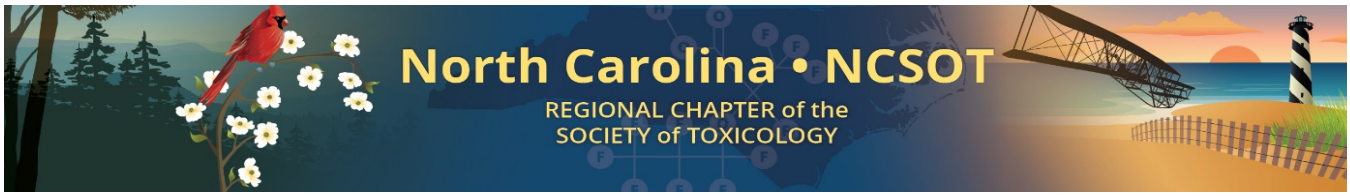


# NCSOT Annual Meeting Program

## *From Dose to Risk: A Class-based Exploration*

September 10, 2025

***Index***

## *Welcome Message from NCSOT President, AtLee Watson*

On behalf of the NCSOT Executive Committee, it is my pleasure to welcome you to the 2025 NCSOT Annual Meeting!

We are grateful to RTI International for once again providing their beautiful event space. We extend our deepest thanks to their team for their gracious hospitality and logistical support. I would also like to acknowledge and thank Dr. Samantha Snow, NCSOT Vice President and 2025 Meeting Organizer, for her leadership in planning this year's meeting. Our incredible program would not be possible without her hard work and dedication over the past several months.

Today's plenary session features *"locally sourced"* yet globally renowned experts, Drs. Cynthia Rider (SOT President), Michael Devito (U.S. EPA), Matt Wheeler (ToxStrategies), and Grace Patlewicz (U.S. EPA). They will discuss how data from a limited set of chemicals can be leveraged to inform risk assessment for broader chemical classes. Their presentations on class-based toxicity evaluations are sure to spark a lively panel discussion. We thank them for generously sharing their time and expertise with the NCSOT community.

Our mid-day and afternoon sessions will highlight new research from our trainees and members. We are excited to showcase more than 50 poster and platform presentations, and we thank all of our presenters for sharing their cutting-edge research. We also appreciate the many attendees who volunteered to serve as judges and table hosts for the "Lunch with an Expert" breakout session. Strong engagement from trainees, presenters, and volunteers is vital to the success of our meetings, past, present, and future. Without question, your enthusiasm and commitment enable our regional chapter to thrive.

We extend our deepest thanks to our sponsors – NIEHS Division of Translational Toxicology, Duke University Superfund Research Program, UNC Curriculum in Toxicology and Environmental Medicine, UNC Marisco Lung Institute Tissue Procurement & Cell Culture Core, Inotiv, and Certara – for making this meeting possible through their generous financial support. We also thank our exhibitors for support our meeting program and encourage all attendees to visit their booths during breaks and mid-day sessions.

We look forward to a productive meeting and hope that today provides meaningful opportunities to learn, connect, and collaborate. Following the meeting close, please join us at The Glass Jug (RTP) to wind down and enjoy an informal networking happy hour with colleagues.

Sincerely,

AtLee Watson

## NCSOT Executive Committee 2025-2026

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Chloe Davis
(NCCU)

| NCSOT Annual Meeting, September 10, 2025, RTI International | | |
|---|---|---|
| **Check-in** | 8:15 – 9:00 | **Holden Building**<br>*Coffee and refreshments provided* |
| **Welcome, Introduction, & Business Meeting** | 9:00 – 9:15 | **Holden Multi-Purpose Room**<br>NCSOT Executive Committee |
| **KEYNOTE SESSION** | | **Holden Multi-Purpose Room** |
| *Which of These Things is not Like the Other: Clustering and Classification* | 9:15 – 9:40 | Dr. Cynthia Rider, Ph.D. |
| *Co-Presentation:*<br>*Development of a database of dose response studies and a weighting scheme for deriving relative potencies for dioxin-like chemicals*<br><br>*Hierarchical Dose-Response Modeling for Dioxin TEF development* | 9:40 – 10:05 | Dr. Mike DeVito, Ph.D., U.S. EPA<br>Dr. Matt Wheeler, Ph.D., ToxStrategies |
| *A chemical category-based approach for selecting and screening PFAS for toxicity and toxicokinetic testing* | 10:05 – 10:30 | Dr. Grace Patlewicz, U.S. EPA |
| Panel Discussion | 10:30 – 11:10 | Cynthia Rider<br>Matt Wheeler<br>Mike DeVito<br>Grace Patlewicz |
| *Morning Break* | *20 minutes* | *Coffee and refreshments provided* |
| **POSTER, LUNCH, and NETWORKING SESSION** | | **Multiple Locations** |
| Poster Session I | 11:30 – 12:30 | Haynes Building |
| Lunch with an Expert | 12:30 – 1:30 | Boxed lunches available 11:45am - 1:15pm in the Holden Building |
| Poster Session II | 1:30 – 2:30 | Haynes Building |
| **PLATFORM COMPETITION SESSION** | | **Holden Multi-Purpose Room** |
| Early-Stage Trainee Platform Competition | 2:35 – 3:20 | Sarah Miller, UNC-CH<br>Kevin Schichlein, UNC-CH<br>Chloe Chou, UNC-CH |
| President's Award for Research Competition (PARC) | 3:20 – 4:05 | Dr. Zakiyah Henry, Ph.D., NIEHS<br>Dr. Michelle Fyle, Ph.D., UNC-CH<br>Dr. Jessie Chappel, Ph.D., UNC-CH |
| *Afternoon Break* | *15 minutes* | *Refreshments provided* |
| **Awards** | 4:20 – 4:45 | NCSOT Executive Committee |
| **Meeting Adjournment** | 4:45 pm | NCSOT Executive Committee |
| Networking Social and Happy Hour to follow at *The Glass Jug* (5410 NC-55, Durham, NC 27713) | | |

## THANK YOU TO OUR 2025 NCSOT SPONSORS!

- Research Triangle Institute (RTI) International (Platinum)
- National Institute of Environmental Health Sciences (NIEHS), Division of Translational Toxicology (Platinum)
- Duke University Superfund Research Center (Gold)
- UNC Curriculum in Toxicology and Environmental Medicine (Gold)
- Inotiv (Gold)
- UNC Marisco Lung Institute Tissue Procurement & Cell Culture Core (Silver)
- Certara (Silver)

## EXHIBITORS

- Research Triangle Institute (RTI) International
- North Carolina Society of Experimental Biology and Medicine
- UNC Tissue Core Facility
- NIEHS Office of Fellows' Career Development
- Certara

## Keynote Speakers and Panelists:

**Which of These Things is Not Like the Other: Clustering and Classification**
Dr. Cynthia Rider
*SOT President*

Cynthia Rider is currently serving as President of the Society of Toxicology. Cynthia's research interests are in evaluating defined mixtures using models of additivity and developing methods to interpret data from complex mixtures. She received her B.S. from Tulane University, New Orleans, LA in Environmental Studies and Biology and her Ph.D. from North Carolina State University, Raleigh, NC in Environmental Toxicology. She completed post doctoral training in the Reproductive Toxicology Branch of the National Health and Environmental Effects Research Laboratory, U. S. Environmental Protection Agency and the Nicholas School of the Environment at Duke University.

**Development of a Database of Dose Response Studies and a Weighting Scheme for Deriving Relative Potencies for Dioxin-like Chemicals**
Dr. Mike DeVito
*U.S. Environmental Protection Agency*

Michael DeVito, Ph.D., has been the Director of the Chemical Characterization and Exposure Division in the Center for Computational Toxicology and Exposure at the USEPA since 2019. In 2009, Dr. DeVito joined the National Toxicology Program at NIEHS as the discipline leader for pharmacokinetic modeling and later became chief of the NTP Laboratory in the Division of National Toxicology Program at the NIEHS from 2014-2019. From 2002-2009 he was Chief of the Pharmacokinetic Branch at NHEERL/EPA. Prior to that from 1995 to 2002, Dr. DeVito was a principal investigator in the Pharmacokinetics Branch. Dr. DeVito was one of the lead health effects researchers on the Dioxin Reassessment from 1991-2009. Dr. DeVito's research has focused on the toxicity of persistent organic pollutants, thyroid hormone disruptors and pyrethroid pesticides. In addition, he has developed quantitative models to understand the exposure, dose and toxicity continuum for individual environmental chemicals as well as for cumulative risk assessments. More recently, Dr. DeVito has begun incorporating transcriptomics and targeted and untargeted metabolomics into these efforts. Dr. DeVito's training includes a B.A. in chemistry from Drew University an M.S. and Ph.D. in Toxicology from the Joint Program in Toxicology at Rutgers, the State University of New Jersey and a post-doctoral fellowship at the USEPA's NHEERL.

**Hierarchical Dose-response Modeling for Dioxin TEF Development**
Dr. Matt Wheeler
*ToxStrategies*

Dr. Matthew Wheeler is a Senior Science Advisor in ToxStrategies' Health Sciences practice. With over twenty years of experience as a biostatistician, Dr. Wheeler's expertise involves analyzing Toxicology data. He is an internationally recognized expert in dose-response risk assessment. His work is used by the U.S. Environmental Protection Agency (EPA), Health Canada, the World Health Organization (WHO), and the European Food Safety Authority (EFSA), among others, to perform chemical risk assessments. He was awarded the Presidential Early Career Award for Scientists and Engineers for his work. His experience includes developing novel statistical methods related to chemical and non-chemical risk assessments; he has most

recently developed biological pathway-based techniques for transcriptomic points of departure. His software is central to BMD Express 3.0 and the EPA's benchmark dose online platform.

### A Chemical Category-based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing

Dr. Grace Patlewicz
*U.S. Environmental Protection Agency*

Grace Patlewicz is a chemist and toxicologist by training, her research interests have been focused on the development and application of (Q)SARs and read-across for regulatory purposes. She has chaired various Industry groups and contributed to the development of technical guidance for (Q)SARs, chemical categories and Adverse Outcome Pathways (AOPs) under various Organisation for Economic Co-operation and Development (OECD) work programmes. She started her career at Unilever United Kingdom, before moving to the European Commission Joint Research Centre in Italy and then to DuPont in the United States. In 2014, she joined US EPA as part of ORD's Centre for Computational Toxicology and Exposure (CCTE) where she has been developing objective read-across (GenRA) and categorisation approaches.

## Early-Stage Trainee Platform Award Speakers:

**Characterizing Individual and Mixtures-based Chemical Contributions to Wildfire Smoke Toxicity through In Vitro Transcriptomics Screening**

Sarah Miller
*UNC-Chapel Hill*

**BACKGROUND AND PURPOSE:** Wildfire incidence and severity have steadily increased in recent decades, leading to increases in smoke emissions that pose risks to public health. Due to the wide variety of chemical mixtures in smoke from wildfires, there is a need to couple high-throughput toxicity screening approaches with computational methods to assess wildfire-relevant mixtures toxicity. This study sought to evaluate transcriptomic responses of respiratory epithelial cells to individual components, defined mixtures, and complex woodsmoke mixtures with two goals: (1) identifying potential modes of action that are shared versus distinct between wildfire components, and (2) quantifying relative impacts across these various mixtures to inform human health risk estimates. **METHODS:** The six chemicals prioritized for this study frequently co-occur across ten different wildfire-relevant burn scenarios (eucalyptus, pine, pine needles, peat, and red oak burned under flaming or smoldering conditions) and were previously positively or negatively correlated with toxicity endpoints in exposed mice through mixtures modeling. Spanning polyaromatic hydrocarbon, methoxyphenol, and metal chemical classes, they included: benzo(a)pyrene, benz(a)anthracene, coniferyl aldehyde, vanillin, sodium dichromate, and copper sulfate. Human bronchial epithelial (16HBE) cells in 12-well plates were exposed at 5 different concentrations to these single chemicals, equimolar binary mixtures, an equimolar defined mixture of all 6 candidate chemicals, and 4 complex biomass mixtures (flaming or smoldering eucalyptus and flaming or smoldering pine). Bulk RNA sequencing was performed in samples collected at 4 hours post-exposure. Differentially expressed genes (DEGs), gene set enrichment, benchmark concentration (BMC) modeling, transcriptomic point of departure (tPOD) derivation, and concentration addition mixtures modeling (CAM) analyses were performed to evaluate transcriptomic response. **RESULTS:** Patterns in DEGs, significantly enriched pathways, BMCs, and tPODs were shared between the complex biomass mixtures, defined mixture, sodium dichromate alone, and sodium dichromate in binary mixture with copper sulfate. Comparisons of observed versus CAM-predicted tPODs revealed that this metals binary mixture and the defined mixture also exhibited synergistic effects compared to their CAM-predicted tPODs (e.g. 0.120 µM observed versus 0.265 µM predicted for metals). Ras/ERK and Ras/PI3K signaling were identified as top ranking enriched pathways highly shared across these exposure conditions. **CONCLUSION:** This study employed a toxicogenomics-based approach to evaluate responses to wildfire-relevant mixtures and identify potential modes of action shared between exposure to these mixtures and individual wildfire smoke components. Ras/ERK and Ras/PI3K signaling were identified as potentially involved in a shared mode of action, and individual molecules involved in these signaling pathways will be further investigated within the dataset. Additionally, sodium dichromate was identified as the individual component under evaluation that shared this mode of action signature with the defined and complex mixtures, suggesting that it may be driving biological response in these mixtures. Interestingly, the sodium dichromate-containing mixtures also exhibited synergy when comparing observed versus modeling-predicted tPODs. Altogether, these results suggest that metals and metal mixtures may be a particular chemical class and/or mixture of public health concern when considering the respiratory health effects of wildfire smoke exposure. [This abstract does not represent the views or policies of the U.S. Environmental Protection Agency.]

**An Open-Source, 3D-Printable On-Plate Aerosol Delivery Array (OPADA) for In Vitro Investigation of Inhaled Pharmaceuticals and Toxicants**

Kevin Schichlein
*UNC-Chapel Hill*

Inhaled therapies and aerosolized exposures are central to both medicine and environmental health research. In vitro aerosol exposure systems have become indispensable for initial drug screening and toxicant evaluation, yet access to robust, reproducible systems remains limited by the proprietary nature and high cost of commercial platforms. Open-source, standardized methods for in vitro aerosol delivery are urgently needed to accelerate innovation in respiratory toxicology and drug development. While differentiated airway epithelial cell cultures at air-liquid interface (ALI) effectively model the respiratory tract, conventional exposure methods—adding dissolved compounds to culture media or bulk application to the apical surface—fail to replicate physiological aerosol deposition. These approaches either ignore polarized cellular responses or disrupt the ALI, causing hypoxic conditions that may alter toxicity and efficacy outcomes. Therefore, systems capable of delivering aerosols directly to ALI cultures are crucial for accurate modeling. While several commercial in vitro aerosol exposure systems are available, they can be cost-prohibitive, contain proprietary components, and have limited throughput. To address these limitations, we developed OPADA (On-Plate Aerosol Delivery Array), a novel open-source system for in vitro evaluation of inhaled drugs and toxicants. OPADA advances previous open-source designs by increasing deposition efficiency and enabling simultaneous multi-well, multi-compound exposures, thus enhancing throughput and experimental flexibility. We validated OPADA through proof-of-principle experiments demonstrating uniform and reproducible deposition using three model aerosols: an aqueous solution (fluorescein disodium salt), a polymer solution (FITC-dextran), and a nanoparticle suspension (polystyrene microspheres). Fluorometric analysis revealed a 20% deposition efficiency across experimental conditions, demonstrating OPADA's utility for dose-response studies. Furthermore, we confirmed OPADA's biological relevance using a model inhaled drug (calcitriol) and toxicant (cinnamaldehyde) in airway epithelial cells at ALI, with aerosol delivery producing gene expression changes comparable to prior inhalation studies. These findings establish OPADA as an effective, reproducible platform for in vitro evaluation of inhaled compounds. By providing an accessible, open-source alternative to commercial systems, OPADA promotes transparency and reproducibility in aerosol research while reducing barriers to entry for laboratories studying respiratory toxicology and drug delivery.

