflective of electron transport chain dysfunction, energy utilization changes, or imbalance with glycolysis. Preliminary results indicate that exposure to 6PPDQ results in non-monotonic dose-dependent effects on total ATP levels in dopaminergic neurons in *Caenorhabditis elegans*, however ATP:ADP ratios remain consistent at all concentrations. This result is not consistent with mitochondrial uncoupling. In ongoing work we are seeking to better characterize the effects of 6PPDQ on the redox state of muscle cells, which may provide insight into the potential for oxidative stress and locations of oxidative stress signaling. Our findings show that (1) despite high inter-species variability in susceptibility, the model organism *C. elegans* is responsive to 6PPDQ exposure and (2) additional work is needed to mitochondrial mechanisms of action of 6PPDQ toxicity.

Knapp, Bridget  
ORISE & US EPA  
**Identifying Negative Control Chemicals to Improve the Sensitivity and Specificity of Larval Zebrafish Behavior Assays**

Identifying reliable positive and negative control compounds is integral to determining the sensitivity and specificity of chemical exposure assays. Our laboratory assesses chemicals for developmental neurotoxicity potential using zebrafish screening assays that measure larval zebrafish locomotor behavior in response to visual stimuli. For such assays, there are established positive controls, however, identification of negative controls is lacking. Martin and coworkers (PMID: 35908584) conducted a literature review that identified nine chemicals that appeared to have no evidence of developmental neurotoxicity in mammals. To test if these chemicals would be appropriate negative controls for a zebrafish behavioral assay, we first performed a range-finding study to assess their lethality and teratogenic potential (≤100μM). Doses that were found to cause death or gross developmental malformations were excluded from further testing. Second, after exposure to each chemical, locomotor activity was assessed at 6 days post fertilization using a light/dark transition assay and analyzed using 13 different aspects of the locomotor profile (e.g., average speed, habituation, maximum and minimum activity, aspects of the startle response, and area under the curve (AUC)). Developmental exposure to the positive control (fluoxetine) decreased total average speed and average speed in the light and dark and...
increased the dark AUC:light AUC ratio. Developmental exposure to many of the postulated negative controls (i.e., L-ascorbic acid, D-mannitol, saccharin, sodium benzoate, or metformin hydrochloride) did not affect any endpoint; however, selegiline hydrochloride decreased average speed in the light and increased the dark AUC:light AUC ratio. Therefore, selegiline hydrochloride may not be an appropriate developmental neurotoxicity negative control for this zebrafish behavioral assay. Standardizing a set of negative control chemicals is essential for researchers to properly evaluate the behavioral results obtained from unknown chemicals and will assist with assessing new chemical screening methods. This abstract does not reflect the official policy of the US EPA.

Korol-Bexella, Evgenia
ORISE & US EPA
Refining High-Throughput In Vitro - In Vivo Extrapolation Modeling Through Incorporation of Intestinal Toxicokinetics

Under the Toxic Substances Control Act (TSCA) and other statutes, the US Environmental Protection Agency (US EPA) is authorized to regulate commercial chemicals that do not fall under the jurisdiction of other US federal agencies. With 42,170 chemicals listed on the 2023 TSCA active inventory, there is a clear need for a high-throughput (HT) risk-based prioritization and assessment. HT screening (HTS) for toxicity and toxicokinetic (TK) data are often used in combination with HT in vitro-in vivo extrapolation (IVIVE) modeling to allow the conversion of in vitro points of departure (POD) and steady state blood concentrations (Css) to an administered equivalent dose (AED) in mg/kg/day. This represents the external dose required to achieve Css values equal to the in vitro POD, in effect serving as an estimate for in vivo POD. The generic HTTK modeling approach exhibits a lack of concordance between predicted AEDs and actual in vivo low effect levels (i.e., PODs) observed in animal studies. This is partially because the HTTK approach primarily considers plasma protein binding and hepatic and nonmetabolic renal clearance. By excluding extrahepatic metabolism, whole body clearance processes are underestimated, resulting in Css overestimations. The model proved to be, on average, 100-fold more conservative across all comparisons of predicted AEDs to in vivo-derived PODs. This study evaluates the impact of incorporating intestinal clearance data on AED-in vivo POD concordance through consideration of CYP3A4 enzymatic contribution. Since over 80% of intestinal cytochrome P450 (CYP) metabolism is due to CYP3A4, incorporating CYP3A4 contribution to clearance could serve as an efficient surrogate for intestinal clearance. CYP3A4 enzymatic contribution to clearance was experimentally measured for 12 chemicals using human liver and intestinal microsomes incubated with and without a specific CYP3A4 inhibitor. The addition of CYP3A4 enzyme kinetics altered the oral bioavailability (Foral), Css and fraction escaping gut metabolism (Fg) predictions made by the HT IVIVE model. Css predictions typically decreased with increasing contribution of CYP3A4 in chemical clearance. Estimates of Fg for diazinon, acetochlor, azoxyxystrobin and difenoconazole dropped from a default of 1 to as low as 0.76 (difenoconazole) after CYP3A4 enzyme kinetics were incorporated in the calculations. Incorporation of Caco-2 data to adjust fraction absorbed through the intestine resulted in even lower Foral and Css values. Moreover, the results showed a consistent increase in the predicted AEDs, confirming greater agreement with available in vivo PODs. Future work will evaluate ways in which CYP3A4 contribution and intestinal absorption can be incorporated into the HTTK model to adjust Foral, Fgut, and fraction absorbed (Fabs). Ultimately this will result in a more accurate risk-based prioritization strategy of the existing commercial chemical space. This abstract does not necessarily reflect the views of the US EPA.

Krone, Paul
ORISE & US EPA
A Cheminformatics Workflow for Higher-Throughput Modeling of Chemical Exposures from Biosolids

Biosolids are treated sewage sludge produced as a byproduct of the wastewater treatment process, often applied to land or disposed of in landfills. Under the Clean Water Act, the US EPA Office of Water (OW) has the responsibility to protect human health and the environment from adverse effects of pollutants that may be present in biosolids. US EPA has identified over 400 chemicals found in biosolids in previous National Sewage Sludge Surveys (NSSS). To prioritize these chemicals for further risk screening and assessment, OW has developed the Biosolids Screening Tool (BST), a software tool implementing a model for potential human and ecological exposures to biosolids chemicals. The BST requires many chemical-specific input parameters, including physico-chemical and fate and transport properties, which would ordinarily need to be collected and entered manually.
for one chemical at a time — a time-consuming and potentially error-prone process. Instead, we have developed an automated workflow that efficiently parameterizes the BST for hundreds of NSSS chemicals by integrating chemical information from publicly available cheminformatics databases and tools: the US EPA CompTox Chemicals Dashboard; the OPEn (Quantitative) Structure-activity/property Relationship App (OPERA); the ClassyFire chemical-classification tool; and the httk R package. The R-based workflow interfaces with the existing Microsoft Access implementation of the BST via Microsoft Excel input/output. Using the newly-developed workflow, we parameterized and evaluated the BST for hundreds of NSSS chemicals in about 30 hours. The workflow is transparent, reproducible, and can be applied to additional chemicals to rapidly predict exposures as new measured or predicted biosolids concentration data become available. This workflow demonstrates the power of cheminformatics to facilitate rapid chemical prioritization to protect human health and the environment. The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.