**Developing a Mixture of Combustible Materials in American Homes and Fire-prone Biomes to Elucidate the Toxicity of Wildland-Urban Interface Fires**

Chloe Chou
*UNC-Chapel Hill*

Wildfires in the Wildland-Urban Interface (WUI) are an increasing concern, with approximately 39% of homes in the U.S. now located in the WUI and thus at elevated risk of burning during wildfires. WUI fires are unique as they emit a complex mixture of inorganic and organic compounds derived from the combustion of anthropogenic materials in addi4on to those produced by biomass-only wildland wildfires. A standardized fuel mixture could be used to characterize exposure chemistries and toxicological impacts of WUI-relevant smoke exposures. We developed two novel mixtures representing WUI materials and a mixture of biomass common to California’s plant biome. To develop the WUI mixture, we first integrated 61 combustible materials typically found in the average American WUI home, a 2,016 sq. ft. single-family home with four bedrooms and three occupants. Materials included structural, plumbing, wiring, paint, appliances, furniture, and furnishing materials. We found that the total combustible mass of an

average American family home was around 46,500 kilograms, including 81% wood materials, 10% petroleum-based products (asphalt shingles), 6% plastics, and 2% metals. To develop the California biomass mixture, we identified the vegetation types and their proportions in the chaparral biome, which represents regions in California where some of the most destructive wildfires have occurred. The chaparral mixture consisted of 60% coastal sage, 30% chamise, and 10% eucalyptus. Each mixture was processed, homogenized, then burned in a lab-scale furnace system to generate WUI and chapparal smoke condensate from smoldering and flaming combustion conditions. The smoke condensates were then evaluated for toxicity in primary human airway epithelial cells cultured at air-liquid interface in 12-well plates. At maturity, 250 uL of smoldering WUI smoke condensate was applied as an aqueous dilution to the apical compartment for four-hours at concentrations of 1, 3, 10, and 30 ug/cm$^2$ for three donors, following which cell cytotoxicity and changes in mRNA expression were evaluated. Initial dose responses performed with the smoldering WUI condensate found no significant cell cytotoxicity at any of the doses used. Initial gene-specific RT-qPCR indicated that IL-6 mRNA release was significantly increased at a dose 30 ug/cm$^2$ of WUI condensate, with chaparral results pending. Transcriptomic signatures are currently being measured to inform more global disruptions in cellular biology resulting from WUI fires. Collectively, the WUI and chaparral biome fuels can serve standardized fuel mixtures representing WUI fires that can be used to investigate chemical and mechanistic determinants to health effects of exposure to smoke. (This abstract of a proposed presentation does not reflect EPA policy.)

## President's Award for Research Competition Postdoctoral Award Speakers:

### 3-month Dosed-Feed Toxicity Study (including Perinatal Exposure) of *Garcinia cambogia* Extract in Sprague Dawley Rats

Zakiyah Henry
*National Institute of Environmental Health Sciences*

*Garcinia cambogia* extract (GCE), also known as *Garcinia gummi-gutta*, is a botanical weight loss supplement ingredient marketed with purported benefits such as appetite suppression, fat burning via thermogenesis, and inhibition of lipid synthesis. The active and predominant ingredient (30-70% by weight) found in the fruit's rind is hydroxycitric acid (HCA), a potent inhibitor of a fatty acid synthesis enzyme, ATP citrate lyase. The effects of GCE on weight loss are inconsistent and cases of liver toxicity have been reported. Adequate toxicological data is limited; therefore, the objective of this study was to evaluate the toxicity of GCE (65.1-66.1% HCA) using oral administration through dosed feed to mimic human exposure. Time-mated $F_0$ Hsd:Sprague Dawley® SD® dams were offered dosed feed with 0, 300, 1000, 3000, 10000, 30000 ppm of GCE in feed (ad libitum) beginning on gestation day (GD) 6 and continuing through post-natal day (PND) 28. Pups received GCE via in utero exposure, followed by approximately 4 weeks of exposure via dam milk and/or dosed-feed consumption prior to weaning and randomization for the start of the 3-month exposure. Animals ($F_1$) selected to continue onto the 3-month exposure phase were provided the same GCE concentration received during the perinatal phase. The following endpoints were measured and/or assessed in the $F_0$ dams: HCA internal concentration, survival, clinical and maternal care observations, body weight, food consumption, and reproductive performance. The following endpoints were assessed in the $F_1$ offspring: HCA internal concentration, survival, clinical observations, body weights, food consumption, evaluation of sperm motility and counts, vaginal cytology for estrous cycling, clinical pathology, organ weights, and histopathology. As expected, HCA internal concentrations increased with increasing exposure concentration. There were no GCE-related effects on dam survival, maternal care, or gestation length. Decreases in dam body weight and food consumption were observed at 30000 ppm during gestation. GCE exposure decreased litter size, viability, and survival of offspring at 30000 ppm. Litter size at birth (PND 0) was significantly decreased with a mean of 9.4 compared to

the control mean of 12.7. A decrease in viability at birth was manifested as a lower number of live pups per litter on PND 0 (mean of 8.4 vs 12.4 controls). Furthermore, pup weights were significantly decreased at 30000 ppm throughout the entire postnatal period. GCE-related reproductive effects were primarily observed in the $F_1$ male rats. GCE in feed led to significantly lower spermatid and sperm counts (with few to no motile cells in 4/10 males) and decreases in epididymis and testis weights. Corresponding histopathological findings in male rat reproductive tissue included testis germinal epithelium degeneration, epididymis hypospermia and exfoliated germ cells observed in males dose-fed 30000 ppm GCE, and chronic inflammation of the preputial gland (≥10000 ppm). In female rats, chronic inflammation of the clitoral gland was detected at 30000 ppm. Administration of GCE resulted in altered estrous cyclicity in the females at 30000 ppm with a significant decrease in estrus length. There were no hematological or serum biochemical effects of GCE exposure. Also, no signs of liver toxicity were observed. Hormonal assessment of insulin, leptin, and adiponectin demonstrated dose-related decreases in insulin and leptin. No changes were seen in adiponectin. In summary, most adverse effects seen at the highest concentration of 30000 ppm, which were primarily reproductive and developmental. GCE exhibited the potential to be a reproductive toxicant, mainly in the $F_1$ male rats. Hormonal assessment of insulin and leptin suggests an impact of GCE on metabolic homeostasis. The lack of liver toxicity observed in the rats does not reflect the adverse event reports of liver toxicity in humans.

**TRPV1 Modifies Cardiovascular and Autonomic Responses to Extreme Heat in a Housing-specific Manner**

Michelle Fyle
*UNC-Chapel Hill*

Extreme heat events are classified as the leading cause of weather-related death in the United States and are associated with increased cardiovascular morbidity and mortality, both in acute and chronic conditions. Further, it is known that these adverse effects do not occur proportionately across populations, indicating that living conditions might have an effect on the physiological and adaptive responses to heat stress. The synergistic effects of housing (i.e.. psychosocial stress) and extreme heat remain understudied, and the role of transient receptor potential vanilloid 1 (TRPV1) in modulating these responses represents a novel mechanistic evaluation of these non-chemical stressors. Female C57BL6/J mice surgically implanted with biopotential radiotelemeters that measured continuous heart rate (HR), core body temperature (Tco), activity, and electrocardiograms (ECG) were randomly separated into either enriched (EH) or depleted housing (DH) for 4 weeks. Mice then underwent a single intraperitoneal injection of either a vehicle-control or a TRPV1 antagonist (BCTC) 30-min before an exposure to a 1-hr normal temperature (22°C) control (NT), followed by three consecutive days of 1-hr extreme heat exposures (~36°C). Before exposures, mice in DH had a higher resting HR, lower Tco and activity levels, and shorter RR-interval and QRS complex on the ECG. The TRPV1 antagonist prevented an increase in HR in DH-mice during the NT-control sham, however the DH-BCTC mice had significantly higher HR and RR-intervals during the first and third day of EHE. BCTC also induced a decrease in heart-rate variability (HRV) throughout the NT-control and the first and second day of EHE, followed by a significant increase in HRV on the third day of EHE. Further, TRPV1 antagonism resulted in significant and sustained elevations in Tco and activity during and after heat exposures, that persisted up to 1-wk postexposure. Overall, our results portray a complex relationship between housing conditions, extreme heat, and the role of TRPV1 in cardiovascular response. In addition, radiotelemetry revealed signs of increased autonomic and thermoregulatory perturbations when TRPV1 was blocked in DH mice, which was not present with EH. Our findings show that TRPV1 plays a role in the response to extreme heat in a housing-specific manner, and further work is needed to characterize whether the changes are related to epigenetic, gene expression, immunological, or other mechanisms that might confer vulnerability to heat stress. (This abstract does not reflect EPA policy).

**Interpretable Machine Learning to Understand Wildfire Toxicity: Bridging Chemicals, Omics, and Health Outcomes via Symbolic Regression with Novel Feature Scoring**

Jessie Chappel
*UNC-Chapel Hill*

Wildfire smoke exposures are increasingly common, consisting of complex mixtures of gases and particulates known to cause diverse pulmonary health effects. While health outcomes are regularly studied, the relationships between chemical concentrations in smoke and toxicological outcomes remain poorly quantified, representing a critical gap in understanding wildfire smoke health risks. This study explores symbolic regression (SR) as an interpretable artificial intelligence / machine learning (AI/ML) method to generate closed-form mathematical models linking chemical exposure to biological responses relevant to wildfire smoke. Prior to application on wildfire relevant datasets, we evaluated three Python-based SR packages on simulated data, assessing performance across varying noise levels and operator complexities. Insights from these simulation tests, such as the importance of including necessary operators, were incorporated when applying SR to lab-generated wildland fire exposure-toxicity data. This dataset included chemical characterizations of biomass smoke exposures and corresponding pulmonary responses in female CD-1 mice. Specifically, we evaluated the ability to predict a lung injury marker using 1) targeted chemical concentrations measured from smoke and 2) lung tissue transcriptomic signatures. To aid model interpretation, we developed directional ensemble contribution scores (DECS), a novel feature importance scoring method that quantifies the direction and magnitude of predictor contributions across well-performing models. Expert toxicologists also contributed to model prioritization, integrating a “biologists-in-the-loop” approach. Results highlighted polycyclic aromatic hydrocarbons and inorganic atoms and molecules as drivers of lung injury (i.e., incorporated addition and multiplication operators) and methoxyphenols as suppressors of lung injury (i.e., incorporated subtraction and division operators). Transcriptomic analyses highlighted key genes driving model predictions, which have roles in metabolism, cell proliferation, immune regulation, and oncogenic processes. This study is among the first to apply SR in toxicology, demonstrating its value as an interpretable AI/ML tool that enhances understanding of complex environmental exposures and their biological impacts.

## Poster Presentations by Number:

* = trainee poster

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**Bettini, Dominik**
Appalachian State University
*Monitoring the Recovery of the Pigeon River Following the Permanent Closure of the Pactiv Evergreen Paper Mill in Canton, North Carolina*

Regarding riverine pulp and paper mills, research on the recovery of stream ecosystems after the cessation of upstream paper production is scarce. As the developed world moves away from single-use paper products, more research is needed to determine the rate of recovery for affected ecosystems. For over a century, the Pigeon River in Haywood County, North Carolina received a large quantity and variety of chemical effluent from the Pactiv Evergreen Paper Mill. Despite overall improvement of the river's health due to the cessation of dioxin discharge in the 1990's, biomonitoring conducted by the North Carolina Department of Environmental Quality and Duke Energy has demonstrated that pollutants continued to impair the macroinvertebrate biota prior to the mill's closure in 2023. Although the NCDEQ and Duke Energy continue to conduct regular water quality assessments on the Pigeon River every 3-5yrs, the frequency of their biomonitoring is insufficient to capture the temporal scale needed to determine the timetable of full recovery of the system following the closure of a major contributor of point-source pollution. Using benthic macroinvertebrates as bioindicators, my research aims to determine if upstream and downstream sites support similar macroinvertebrate assemblages >7 months post-production, the rate of recovery for the benthic community, and the abundance of pollution-sensitive taxa. Measurements of specific conductivity, dissolved oxygen saturation, turbidity, pH, temperature, and the concentrations of chloride and nitrates at each site will be taken by a YSI ProQuatro multi-parameter water quality meter and Vernier GoDirect ion-selective probes. Additionally, water and sediment samples will be taken at each sampling site and analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy to provide more context as to what physicochemical properties are significantly different between upstream and downstream sites, if any at all.

**Bomstein, Zach**
UNC-Greensboro
*Combined Effects of Environmentally Relevant Di(2-Ethylhexyl) Phthalate (DEHP) Exposure and a Western-Style Diet on Colonic Inflammation*

Diets rich in fat and ultra-processed food (Western-style diets; WD) increase the risk of colorectal cancer (CRC) through the initiation and perpetuation of colonic inflammation. Di(2-ethylhexyl) phthalate (DEHP) is an endocrine-disrupting and reprotoxic phthalate widely used in plastic food packaging and ultra-processed and fat-rich foods are most susceptible to contamination. Previously, our lab demonstrated that DEHP disrupts homeostatic cellular responses in colonocytes in vitro. This study aimed to determine the effects of DEHP in combination with a WD on colonic inflammation in vivo. Female, C57BL/6 mice consumed a diet with or without DEHP for 13 weeks. At week 5, one set of mice was transitioned to a WD for the remainder of the study. At week 11, a subset of mice from each group received a colitis-inducing agent (DSS) to examine the effects of exposure on acute colitis. Histological analysis and gene expression was used to evaluate intestinal injury, inflammation & repair following DSS Exposure. WD/DEHP combined exacerbated clinical symptoms of DSS-induced colitis while DEHP alone significantly worsened DSS-induced injury to the distal colon. DSS/WD/DEHP significantly increased colonocyte proliferation without affecting crypt height or transcriptional indices of repair-mediated proliferation (c-MYC, RSPO3). DSS/WD alone significantly increased mucosal IL-6 expression relative to DSS/Control Diet (CD) while DSS/WD/DEHP significantly increased mucosal IL-10 expression relative to DSS/CD only. In mice without DSS (healthy), no significant effects on colonocyte proliferation were observed, but DEHP alone, WD alone, and WD/DEHP combined significantly reduced crypt height relative to CD. WD/DEHP significantly decreased mucosal ZO-1 expression without significantly affecting the expression of genes TNF-a, IL-6 or IL-10. These data demonstrate that WD and DEHP consumption exacerbate chemically-induced colitis in an exposure specific manner, while WD/DEHP exposure in the absence of chemical colitis downregulates the expression of genes implicated in gut barrier function.

**Boney, Sydney**
National Institute of Environmental Health Sciences
*First Evidence of Legacy and Emerging PFAS in the Follicular Fluid of a Cohort of North Carolina IVF Patients*

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants with reported reproductive toxicity. Despite widespread PFAS contamination in North Carolina (NC) and detection in drinking water and serum samples, little is known about PFAS accumulation in human follicular fluid (FF)—a critical microenvironment for oocyte maturation. Therefore, the primary aim of this study was to quantify PFAS concentrations in FF collected from 86 women undergoing in vitro fertilization (IVF) in NC. Using targeted ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), we measured 23 PFAS analytes encompassing legacy compounds (e.g., PFOS, PFOA), short-chain alternatives, and emerging ether-based PFAS such as hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). PFAS were detected in all patient FF samples, with perfluoroalkyl sulfonic acids (PFSAs) such as PFHxS and PFOS exhibiting the highest median concentrations (PFHxS: 2.36 ppb; PFOS: 1.28 ppb). Detection frequencies varied by chemical class, with long-chain perfluoroalkyl carboxylic acids (PFCAs) and PFSAs more frequently detected than short-chain fluorinated alternatives. Interestingly, HFPO-DA was detected in 88.4% of the samples and the alternative PFAS 6:2 fluorotelemer sulfonic acid (6:2 FTS) was detected in 47.7 % of the samples. The cumulative PFAS concentrations ranged from 0.49 to 12.78 ppb across individuals, with 3 to 16 analytes detected per patient. The cumulative PFAS load was not significantly different between patients with and without an infertility diagnosis ($p = 0.88$), though individual variability was high. In addition, no significant correlations were observed between PFAS load and age or BMI. This is the first study to examine PFAS in FF in NC and confirms that legacy and shorter chain alternative PFAS can cross the blood–follicle barrier to accumulate in FF. The consistent detection of replacement PFAS compounds such as HFPO-DA in most of the patients underscores the evolving nature of human exposure as the legacy chemicals are phased out. Given the critical role of FF in oocyte development and recent reports of PFAS effects on ovarian outcomes, future studies are warranted to elucidate the effects of the alternative PFAS on ovarian function. This abstract does not necessarily reflect EPA policy.

**Courtney, Isabel**
North Carolina State University
*Examining the Neurotoxic Effects of Cultured Cyanobacteria Exposure in a Larval Zebrafish Model*

Cyanotoxins are chemical compounds produced by photosynthetic organisms found in aquatic environments called cyanobacteria. As anthropogenic interference increases the occurrence of harmful algal blooms that produce cyanotoxins, the human population at risk of exposure to cyanotoxins is increasing. Exposure to the cyanotoxin betamethylamino-L-alanine (BMAA), a non-proteinogenic amino acid has been linked through several case studies to the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS), although this link is still controversial, and the mechanisms by which BMAA mediates neurodegenerative processes are unclear. In addition, cyanotoxins naturally cooccur as a mixture of many cyanotoxins. Thus, there is great concern over the effect of mixture exposures on the development of neurodegenerative diseases. In a previous study, we found that a mixture of BMAA and the most abundantly produced cyanotoxin, microcystin leucine arginine (MCLR), synergistically increased larval zebrafish acoustic startle responses relative to the effects of either toxin alone. This increase in startle responsiveness was also observed in mixture-exposed fish 2 years after the exposure, highlighting the long-term impact of developmental cyanotoxin exposure. Additionally, preliminary results from cultured cyanobacteria isolated from North Carolina water samples demonstrate that exposure to specific strains of cyanobacteria also increases acoustic startle sensitivity in larval zebrafish. In the present study, we aim to elucidate the mechanisms by which environmental exposure to cyanobacteria and their toxins mediates neurodegeneration. We will test exposures of isolated cyanotoxin mixtures and cultured cyanobacteria using behavioral assays and whole-brain imaging as readouts for neurotoxic effects and explore potential gene-by-environment interactions between cyanotoxin exposure and known ALS risk genes. Through these studies, we will define how environmental exposure to cyanotoxins impacts brain development and behavior, providing key insights into factors that contribute to neurodegenerative disease.

**Cuevas Mora, Karina**
Duke University
*The Impact of PFAS Exposure from Drinking Water on the Immune Response to Maternal Tdap Vaccination*

Per- and polyfluorinated substances (PFAS) are chemical compounds abundant in the environment. Efforts have been made to replace legacy compounds (PFOA and PFOS) with new, shorter carbon chain compounds with similar properties. However, PFAS continues to be found in our drinking water due to both policies surrounding permissible exposure and the inadequacy of many public water systems to remove these chemicals. This means that people are exposed to a mixture of PFAS compounds every day. Legacy PFAS compounds, PFOA and PFOS, have been associated with immunotoxicity, specifically reducing antibody response in children. However, to our knowledge, epidemiological and causal data on the effect of PFAS exposure on antibody response to maternal vaccines in expecting mothers are unknown. Maternal vaccinations such as Tdap during pregnancy are important to provide passive immunity to the fetus and protect a newborn child from neonatal infectious disease. The goal of our study was to assess whether exposure to a PFAS mixture affects the effectiveness of Tdap vaccination in pregnant females. Six-month-old female rabbits were given clean water or water contaminated with a mixture of PFAS compounds to drink ad libitum for 30 days prior to pregnancy and throughout gestation. The PFAS mixture was formulated to mimic commonly detected PFAS compounds in North Carolina. Rabbits were given two doses of a Tdap vaccine; the first dose was given seven days before pregnancy (GD-7), and the booster was given during pregnancy at GD11. Dams and their kits were necropsied at GD25. Serum samples were collected at GD-7 and GD25. Antibodies to the Tdap vaccine were measured using ELISA, and antibody titers were calculated as the dilution necessary to achieve a 3-fold difference between GD-7 and GD25. Peripheral blood mononuclear cells (PBMCs) were analyzed using single-cell RNA sequencing (scRNA-seq). We observed a significant decrease in dam serum antibody titer in rabbits exposed to PFAS-contaminated water versus those given deionized water, as well as an inability of serum antibodies to neutralize diphtheria in vitro compared to serum antibodies from the controls. Notably, scRNA-seq revealed a significant increase in myeloid lineage cells and premature immune cell aging with PFAS exposure. Specifically, exposure to the PFAS mixture downregulated the B-cell receptor regulator CD79b, dysregulated apoptosis, and reduced regeneration capacity. Taken together these results indicate premature aging of B cells and reduced efficacy of IgG antibodies. This study demonstrates that exposure to a PFAS mixture can alter the health of B cells from the mother and impact antibody production during gestation, thus potentially reducing the effectiveness of prenatal vaccination to protect offspring from neonatal infectious diseases.

**Dagenhardt, Kristina**
UNC-Chapel Hill
*Maternal Socioeconomic Traits and Community-indices as Predictors of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) Exposures within the Extremely Low Gestational Age Newborns (ELGAN) Cohort*

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are present throughout the environment and accumulate within water, food, and humans. PFAS exposure is associated with higher serum cholesterol, disrupted immune function, higher risk of preeclampsia, and lower birth weight. Understanding demographic and socioeconomic variables associated with higher exposure to PFAS can inform interventions for decreasing exposure. In this study, we investigated associations between socioeconomic and demographic variables and four PFAS: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS) among 181 mothers who delivered babies in the Extremely Low Gestational Age Newborns (ELGAN) cohort from 2002-04. PFAS exposure was quantified utilized liquid chromatography tandem mass spectrometry in maternal blood spots taken during the perinatal window (2 weeks before or after birth). Demographic variables include insurance type, occupation, use of food stamps, education status, smoking during pregnancy, maternal BMI, delivery type, and birth region, based on medical records and self-reported data. We also utilized the Centers for Disease Control and Prevention's Social Vulnerability Index (SVI) total percentile, based on reported residence during pregnancy, to examine the influence of community-level socioeconomics. We assessed differences in PFAS exposure by key demographic variables using Wilcox and Kruskal-Wallis tests. If there was a statistically significant result from the Kruskal-Wallis test, then we utilized Dunn's test to determine which category was significantly different ($p \leq 0.05$). A linear regression model was used to assess the relationship between SVI and PFAS exposure. Over 98% of blood samples had detectable levels

of the four PFAS compounds. PFOS had the highest measured exposure (mean: 7.58 ng/mL), followed by PFHxS (1.58 ng/mL), PFOA (1.17 ng/mL), then PFNA (0.42 ng/mL). Participants in North Carolina had higher PFNA, PFOS, and PFOA exposure than those in the Great Lakes Region and in New England. PFNA exposure was greater in participants who used Medicaid (mean: 0.49 ng/mL, SD: 0.36, p-value: 0.02) in comparison to those not on Medicaid (mean: 0.37 ng/mL, SD: 0.25). PFNA exposure was moderately higher in the overweight maternal BMI category (0.50 ng/mL, SD: 0.28, adjusted p-value: 0.09) when compared to the normal BMI category (mean: 0.39 ng/mL, SD: 0.35). PFOA exposure was higher in the obese level (mean: 1.28 ng/mL, SD: 0.53, adjusted p-value: 0.03) in comparison to the normal weight category (mean: 1.04 ng/mL, SD: 0.71). There were no statistically significant associations with maternal occupation, delivery type, food stamp usage, smoke exposure, and education. Increased social vulnerability was moderately associated with lower PFNA exposure (Beta= 0.136, p-value= 0.096). Future analysis will investigate if close proximity to Formerly Used Defense sites and Superfund National Priorities List sites is associated with higher maternal PFAS exposure. Through understanding what individual and area-level factors drive PFAS exposure, these results can be used to help inform targeted interventions to decrease maternal exposure to legacy PFAS compounds.

**DeLuca, Nikki**
RTI International
*Hold My Beer: A Characterization and Risk Assessment of PFAS in Beer and the Linkage to Municipal Water*

**Background:** Beer has been a popular beverage for millennia, historically having been considered safer to drink than water as boiling and fermentation during the brewing process killed pathogens. As water is a main component of beer and the brewing process, we surmised that PFAS presence and spatial variability in drinking water systems are a PFAS source in beers and a source of PFAS exposure for consumers. **Methods:** This is the first study to adapt EPA Method 533 to measure PFAS in beer from various regions, brewery types, and water sources. Statistical analyses were conducted to correlate PFAS in state-reported drinking water with analyzed beers by brewing location. Hazard quotients and incremental lifetime cancer risks were calculated for low, medium, and high beer drinking scenarios using U.S. EPA toxicity assessment reference dose and cancer slope factor values to determine whether there could be non-cancer or cancer health risks from regular consumption of PFAS in the beers we tested. **Results:** PFAS were detected in most of the beers we tested, particularly from smaller scale breweries located near drinking water sources with known PFAS. Perfluorosulfonic acids, particularly PFOS, were most frequently detected, and PFOA or PFOS were above U.S. EPA's Maximum Contaminant Limits in some beers. Hazard quotient calculations revealed that 1 beer consumed per day could pose non-cancer health risks from PFOA, with higher consumption scenarios also posing non-cancer risks from PFOS. Carcinogenic risks were not observed. There was a county-level correlation between total PFAS, PFOA, and PFBS concentrations in drinking water and beers. **Conclusion:** Given that approximately 18% of U.S. breweries are located within zip codes with detectable PFAS in municipal drinking water, our findings which link PFAS in beer to the brewery water source and reveal non-cancer health risks from frequent consumption of some beers, are intended to help inform data-driven policies on PFAS in beverages for governmental agencies, provide insights for brewers and water utilities on treatment needs, and support informed decision-making for consumers.

**Dillon, Meghan**
North Carolian State University
*Precision in a Pinprick: Biomonitoring from a Dried Blood Strip*

In 2022, the International Agency for Research on Cancer (IARC) classified firefighting as a Group 1 carcinogen. Firefighters are routinely exposed to various toxicants in responding to diverse fire (structure, wildland, vehicle) and non-fire events, such as vehicle accidents, medical incidents, and building collapses. For example, firefighters are among those at highest risk for exposure to per- and polyfluoroalkyl substances, or PFAS, due to their presence in heat-resistant turnout gear, aqueous film-forming foams (AFFFs), and air at the scene of fires and building collapses. Further, though PFAS have been associated with numerous human health effects, including adverse developmental and reproductive outcomes, altered immune, liver, and thyroid function, and various cancers, additional research is needed to characterize the early adverse health consequences of chronic use/contact. As such, this work aims to discover if dried blood strips (DBS) can be used to quantitate the

biological biomarkers associated with occupational PFAS exposure. As part of ongoing pilot projects, a minimally invasive Drawbridge Health OneDraw™ device was used to collect DBS (150 uL, matrix paper) from a convenience sample of anonymous individuals who simultaneously donated a vial of venous blood (VBS; 6 mL, EDTA). VBS will be used for DNA damage evaluations (Comet assay and long PCR), DNA methylation and hydroxymethylation analysis (bisulfite sequencing), miRNA profiling (cancer-focused PCR panels), oxidative lipid characterization (LC-MS), and thyroid hormone quantification (chemiluminescent immunoassay). DBS will be extracted to isolate DNA, RNA, lipids, and hormones, which will be analyzed using similar methodological approaches to enable direct comparisons with matched VBS. Biomarkers, regardless of original source, will be evaluated for associations with PFAS measured via UHPLC-coupled mass spectrometry. Our current work has optimized extraction of DBS for measurement of approximately 27 PFAS, demonstrating that PFAS with detection frequencies >10% in DBS are strongly predictive of levels in plasma. Ongoing research will further optimize DBS protocols for genomics, epigenomics, and metabolomics. This project will 1) develop cellular and health biomarkers indicative of PFAS exposure using non-invasive and easy to store DBS, 2) further characterize DBS-based biomarkers of occupational PFAS exposure, and 3) enable longitudinal health and exposure monitoring of military and civilian firefighters.

**Dolphin, Shane**
UNC-Greensboro
*Diisononyl Phthalate Alters Cell Growth in Nonmalignant Colonocytes*

Diisononyl phthalate (DiNP) is a commonly used plasticizer in both industrial and consumer products, including food packaging. Human exposure to DiNP is estimated to be between 1-2 µg/kg/bw/day on average, with estimates of 5-10 µg/kg/bw/day at the 95th percentile. It has been estimated that up to 61-71% of DiNP exposure comes from ingestion of food, so the potential effects of DiNP in the gastrointestinal tract are important to understand. To investigate this, young adult mouse colonocyte (YAMC) cells were exposed to varying concentrations of DiNP between 0.01 µM and 100 µM and counted at baseline, 24, 48, and 72 hours. Reductions in cell number were observed at 10 µM and 100 µM after 72 hours. To better define the cellular response of YAMCs to DiNP, we assessed the influence of the compound on cell proliferation using a bromodeoxyuridine (BrdU) cell proliferation assay. A significant reduction in proliferation was seen at 100 µM. To further investigate the mechanisms behind the reduction in proliferation, YAMCs were exposed to DiNP for 24 hours before RNA extraction to measure gene expression via RT-qPCR. Significant increases were seen in the expression of multiple genes regulating the cell cycle (P21 and cyclin D1) and apoptosis (bcl-2). Ongoing analyses include the effects of the primary metabolite (MiNP) on cell growth and further tests on both compounds to assess cytotoxicity and their effects on apoptosis to further evaluate the potential impact of DiNP in the gastrointestinal tract.