Ledbetter, Victoria
US EPA
A New Approach Methodology (NAM) to the Prediction of Tumorigenesis
Current methods for cancer risk assessment are resource-intensive and not feasible for the vast majority of the thousands of untested chemicals. In earlier studies, we developed a new approach methodology (NAM) to identify liver tumorigens using gene expression biomarkers and associated tumorigenic activation levels (TALs) after short-term exposures in rats. The biomarkers are used to predict the six most common rodent liver cancer molecular initiating events (MIE). In the present study, we wished to confirm that our approach could be used to identify liver tumorigens at only one time point/dose and if the approach could be applied to (targeted) RNA-Seq analyses. Male rats were exposed by daily gavage for 4d to 15 chemicals at doses with known chronic outcomes; liver transcript profiles were generated using Affymetrix arrays. Our approach had 75% or 85% predictive accuracy using TALs derived from the TG-GATES or DrugMatrix studies, respectively. In a dataset generated from the livers of male rats exposed to 16 chemicals at up to 10 doses, we found that our NAM coupled with targeted RNA-Seq (TempO-Seq) could be used to identify tumorigenic chemicals with predictive accuracies of up to 91%. Overall, these results demonstrate that our NAM can be applied to both microarray and (targeted) RNA-Seq data generated from short-term rat exposures to identify chemicals and their doses that would induce liver tumors, one of the most common endpoints in rodent bioassays. (This abstract does not represent US EPA policy.)

Masood, Syed
UNC-CH
Detecting Protein Sulfenylation in Human Airway Epithelial Cells (HAEC) Exposed to Environmental Peroxides
Exposure to airborne fine particulate matter (PM2.5) is a leading cause of morbidity and mortality worldwide. A major progenitor of PM2.5 is derived from the atmospheric oxidation of isoprene by hydroxy radical to form isoprene hydroxy hydroperoxide (ISOPOOH). ISOPOOH formation is well understood however relatively little is known about the adverse health effects of ambient ISOPOOH exposure. We previously showed that ISOPOOH exposure induces oxidative stress by inducing glutathione oxidation independently of the generation of intracellular H2O2 in HAEC. We have also demonstrated that ISOPOOH exposure alters intracellular NAPDH levels in HAEC. NAPD+ is reduced to NAPDH by the metabolism of glucose in the pentose phosphate pathway. The shifting of glucose metabolism from glycolysis to pentose phosphate pathway requires the sulfenylation of GAPDH. Our current hypothesis is that in addition to ISOPOOH induced glutathione oxidation, ISOPOOH exposure of HAEC leads to protein sulfenylation specifically GAPDH sulfenylation which, in turn, leads to shunting of glucose to the pentose phosphate pathway. Our experimental approach primarily uses dimedone-based reagents and copper catalyzed azo-alkynyl cycloaddition reactions to tag intracellular protein sulfenic acids. Low micromolar exposure of H2O2 and ISOPOOH induces dose-dependent protein sulfenylation of GAPDH. Exposure of GAPDH to H2O2 or ISOPOOH in the presence of glutathione leads to glutathionylation of GAPDH. Fluorescent images of intracellular protein sulfenylation were detected in HAEC exposed to ISOPOOH. Furthermore, ISOPOOH exposure of HAEC induces hyperoxidation of intracellular proteins. These findings demonstrate that ISOPOOH is cause structural changes in intracellular proteins and is a potent environmental
hydroperoxides capable of inducing oxidative stress through mechanisms involving glutathione oxidation and selenylation regulatory proteins in HAEC.

McLean, Zachary
NCSU

*Haemogregarina crocodilnorum* Infection as a Biomarker of Adverse Health Impacts of PFAS in American Alligators (*Alligator mississippiensis*)

Per- and polyfluorooalkyl substances (PFAS) are persistent contaminants linked with adverse immune health outcomes in humans and animals. We demonstrated that increased concentrations of PFAS are associated with increased innate immune activity and autoimmune-like phenotypes in American alligators. We sought to systematically quantify numbers of *Haemogregarina crocodilnorum*, an apicomplexan blood parasite, and evaluate whether parasite infection was associated with PFAS concentrations and adverse phenotypes. Because some human auto-immune diseases are associated with low parasite infection, and host-parasite dynamics can be impacted by environmental factors and health status of the host, we hypothesized that *H. crocodilnorum* would be decreased in alligators with elevated blood PFAS concentrations. To address this hypothesis a comparative study was conducted by collecting blood samples from animals at Greenfield Lake, a site connected to the Cape Fear River, and Lake Waccamaw (LW), a site located in the adjacent Lumbar watershed with low PFAS contamination. Following quantification of blood PFAS concentrations, whole blood slides were stained, and *H. crocodilnorum* infected red blood cells were scored. *H. crocodilnorum* infections in each infected animal were significantly lower in alligators from Greenfield Lake. Correlation analysis revealed a negative correlation between PFAS concentrations and *H. crocodilnorum*. This work provides novel evidence that PFAS exposure can alter health biomarkers and host-parasite dynamic, important groundwork for establishing the utility of *H. crocodilnorum* as a biomonitoring tool of organismal and ecosystem health. Ongoing analysis is evaluating correlations between *H. crocodilnorum* abundance, PFAS concentrations and health-related endpoints in alligators.

McNell, Erin
NIEHS

Gestational Exposure to Phthalates and Replacements may Increase Maternal Blood Pressure Throughout Pregnancy

Phthalate plasticizers are found in numerous consumer products, many of which are used daily. Phthalates have endocrine-disrupting properties, thus gestational exposure may disrupt hormonal balance and contribute to pregnancy complications, including hypertensive disorders such as gestational hypertension and preeclampsia. In addition to traditional phthalates, replacement compounds with unknown health effects are emerging. This study aims to investigate the prospective association between gestational exposure to phthalates and replacements and blood pressure throughout pregnancy within the Human Placenta and Phthalates Study. The study included 291 pregnant persons from Norfolk, VA and Galveston, TX, recruited between 2017-2020. Urine samples were obtained at up to 8 visits throughout gestation, and metabolites of eight phthalates and two replacements were quantified. For compounds with multiple metabolites, molar sums were calculated as exposure biomarkers. Geometric mean metabolite or exposure biomarker concentration was calculated from repeated measures in early gestation (12-15 weeks) and across pregnancy (12-38 weeks). Maternal systolic and diastolic blood pressure was measured at routine obstetric visits. Linear mixed effects (single pollutant) and quantile g-computation (multi-pollutant) models, adjusted for several maternal characteristics, were used to estimate the difference in longitudinal blood pressure for a one-interquartile range (IQR) increase in early pregnancy and pregnancy-average phthalate and replacement exposure biomarkers. Single pollutant models showed that a one-IQR increase in the sum of diisononyl phthalate metabolites in early pregnancy is associated with a 1.31 mmHg increase in systolic blood pressure ([95% confidence interval: 0.25, 2.37]; p=0.016) and a 0.90 mmHg increase in diastolic blood pressure ([0.16, 1.65]; p=0.018). In multi-pollutant models, a simultaneous one-IQR increase in exposure biomarker concentrations for several phthalates or replacements was associated with increased blood pressure. Our findings suggest that gestational exposure to phthalates and replacements, especially when exposed to multiple compounds as a mixture, may contribute to increased maternal blood pressure throughout pregnancy.
Miller, Sarah
UNC-CH

Transcriptomics Similarity Scoring Using Toxicological Databases to Anchor In Vivo Wildfire Smoke Exposure Data to Human Pulmonary Disease Outcomes
Incidence and severity of wildfire events continue to increase, putting human populations at greater risk for development and exacerbation of pulmonary disease. Difficulties persist in wildfire health research due to the wide variety of exposure conditions that can be generated during wildfire events (highly dependent upon fuel type and combustion variations), coupled with the wide variety of pulmonary outcomes that can be impacted by such exposures. These variations have made it difficult to establish which types of wildfires impact specific pulmonary health outcomes. To address this knowledge gap, this study set out to couple database mining methods with our recently developed transcriptomic similarity scoring approach to better understand exposure disease linkages covering a wide landscape of wildfire relevant exposures. Specifically, we mined the Computational Toxicogenomics Database (CTD) and Molecular Signatures Database (MSigDB) for human consensus gene signatures of pulmonary diseases that have been putatively linked to wildfire exposures. These gene signatures were then compared to lung transcriptional changes from mice exposed to ten unique biomass burn conditions in a lab setting; namely eucalyptus, pine, pine needles, peat, and red oak burned under flaming (high temperature) as well as smoldering (low temperature) combustion conditions. Transcriptomic similarity scoring, primarily reliant on Jaccard indices, was performed to quantify gene response similarities between human pulmonary disease profiles and mouse exposure profiles. Scoring yielded groups of signatures with extensive similarities, such as those present in interstitial lung disease and induced by exposure to flaming red oak smoke condensates, suggesting that red oak biomass fuel burns may impact this specific disease outcome. In conclusion, this study leverages in silico modeling, human disease databases, and advanced lab-based wildfire exposure studies to uncover needed insights between wildfire smoke exposures and human disease outcomes.

Moore, Nia
ECU

Developmental Toxicity and Immunotoxicity of Perfluoropentane Sulfuric Acid
Perfluoropentane sulfuric acid (PFPeS) is a synthetic organic chemical that belongs to the class of per- and polyfluoroalkyl substances (PFAS). PFPeS is used in older aqueous film-forming foams (AFFF) agents essential for fire suppression. PFPeS has been introduced into the environment through AFFF uses with the potential of contaminating drinking water. The health effects of other PFAS that have been studied include negative impacts on the developing immune system. To evaluate the developmental immunotoxicity potential of PFPeS, pregnant C57BL/6 dams were exposed via gavage for 17 days to concentrations of PFPeS: 0, 0.05, 0.5, or 1 mg/kg PFPeS in utero. Dam body weights, offspring body weights, and anogenital distance were collected during the study. Biomarkers consistent with PFAS exposure include liver peroxisomal enzyme activity and the T-cell dependent antibody response (TDAR). In the 0.05 mg/kg group, female offspring body weights were lower than controls up until postnatal day (PND) 10. In the 0.5 mg/kg group, male offspring anogenital distance was lower than controls up until PND7. In the 1.0 mg/kg group, female offspring peroxisomal activity measured in the livers was higher compared to controls. The TDAR in offspring did not differ statistically among dose groups in either sex. While some of the developmental markers differed, most biomarkers evaluated in this study did not differ statistically, indicating that at administered doses during the developmental window examined, PFPeS was not developmentally toxic or toxic to the developing immune system.

Moreira Filho, Jose Teofilo
NIEHS

MOVIZ: Democratizing Cheminformatics with a User-friendly and Guided Platform
Computational methods, especially machine learning (ML), have revolutionized chemogenomics research and chemical safety assessment, streamlining processes and cutting costs. However, limited accessibility and programming prerequisites hinder the widespread use of publicly available ML models. To democratize the use of these tools among non-experts, we are building a comprehensive modeling and visualization platform (MOVIZ), leveraging the free and open-source no-code/low-code data analytics environment of Konstanz.
This presentation outlines the design and implementation of MOVIZ, which offers an intuitive interface and extensive documentation for tasks such as data access, storage, mining, curation, analysis, modeling, and visualization. The MOVIZ pipeline will be showcased in an example of chemical grouping via supervised and unsupervised ML methods. This workflow enables users to input labeled or unlabeled chemical data and undergo preliminary analyses. For unlabeled data, users can manually select features and apply unsupervised clustering algorithms with automatically optimized hyperparameters. Visualization of the clusters in reduced dimensions can be achieved through Uniform Manifold Approximation and Projection (UMAP), Principal Component Analysis (PCA), or t-Distributed Stochastic Neighbor Embedding (t-SNE). For labeled data, automated feature selection is facilitated using Recursive Feature Elimination, Genetic algorithms, or Simulated annealing. Subsequently, users can opt for a supervised ML method to train a statistical model. SHapley Additive exPlanations (SHAP) values can be calculated to assess the significance of molecular descriptors for the modeled endpoint. At the end, the workflow provides options for visualization of the unsupervised clusters or the supervised classes as well as the most important molecular descriptors and structural features for the studied data. Additionally, the GPT-3.5 large language model is employed to auto-generate a natural language interpretation of the results. A comprehensive report is also available for download. This user-friendly chemical grouping workflow has been integrated into the NIEHS KNIME WebPortal, forming part of the NIEHS/DTT cyber-infrastructure.

Morton, Katherine
Duke University
High Sugar Diets Alter Toxicokinetics of 6-OHDA Uptake to Reduce Oxidative Stress Induction and Subsequent Dopaminergic Neurodegeneration
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 component of PD pathology, and that dietary factors such as a western diet increase risk for PD development. However, the mechanism by which western diets and mitochondrial toxicants induce PD remains unclear. In contrast to previous evidence that western diets increase PD risk, we show that with our experimental paradigm, a portion of a western diet, high sugar consumption, is protective from 6-hydroxydopamine (6-OHDA) induced dopaminergic neurodegeneration. Utilizing 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. 6-OHDA uptake into the neurons is dependent on the DAT-1 dopamine transporter, leading to our discovery that high sugar diets cause dat-1 downregulation, supporting the likelihood for lower 6-OHDA uptake. 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 over bioenergetic inhibition.

Nguyen, Helen
ORISE & US EPA
Paternal Eucalyptus Smoke Exposure Alters Metabolic Responses to Heat in Offspring
Paternal exposures to environmental stressors may program offspring susceptibility to metabolic deficits, in part, through epigenetic sperm alterations. We recently demonstrated that laboratory-generated wildfire smoke alters sperm motility and non-coding RNAs. Considering increasing wildfire frequency, our objective was to determine whether paternal smoke exposure alters metabolism in offspring exposed to heat, a prototypic metabolic stressor. 14-week-old male Long-Evans rats were episodically exposed to filtered air (FA) or eucalyptus smoke (WF; 4-5 mg/m3 PM, 10 ppm CO) for 1 hour/day over 2 weeks during sperm maturation. 24 hours following the final exposure, male rats were bred with estrus-synchronized female rats. Offspring were litter-standardized on PND 4 and weaned on PND 19. At approximately 5-months-old, offspring were exposed to standard vivarium temperature (N; 72oF) or heat (H; 78oF) for 2 weeks. Offspring were then euthanized, and serum was collected.

NIH National Institute of Environmental Health Sciences Division of Translational Toxicology RTI International inotiv Duke SP
for further assessments. Results revealed no significant differences in final rectal temperature, despite increased surface body temperature in heat-exposed males and females. Pre- and post-heat exposure body weights were also not significantly different between groups. Body weight gain, however, was significantly reduced by 31% in WFH males compared to FAN controls after 1 week and nearly significant at 2 weeks (p = 0.08). Furthermore, serum glucose levels were significantly higher in FAH (20%) and WFH (10%) males compared to FAN, suggesting heat alone impacted offspring glucose metabolism. Serum low-density lipoprotein cholesterol, a marker of dyslipidemia, was also 59% higher in WFH compared to FAN. Females showed no significant differences in body weight gain or serum clinical chemistry endpoints, regardless of paternal exposure or temperature condition. In summary, paternal smoke exposure resulted in sex-specific metabolic alterations in offspring exposed to heat. Our data suggest paternal exposure to air pollutants may program metabolic risk related to postnatal stressors. Abstract does not reflect US EPA policy.