**Farmer, Kyra**
North Carolina Central University
*Neurobehavioral Effects of Fluoranthene and Tocofersolan on Zebrafish (Danio rerio)*

Polycyclic aromatic hydrocarbons (PAHs) are long-lasting environmental pollutants linked to developmental and neurological effects in aquatic species. Fluoranthene, a common PAH, disrupts nerve development through oxidative stress and mitochondrial dysfunction. However, possible rescue agents have not been well studied. This research investigated the neurobehavioral effects of fluoranthene exposure in zebrafish (Danio rerio) and the potential of the antioxidant vitamin E (tocofersolan) to rescue these effects. Embryos were exposed from 6 to 120 hours post-fertilization (hpf) to different concentrations of fluoranthene, vitamin E, or both. Larval movement in light and dark conditions was measured one day after the end of exposure using the DanioVision behavioral assay. Fluoranthene exposure caused hypoactivity, especially during dark periods. When combined with vitamin E, there was a dose-dependent rescue of the fluoranthene-induced hypoactivity. The strongest rescue occurred with 3 µM fluoranthene and 0.1 µM vitamin E, where activity levels were close to those of control groups. However, there were no improvements with higher concentrations of fluoranthene. Higher concentrations of vitamin E only caused partial recovery. These findings suggest that fluoranthene exposure causes neurotoxicity, potentially through oxidative stress and that vitamin E can reduce these effects in a dose-dependent manner. This study highlights zebrafish as a useful model for studying environmental neurotoxicity

and points to antioxidant co-exposure as a possible way to lessen PAH-related developmental problems. This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P42ES010356 (Duke University Superfund Research Center – Developmental Co-Exposures: Mechanisms, Outcomes, and Remediation). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**Frey, Jenna**
UNC-Chapel Hill
*Individual and Joint Effects of Prenatal Exposure to Trace Metals and SES Adversity on Adolescent Well-being Among Those Born Extremely Preterm*

Exposure to chemical (e.g. toxic metals) and nonchemical stressors (e.g. socioeconomic adversity)—particularly during critical windows of susceptibility such as in utero—have been separately and jointly associated with adverse health outcomes. Aiming to identify associations between prenatal exposure and adolescent health, we hypothesized that (1) higher levels of toxic metals and SES adversity would be associated with lower levels of well-being and increased internalizing and externalizing behaviors; and (2) that combined exposure to all stressors would have an amplified effect on these outcomes. Participants in this analysis (n=159) were part of the Extremely Low Gestational Age Newborn (ELGAN) cohort which comprises individuals born before 28 weeks of gestation in five US states (North Carolina, Massachusetts, Michigan, Illinois, and Connecticut). Chemical stressors (11 metals/metalloids measured in umbilical cord samples) and nonchemical stressors (SES composite variable including <12 years of educational attainment, Medicaid insurance use during pregnancy, “single” marital status, and food stamp use during pregnancy, based on maternal interview at childbirth) were assessed. Three outcomes were measured at age 15: (1) overall wellbeing using the Patient-Reported Outcome Measurement Information System (PROMIS) Global health self-report measure; (2) internalizing behaviors through the Youth Self-Report (YSR) form, the child-report component of the Child Behavior Checklist (CBCL); and (3) externalizing behaviors, also measured using the YSR. Metals were natural log-transformed for normality. Linear regression models were fit for each of the 11 metals and the SES variable for each outcome to determine the individual effect of the exposures on the outcomes and quantile g-computation was utilized to determine the overall mixture effect of all exposures on each outcome. All models were adjusted for birthing parent age at birth and any active or passive smoking before or during pregnancy. In adjusted regression models, three metals were positively associated with overall well-being: zinc ($\beta$=4.77, p=0.007), strontium ($\beta$=2.75, p=0.04), and barium ($\beta$=2.90, p=0.007). A quartile increase in all chemical and nonchemical exposures was associated with an 8.31 point (95% CI: 1.03, 15.59, p= 0.03) increase in internalizing behaviors and a 9.02 point (95% CI: 3.16, 14.91, p < 0.01) increase in externalizing behaviors. The overall mixture effect of all exposures on overall wellbeing score was -2.26 (95% CI: -8.09, 3.57, p= 0.45). For each of the three outcomes, the SES component was the largest contributor of the exposures in the direction of the overall effect with weights of 0.74, 0.69, and 0.63 for wellbeing, internalizing behaviors, and externalizing behaviors, respectively. The overall mixture effects supported our hypothesis that prenatal exposure to chemical and nonchemical stressors will be associated with higher levels of problem behavior and lower levels of wellbeing in adolescence and that combined exposure to stressors has an amplified effect compared to individual exposures alone. Further, our findings highlight the importance of including both chemical and nonchemical factors when characterizing the health effects of early life exposure to stressors.

**Gaballah, Shaza**
Duke University
*Associations with Placental Brominated Flame Retardants and Thyroid Hormones in a North Carolina Prospective Birthing Cohort*

Thyroid hormones (THs) are major regulators of fetal growth and development during pregnancy. Gestational TH disruption is associated with adverse pregnancy outcomes such as preterm birth and intrauterine growth restriction, as well later life fetal health effects including metabolic diseases and several cancers. Legacy and alternative brominated flame retardants (BFRs) have been demonstrated to disrupt TH regulation through multiple pathways, and in utero BFR exposure is associated with altered maternal and fetal TH levels and adverse fetal birth outcomes. Some BFRs, including polybrominated diphenyl ethers (PBDEs), have been

demonstrated to differentially accumulate in the maternally- and fetally-derived placental tissues. However, to date, no studies have assessed the relationship between THs, legacy and replacement BFRs, and birth outcomes separately in the maternal and fetal placenta. Therefore, we conducted a study using paired maternal and fetal placentas (n=200) from a prospective birthing cohort in Durham, NC. Placentas were collected at delivery, microdissected into the maternal and fetal tissues, and analyzed using mass spectrometry for T3, rT3, T4, 10 PBDEs, and several alternative BFRs, including decabromodiphenyl ethane (DBDPE), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)tetrabromophthalate (BEH-TBP). BDE 47 was the most frequently detected (59%) BFR, followed by BDE 99, 153, and 209. DBDPE was the most frequently detected alternative BFR (20%). rT3 and T4 were detected in all samples, while T3 was detected above the method detection limit (MDL) in 43% of tissues. For analyses with BDE 47, a multivariate imputation by chained equations (MICE) approach was used to impute values below the MDL. For analyses looking at associations with birth weight, birthweight-for-gestational age percentiles (z-score BW) where calculated separately for male and female babies. Spearman correlations were used to assess the relationship between BFRs, THs, and fetal birth outcomes. BDE 47 and rT3 concentrations were higher in the fetal placenta than maternal placenta ($p<0.0001$). BDE 47 concentrations were weakly positively correlated with T4 in maternal ($rs=0.19$) and fetal tissues ($rs=0.11$); however, this correlation was stronger in male-associated maternal and fetal placentas ($rs=0.19$; $rs=0.11$, respectively). Similarly, both Spearman analyses and linear regression analyses revealed that maternal and fetal placental rT3 levels were positively associated with z-score BW ($\beta=0.4$), including in male-associated tissues ($\beta=0.5$-$0.7$). Based on the average male-associated maternal and fetal beta-coefficient of 0.6, at 40 weeks gestation, a 1 ng/g increase in placental rT3 corresponds with a 253 g increase in male birthweight. This is the first placenta study to report detecting EH-TBB and BEH-TBP, and the second to detect DBDPE. These data highlight associations between gestational exposure to legacy and alternative BFRs and placental TH levels, including sex-specific differences with fetal birth outcomes, and may be indicative of adverse impacts on placental TH regulation and fetal health. This work was supported by a grant from the NIEHS (RO1 ES031419; HMS, Duke; RO1 ES028110; HP, NCSU) and T32-ES021432.

**Heckman, Morgan**
Duke University
*Lipids Adsorbed on the Surface of Nanoparticles Enhance Protein Corona Formation*

Nanomaterials have become widely used in industrial applications, creating environmental and health hazards. The risks of inhalation exposure for those working in these industries are not well understood. Inhaled nanomaterials interact with a variety of biomolecules, including proteins and lipids, that are present in lung fluid. These biomolecules form a corona on the surface of the nanomaterial. While the protein corona is well-studied, there have been few studies on the lipid corona and how lipids may influence the protein corona. We examined the bio-corona that forms on TiO2 nanoparticles, SiO2 nanoparticles, Ag nanoparticles, and multi-walled carbon nanotubes as a function of L-alpha-phosphatidylcholine (LAP). LAP was selected as a representative lipid due to the high concentration of phosphatidylcholines in lung fluid. The nanomaterials were characterized using dynamic light scattering to measure hydrodynamic diameter, polydispersity index, and zeta potential, and TEM to measure primary diameter. The presence of LAP increases both the lipid and protein concentration in the bio-corona formed on the nanomaterials. Current work is examining the interaction between the lipid and protein in solution, as well as the impact on macrophage response to these combined lipid and protein coronas. Overall, we hope that understanding the bio-corona that forms on the nanomaterials, and the downstream effect on cells, will give us a greater ability to assess the risks of inhalation exposure to these nanomaterials.

**Hepworth, Emma**
North Carolina State University
*Investigating How Per- and Polyfluoroalkyl Substances (PFAS) Affect Neutrophil Metabolism*

Per- and polyfluoroalkyl substances (PFAS) are widespread and persistent pollutants and can be detected in the serum of an overwhelming majority of people in the U.S. There is substantial evidence that PFAS alter immune function. Two particular PFAS, perfluorohexanoic acid (PFHxA) and hexafluoropropylene oxide dimer acid (HFPO-DA or GenX), have been found to suppress the neutrophil respiratory burst, a key neutrophil function and critical process in the innate immune response. However, it is not yet known which aspect of neutrophil

biology the PFAS are disrupting. Compared to some other innate immune cells, the characteristic metabolic profile of neutrophils is primarily glycolytic, in part because glycolysis supplies the cells with the burst of energy necessary to rapidly produce the reactive oxygen species (ROS) used to perform the respiratory burst. In addition, there is emerging evidence that mitochondrial metabolism plays an important role in many neutrophil functions. Therefore, it is possible that PFAS suppress neutrophil function by altering cellular metabolism. Real-time cell metabolic analysis with an Agilent Seahorse Analyzer was used to investigate if neutrophil-like HL-60 cells exposed to either PFHxA or GenX had altered cellular metabolism after 24 and 96 hours of exposure. The Glycolytic Rate Assay and Cell Mito Stress Test measured changes in glycolysis and mitochondrial respiration, respectively. While changes in many of the measured parameters were subtle, several trends were observed such as, that GenX and PFHxA may induce different metabolic responses, and these responses may also change with exposure duration. Uncovering changes in the metabolism of neutrophils exposed to PFAS will improve understanding of the mechanisms behind PFAS-induced suppression of immune cell function.

**Huber, Erin**
RTI International
*Modeling Repeated ENDS Exposure Trajectories in Adolescents Using a Co-culture Model of the Bronchial Epithelium*

**Background and Purpose:** The use of primary in vitro models for inhaled chemical testing often relies on single cell models, specifically, differentiated primary human bronchial epithelial cell (dpHBEC) cultures. While dpHBECs hold physiological relevance based on their ability to form cilia, tight junctions, and mucus production, they are only one of many cell types in the bronchial epithelium. Incorporating multiple cell types increases biological complexity of in vitro models while also providing added context of interactions occurring in vivo to better predict human health outcomes. Additionally, primary in vitro models from human donors may capture heterogenous responses to toxicant exposures, including response trajectories in susceptible populations. The rapid popularity of Electronic Nicotine Delivery Systems (ENDS) in adolescents had led to a knowledge gap in understanding the cumulative and long-term health impacts in humans. The effects and underlying mechanisms of inhaled test articles, such as ENDS aerosols, are often modeled using acute exposures and only one cell type commonly from human immortalized cell lines. In realistic exposures, ENDS usage typically involves repeated exposures over time and interaction of numerous cell types in the bronchial epithelium. Therefore, to increase physiological relevance and replicate real-world exposures in adolescents, we conducted repeated exposures to ENDS aerosols using a co-culture model containing dpHBEC air-liquid interface (ALI) cultures and donor-matched primary human lung fibroblasts (pHLFs) from adolescent donors, known as the trans-epithelial exposure model (TEEM). **Methods:** We assessed impact of repeated ENDS exposures in the TEEM using 6 sex-balanced adolescent donors with no known health issues or history of smoking. The ENDS exposure system utilized CelTox exposure units, with a flow rate of 1.1 L/min using zero air, 5% $CO_2$ gas, and connected to an e-cigarette tank that housed an SVS 250 e-cigarette device affixed with a controller. ENDS puff profiles were time triggered with a 3 second puff and 27 second pause for a total of 15 puffs. Cultures were exposed to either vehicle control containing propylene glycol: vegetable glycerin (PG:VG) or ENDS aerosol containing benzaldehyde and nicotine for 1 or 5 days in CelTox units. Exposure groups were compared against a matched ALI control. To determine uniformity, glycerol deposition efficiencies and total aerosol mass were quantified in blank cell culture inserts using electrostatic precipitation or sedimentation in CelTox units with glycerol free reagent. Following repeated exposures, we evaluated impacts on common in vivo-relevant endpoints, such as ciliary beat frequency (CBF), trans-epithelial electrical resistance (TEER), cytotoxicity using the adenylate kinase assay, MUC5AC secretion, and pro-inflammatory cytokine secretion: interleukin 1a (IL-1a), IL-6, and IL-8. We assessed transcriptional changes using RNAseq. **Results:** ENDS aerosol exposure for 5-days led to increased cytotoxicity among all donors when compared against both ALI and vehicle controls. TEER was not impacted by exposures. CBF and MUC5AC had altered responses following 1 and 5-days of exposure. Donor specific increases were seen in pro-inflammatory cytokine secretion with two donors classified as responders to ENDS aerosol effects. Phenotypic endpoints captured donor specific sensitivity from repeated ENDS aerosol exposures. RNAseq revealed distinct gene expression patterns between 1 and 5-day exposures, involving oxidative stress, DNA damage, fibrosis, and lung cancer pathways. Cell type specific effects were observed with increased differential gene expression in dpHBECs reflecting direct exposure impacts, while pHLFs reflected the cumulative impact of repeated exposures to ENDS aerosols. **Conclusions:** This study strengthens the need to

characterize primary in vitro systems through understanding inter-individual variability by including susceptible population groups and increased donor numbers in toxicological studies to capture response trajectories that represent the human population. Utilization of the TEEM and repeated exposures to ENDS aerosols was able to capture complex and cumulative biological responses that single-cell or single-exposure models may miss, which better predicts adverse health outcomes in humans.

**Jacobs, Savannah**
North Carolina State University
*The Effects of Volatile Organic Compound Mixtures on Liver and Mammary Gland Toxicity in Male and Female Rats*

**Objective:** Volatile organic compounds (VOCs) are ubiquitous anthropogenic chemicals linked to numerous cancers. At Camp Lejeune, North Carolina (NC; 1953-1987), United States Marine families exposed to VOC-contaminated drinking water developed adverse outcomes including male and female breast cancer. While this exposure resulted in the largest male breast cancer cluster to-date, mechanisms driving this relationship are unknown. Thus, we evaluated the effects of a VOC mixture in male and female rat offspring following exposure during pregnancy and adolescence. **Material and Methods:** Timed-pregnant Sprague Dawley rats were exposed from gestational day 13 (GD13) through postnatal day 48 (PND48) to drinking water containing a VOC mixture mirroring that measured at Camp Lejeune. Upon necropsy at pre/post-pubertal timepoints (PNDs 23, 30, and 48), mammary gland (MG) and livers from male and female offspring were analyzed for RNA expression and morphology, and VOC concentrations and half-lives were quantified. **Results:** Early-life VOC exposure resulted in decreased body and liver weights in both sexes at PNDs 30 and 48, and decreased liver P450 enzyme-related RNA expression at PND 48. Further, in both sexes, we observed a dose-dependent increase in MG developmental scores at PNDs 30 and 48. Following VOC mixture exposure, blood and MG tissue contained elevated benzene levels with extended half-lives relative to other VOCs in the mixture. However, a benzene-only sensitivity analysis revealed no effects on aforementioned endpoints. **Conclusion:** Using a rat model, these data suggest that early-life VOC mixture exposure carries elevated risks for liver toxicity and advanced MG growth in males and females. **Funding:** National Institute of Environmental Health Science P30ES025128 to the Center for Human Health and the Environment; Molecular Pathways to Pathogenesis in Toxicology T32ES007046-41 to NC State University. **Acknowledgements:** The authors thank Dr. Ben Blout, Center for Disease Control and Prevention, for his role in developing mixtures for this study.