Paul, Oindrila
NIEHS

Transcriptomic Responses in a Co-Culture Lung Cell System Indicate Unique Cell Type Transcriptional Responses to Acrolein Exposure

Exposure to inhaled compounds is ubiquitous in modern life. Understanding whether these exposures are of concern is a key goal of public health research. Understanding the impacts of these exposures has relied on human population studies, animal exposure studies, and in vitro cell culture studies; however, most in vitro studies have focused on cultures composed of a single cell type. Thus, the roles of various cell types as targets and/or mediators of exposure effects in tissue microenvironments remains poorly understood. Here, we tested the hypothesis that dose and donor impacted the transcriptional response to the model inhaled toxicant acrolein in differentiated primary human bronchial epithelial cells (dpHBECs) and adjacent primary human lung fibroblasts (pHLEF) in a donor-matched co-culture model of the human bronchial epithelium. We observed that acrolein exposure resulted in distinct transcriptomic profiles in the two different cell types specifically, only five genes overlapped between cell types. Further, when evaluated by principal component analysis, exposure-associated transcriptional changes in epithelial cells were influenced by differences in dose, but not donor, while the response in fibroblasts was influenced by donor, but not dose. Ingenuity Pathway Analysis of differentially expressed genes indicated that xenobiotic metabolism AHR signaling pathway, glutathione redox reactions I, and glutathione-mediated detoxification, were the most significantly alternatively regulated biological pathways in both cell types. Despite the differences, this similarity, the alternative regulation of these pathways were attributed to the activity of different transcriptional regulators in epithelial cells (ASB2, LEO1, and PAF1) and fibroblasts (NRF2, CRTC3, and ASB2). These findings show that acrolein elicits different transcriptomic responses in different cell types and that the effect of exposure within each cell type is predicated upon the baseline characteristics of the cell. Taken together these data demonstrate the utility of a diverse donor-derived multi-cellular in vitro systems for inhalation toxicology.

Pearson, Kashenya
NC A&T

Exploring the Effects of Doxorubicin on Triple Negative Breast Cancer Cell Cytotoxicity and Cell Viability

In this preliminary study, we aimed to investigate the cytotoxic effects of doxorubicin (DOX) in triple negative breast cancer (TNBC) cell line, HCC1806. The response of HCC1806 to DOX was examined after exposure to 0.5μM, 1.5μM, and 2.0μM concentrations of DOX for 3h and 3h DOX followed by 24h fresh medium. The cellular physiology, cell growth, and cell viability were evaluated at 0h, 48h, and 72h after passage through light microscopy and trypan blue exclusion assay, respectively. After DOX exposure, HCC1806 begun to exhibit morphological alterations that may be caused by the activation of apoptotic mechanisms as compared to the control cell groups. The trypan blue exclusion assay demonstrated the ability of DOX extracts to efficiently inhibit the growth potential of triple negative breast cancer cells with results also confirmed in viability. In each of the treatment conditions tested, all concentrations of DOX extracts demonstrated an equal or greater decrease of cell proliferation than the control groups. There was approximately a 4-fold change in cells treated for 3h followed by 24h in fresh medium and a 3-fold change in cells exposed to DOX for 3h compared to the cells in the control groups. These data suggest that DOX extracts have a greater capacity to prevent TNBC cell proliferation at
lower concentrations. The results also showed a significant decrease of approximately 40-50% of viable cells in HCC1806 cells after exposure to DOX extracts compared to cells not exposed to DOX. Our results indicate that treating TNBC with lower concentrations of DOX reduces tumor cell growth and significantly inhibits proliferation and viability. However, further evaluation of the molecular features of the viable cells can identify potential drug resistance pathways as well as molecular targets that may help to develop biomarkers for potential drug treatments to overcome this resistance mechanism.

Preston, Sydney
NCSU
Discovery and Characterization of a Novel UVB-Inducible Long Noncoding RNA that Suppresses p53 and Trp53cor1-Mediated Apoptosis
Long noncoding RNAs (LncRNAs) have important roles in disease and normal cellular function. LncRNAs also play key roles in the DNA damage response. We have identified a novel IncRNA that is upregulated by UVB-treatment in mouse and human skin, and keratinocytes in culture. In mice, the novel IncRNA transcript is transcribed from the (+) DNA strand on chromosome 17 in a region antisense to a portion of the pro-apoptotic IncRNA, Trp53Cor1 (aka lincRNA-p21), which is transcribed from the (-) DNA strand. Further characterization of the natural antisense transcript (NAT) to Trp53cor1 revealed it partially aligned with a computationally predicted IncRNA, called Gm41556. A 15-30-fold increase in the novel transcript is seen in both mouse keratinocytes and mouse epidermis when treated with UVB. RNAseq, PCR tiling, 3’ RACE, and IsoSeq analysis shows the inducible transcript has two exons ~3200 RNA bases in length separated by an exon of 2405 base pairs. Decreased induction of Gm41556 was observed in UVB-treated mouse keratinocytes treated with an ATM inhibitor, suggesting it is regulated in an ATM-dependent manner. Knockdown of Gm41556 in UVB-treated mouse keratinocytes results in increased p53 protein, cleaved-caspase 3 protein, and Trp53cor1 transcripts. These results suggest Gm41556 suppresses p53 protein levels and also suppresses Trp53cor1’s pro-apoptotic activity, possibly through a natural antisense transcript mechanism. Finally, in the human genome, genomic synteny shows a possible Gm41556 syntolog that partially aligns with a predicted IncRNA on chromosome 6, called XR_926759.2. Like the novel transcript, this IncRNA is expressed in response to UVB and has a ~27-fold increase in transcription in human keratinocytes. In summary, a novel IncRNA has been identified that is highly UVB-inducible and our results suggest it suppresses p53 protein and Trp53cor1-mediated apoptosis possibly through an antisense mechanism.

Ranganath, Dhruv
UNC-CH
Streamlining Chemical Grouping with a User-Friendly Automated Workflow
Computational methods like machine learning can help uncover meaningful structural and functional relationships in chemical datasets. However, applying these sophisticated algorithms can be daunting due to the level of expertise required. To overcome this challenge, a web application was developed using the open-source low-code data analytics platform called Konstanz Information Miner (KNIME) to guide users through the process of grouping and analyzing chemicals with machine learning algorithms. This workflow packages several features, including molecular fingerprint calculation, dimensionality reduction, supervised and unsupervised chemical grouping methods, visualization, and interpretation. The aim of this work was to test and improve this web application, making it more user-friendly for individuals with limited cheminformatics expertise. The main focus was on expanding the in-app documentation and refining the user interface to effectively present the diverse functionalities and options in a comprehensible manner. Certain backend scripts, such as those responsible for molecular fingerprint calculation, were optimized for greater efficiency, resulting in a significant reduction in execution time. An additional option was added that allows the user to save their selected settings in a configuration file and quickly reapply them to a new dataset. Interpretability through SHapley Additive exPlanations (SHAP) was also extended with the OpenAI GPT-3.5 Large Language Model, adding dynamically generated textual interpretations of feature importance. Finally, a tutorial video created using the AI tool Synthesia was added to offer a concise visual overview of the whole application. These features were all evaluated and optimized for both local and remote execution on the KNIME WebPortal. Overall, this web application offers a user-friendly and efficient approach for chemical grouping, contributing to the NIEHS/DTT cyber-infrastructure and benefitting researchers in various chemical data analysis tasks.
Rodriguez, Marc  
UNC-CH  
Sex Dependent Human Bronchial Epithelial Cell Effects Induced by Wood Smoke Particle Exposures  
Wildfires are an increasing threat to public health and source of particulate matter (PM) with climate change expected to amplify their prevalence. PM exposure from biomass burning is linked to outcomes such as reduced lung function, increased infection susceptibility, and higher inflammation levels, but the drivers of varied susceptibility to PM are not well understood. Composition of wildfire derived-PM varies by fuel type, which differs across the United States and could influence toxicity. Individual characteristics, such as sex, also modulate the effects of biomass smoke exposure, however mechanisms are unknown. We aimed to address the hypothesis that both fuel type and sex play a role in modulating observed respiratory health effects of smoke exposure. First, optimum exposure dose was determined using human bronchial epithelial cell line, 16HBE14o-, which was grown in submerged cultures and at air-liquid interface (ALI). Cultures were exposed to peat, pine, eucalyptus, and red oak-derived wood smoke particles (WSPs) in PBS for 4 hours (5μg/cm2 to 100μg/cm2). Cultures grown at ALI demonstrated dose-dependent increases in IL-8 and IL-6 responses. Submerged cultures showed an increase in IL-8 for all WSPs up to 25μg/cm2 and up to 50μg/cm2 for peat, but decreased at higher doses for all WSPs, suggesting potential cytotoxicity. Similarly, an increase in IL-6 was observed up to 50μg/cm2. Overall, these data suggest differential inflammatory effects of fuel type. Second, the optimized doses were used to evaluate the effect of sex on response in primary cells. Primary cell cultures (n=4 Male, n=3 Female) were grown at ALI and exposed to WSPs at 5μg/cm2 and 25μg/cm2 for 4 hours. Downstream analyses are still being conducted and cytotoxicity, inflammatory cytokines, and gene expression will be assessed. These data will impact the field of toxicology by uncovering new mechanisms of individual susceptibility and potential regional specificity of wildfire smoke outcomes.

Rogers, Jesse  
US EPA  
Integrating High Throughput Transcriptomics into a Tiered Framework to Prioritize Chemicals for Toxicity Testing  
US EPA is developing a tiered assessment strategy for chemical toxicity testing by integrating multiple data streams. Pairing high content assays such as high-throughput transcriptomics (HTTr) with high-throughput screening (HTS) of specific molecular targets may improve confidence when assessing key hazards. Here, we used HTTr screening data to generate new signatures representing known molecular targets by applying a univariate potency analysis to select genes uniquely responsive to reference chemicals based on their assigned mechanisms-of-action. Signature-level potency estimates were integrated with orthogonal HTS assays as a proof-of-concept framework for chemical prioritization. Transcriptomic profiles generated via the TempO-Seq platform in both HepaRG and U-2 OS cell lines were used to develop signatures comprised of genes selectively responsive to reference chemicals for one of 13 distinct molecular targets. Of 1,201 chemicals screened in HTTr to date, 232 chemicals produced a specific change in one or more reference signatures at concentrations lower than the bulk of transcriptional activity indicative of non-selective effects. Of these HTTr-predicted perturbagens, 182 chemicals had existing orthogonal HTS assay data from US EPA’s ToxCast program which were examined further, and 74 of these chemicals were confirmed as selective AHR, GR, or RAR/RXR nuclear receptor agonists in at least one HTS assay. Our work demonstrates that HTTr data identifies putative molecular targets to inform chemical selection for further screening in a framework to support chemical risk assessment. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.
Scherer, Meredith  
ORISE & US EPA  
*In Vitro* Distribution Model Evaluation  
To establish toxicity reference values, risk assessments require reliable methods with well-characterized uncertainty. Next generation risk assessment will require *in vitro* to *in vivo* extrapolation (IVIVE) to translate observed cellular responses to whole organisms. IVIVE informs quantitative dose-response using mathematical modeling and *in vitro* bioactivity assay data. *In vitro* disposition is an important part of IVIVE and refers to the way that a given chemical partitions within the *in vitro* system via binding to the plate wall, media, proteins, cells, and volatilization to air. The distribution of a chemical dictates the difference between the nominal and bioavailable chemical concentration that causes any observed effects. Chemical distribution varies across chemicals as a function of both inherent chemical properties and *in vitro* test conditions. Given that *in vitro* bioactivity screening has been performed across large chemical libraries (for example, ToxCast and Tox21), there is a critical need to accurately predict *in vitro* distribution for many chemicals. Currently, most IVIVE models only use the nominal values. To address this gap, the available literature was evaluated to find studies that reported experimentally derived intracellular concentrations in *in vitro* tests. Information characterizing the experimental conditions was then input to the Armitage et al. (2014) *in vitro* disposition model as implemented within R package “httk”. The Armitage model predictions correlate with the measured experimental values but tend to overpredict the intracellular concentrations. As a result, other models that are relying on these predictions could be underpredicting toxicity. This would have ramifications for both risk assessment and decision making.

Singh, Anisha  
NIEHS  
**Becoming aWARE: The Development of a Web-based Tool for Autism Research and the Environment**  
A sharp rise in autism spectrum disorder (ASD) prevalence estimates, beginning in the 1990s, suggested etiological factors beyond genetics. This stimulated increased research investment in nongenetic factors, including the study of environmental chemical exposures, diet, nutrition, lifestyle, social factors, and maternal medical conditions. Consequently, both peer- and non-peer reviewed bodies of evidence investigating environmental contributors to ASD etiology have grown significantly, resulting in a large and heterogenous body of evidence. Our objective is to identify and characterize published literature relevant to environmental exposures associated with ASD and summarize/map the research gaps and knowledge clusters. Therefore, we propose to develop a Web-based tool for Autism Research and the Environment (aWARE) using a systematic evidence map (SEM) approach to comprehensively aggregate and characterize this highly variable and often conflicting data. Here, formulation of a single and tightly focused research question is challenging, and of limited utility. However, SEMs specifically address these issues by displaying complex evidence in an interactive format that presents the opportunity to explore multiple research questions within the scope of the SEM. SEMs combine visual presentation with a methodologically rigorous approach to 1) collate data from different sources; 2) extract and store unstructured data in structured format; and 3) categorize data based on controlled vocabulary codes enabling meaningful comparison of evidence regardless of its heterogeneity. Hence, the SEM-based aWARE tool would be a comprehensive queryable database which will enable users with computational access to explore, organize, compare, and analyze the evidence base. Throughout tool development, listening sessions and workshops will be used to seek perspectives from the broader autism community. Thus, active community engagement is a unique foundation of this SEM-based tool. New evidence will be indexed in the tool annually, which will serve as a living resource to investigate the association between environmental factors and ASD.
Adopting Duplex Sequencing Technology for Genetic Toxicity Testing: A Proof-of-Concept Mutagenesis Experiment with N-Ethyl-N-Nitrosourea (ENU)-Exposed Rats

Duplex sequencing (DuplexSeq) is an error-corrected next-generation sequencing (ecNGS) method in which molecular barcodes informatically link PCR copies back to their source DNA strands, enabling computational removal of errors by comparing grouped strand sequencing reads. The resulting background of less than one artifactual mutation per 10^7 nucleotides allows for direct detection of somatic mutations. TwinStrand Biosciences, Inc. has developed a DuplexSeq-based mutagenesis assay to sample the rat genome, which can be applied to genetic toxicity testing. To evaluate this assay for early detection of mutagenesis, a time-course study was conducted using male Hsd:Sprague Dawley SD rats (3 per group) administered a single dose of 40 mg/kg N-ethyl-N-nitrosourea (ENU) via gavage, with mutation frequency (MF) and spectrum analyzed in stomach, bone marrow, blood, and liver tissues at 3 h, 24 h, 7 d, and 28 d post-exposure. Significant increases in MF were observed in ENU-exposed rats as early as 24 h for stomach (site of contact) and bone marrow, blood, and liver tissues at 3 h, 24 h, 7 d, and 28 d post-exposure. The canonical, mutational signature of ENU was established by 7 d post-exposure in all four tissues. Interlaboratory analysis of a subset of samples from different tissues and time points demonstrated remarkable reproducibility for both MF and spectrum. These results demonstrate that MF and spectrum can be evaluated successfully by directly sequencing targeted regions of DNA obtained from various tissues, a considerable advancement compared to currently used in vivo gene mutation assays.