**Javier, Huayta**
Duke University
*Inhibition of Mitochondrial Complex III in Caenorhabditis elegans Leads to Dopaminergic-specific Neurodegeneration*

**Background and purpose:** Environmental factors are important contributors to Parkinson's Disease (PD). Laboratory, clinical, and epidemiological studies have demonstrated a role for several chemical exposures. All these chemicals affect mitochondria. However, there is strong evidence for association with PD for only a few chemicals, and because relatively few people are exposed to significant amounts of those chemicals, they collectively likely explain only a small fraction of PD. It is not feasible to test all the chemicals that induce mitochondrial dysfunction. Our approach is aimed at assessing the mechanisms of toxicity for these chemicals. These include inhibition of all four electron chain complexes, ATP synthase, and Krebs cycle enzymes; redox cycling; mtDNA damage; and uncoupling of ATP production from oxygen consumption. We are working to define which forms of mitochondrial dysfunction result in dopaminergic neurodegeneration, as well as whether oxidative stress, and ATP depletion, are required for dopaminergic neurodegeneration. This information should help narrow the focus of efforts to identify chemicals that could contribute to PD. Here, we report results from inhibition of Complex III. **Methods:** We are using the model organism *C. elegans* to evaluate the in vivo effects of toxic mitochondrial exposures on dopaminergic neurodegeneration (based on morphological and behavioral changes), ATP levels, and redox state. We exposed *C. elegans* embryos to chemicals toxic to mitochondrial until the L4 larval stage. We acquired images of the animals' heads using fluorescence microscopy, capturing the 4 cephalic dopaminergic neurons. Neurodegeneration was quantified by assigning a score of 0 to 6 to each neuron, depending on the type of damage. **Results:** Complex III inhibitors Antimycin A and Pyraclostrobin -which are

environmentally relevant pesticides- caused dopaminergic neurodegeneration in a dose dependent manner, and significant dopaminergic neurodegeneration was observed for concentrations of 500 nM and 50 µM respectively. Complex III inhibitors may also induce neurodegeneration in glutamatergic and cholinergic neurons, and in neuronal sheath cells, but not in serotonergic or GABAergic neurons. Additionally, both Antimycin A and Pyraclostrobin increased the ratio of oxidized to reduced roGFP, a reporter of redox state in dopaminergic neurons. Rescue experiments with an antioxidant (N-acetylcysteine), a signaling modifier (dichloroacetate), and a blocker of reactive oxygen species (ROS) specific to Complex III (S3QEL-2) indicate that neurodegeneration linked to Complex III inhibitors exposure is caused by an increased production of mitochondrial ROS. **Conclusions:** Specific inhibition of mitochondrial Complex III using pesticides Antimycin A and Pyraclostrobin during larval development of *C. elegans* lead to degeneration of dopaminergic neurons. These deleterious effects are linked to increased mitochondrial ROS production at Complex III. These results will serve to elucidate the mechanistic aspects of chemicals exposures leading to PD, steering future research in vertebrate models.

**Khan, Ajmal**
UNC-Greensboro
*Integrated Transcriptomics, Metabolomics, and Epi-transcriptomics (RNA Modification) Reveal the Role of PS-MPLs in Cardiovascular Diseases, Especially Atherosclerosis*

**Background/Goal:** Atherosclerosis is a significant chronic inflammatory form of cardiovascular diseases (CVDs), which continue to be the world's leading cause of death. Recently, environmental pollutants such microplastics and nanoplastics (M-NPLs) have been recognized as new toxicants that may influence the cardiovascular system. Particularly prevalent, polystyrene microplastics (PS-MPLs) have been linked to endothelial dysfunction, oxidative stress, inflammation, and alteration of lipid metabolism, all of which are key factors in atherosclerosis. However, little is known about the molecular pathways through which PS-MPLs contribute to the pathophysiology of CVDs. To clarify the molecular involvement of PS-MPLs in endothelial dysfunction and the development of atherosclerosis, this study proposes an integrated multi-omics strategy that combines transcriptomics, metabolomics, and epitranscriptomics. **Methods:** PS-MPLs (0.08 µm, 0 and 120 µg/mL) were administered to human aortic endothelial cells (HAECs) in a controlled environment. Fluorescence microscopy, flow cytometry, and Mitotracker co-localization were used to evaluate cellular uptake and subcellular localization. Differentially expressed genes (DEGs) and enriched pathways were found using bulk RNA sequencing. This was followed by a comparative transcriptome study using publicly accessible datasets of human atherosclerotic plaques. To measure RNA modifications and assess the expression of enzymes linked to modifications (writers, readers, and erasers), epitranscriptomic profiling was carried out using UPLC-MS. Integrative gene–metabolite network analyses were performed after global metabolic changes were characterized using untargeted LC–MS metabolomics. A difference of $p < 0.05$ between biological replicates was considered statistically significant. **Results:** The dose-dependent internalization of PS-MPLs by HAECs and their colocalization with mitochondria suggested interactions specific to particular organelles. According to transcriptomic analysis, genes related to inflammatory signaling, vesicular trafficking, endocytosis, and NF-κB activation were significantly upregulated. 457 DEGs were found to be shared between PS-MPL-treated HAECs and human atherosclerotic plaques, with enrichment in pathways such focal adhesion, PI3K-Akt signaling, and fluid shear stress and atherosclerosis. Epitranscriptomic profiling revealed differential regulation of their enzymatic modulators and notable changes in conventional RNA modifications, such as elevated levels of m1A, m5C, m3C, and pseudouridine. Different fingerprints in oxidative stress pathways, bioactive sphingolipid signaling, and lipid and amino acid metabolism were found by metabolomic profiling. Integrative network analysis revealed that the metabolism of arginine, lysine, and glutathione served as key hubs linking transcriptome and metabolomic changes to pathways linked to inflammatory and cardiovascular disorders. Conclusions: This study shows that PS-MPLs change transcriptional programs, RNA modification landscapes, and metabolic networks, hence upsetting endothelium homeostasis. The results show convergent pathways of inflammation, vascular remodeling, and metabolic dysregulation, offering mechanistic insights connecting PS-MPLs exposure to endothelial dysfunction and atherosclerosis. The integrated multi-omics approach finds possible biomarkers and treatment targets for environmentally caused CVDs and provides a systems level knowledge of PS-MPLs induced cardiovascular damage.

**Lewis, Ashley**
U.S. Environmental Protection Agency
*A Single Oral Exposure to Polyethylene Terephthalate Microplastics Causes Mild Metabolic and Gastrointestinal Disruption: Dose and Sex Determinants*

Microplastics, derived largely from degraded household and commercial plastics, have become a pervasive environmental concern, and may pose health risks resulting from ingestion and accumulation in living organisms. Polyethylene terephthalate (PET) microplastics, commonly found in consumer products such as plastic bottles and clothing, have been detected in human arteries and brains among other tissues—although their health impacts remain unknown. This study aimed to evaluate the biological effects of a single oral exposure to PET microplastics in female and male rats. Three-month old Sprague-Dawley rats were exposed once via oral gavage to sterile water vehicle or 5 or 50 mg/kg of PET microplastics derived from cryomilling of plastic nurdles. Animals were monitored for ~ 18 hours in an indirect calorimetry apparatus beginning immediately after gavage for changes in metabolic rate. Tissue samples were collected the following morning after gavage to measure systemic indicators of inflammation and injury, changes in hormones related to metabolic function, and changes in gene expression in the liver and gastrointestinal tissue. Males exposed to low PET had significant decreases in metabolic rate, respiratory exchange ratio, and blood insulin, and significant changes in gene expression in the duodenum, whereas PET caused dose-dependent increases in serum platelets, LDL cholesterol and glucose. By contrast, females exposed to PET were limited to decreased metabolic rate with the high dose, and dose-dependent increases in serum LDL. These findings suggest that exposure to PET has the potential to cause mild metabolic dysfunction and systemic and organ toxicity in a dose- and sex-specific manner (Abstract does not reflect U.S. EPA policy).

**Liu, Ying**
National Institute of Environmental Health Sciences
*Chemical Effects in Biological Systems (CEBS): A Curated Toxicology Database with Extensive Data, Integrated Datasets, and User-friendly Interface*

The Chemical Effects in Biological Systems database (CEBS) contains extensive data from toxicology studies in support of the Division of Translational Toxicology (DTT) at the National Institution of Environmental Health Sciences (NIEHS), along with data from other studies of environmental health relevance. It encompasses a wide range of in vivo and in vitro data types at the individual animal/subject level, summary level, and conclusion level. CEBS incorporates growing internal databases into a central data repository and builds high quality curated datasets with rich standardized metadata to better serve the scientific community. This effort includes harmonizing legacy terms and metadata with current standards and aligning terms to OBO (Open Biological and Biomedical Ontology). CEBS grants public access to data from over 10,000 studies for 12,750 test articles (chemicals, environmental agents). The CEBS user interface features a single search bar to query test articles, study identifiers, and other keywords related to study metadata and publications. Search results are consolidated onto dedicated pages for each test article, including study data, protocol, conclusions, publications, datasets, and links to related articles and projects. Data are also organized by endpoint into data collections and are accessible through a filterable and downloadable application at https://cebs-ext.niehs.nih.gov/datasets/, as well as in flat file format for computational analysis and AI applications at https://doi.org/10.22427/NTP-DATA-110-021-001-000-6. CEBS also offers guided applications for exploring and analyzing specific data types, such as gene expression, pathology etc. High-interest datasets, including TOX21 data, studies containing both genomic and apical endpoints with benchmark dose (BMD) analysis, and expression data, are readily accessible.

**Masood, Syed**
UNC-Chapel Hill
*Molecular Profiling of Thiol Oxidation in Idiopathic Pulmonary Fibrosis*

**Background:** Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by the scarring of the lung interstitium and destruction of airspaces. Many risk factors such as environmental exposures, genetics, and pathogens are associated with IPF development and progression. However, the precise mechanisms underlying the initiation and progression of IPF have yet to be fully elucidated. One such mechanism is the alterations in redox signaling caused by the dysregulation of the endoplasmic reticulum (ER).

Additionally, airway epithelial cells and fibroblasts in IPF lungs have reduced capacity to prevent oxidative damage due to reduced expression of antioxidants and oxidoreductases such as glutathione peroxides and glutaredoxin. Therefore, the oxidative environment formed through ER stress, which is induced by the accumulation of misfolded proteins, is potentially critical in the initiation and progression of IPF. In the present study, we hypothesize that ER stress induced by toxicological exposure will alter redox signaling and homeostasis in IPF airway epithelial cells. **Methods:** Using maleimide fluorescent probes to specifically tag reduced sulfhydryl groups in combination with western blotting, we monitored total intracellular thiol oxidation in human bronchial epithelial cells (HBECs) isolated from healthy and IPF lungs. **Results:** Hydrogen peroxide exposure of HBECs induced a dose-dependent increase in intracellular total thiol oxidation. Total thiol oxidation induced by hydrogen peroxide exposure was increased in IPF compared to healthy HBECs. **Conclusions:** These data indicate that accumulation of extracellular peroxide induces oxidative stress and modifies intracellular protein residues. The data further suggest that the reduced antioxidant capacity of IPF cells increases their susceptibility to peroxide induced alteration in redox homeostasis. Taken together, these findings illustrate a potential mechanism by which environmental exposures that result in peroxide formation cause dysregulated redox signaling in IPF.

**McLean, Zachary**
North Carolina State University
*In Vitro and In Silico Assessment of Calycin Protein Binding of Per- and Polyfluoroalkyl Substances*

Per- and polyfluoroalkyl substances (PFAS) are a structurally diverse class of synthetic chemical pollutants that are ubiquitous in the environment and can act as endocrine disrupters, immuno- and hepatotoxicants. PFAS found in both drinking water and the food supply, including dairy, are a major route of human exposure. B-lactoglobulin (β-Lg) is the major whey protein in bovine milk and a member of the lipocalin protein family. Lipocalins are known for binding endogenous ligands, such as fatty acids, vitamins, and hormones, and have important physiological functions in the endocrine and immune systems, which are known to be impacted by PFAS exposure; thus, consumption of PFAS-contaminated milk and dairy products is concerning, especially for the health of infants and children. Here, we quantitatively analyzed PFAS binding to β-Lg using differential scanning fluorimetry to determine the binding affinities for a test set of 17 PFAS. Further, computational molecular-docking was used to simulate and predict the location and type of molecular interactions responsible for the binding of PFAS to β-Lg and across the calyx domain superfamily of lipocalins and fatty acid binding proteins, as the tertiary structure is conserved. In vitro, PFAS binding to β-Lg was determined for all compounds except fluorotelomer alcohols, and the binding of PFAS with less than 8 carbon-chain lengths (C8) was also discovered. An inverted J-shaped trend was found for the relationship between binding affinity and chain length for C2-C11 carboxylic acids, with C2, trifluoroacetic acid, having the lowest binding affinity (Kd = 8.6 mM) and C9, perfluorononanoic acid, having the highest (Kd = 0.06 mM). Molecular docking simulations predicted all calyx domain proteins were predicted to bind PFAS with changes in Gibbs free energy of binding (ΔG) ranging from -5.3 kcal/mol, for β-Lg binding of perfluorobutanoic acid to, to -9.4 kcal/mol, for human liver fatty acid binding protein binding to perfluorooctanoic acid (PFOA). These DG values are equal to or greater than the DG observed for serum albumin or liver fatty acid binding protein. For all calyx domain proteins, the primary binding site was identified as the calyx binding site, where endogenous ligands, such as retinol or vitamin D, bind. Energetically, van der Waals and hydrophobic interactions make up the majority of the DG values predicted, as hydrogen bonds were only predicted in 7 out of 14 of the top conformations and did not show a significant increase in DG. For example, RBP4 bound PFOA with the highest DG for the lipocalins at -9.1 kcal/mol, with no predicted hydrogen bonding. Overall, this study has identified calyx domain proteins, many with well-characterized impacts on regulating key immune and endocrine functions, as PFAS binding proteins, identifying new modes of action for PFAS toxicokinetics and toxicity.

**Moore, Nia**
U.S. Environmental Protection Agency
*Prenatal PFOA Exposure and Long-term Health Impacts*

Per- and polyfluorinated substances (PFAS) are known developmental toxicants that affect physiology, growth, and immune function. Perfluorooctanoic Acid (PFOA) has been linked to adverse impacts on biochemical

pathways involved in glucose metabolic regulation and kidney function. Kidney disease is one the of well-established diseases associated with PFAS exposure. The environmental etiology of kidney disease and mechanisms that contribute to the disease have not been well studied and are largely unknown. The purpose of this study is to understand the impacts of prenatal PFOA exposure on metabolic endpoints and measures of kidney function in adulthood. Pregnant Sprague-Dawley rats were dosed via oral gavage with PFOA from gestation day (GD) 9-21, and their prenatally exposed offspring were examined for measures of glucose metabolism and kidney function in adulthood. To understand the impacts of prenatal PFOA (80mg/kg daily, GD9-21), intraperitoneal glucose tolerance testing (IPGTT) was used to monitor the glucose response in offspring at two time periods (PND38-PND40 and at PND 88 - 90). At PND 90, offspring were assessed for impacts on kidney function with serum measurements of blood urea nitrogen (BUN), creatinine, urea, and serum proteins (albumin and globulin), proteins that may be important in immune function. Prenatal PFOA exposure caused an elevated spike in glucose immediately after injection, but this response returned to normal in animals aged PND38-PND40. Prenatal PFOA exposure caused elevated peak glucose levels versus control in animals of age PND38 – PND40, an effect that was not present in older animals (PND 88 – 90). Also, prenatal PFOA exposure was associated with elevated glucose area under the curve. Other time points in the glucose tolerance testing were unchanged in PFOA-exposed animals. Kidney function measures from adult F1 adult offspring (PND 90) showed significant effects at $p<0.001$ for BUN, urea, and BUN/creatine ratio. PFOA-dependent significant elevations versus control were seen for BUN, urea, and BUN/creatinine ratio. Serum creatinine and serum albumin were unchanged with PFOA exposure. Adult outcomes including kidney and metabolic function are adversely impacted by prenatal PFOA exposure. Prenatal exposure to PFOA significantly impacted various serum measures of kidney function (BUN, BUN: creatinine ratio, and urea). Metabolic function, as measured by glucose tolerance testing, was adversely impacted by prenatal PFAS exposure. This study shows that developmental exposure to PFOA is associated with adverse health outcomes in adulthood. The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

**Moyd, Sarahna**
National Institute of Environmental Health Sciences
*Prenatal Environmental Exposure to Phthalates and Fetal Growth in the LIFECODES Fetal Growth Study*

**Introduction:** Phthalates are a class of endocrine-disrupting chemicals used in various everyday consumer and industrial products. Exposure to phthalates has been associated with several pregnancy and developmental endpoints such as preterm birth and altered fetal growth. As fetal development is an intricate process that sets the stage for optimal delivery outcomes as well as early life development, characterizing associations with fetal growth across pregnancy, and not only at birth, may provide greater insight into growth dynamics. Therefore, our objective was to investigate associations between environmental exposure to phthalates and their replacements with ultrasound and delivery measures of fetal growth. **Methods:** The LIFECODES Fetal Growth Study is a case-cohort study with 248 small-for-gestational-age (SGA), 240 large-for-gestational age (LGA), and 412 appropriate-for-gestational-age (AGA) births. Biomarker concentrations of 12 phthalates and 4 replacement chemicals were quantified in urine sampled at 3 time points across gestation (median 10-, 24-, and 35-weeks gestation). We estimated associations between pregnancy-average biomarker concentrations and fetal growth using repeated ultrasound measures (median 4 per participant), birthweight z-scores, and case status (i.e., SGA or LGA vs. AGA birth). Effect measure modification by fetal sex was also assessed. Quantile g-computation was used to estimate joint associations (i.e., mixture effects) between low and high-molecular weight as well as overall phthalate mixtures and each measure of fetal growth. **Results:** The LMW and overall phthalate mixture were both inversely associated with head circumference z-scores specifically in male births. Conversely, certain HMW phthalates, including MCOP and MCNP, were associated with higher ultrasound measures across all multiple parameters, mostly observed in female fetuses. For example, an interquartile range increase in MCOP was associated with higher head circumference (0.12, 95% CI: 0.01, 0.23), abdominal circumference (0.14, 95% CI: 0.01, 0.26), femur length0.18, 95% CI: 0.06, 0.30), and estimated fetal weight 0.15, 95% CI: 0.02, 0.28) in females, while comparable associations in males were all null. Fewer associations were observed for remaining delivery measures. **Discussion:** Effects of LMW phthalates were most pronounced among male births and

effects were specific to head circumference. Estimates of select HMW phthalates, including MCOP and MCNP, were most pronounced among female births across all ultrasound measurements. Although these differences did not manifest at birth, gestational associations support biological effects of phthalate exposure in utero that may have long-term consequences to the developing fetus.