Evaluation of the Herbicide Glyphosate, (Aminomethyl)phosphonic Acid, and Glyphosate-Based Formulations for Genotoxic Activity Using In Vitro Assays

Glyphosate, the most heavily used herbicide world-wide, is applied to plants in complex formulations that promote absorption. The National Toxicology Program reported in 1992 that glyphosate, administered to rats and mice at doses up to 50,000 ppm in feed for 13 weeks, showed little evidence of toxicity, and no induction of micronuclei was observed in the mice in this study. Subsequently, mechanistic studies of glyphosate and glyphosate-based formulations (GBFs) that have focused on DNA damage and oxidative stress suggest that glyphosate may have genotoxic potential. However, few of these studies directly compared glyphosate to GBFs, or effects among GBFs. To address these data gaps, we tested glyphosate, glyphosate isopropylamine (IPA), and (aminomethyl)phosphonic acid (AMPA, a microbial metabolite of glyphosate), 9 high-use agricultural GBFs, 4 residential-use GBFs, and additional herbicides (metolachlor, mesotrione, and diquat dibromide) present in some of the GBFs in bacterial mutagenicity tests, and in human TK6 cells using a micronucleus assay and a multiplexed DNA damage assay. Our results showed no genotoxicity or notable cytotoxicity for glyphosate or AMPA at concentrations up to 10 mM, while all GBFs and herbicides other than glyphosate were cytotoxic, and some showed genotoxic activity. An in vitro to in vivo extrapolation of results for glyphosate suggest that it is of low toxicological concern for humans. In conclusion, these results demonstrate a lack of genotoxicity for glyphosate, consistent with observations in the NTP in vivo study, and suggest that toxicity associated with GBFs may be related to other components of these formulations.

Lipidomic and Proteomic Plasma Evaluations Reveal Biomarkers for the Diagnosis of Domoic Acid Toxicosis in California Sea Lions

Domoic acid is a neurotoxin commonly found in the ocean and secreted by the marine diatom genus, Pseudo-nitzschia spp. Unfortunately, California sea lions (Zalophus californianus) are readily exposed to domoic acid through its accumulation in small fish. This exposure contributes to a high rate of domoic acid toxicosis (DAT) which presents as sea lions being stranded on the beach with cardiomyopathy and neurological dysfunctions such as disorientation and seizures. A previous proteomic study revealed three apolipoproteins (apolipoprotein E, B-100, and C-III) as candidate biomarkers for DAT in the sea lions. As this suggests lipid dysregulation, we performed lipidomic analyses of the same sea lion plasma to determine potential lipid biomarkers of DAT for rapid diagnosis and treatment. This retrospective sample set is comprised of plasma from 31 sea lions taken at
or near the time of admission at The Marine Mammal Center. Fourteen were diagnosed with DAT based on clinical signs and histological examination of brain tissue and the seventeen that were not are referred to as the non-DAT group. In this study, lipids were extracted using a modified Folch extraction and were analyzed using a platform combining liquid chromatography, ion mobility spectrometry, collision-induced dissociation, and mass spectrometry (LC-IMS-CID-MS). Lipid identification was performed using Skyline software with a library containing 994 lipids. Statistically significant differences in lipid identities were determined by comparing those in the two groups. Almost 400 lipids have been confidently identified in the plasma samples. At present, additional data analysis is being performed on the samples and then statistical comparisons will be made on the DAT and non-DAT groups to investigate headgroup and fatty acyl trends corresponding to the statistically significant lipids.

Sriram, Anuragh
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Evaluation of the Potential for Strobilurins to Modify Neuroinflammatory Responses
Strobilurins are a class of commercial fungicides used globally, and work by dysregulating oxidative phosphorylation. Recent studies have implicated strobilurins as a potential developmental neurotoxicant, as their exposure in vitro has yielded gene expression profiles in cortical neurons that mimic profiles observed in neuroinflammation, neurodegeneration, and neurodevelopmental disorders. One cellular mechanism underlying inflammatory regulation involves the activation of an inflammasome, a multi-protein complex utilized for the detection of pathogenic microorganisms and sterile stressors. One such inflammasome, the NLRP3 inflammasome, follows an activation sequence that involves priming mediated by various substrates, succeeded by a secondary, sterile, triggering stimulus (e.g., ATP). Activation leads to the release of mature interleukin-1β (IL-1β) and IL-18, along with a protein aggregate called ASC Speck protein, which can have subsequent effects on surrounding tissue. In the brain, this process occurs within microglial cells, the brain resident immune cell. Given the hypothesized association between mitochondrial dysregulation and NLRP3 inflammasome activation, this study aimed to investigate the potential for strobilurins to function as secondary triggers for NLRP3 inflammasome activation, thereby characterizing their inflammatory potential. Murine BV-2 ASC-Cerulean reporter cells were used to examine the production of the ASC Speck complex. Cells were primed with a low dose of lipopolysaccharide (LPS) prior to exposure with apyrase and trifloxystrobin, a strobilurin experimentally shown to get into the brain. The formation of fluorescent ASC-Speck aggregates was quantitated using live-cell imaging. Trifloxystrobin was able to function as a potential secondary trigger for NLRP3 inflammasome activation, with a dose response across the range of 62.5 nM – 250 nM of trifloxystrobin. This was accompanied by an increase in the number and distribution of ASC Specks. This data supports the potential for neurotoxicity being proposed for these compounds, especially in cases of neurodegeneration and neurodevelopmental disorders, as they have some underlying etiology associated with the dysregulated neuroinflammatory response of microglia.

Starnes, Hannah
NCSU
In Vitro and In Silico Methods for the Assessment of Serum Albumin Interactions with PFAS
Per- and poly-fluorinated compounds (PFAS) are a diverse class of over four thousand man-made toxic chemicals that can bioaccumulate in humans and wildlife. Most toxicokinetic data describing PFAS is limited to a small subset of compounds, despite the chemical diversity of the class. Data describing PFAS-protein binding kinetics are critically needed to maximize the predictive power of physiologically based pharmacokinetic modeling approaches. We have demonstrated the utility of the in vitro differential scanning fluorimetry (DSF) assay for rapid, high-throughput determination of relative binding affinities of PFAS for serum albumin. Comparative assessment of species differences in PFAS binding between humans and experimental and livestock animals is needed to understand differences in PFAS absorption and distribution across species. In this study, we utilize DSF to compare binding affinities of serum albumin from human and experimental and livestock animals (bovine, porcine, and rat) to PFAS, to define differences in concentration response relationships and dissociation constants (Kd) for individual compounds across species. This comparative assessment of binding kinetics across species will strengthen the relevance and applicability of non-animal models of PFAS binding and elucidate potential factors involved in differential transport, bioaccumulation, and resulting toxicity. These data will contribute to the increased accuracy of computer-based modeling of PFAS behavior in the body. Additionally, through coupling of DSF-generated data with machine learning methods, we
are working to predict albumin binding affinities for thousands of PFAS congeners in which no experimental toxicokinetic data currently exists.

Stuckey, Kristina
UNC-CH
Exploring Associations of Prenatal Per- and Polyfluoroalkyl Substances Exposure to Autism Spectrum Disorder and Differential Gene Expression in Extremely Low Gestational Aged Newborns

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous and pose great risks. Potential adverse maternal and fetal health outcomes related to PFAS exposure include decreased birth weight, higher rates of preterm birth and preeclampsia, and low birth weight. However, there is limited research of the impacts of exposure in infants born before 28 weeks’ gestation. The study aimed to analyze how PFAS concentrations in maternal blood affect (1) gene expression changes in the placenta and (2) the development of ASD at age 10. These analyses utilized information from the Extremely Low Gestational Aged Newborns (ELGANs) cohort. The PFAS measured included Perfluorooctane sulfonic acid (PFOS), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), and Perfluorohexane sulfonic acid (PFHXS). A standard least squares regression analysis was performed to measure placental differential gene expression. Individually, PFOS, PFOA, PFNA, and PFHxS were associated with 363, 28, 149, and 20 differentially expressed genes (DEGs), respectively. There were 10 DEGs shared between two PFAS compounds (2 between PFOA and PFHxS, 4 between PFOA and PFOS, 1 between PFOS and PFNA, 3 between PFNA and PFOA). The 10 shared genes related to transcription factors and neurocognition pathways. Similarly, for the individual DEGs, many of the pathways related to transcription factors, neurocognition, and inflammation. A nominal logistic regression analysis was used to measure associations between individual PFAS exposure and ASD. No statistically significant associations between any individual PFAS and ASD were found. These results suggest that individual PFAS exposure could potentially elicit different molecular responses and health outcomes, however, further research is necessary for elucidating the potential impacts of prenatal PFAS exposure to infants born extremely premature.

Sutton, Khiry
WFIRM
Airway Organoids for Disease and Toxicity Modeling and Countermeasure Development

Currently, research studies lack the capacity to efficiently model the in vivo environment and functionality of tissues/organs in response to infection, disease, drugs and medical countermeasures. The goal of our study was to develop a multicellular lung airway organ tissue equivalent (OTE) that can be integrated into a microfluidic device at air-to-liquid interface (ALI) for modeling aerosolized toxins and possible countermeasures. This organoid model is expected to behave more physiologically relevant with respect to the human pulmonary environment. The 3D airway organoids were constructed by seeding lung fibroblasts within an interstitial hydrogel with a monolayer seeding of microvasculature endothelial cells and lung bronchial epithelial cells on the basal and apical sides, respectively. Cultures are fabricated within a microfluidic device and can be maintained through peristaltic flow. The microfluidic device is adapted to connect to a modified toxic gas exposure system allowing for a precise control of final airway exposure. Organoids can be successfully exposed to a wide range of toxic gases at varying doses and analyzed through chemical (ph, ROS, and chlorinated macromolecules), biological (viability, inflammation, and cell function) as well as multi-omics cellular assays. Both gap junction staining, and TEER measurements were utilized to show the physiological barrier function of the primary epithelial cells with significant increases in TEER values when the culture is switched and maintained in ALI culture. The multicellular airway organoid will more accurately mimic the biological environment of the human upper airway tract compared to current 2D models allowing for more significant physiological responses to infection, toxicity and medical countermeasures. Furthermore, this model accurately displays cellular responses based on desired concentrations of toxic gases. The cell types behave differently than in standard 2D culture showing the importance of both a 3D environment and ALI culture conditions.
Szabo, Gillian  
NCSU  
Environmental Circadian Disruption Heightens Immuno- and Hepatotoxicity of PFOA in SKH-1 Outbred Mice  
Contact author for more information.

Tisch, Logan  
NCSU  
Proteinase-Activated Receptor-2 Regulates the Production of Fibrotic Mediators by Murine Bone Marrow-Derived Macrophages, Ex Vivo Alveolar Macrophages, and Mouse Lung Fibroblasts in Response to Multiwalled Carbon Nanotubes in an Asthma-like Microenvironment  
Proteinase-activated receptor 2 (PAR2), a 7-transmembrane G protein-coupled receptor, is implicated in immune regulation and the pathogenesis of various inflammatory diseases, including pulmonary fibrosis and asthma. PAR2 is activated by proteolytic cleavage of the N terminus by serine proteases, including those found in the extract of house dust mites (HDM). We previously reported that co-exposure to HDM extract and multiwalled carbon nanotubes (MWCNTs) synergistically enhanced allergic lung inflammation in mice and that Par2 KO mice had reduced airway fibrosis and arginase-1 (ARG-1) expression compared to wildtype (WT) mice. In this study, we explored the effects of PAR2 on profibrotic cytokine production and intracellular signaling in primary bone marrow-derived macrophages (BMDMs), mouse lung fibroblasts (MLFs), and murine ex-vivo alveolar macrophages (mexAMs) in response to MWCNTs in an asthma-like microenvironment consisting of the Th2 cytokines IL-4 and IL-13. Additionally, MWCNT-induced polarization of BMDMs and murine ex-vivo alveolar macrophages (mexAMs) was investigated using flow cytometry. In both Par2 -/- BMDMs and MLFs, the secretion of profibrotic cytokines osteopontin (OPN) and transforming growth factor-β1 (TGF-β1) was enhanced compared to WT cells. Additionally, the expression of collagen mRNAs (col1a1 and col1a2) was upregulated in Par2 -/- MLFs compared to WT MLFs stimulated with TGF-β1. Interestingly, treatment with MWCNTs in the presence of IL-4 and IL-13, increased ARG-1 production in WT compared to Par2 -/- BMDMs. MWCNTs increased the polarization of BMDMs to an M2 or profibrotic phenotype for both WT and Par2 -/- cells. However, M2 polarization of mexAMs was increased in Par2 -/- cells compared to WT cells. These findings indicate that PAR2 regulates the production of fibrotic mediators by MWCNTs in macrophages and fibroblasts in a cell-type-specific manner. Additionally, MWCNTs further facilitate the production of fibrotic mediators through the polarization of macrophages.

Tobin, Emma  
NCSU  
C/EBPβ Mediates Keratinocyte Apoptosis After UVB-Induced DNA Damage via Regulation of the Type I IFN Response and Extrinsic Apoptosis  
The human epidermis is routinely subjected to DNA damage induced by solar radiation (UVB). Keratinocytes have developed intricate mechanisms to respond to UVB-induced DNA damage, including cell cycle checkpoints, DNA repair, and cell death pathways. The decision to undergo regulated cell death (also known as apoptosis) after DNA damage is vital in preventing damaged cells from progressing into cancer cells. The basic leucine zipper transcription factor CCAAT-enhancer/binding protein β (C/EBPβ) is highly expressed in epidermal keratinocytes. Our previous studies have reported that C/EBPβ is a suppressor of epidermal keratinocyte apoptosis in response to UVB-induced DNA damage and that C/EBPβ is required for skin tumor formation and skin tumor survival. The precise mechanism by which C/EBPβ regulates apoptosis in keratinocytes is still unclear. Here we utilize siRNA-mediated gene knockdown and pharmacological inhibition to examine the role of C/EBPβ in regulating UVB-induced apoptotic cell death. We found that C/EBPβ regulates caspase-3 mediated apoptosis that is dependent on cytochrome c release following mitochondrial outer membrane permeabilization (MOMP) and is regulated by the pro-apoptotic protein BH3 interaction-domain death agonist (Bid). In C/EBPβ deficient keratinocytes, the activation of Bid is driven by an extrinsic apoptosis pathway that is surprisingly dependent on the activation of the type I interferon (IFN-I) response. In addition to anti-microbial and anti-viral responses, the IFN-I response also mediates diverse cellular and biological responses such as proliferation, apoptosis, senescence, and the DNA damage response. Our results identify C/EBPβ as a critical regulator of a novel cell death pathway involving UVB-induced DNA damage, the IFN-I response, and extrinsic apoptosis.
Defining the role of C/EBPβ in regulating cell survival could identify novel therapeutic targets to restore regulated cell death pathways and prevent and treat cancer.

Vaca, Kain
UNC-G

**In Vitro Cytotoxicity of Nanoplastics in Human Aortic Endothelial Cells**

Plastics have seamlessly integrated into our daily lives; however, the consequential predicament revolves around their associated adverse impacts. Plastics degrade into micro and nanoplastics (MNPLs), which infiltrate the human body via ingestion through food and water and inhalation of airborne particulate matter. However, whether MNPL triggers cytotoxicity needs to be further studied. Here, we investigated the effect of MNPL exposure on the release of lactate dehydrogenase (LDH), a biomarker of cytotoxicity. Human aortic endothelial cells (HAECs) were exposed to varying sizes of MNPLs (0.08μm, 0.8 μm, 10 μm, 100 μm) and different doses (0, 20, 120, 240, 480 μg/mL). Our results showed that nanoplastic treatment (0.8μm & 0.08μm) exhibited pronounced cytotoxic effects at 120, 240, and 480 μg/ml doses when compared to both control and 20 μg/ml conditions. In contrast, HAEC cells exposed to microplastics (100 μm & 10 μm) displayed negligible cytotoxic effects across all doses compared to the control. In conclusion, the study establishes the cytotoxicity of nanoplastics on HAECs, highlighting their stark contrast to microplastics. This observation suggests that these minute yet perilous particles could potentially induce arterial damage within the human body. Given cardiovascular disease's status as a global leading cause of mortality, this pioneering research underscores the urgency of delving deeper into the ramifications of omnipresent nanoplastics on heart health.