**Nowell, Crystal**
North Carolina Central University
*Enhancing Biodegradation of Trichoderma Using Ionic Gelation Encapsulation*

A major contributor to the persistent pollution in the Elizabeth River, a designated Superfund site, is creosote, a coal-derived chemical widely used at former wood treatment facilities along the river. While creosote is effective at preserving wood and increasing its durability, it contains numerous harmful pollutants, a major group being polycyclic aromatic hydrocarbons (PAHs). These compounds can be toxic and bioaccumulate in organisms that do not have excretion pathways. They can bind to DNA resulting in mutation and some can have carcinogenic properties. Dredging is a common PAH remediation approach that excavates the sediment bed and moves it to a secondary location. While this practice has been utilized in the past, it is costly and ecologically invasive, resulting in habitat loss for organisms in the soil, disruption of nutrient cycling, and impacting benthic species. Bioremediation using Trichoderma is an emerging strategy as it can biodegrade several forms of PAHs. Trichoderma is a genus of fungi found in soils worldwide, making it highly adaptable to a variety of environments. It is well-known for its strong colonizing ability and natural antibiotic properties. Improved speciation of Trichoderma will be conducted during this research to explore characteristics across the fungus, with a focus on Trichoderma deliquescens. To further optimize biodegradation methods, Chitosan coated calcium alginate encapsulation is hypothesized to provide the fungi an extra layer of protection in harmful environments. Chitosan coating is preferred due to its ability to improve bio adhesivity. Exploring the effects of 1% - 2% alginate solutions on fungi CFU concentrations will also be performed. This protection is hypothesized to house the fungi while it grows to reach optimal density, potentially provide growth materials resulting in higher CFU concentrations, and provide increased adsorption of PAH, mediated by physical and chemical reactions. These attributes can yield more precise remediation and is a more cost-effective strategy to PAH remediation. Hazards in environments that need remediation can negatively affect these introduced biotic remediators and ultimately interfere with their growth and bioactivity. Bioremediation may fail in some environments due to severe resource competition. To address this, encapsulation may provide the fungi an extra layer of protection. This protection will house the fungi while it grows to reach optimal density and potentially provide growth materials resulting in higher CFU concentrations. Encapsulation can be the change in how active and transformative trichoderma deliquescens can be in hazardous communities. Chitosan coated calcium alginate encapsulation is the method utilized in this research. Chitosan coating is preferred due to its ability to improve bio adhesivity. Calcium alginate can hold the fungi well and provides a matrix for use. Biodegradation using Trichoderma is a promising approach in this field. Trichoderma is a genus of fungi found in soils worldwide, making it highly adaptable to a variety of environments. It is well-known for its strong colonizing ability and natural antibiotic properties, often serving as a biocontrol agent. Novel research has shown that multiple species of Trichoderma can degrade PAH. Its proven capacity to break down complex organic compounds and its environmental resilience make Trichoderma a highly promising candidate for PAH bioremediation efforts. To further optimize biodegradation efforts of Trichoderma, observing bioactivity in different species of these fungi can provide in -depth understanding of Trichoderma's abilities and enhance remediation.

**Padilla Mercado, Gilberto**
U.S. Environmental Protection Agency/ORISE
*Leveraging In Vivo and In Vitro Data to Estimate Metabolic Clearance*

For many new and existing chemicals there is little to no in vivo data to evaluate effects of exposure. The EPA's Computational Toxicology and Exposure has developed an informatics ecosystem to support physiologically-based pharmacokinetic (PBPK) modeling through the use of in vitro data and quantitative structure-activity relationship models However, there remains a need to refine estimations of PBPK model parameters that have the most impact in improving in vitro-in vivo extrapolation. One such parameterization is the decision to model hepatic metabolism as restrictive (dependent on plasma protein binding) or non-restrictive. Pharmaceutical

compounds are often modeled as undergoing restrictive metabolism, while some commercial chemicals are more accurately modeled as non-restrictive. Here we analyze a large, multi-chemical database of in vivo concentration vs. time data to categorize chemicals with respect to restrictive clearance. We developed clearance-aware one-compartment models using the open-source R packages invivoPKfit and httk to estimate whether each chemical follows restrictive or non-restrictive clearance. These models allow us to calculate the relative likelihood of restrictive hepatic metabolism when benchmarked against in vivo data. Because the majority of in vivo experimental data is created for rodent models while the bulk of in vitro work results from human cell culture experiments, we also compared results from within-species estimations and cross-species estimation, where the latter uses human physiochemical data to initialize parameters in our clearance-aware models. We compare the initial (in vitro) parameter estimates to model parameters optimized to fit in vivo data. We conducted within-species comparisons for 52 chemicals and cross-species comparisons for 171 chemicals and can identify likely restrictively cleared chemicals based on these combined assessments. We find that using human in vitro data as a surrogate for rat in vitro data introduces noise with 3-fold root mean square log10 error. Although accurately predicting and modeling metabolism remains a challenge, this workflow outlines a targeted assessment of uncertainty based on in vitro and in vivo data integration that can inform decisions to model a chemical restrictively or non-restrictively. Sets of chemicals categorized as restrictive or non-restrictive can then serve as training sets for models to predict restrictive clearance for chemicals without in vivo data.

**Paul, Britney**
North Carolina State University
*Uncovering Associations Between Metabolites and PFO5DoA in Women Aged 50 and Older: Results from the GenX Exposure Study*

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of environmental contaminants, among which historically used compounds, like PFOS and PFOA, have been associated with dyslipidemia and metabolic disruption. The Cape Fear River, the primary drinking water source of over 1.5 million people, is impacted by PFAS, including a poorly studied subclass known as perfluoroalkyl ether acids (PFEAs). From at least 1980 to 2017, PFEAs were released into the environment through wastewater from a fluorochemical manufacturing factory. After PFEA-discharge ceased, the GenX Exposure Study assessed PFAS exposure in residents of the lower Cape Fear River Basin, finding elevated serum levels of multiple PFAS, including PFOS, PFOA, and PFO5DoA—a PFEA compound. In experimental models, PFO5DoA induces toxic effects similar to PFOS and PFOA; however, due to a lack of exposure data, its metabolic impacts on humans remain unknown. Recently, using data from 477 GenX Exposure Study participants, we found that PFO5DoA is associated with elevated aspartate aminotransferase (AST), a biomarker of liver injury, suggesting potential toxicity to the liver—an organ central to metabolic regulation. To investigate the metabolic effects of PFO5DoA, we conducted a pilot cross-sectional study on 60 older female participants (ages >49) of the GenX Exposure Study (2017-2019). Non-fasting serum samples underwent untargeted metabolomics profiling via Ultra-Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS/MS). A total of 1223 metabolites were detected in over 50% of participants. To explore global metabolic differences, we stratified participants into tertiles (n = 20 each) based on previously measured PFO5DoA concentrations and performed Partial Least Squares Discriminant Analysis (PLS-DA) comparing the highest and lowest tertiles. These initial results suggest two distinct metabolic profiles between participants in low and high PFO5DoA tertile groups. Multivariate linear regression models, adjusted for age, examined associations between natural-log transformed PFO5DoA concentrations and 1223 serum metabolite measurements. PFO5DoA was significantly associated with 136 metabolites (FDR-adjusted $p < 0.05$), with 88 metabolites classified within lipid pathways. Three lipids exhibited strong associations: two glycerophospholipids were positively associated, whereas one fatty acid was negatively associated with PFO5DoA. Metabolites involved in glutathione metabolism, including cysteine and cysteine-glutathione disulfide, had significant inverse associations with PFO5DoA. Additional associations were observed with metabolites within peptide and nucleotide pathways. To our knowledge, this is the first study to characterize associations between the global metabolome and PFO5DoA. While limited by sample size and cross-sectional design, this preliminary analysis provides insight into the identification of early effect biomarkers of PFO5DoA exposure, which may inform toxic mechanisms and early indicators of harm.

**Resh, Violet**

North Carolina State University
*Investigating How Per- and Polyfluoroalkyl Substances (PFAS) Affect the Neutrophil Transcriptome*

The term per- and polyfluoroalkyl substances (PFAS) denotes a vast collection of highly stable, synthetic chemicals commonly used in a multitude of consumer products such as nonstick cookware, food packaging, stain repellent materials, and fire fighting foams. Their resistance to most forms of degradation has allowed them to become nearly omnipresent, posing a threat to environmental and human health. PFAS now contaminate water resources and farmland, bioaccumulate in wildlife, and are estimated to be detectable in the blood of all humans. Concerningly, exposure to PFAS has been shown to impair the immune system, cause developmental delays, increase the risk of cancer, dysregulate hormonal balance, and induce disorders of the liver, kidney, and thyroid in humans. Despite these observations, research on the health risks posed by PFAS remains in its infancy mostly due to a lack of understanding in how different PFAS alter the cellular functions and molecular pathways that culminate in the aforementioned outcomes, as well as the sheer number of PFAS that still require testing. My research aims to utilize transcriptomics data to better understand how exposure to under-researched PFAS impact gene expression and consequently disrupt the molecular pathways vital for a functional immune system. Previous work has shown that exposure to certain PFAS alter the ability of neutrophils to release antimicrobial stores of reactive oxygen species in a process known as the respiratory burst. By coupling this measurable phenotypic endpoint with differential gene expression analyses, this work seeks to demonstrate how PFAS influence the transcriptome and assess how these alterations manifest into disease states. Ultimately, this information will bridge existing knowledge gaps related to PFAS exposure and inform future methodologies.

## Rickard, Brittany

North Carolina State University
*Photodynamic Therapy (PDT) Targets Chemoresistance and Mitochondrial Enhancements in Ovarian Cancer Cells with a History of Chronic PFAS Exposure*

Photodynamic therapy (PDT), which generates reactive molecular species following light-based activation of a photosensitizer, can synergize with traditional chemotherapies to overcome chemoresistance. Such combinations are needed in ovarian cancer, where ~80% of patients develop platinum-resistant disease. Studies have shown that perfluoroalkyl substances (PFAS) induce chemoresistance in a duration-dependent manner in OVCAR-3 cells. PFAS are widespread drinking water contaminants present in the blood of nearly all Americans. The present study evaluated the ability of photodynamic priming (PDP), a sub-cytotoxic variant of PDT, in combination with chemotherapy to overcome chemoresistance in two OVCAR-3 cell cohorts: PFAS chronically-exposed and outgrown (allowed to “recover” from chronic PFAS exposure). Compared to cells that were chronically exposed to PFAS, outgrown cells displayed heightened sensitivity to chemotherapy along with decreased mitochondrial content. Proliferation remained significantly elevated compared to controls in outgrown cells, suggesting that not all PFAS-induced effects were rescued by a recovery period. Effectiveness of benzoporphyrin derivative-(BPD-) or aminolevulinic acid-induced protoporphyrin IX-PDP (ALA-PpIX-PDP) was assessed in combination with carboplatin and doxorubicin. In PFAS chronically-exposed cells, BPD-PDP + carboplatin decreased cellular survival fraction compared to carboplatin alone. Mitochondrial membrane potential also decreased significantly in both cell cohorts, mainly following ALA-PpIXPDP-based combinations. PDP + doxorubicin also successfully overcame chemoresistance arising from chronic PFAS exposure but was less effective than PDP + carboplatin. Together, these findings demonstrate the efficacy of PDP-based combinations for overcoming chronic PFAS exposure-induced chemoresistance and should be further explored in pre-clinical models of ovarian cancer.

## Rodriguez, Marc

UNC-Chapel Hill
*Fuel Type- and Sex-Specific Transcriptional Profiles of Wood Smoke Condensate Exposure in Primary Human Bronchial Epithelial Cells*

Wildfire-derived particulate matter (PM) varies in toxicity by fuel source, and sex may be an important mediator in health outcomes from exposure. PM produced by wood burning is associated with reduced lung function and increased infection susceptibility which are potential markers for lung injury and has become a global concern due to escalating prevalence and severity of wildfires. Smoke from fuel types across the United States, such as

red oak (eastern and central US), peat (peatlands/coastal, midwestern and southeastern US), ponderosa pine (western US), and eucalyptus (chaparral, southern California), have shown differential mutagenic and toxic effects in animal models, but effects by fuel type in human-derived models have not been investigated. Individual characteristics, such as sex, are also known to modulate the effects of biomass smoke exposure, though the mechanisms remain unclear. We aimed to fill these knowledge gaps by addressing the hypothesis that both fuel type and sex play a role in modulating respiratory health effects of smoke exposure using an in vitro model. Primary human bronchial epithelial cell (hBEC) cultures (n=4 Male, n=4 Female) were grown at air-liquid interface, and exposed apically to wood smoke condensates (WSCs) generated from red oak, peat, pine, and eucalyptus biomass at 5 μg/cm2 (low dose) and 25 μg/cm2 (high dose) for 4 hours. Apical washes, basolateral supernatants, and cell lysates were collected and analyzed for cytokine production via multiplex ELISA and transcriptional changes via RNA sequencing. Across all WSCs, there was a consistent induction of IL-1β, IL-8, and IL-6, while eucalyptus and peat exposures were uniquely associated with IL-2 induction. Peat, pine, and red oak exposures had unique IL-13 induction. When analyzed by sex, male hBECs exhibited a consistent IL-1β response across all WSCs and a unique IL-2 response to eucalyptus and peat WSCs. In contrast, female hBECs showed IL-8 induction specifically in response to eucalyptus and peat exposures. Overall, in vitro WSC exposure results in distinct inflammatory cytokine profiles based on fuel type and sex. Our RNA-seq analysis identified 202 differentially expressed genes (DEGs) across fuel types, with 51 DEGs overlapping between fuel types. Sex-specific analysis revealed 351 DEGs in male cultures and 108 DEGs in female cultures. Transcriptional pathway analysis utilizing GSEA and Hallmark MSigDB revealed upregulation of ROS production and fatty acid metabolism across all exposures, indicating cellular stress responses. Eucalyptus exposure downregulated protein secretion, while peat and red oak upregulated unfolded protein response, indicating endoplasmic reticulum stress. Downregulation of Notch signaling in both high dose pine and red oak exposures may reflect reduced cell proliferation and repair. Eucalyptus and pine exposure both led to upregulation of IL6/STAT3 signaling, while peat exposure caused upregulation of xenobiotic metabolism and cell proliferation. When disaggregated by sex, glycolysis was downregulated only in female hBECs for eucalyptus and peat exposure, while G2M checkpoint was downregulated in females for eucalyptus and peat and in males for eucalyptus and pine. Estrogen response was downregulated in females following eucalyptus and peat exposure, while only downregulated in males for pine exposure. Androgen responses were downregulated for multiple exposures across both sexes. Oxidative phosphorylation had opposite directions of induction for pine exposures between male and female hBECs. This research has the potential to advance the field of toxicology by revealing new insights into individual susceptibility and potential geographical variations in the respiratory health effects of wildfire smoke exposure. Mechanistic insight into fuel type or sex-based susceptibility can provide a basis for personalized treatments and targets for mitigating the toxic effects of wildfire smoke.

**Sarkar, Arunabh**
Duke University
*An Electron Transport Chain Complex V Inhibitor Attenuates Mitochondrial Energetics and Causes Dopaminergic Neurodegeneration*

Growing incidences of Parkinson's Disease (PD), characterized by loss of dopaminergic neurons, are reported in ageing populations. Epidemiological studies suggest that environmental factors like many specific chemical exposures are contributors to PD. Many of the implicated chemicals are mitotoxicants, but it is not clear if all mitotoxicants play a role. One less-studied mechanism of mitochondrial toxicity is Complex V inhibition, for which little is known about downstream molecular events. Therefore, as neurons have high energy demands to perform their functions, we are interested in investigating whether Complex V inhibitors that attenuate mitochondrial energetics trigger neuropathology in dopaminergic neurons. We found that developmental exposure to one the Complex V inhibitor dicyclohexylcarbodiimide (DCCD) caused dopaminergic neurodegeneration in a transgenic strain of *C. elegans* that permits in vivo imaging of neuronal morphology. The severity of neurodegeneration (ND) was exacerbated when DCCD exposure was combined with secondary neuronal stressors like 6-hydroxydopamine (6-OHDA, a specific dopaminergic neurotoxicant) or human α-synuclein (a protein that forms neuronal aggregates in PD) expression in dopaminergic neurons in *C. elegans*, suggesting a cumulative effect of stressor exposure in ND. We have also observed that DCCD exposure reduces dopaminergic ATP:ADP ratios and caused an upsurge in dopaminergic neuron oxidation state in adult worms. A partial rescue of dopaminergic ND was evident when we mitigated these elevated ROS levels using the MnSOD/catalase mimetic EUK-134.

We also observed that compromised lysosomal function due to mitotoxicant-mediated ATP depletion augmented dopaminergic ND. We further found that DCCD exposure reduced the acidity of lysosomes, and that when we specifically inhibited V-ATPases in lysosomes, this caused significant ND in dopaminergic neurons expressing human α-synuclein. Overall, our results suggest that inhibition of Complex V by chemical exposure increases redox stress and decreases energetics which may interfere with lysosomal clearance of protein aggregates, and that both of these contribute to dopaminergic neurodegeneration, in *C. elegans*.

**Scherer, Meredith**
U.S. Environmental Protection Agency/ORISE
*Modeling In Vitro Distribution Improves Accuracy of Bioavailable Dose Estimation*

**Background and Purpose:** Quantitative in vitro to in vivo extrapolation (IVIVE) relies on accurate estimations of the in vivo toxic dose. Use of the nominal effect concentration as proxy for bioavailable effective dose lacks accuracy due to chemical partitioning and binding to in vitro assay components. In vitro chemical distribution is often ignored because the bioavailable dose is not typically measured experimentally. The aim of this project is to adjust large quantities of new approach methodology data for in vitro distribution. Therefore, we are examining the accuracy of an in vitro distribution model in the context of a high throughput approach. This poster includes an additional review of the literature and has identified 86 observations of 36 new chemicals. **Methods:** The Armitage et al. (2014) in vitro distribution model (as implemented within R package "httk") has been modified to include ionization and plastic binding with the goal of increasing the applicability domain and more accurately modelling more chemicals. Paired experimental measurements of in vitro nominal and bioavailable concentrations were extracted from 13 studies in the peer-reviewed literature. The updated Armitage et al. (2014) model was evaluated with parameters corresponding to the experimental conditions of each study. Prediction errors were quantified using root mean squared log10 error (RMSLE). **Results:** Results of this analysis show that the Armitage model's predictions of bioavailable concentrations are more accurate (RMSLE = 2.57) than assuming the nominal concentration is equal to the bioavailable concentration (RMSLE = 3.08). This result is similar when different ionization states are considered; the Armitage model better predicts neutral (RMSLE = 1.81) and acidic chemicals compared to the nominal concentration (RMSLE = 2.87 for neutral chemicals) with only a slight improvement when using the nominal concentration to predict basic chemicals. **Conclusions:** This research suggests that the Armitage chemical distribution model is a more accurate method for predicting bioavailable concentrations for high throughput applications including next generation risk assessments using IVIVE. In this context, the bioavailable concentration is a proxy for tissue concentration in in vivo systems and accurately modeling in vitro distribution is necessary to accurately assess chemical hazards. This abstract does not reflect U.S. EPA policy.

**Shabrang, Mitra**
UNC-Chapel Hill
*Immune Evasion in Malignant Pleural Effusions: Could Smoking Be a Driver?*

Exposure to cigarette smoke, a complex mixture of environmental toxicants, is the primary risk factor for lung cancer. Lung cancer remains a leading cause of cancer-related mortality and a major public health challenge. A common and debilitating complication of lung cancer is malignant pleural effusion (MPE), characterized by the pathological accumulation of fluid in the pleural space, typically resulting from tumor spread to the pleura and/or lymphatic system. MPEs contain a diverse cellular population, including tumor cells, immune cells, mesothelial cells, fibroblasts, and endothelial cells, along with cytokines produced by both immune and tumor cells. Previous studies by Mortaz et al. demonstrated that cigarette smoke extract can modulate dendritic cell function, inducing chemokine release and altering T cell proliferation, thereby influencing immune cell recruitment and activity. Despite high total lymphocyte numbers found within MPE, CD8+ T cells which are the central mediators of anti-tumor immunity appear to lack ability to negate the establishment and maintenance of malignant pleural disease. The primary objectives of this study were to 1) characterize the presence of antigen presenting cells (APCs) in the MPE fluid, and 2) identify and assess antigen-specific CD8+ T cells within the MPE microenvironment. One patient was selected from the Carolina Center for Pleural Diseases (CCPD) biobank for personalized studies. Using tumor genomics data on the LENS platform, we identified 96 neoantigens in this patient's lung cancer cells. Multi-color flow cytometry was then used to assess the presence of APCs within the pleural fluid

mononuclear cell (PFMC) population. Finally, using the MANAFEST assay, T cells were stimulated with the predicted neoantigen peptides, and after 10 days of culture, an IFN-γ ELISpot assay was performed to detect neoantigen-specific CD8+ T cells in the pleural fluid. Flow cytometry analysis of PFMCs identified robust populations of dendritic cells, B cells, and macrophages, all expressing markers indicative of their capacity to participate in antigen presentation. The MANAFEST assay, which promotes the proliferation of neoantigen-specific CD8+ T cells over a 10-day culture period, followed by IFN-γ ELISpot to detect antigen-specific responses, confirmed the presence of neoantigen-specific CD8+ T cells in the MPE fluid, supporting the potential for localized anti-tumor immune responses within the pleural space. Our findings demonstrate that MPE fluid contains both functional APCs and antigen-specific CD8+ T cells. However, despite the presence of these populations, effective intrapleural anti-tumor immunity is not achieved, suggesting factors within the MPE microenvironment may actively suppress CD8+ T cell function or impair their cytotoxic potential. In future studies, we plan to perform cytokine profiling and use mass spectrometry to identify cigarette smoke metabolites and investigate their impact on these immune populations.

**Sharma, Sunil**
Duke University
*Perfluorononanoic Acid (PFNA) Exposure and Photoperiod Differences: A Dual Impact on Developing Eye and Brain Transcriptome in Zebrafish*

Perfluorononanoic acid (PFNA) is a synthetic long-chain ($C \geq 8$) homolog of per and polyfluoroalkyl substances (PFAS) and has broad applications in industrial and consumer products. It is frequently detected in the environment as an emerging contaminant with potential threats to living organisms, like immune, cardiac, endocrine, reproductive and developmental toxicity. This study investigates the developmental toxicity of PFNA using behavioral assay, especially focusing on organ-specific toxicity via transcriptomic analysis. Tropical 5D zebrafish embryos were exposed to PFNA from 2 hours post-fertilization (hpf) to 120 hpf under dark and light: dark conditions at 28 °C. Larval photomotor response (LPR) was conducted to assess the change in behavioral patterns. For organic-specific toxicity, embryos were exposed to 0 and 12.5 µM of PFNA for 120 hpf, and the organs (brain and eyes) were dissected for RNA extraction and sequencing. PFNA exposure led to significant behavioral alterations in zebrafish larvae at 6.25 and 12.5 µM, characterized by hyperactivity during light phases and distinct “O-bend” responses in the LPR assay. This hyperactivity indicates neurotoxic and visual impairments, which directed us to focus on transcriptomic analysis of the brain and eyes. RNA sequencing showed 287 (dark incubation) and 503 (light: dark incubation) differentially expressed genes (DEGs) in the brain, while 683 (dark incubation) and 914 (light: dark incubation) in the eye. GO and KEGG pathways analysis revealed that the commonly affected pathways in both organs were steroid biosynthesis, fatty acid metabolism and PPAR, MAPK and P53 signaling. These results suggest that PFNA affects lipid metabolism and other signaling pathways, potentially contributing to hyperactivity. Additionally, a smaller number of DEGs in the eyes and brain in dark incubation with respect to light: dark suggested that incubation conditions also affect toxicity during early embryonic development.

**Silver, Brian**
National Institute of Environmental Health Sciences
*Characterization of Extracellular Vesicles and miRNA Released by Cerebral Organoids*

Environmental toxicants can contribute to the development of several neurodegenerative diseases. However, the mechanisms behind this pathology are still incompletely understood. Prompt diagnosis of impending neurodegeneration is crucial for early interventions to prevent cognitive decline. Towards this end, accurate biomarkers for early neurodegenerative processes and exposure risk are needed. Extracellular vesicles (EVs) are lipid particles released by cells which contain many bioactive molecules including miRNAs. EVs may serve both as a route of propagating neurotoxic phenotypes and as a source of biomarkers for neurological disease. However, the exact mechanisms though which EVs could spread the deleterious effects of toxicants and the full spectrum of their usage as biomarkers remain unclear. Organoid models have several advantages, including potential for use in high-throughput toxicant testing and applications in personalized medicine and disease models. However, few studies have examined EV release in brain organoids to determine if the EVs could contain useful biomarkers. We employed several technologies to characterize EVs released by human cerebral

organoids and their associated miRNAs. We identified that cerebral organoids consistently release EV-associated miRNA in quantities sufficient for robust analysis with NanoString. Further, pathway analyses revealed that terms related to neurodegenerative disease and nervous system signaling are associated with the recovered miRNAs. Together, these data suggest that cerebral organoids have utility as a tool for the discovery of EV-associated miRNAs involved in neurodegenerative disease and neurotoxicity.

**Soerianto, Winny**
UNC-Chapel Hill
*Wildfire Smoke Attenuates Antiviral Defenses Against Live Attenuated Influenza Virus in Differentiated Human Nasal Epithelial Cells*

Wildfires are occurring with increasing frequency, leading to greater human exposure to woodsmoke and its associated health risks. Co-exposure to wildfire smoke and respiratory infections may amplify adverse health outcomes. Our previous studies have shown that woodsmoke particles (WSP) can alter antiviral host defense responses in human volunteers inoculated with the live-attenuated influenza virus (LAIV) vaccine, as a model for influenza infections. To determine underlying mechanisms, we established an in vitro nasal epithelial cell WSP exposure–infection model using the LAIV vaccine that replicates at the temperature of the nasal passage. Primary human nasal epithelial cells (hNECs) from six healthy adults (3 males, 3 females) were differentiated at the air–liquid interface. Cells were exposed apically to 22 μg/cm² of red oak WSP for 2 hours, followed by infection with 2024/2025 FluMist® (intranasal LAIV) for 24 or 72 hours. In a reverse design, hNECs were first infected with LAIV for 24 hours prior to WSP for 48 hours. WSP composition was analyzed by gas chromatography–mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Barrier integrity was assessed by transepithelial electrical resistance (TEER). IP-10 and IL-6 concentrations were measured by ELISA, and IFNα expression was evaluated by targeted gene expression analysis. MS chemical analysis confirmed the presence of polycyclic aromatic hydrocarbons, phenols, and other respiratory irritants in WSP. TEER measurements showed no significant differences between control, WSP-exposed, or LAIV-infected cells. IP-10 levels increased following LAIV infection, but this response was attenuated when cells were pre-exposed to WSP. Infection with LAIV followed by WSP exposure did not significantly alter IP-10 compared to controls. IL-6 concentrations remained unchanged across all conditions. Gene expression analysis showed a significant decrease in IFNα when WSP exposure preceded LAIV infection. Woodsmoke exposure prior to LAIV infection dampens antiviral responses in the hNEC in vitro model, potentially decreasing effectiveness of intranasal vaccines. Future studies are needed to elucidate the molecular pathways altered by woodsmoke and to identify chemical constituents mediating impaired antiviral signaling.

**Thomas, Mallory**
North Carolina State University
*PFAS Interaction with Gamma Globulin and Lactoferrin: Implications for Immune System Dysfunction*

Per- and polyfluoroalkyl substances (PFAS) constitute a wide variety of man-made chemicals utilized for their many advantageous industrial properties, such as heat-, oil-, and water-resistance. Due to their ubiquity in manufacturing, these chemicals are persistent in the environment with no successful methodology for degradation. Their longevity highlight the necessity for experimental toxicity data regarding human exposure from contaminated waterways and other systems. Here, studies investigated the binding affinity of PFAS to key immune function proteins to determine their toxicity and bioaccumulation in the human body. Two proteins of interest reported are human lactoferrin and the immunoglobulin, gamma globulin. Both are present in bodily fluids, such as breast milk and saliva, and have primary functions as components of the immune system. Utilizing changes in melting temperature measured via a simple, cost-effective thermal shift assay, we can provide valuable insight into the destabilization of these proteins close to, or at, biological temperatures. In addition, these changes in melting temperature reveal relative binding affinities to PFAS and determine the strength of binding in biological systems. Our study aims to determine these relative binding affinities and overall destabilization of both proteins reported herein when exposed to a various PFAS covering a range of functional groups and carbon chain lengths. Preliminary studies reveal mass destabilization of lactoferrin and gamma globulins when bound to all PFAS investigated leading to profound questions on how the immune system can be severely affected by the binding of PFAS.