Waterbury, Carolyn
US EPA

**Targeted RNA-Sequencing of Testes from Fetal Rats Exposed to Dicyclohexyl Phthalate Informs Potency and Adverse Outcome Pathway Development**

Research investigating phthalate-induced demasculinization of male offspring in rats found dicyclohexyl phthalate (DCHP) to reduce overall testosterone production and expression of genes vital to steroid hormone processes in a dose-dependent manner in fetal rat testis. The goal of this study was to apply targeted RNA-sequencing (BioSpyder Technologies) from a short-term in utero exposure to understand DCHP potency while identifying putative molecular events involved in male reproductive tract malformations. Pregnant Sprague Dawley rats received 0 (vehicle), 100, 300, 600 (n=3/dose-level), or 900 (n=2) mg/kg-day DCHP by oral gavage from gestation day (GD) 14 to 18. Messenger RNA extracted from testes pooled by litter at GD 18 underwent targeted RNA-sequencing. Reads were aligned using STAR and counts analyzed for differential gene expression in Partek Flow. Transcriptomic potency estimates were calculated in BMDExpress2 while molecular pathway enrichment was performed using Ingenuity Pathway Analysis. Differential gene expression analysis showed a dose-dependent change in prior-identified biomarker genes, including Insl3, that are integral to male reproductive development. The dataset highlighted additional genes to monitor, including the upregulation of Testin, a Sertoli cell factor associated with disruption of the blood-testis barrier. Benchmark dose (BMD) analysis identified a transcriptomic potency estimate with the median BMD of 40.9 mg/kg-day, which was slightly more sensitive than the apical developmental and reproductive BMDL10 of 68 mg/kg-day. Pathway analysis identified significant downregulation in hormone regulation and synthesis. Upstream regulator analysis predicted activation of NR0B1 and inhibition of NR5A1 as potential mediators of the gene expression results. Both regulators are important in testis development and may be significant in DCHP mechanism of action. Our results show that targeted RNA-sequencing data was able to predict molecular effects consistent with DCHP developmental and reproductive toxicity, which emphasizes its utility in informing chemical potency and mechanisms of toxicity. This abstract does not represent US EPA policy.
The Per- and Polyfluoroalkyl Substances (PFAS) Hexafluoropropylene Oxide Dimer Acid (GenX) Alters Mitochondrial Protein Expression and Function in Placental Trophoblasts

The Per- and polyfluoroalkyl substances (PFAS) hexafluoropropylene oxide dimer acid (GenX) alters mitochondrial protein expression and function in placental trophoblasts. Per- and polyfluoroalkyl substances (PFAS) are found in many industrial and household products due to their popular water-resistant abilities. Although some PFAS have been banned, hexafluoropropylene oxide dimer acid (HFPO-DA, referred to as “GenX”) is a newer and widely used PFAS found in a variety of products including non-stick items, surfactants, and Teflon™ coatings. Of broad public health concern, PFAS have been shown to bioaccumulate in the body. Of specific concern for pregnant populations, PFAS accumulate in the placenta and have been associated with increased risk for adverse pregnancy outcomes such as preeclampsia. Preeclampsia, a leading cause of maternal mortality, has been tied to impaired placental function. Proper placental development is dependent upon the ability of placental trophoblast cells to invade and migrate through endometrial tissue and promote spiral artery formation. Related to placental function, we have shown that HFPO-DA alters trophoblast migration and invasion and impacts the mRNA expression of migratory and inflammatory signals. However, the impact of HFPO-DA on protein expression in trophoblast is unknown. To address this, we tested the effects of HFPO-DA at 10, 100, and 1000ng/ml for 24hrs on human trophoblast protein expression and mitochondrial function in vitro.

Proteomic analysis showed 100, 180, and 561 significantly altered proteins (p< 0.05) in relation to 10, 100, and 1000ng/ml HFPO-DA respectively. Among these proteins, mitochondrial function, heme regulation, and cellular signaling pathways were differentially expressed. Preliminary data on mitochondrial function tested through Seahorse mitochondrial stress tests shows decreases in maximal respiration in all HFPO-DA exposed groups. Taken together these data highlight the effect of HFPO-DA on trophoblasts and have implications for understanding the effects of PFAS on placentation and pregnancy outcomes.

Mutant Nrf2E79Q Enhances the Promotion and Progression of a Subset of Oncogenic Ras Keratinocytes and Skin Tumors

Squamous cell carcinomas (SCCs) in a variety of epithelial tissues, including skin SCCs (sSCCs), often display constitutive activation of the KEAP1-NRF2 pathway. This constitutive activation can be achieved via numerous mechanisms, including gain-of-function mutations in NRF2, aka NFE2L2, of which the NRF2E79Q mutation is one of the most common. To determine the functional consequences of mutant Nrf2E79Q on skin tumor promotion as well as on progression of pre-existing squamous papillomas to sSCC, we utilized; 1) K14CreERtam;LSL-Nrf2E79Q/wt mice where the mutant Nrf2E79Q allele was knocked into the endogenous Nrf2 locus and silenced by lox- stop-lox (LSL) cassette until removed by CreERtam and 2) the mouse skin multistage carcinogenesis model utilizing the DMBA- initiation/TPA-promotion protocol where the Hras A->T mutation (Q61L) is the signature driver mutation. Using these two techniques Nrf2E79Q was temporally and conditionally unsilenced in the epidermis by tamoxifen treatment at two tumor stages; 1) after DMBA initiation but before cutaneous tumor development and 2) in pre-existing DMBA-initiated/TPA promoted squamous papillomas. Expression of Nrf2E79Q in the epidermis after DMBA initiation but before tumor occurrence inhibited the development/promotion of 70% of squamous papillomas, however the papillomas that did develop displayed a high frequency of non-signature Hras and Kras mutations, enhanced progression to sSCCs, and an enrichment in expression of Nrf2 target genes. When Nrf2E79Q was expressed in pre-existing tumors it caused rapid regression of over 50% of papillomas, however the papillomas that were resistant to regression displayed enhanced progression to sSCCs, enriched expression of Nrf2 target genes, and a high frequency of the expected signature Hras A->T mutation (Q61L). Taken together these results indicate that mutant Nrf2E79Q enhances the promotion and progression of a subset of oncogenic Ras keratinocytes to becoming skin tumors.
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Reflected Generalized Concentration Addition and Bayesian Hierarchical Models to Improve Chemical Mixture Prediction  

Environmental toxicants overwhelmingly occur together as mixtures. The variety of possible chemical interactions makes it difficult to predict the danger of the mixture. In this work, we develop a novel algorithm enhancing the classical two-step model for the cumulative effects of mixtures, which assumes a combination of generalized concentration addition (GCA) and independent action (IA). This is purely predictive because mixture toxicity observations are not required. We propose a geometric technique based on a reflection argument for estimating 3+ parameter Hill model inverse functions that extends GCA by removing parameter restrictions. Our approach incorporates a Bayesian hierarchical model with a Dirichlet Process (DP) clustering prior that allows for uncertainty quantification with the two-step approach. The DP prior is applied to the slope parameters of the individual chemical Hill models as a proxy for clustering by toxicity mode of action and does not require specification of the number of groups. We compare our technique to the IA and GCA models and show in a simulation study that the standard methods are inadequate under our assumptions. We then apply our method to a challenging data set of individual chemical and mixture responses where the target is an androgen receptor (Tox21 AR-luc). Our results show significantly improved predictions for larger mixtures. Our work complements ongoing efforts to predict environmental exposure to various chemicals and offers a starting point for combining different exposure predictions to quantify a total risk to health. Lastly, we provide future research directions for integrating synergy or antagonism based on physico-chemical properties.