**Tobin, Emma**
North Carolina State University
*Assessing the Impact of PFAS Exposure on Doxorubicin Uptake and Doxorubicin-induced DNA Damage Repair*

Per- and polyfluoroalkyl substances (PFAS) are a widely distributed class of fluorinated, synthetic chemicals. Exposure to some PFAS is associated with cancer but the mechanisms of carcinogenesis are not fully understood. A common characteristic of carcinogenic chemicals is effects on DNA damage repair, but this endpoint is largely understudied for PFAS. Few previous studies have indicated exposure to perfluorodecanoic acid (PFDA ) and perfluorooctanoic acid (PFOA) can lead to inhibition of the repair of double strand DNA breaks, but this work is limited in scope – assessing effects of individual PFAS only. The prior studies also did not assess bioavailable PFAS concentrations or test whether PFAS exposure altered DNA damaging agent uptake. We are using high throughput techniques to assess the impact of exposure to multiple PFAS, individually and in mixtures, on DNA damage repair. We are incorporating analytical methods to measure bioavailable PFAS concentrations and intracellular concentrations of DNA damaging agents, increasing our research results translation. We hypothesize; if PFAS exposure interferes with baseline DNA repair processes, then exposing mammalian cells to PFAS prior to and during DNA damage will result in reduced DNA damage repair rate. We are validating the use of a high throughput alkaline comet assay (HT CometChip assay, Cell Array). The 96-well HT CometChip format allows us to assess a wide range of PFAS and environmentally informed PFAS mixtures to screen for impacts on DNA repair. Each of the 96 wells contains ~300 microwells in which cells are embedded, allowing many comets to be quickly imaged and scored using Comet Analysis Software (Trevigan, 4260-000-CS). We are conducting initial method validation and hypothesis testing with two PFAS; perfluorooctanoic acid (PFOA) and trifluoroacetic acid (TFA). The DNA-damaging agent is doxorubicin which can induce both single and double strand DNA breaks. As it is not understood if PFAS exposure affects the uptake of doxorubicin, potentially impacting the initial severity of DNA damage, we are quantifying the impact of PFAS exposure on intracellular doxorubicin concentration using fluorescence spectrometry. Our overarching study design includes pre-exposing human hepatoblastoma cells (HepG2) and Chinese hamster ovary cells (CHO-K1) to PFAS individually or in mixtures for 24 hours, initiating DNA damage with doxorubicin treatment (0.125μM – 2μM), then removing doxorubicin and monitoring the repair of damaged DNA in the presence of PFAS over a 24 hour period utilizing the HT CometChip assay. Prior to completing initial hypothesis testing, we are assessing the precision of the HT comet chip assay by comparing within-sample comet size variability across wells and across comet chips. After exposure of CHO-K1 cells to 2μM doxorubicin for 24 hours we found the average coefficient of variation (CV) across comet size measurements in wells containing technical replicates to be 33%. The exclusion of wells on the perimeter of the comet chip from analysis reduced the CV to 26%, indicating the location on the HT CometChip impacts comet size. Different analytics of comet size exhibited different variability, with DNA fragmentation (a measure of total fragmentation in both head and tail) being least variable (13% CV), and moment of inertia (a measure incorporating tail size and comet intensity) being most variable (45% CV). Doxorubicin uptake and PFAS-exposure experiments are ongoing. This work will assess the impact of PFAS on an understudied but potentially sensitive endpoint of PFAS exposure (DNA damage repair). We aim to generate translatable data describing the impact of environmentally relevant PFAS exposure paradigms.

**Toler, Sydnie**
UNC-Chapel Hill
*Increased Living Temperature Alters the Cardiopulmonary Response of Male and Female Mice to Benzene-toluene-ethylbenzene-xylene (BTEX)*

Benzene, toluene, ethylbenzene, and xylene (BTEX) compounds are ubiquitous in the environment, originating from both natural and anthropogenic sources. Despite the broad presence of BTEX in the environment, extensive use of petroleum products and improper maintenance of underground storage tanks (USTs) have increased the risk of exposure to these compounds. Hazardous substances stored in USTs, like BTEX, may leak into groundwater, soil, and buildings and homes over time. Furthermore, higher temperatures exacerbate the release of VOCs. Therefore, the purpose of this study was to characterize the cardiopulmonary effects of inhaled BTEX in female and male C57BL6J mice and assess the role of living temperature. Eight-week-old mice were housed at normal temperature (NT - 22°C) or high temperature (HT - 32°C) for five weeks. Mice were then exposed to either filtered air (FA) or BTEX (316ppm, nose-only) for four hours per day for two consecutive days. Ventilatory

function was assessed via whole-body plethysmography (WBP) three days before and immediately after the first exposure. High-frequency echocardiography (HF-echo) was performed the week prior to exposure and again immediately following the second exposure. WBP and HF-echo measurements were compared from pre- to post-exposure. When compared to NT, HT significantly increased tidal volume (TV), peak expiratory flow (PEF) peak inspiratory flow (PIF), and relaxation time (RT), and decreased enhanced pause (Penh) in female and male mice. BTEX increased frequency (f) and decreased expiratory and inspiratory times (Te and Ti, respectively) as well as Penh in female NT mice but had no effect on male NT mice. At HT, BTEX significantly decreased f and Penh and increased TV, Te, Ti, and RT in female mice, and only increased TV, PEF, PIF in males. Thus, the impact of HT and BTEX on female mice appeared to be greater than on males. Female HT-FA mice had a significant decrease in heart rate (HR) compared to NT, while BTEX caused an increase. On the other hand, male FA mice experienced an increase in HR at HT. No significant differences in cardiac output, stroke volume, fractional shortening, ejection fraction, end systolic volume, or end diastolic volume were observed between the groups. These results indicate that higher living temperature alters cardiopulmonary function in female and male mice in a differential manner. Although breathing changes seem to be largely driven by temperature, it is worth noting that HT might contribute to a higher inhaled BTEX dose, and therefore, increased risk. Long-term exposure assessments are needed to clarify these effects further. (This abstract does not reflect EPA policy.)

**Tu, Litao**
UNC-Chapel Hill

*Differentiation Media and Substrate Choice Shape Morphology and Electrophysiology of Air-Liquid Interface Primary Human Airway Epithelial Cell Cultures*

**Background:** Primary human bronchial epithelial cell (hBEC) air-liquid interface (ALI) cultures are widely used to: 1) investigate respiratory biology, 2) model inhalation exposures, and 3) develop airway New Approach Methodologies (NAMs). These in vitro models mimic key features of the in vivo airway epithelium, including mucociliary differentiation, barrier formation, and ion transport, increasing physiologic relevance for toxicology studies. Despite their broad use, it remains unclear how specific culture conditions shape epithelial architecture and function, which complicates data interpretation and reproducibility. To address this gap, we compared four commonly used differentiation media formulations: 1) University of North Carolina ALI (UNC-ALI), 2) PneumaCult® ALI (PC-ALI), 3) Vertex ALI (VALI), and 4) a modified Lonza® (ML) formulation on two porous support substrates. Our goal was to characterize morphological and functional differences between cultures to inform best-use cases for airway models, improve reproducibility across laboratories, and guide regulatory understanding of different in vitro systems for respiratory toxicology. **Methods:** Primary hBECs from three donors were expanded using conditionally reprogrammed cell (CRC) methods to passage 3, then differentiated at ALI on either 12-mm Millicell®-CM inserts (polytetrafluoroethylene, 0.4 μm pore size) or 6.5-mm HTS Transwell® inserts (polyester, 0.4 μm pore size). Between 28 and 35 days, barrier function of Millicell® cultures was determined by measuring transepithelial electrical resistance (TEER) with an EVOM Manual™, while Transwell® cultures were evaluated using a 24-channel transepithelial current clamp amplifier (TECC24). Epithelial sodium channel (ENaC) activity was assessed in Transwell® cultures using benzamil-sensitive current measurements. For morphological analysis, cultures were fixed and stained with hematoxylin and eosin (H&E) and Alcian Blue-PAS (AB-PAS) to quantify epithelial thickness, cell numbers, goblet cells, and ciliated cells. **Results:** Media and substrate conditions produced striking differences in epithelial architecture and function. Key differences were that PC-ALI/Millicell® cultures were thicker than all other conditions. PC-ALI media yielded the highest ciliated cell counts on both substrates and PC-ALI/Millicell® cultures had lower TEER than all other medias tested. VALI/Millicell® cultures had more goblet cells than any other media/substrate combination, an effect not observed in VALI/Transwell® cultures. Broadly, Millicell® supports produced thicker cultures with more goblet cells than Transwell® supports. TEER was highest in UNC-ALI/Millicell® cultures but was only significantly higher than VALI/Millicell® cultures. TEER values in other media and substrate conditions were not significantly different. Benzamil-sensitive ENaC activity was greatest in PC-ALI/Transwell® and VALI/Transwell® cultures, and lowest in UNC-ALI/Transwell® cultures. **Conclusions:** These findings demonstrate that both differentiation medium and substrate strongly influence airway epithelial model parameters, including morphology, cell-type composition, barrier properties. These features are likely to shape airway model responses to airborne toxicants. Thus, careful selection and transparent reporting of model parameters are essential to ensure reproducibility across laboratories. These data support the thoughtful application of primary human airway models as NAMs to

evaluate inhaled toxicants and to guide the design of in vitro systems that more accurately predict human outcomes. Importantly, this comparative dataset will contribute to ongoing efforts by agencies and consortia to advance airway NAMs for regulatory decision making.

**Wang, Amy**
National Institute of Environmental Health Sciences
*Where Text Mining Helped and Where it Did Not: A Case Study of Building a Systematic Evidence Map of Mechanistic Information for Polycyclic Aromatic Hydrocarbons (PAHs)*

**Background and purpose:** It is critical to consider mechanistic information in high quality cancer hazard evaluations. However, systematically organizing mechanistic information manually is time and labor intensive such that it can be impractical for data-rich chemicals. We aimed to efficiently organize mechanistic information for polycyclic aromatic hydrocarbons (PAHs) by key characteristics of carcinogens (KCCs) via text-mining, manual review, and survey sampling prediction to generate systematic evidence maps (SEMs) to support future hazard evaluations. **Methods:** A literature search for PAHs known to have animal cancer data was conducted in PubMed, Scopus, and Web of Science. We evaluated several methods to identify and label references by chemical (PAH) and by mechanism (KCCs) before building SEMs. Because cytotoxicity was ubiquitously measured, studies were only considered for KCC10 if cell death was decreased (KCC10b). All other KCCs were based on what was investigated (regardless of outcome). Stage A: We compared manual identification and labeling to text-mining for PAHs and KCCs. The identification of PAHs by text mining used PAH official names and synonyms. For KCCs , we tested three automated text-mining approaches to identify studies using syntax developed based on RoC literature search terms for the KCCs. The results underwent extensive comparative analysis to evaluate the accuracy and comprehensiveness of the identification/labeling compared to human reviewers. Stage B: We developed and validated performance of survey sampling prediction of number of references for KCCs by comparing predictions to the human tagging results for five PAHs. Stage C: The best-performing methods from earlier stages were then used to develop an interactive SEM of studies with mechanistic data on 14 priority PAHs that had animal cancer data from human-relevant exposure routes. Metabolites of PAHs were added to the text-mining syntax for PAH identification. **Results:** Stage A: Identification of PAHs by text mining was more accurate than by human reviewers, while determination of KCCs was more accurate by human reviewers than by any of the three text-mining approaches. We recommend PAH identification by text mining followed by KCC determination/labeling manually. Stage B: Survey sampling prediction produced estimates of the distribution of references across KCCs per PAH closely aligned with the results of human reviewers. We recommend survey sampling prediction for PAHs with over 500 references per PAH for appreciable time savings. Stage C: Over 16,000 references were identified by text mining as having studied one or more of the 14 priority PAHs. For the three PAHs (benzo[a]pyrene, fluoranthene, and naphthalene) that had over 500 references each, the distribution of KCC information per PAH was based on both human reviewer determination and survey sampling prediction. The distribution of KCC information for all other PAHs was based on human reviewers' assessment for every reference. The most frequently studied KCCs were KCC1 (electrophilicity), KCC2 (genotoxicity), KCC5 (oxidative stress), and KCC8a (AhR-mediated effects). An interactive SEM of studies on these 45 PAHs can be used to identify studies that provided data across all 10 KCCs and search, sort, or filter the data by chemical structure features, KCCs, bibliography information, etc. **Conclusion:** Evaluating the performance of new methods helps build confidence in approaches that differ from standard procedures. We showed the development, evaluation, and validation of tagging approaches and survey sampling prediction, and how using text mining and survey sampling prediction in selected topic areas can expedite organizing voluminous mechanistic information into an SEM. This research was supported by the National Institute of Health, National Institute of Environmental Health Sciences.

**Williams, David**
RTI International
*A Mechanistic Use Case to Support Data Interoperability Across the Source to Outcome Continuum in Environmental Health Science*

**Background**: Environmental Health Science (EHS) encompasses multiple interconnected research domains with components that span the entire source to outcome (S2O) continuum. This continuum encompasses

domains ranging from external exposure, to dosimetry, to mechanistic pathway responses, culminating in biological and population outcomes. Integration of data from across these research domains strengthens the conclusions in EHS by leveraging multiple available information streams. However, each data type is nuanced, and researchers from one domain may not have a detailed understanding of how the methodologies used impact the integration of their data with other EHS sources. Additionally, studies may not record these nuances in such a way that they are accessible or clear. For example, researchers conducting in vivo experiments may look to in vitro experiments and analyses to inform dosing regimens but may not be able to determine how the laboratory exposure concentrations relate to their model organism. This work focuses on improving the integration of EHS data from across the S2O continuum through the development and implementation of data standards. To accomplish this goal, we develop a pilot use case focused on PM2.5 exposure leading to decreased lung function to identify deficiencies in interoperability, test the functionality of improved standards, and demonstrate the benefits of integrated analyses. We present the preliminary work for the pilot study describing the “outcomes” portion of the S2O continuum. **Methods**: We leverage the Adverse Outcome Pathway (AOP) framework to construct our pilot use case. Specifically, we use existing molecular initiating events, key events and key event relationships, and adverse outcomes from the AOP Wiki site (numbers 148, 411, and 425) to construct an AOP network describing the mechanistic pathways that lead from PM2.5 deposition in the lungs to decreased lung function. The two molecular initiating events, EGFR activation and oxidative stress, and key events, including decreased FoxJ1, decreased cilia number/length, decreased ciliary beat frequency, increased goblet cell production, decreased mucociliary clearance, and increased mucin production, form the pathways leading to the adverse outcome of decreased lung function. We identify computational models to describe the mechanistic steps in this network, map data types to each step in the AOP network, and identify key inputs and outputs. These computational models are combined in a series of relationships to form an AOP Bayesian Network (AOPBN) to inform predictions of decreased lung function. Using these models, we identify where standards and metadata are needed for integration. **Results:** We present models, preliminary findings, and anticipated results for the computational pilot use case focused on the “outcomes” portion of the S2O continuum. Specifically, we highlight inputs and outputs from each model, limitations of parameter sources, and the initial needs identified by this work to promote interoperability among EHS data streams. These needs include 1) the contextualization of parameter values regarding experimental conditions (e.g. cell line), 2) the development of response-response models to quantitatively link causal mechanistic steps and inform probabilistic relationships, and 3) the harmonization of model parameters, inputs, and outputs. We demonstrate the benefits of integrating multiple EHS data sources through comparative analysis of initial model outcomes, enabling a scalable and reusable framework for modeling, analysis, and decision-making. This approach builds a foundation for more trustworthy EHS science. **Conclusions**: This work highlights the concerns and considerations that are relevant when performing computational modeling with secondary data use. Computational models provide approximations based on assumptions about the best available data, which may be assembled from multiple disparate studies. Thus, it is critical that the data used to parameterize computational models must be used in appropriate contexts. Data standards are essential to ensuring that contextualizing information is available for secondary data use. Additionally, standards can allow for increased machine readability to support both data discovery and implementation through emerging technologies such as Large Language Models and Artificial Intelligence. As EHS continues to develop as a field, it is critical that data interoperability be prioritized to enable stronger inferences that leverage knowledge from multiple study domains.

**Yoo, Brendan**
U.S. Environmental Protection Agency/ORISE
*Differential Cardiometabolic Impacts of Maternal Ozone Exposure in Adult Long-Evans Offspring*

Cardiovascular disease (CVD) and metabolic disorder are both substantial contributors to global mortality, with their pathologies closely intertwined. Exposure to ambient air pollutants (AAPs) has been shown to negatively impact offspring cardiometabolic health, linked with the development of cardiac hypertrophy and dyslipidemia, among others. While environmental factors influence the pathogenesis of cardiometabolic conditions, adverse exposures during critical developmental windows may also contribute to disease risk. We have previously shown that gestational exposure to the AAP ozone (O3) compromises fetal growth and predisposes offspring for susceptibility to metabolic and pulmonary perturbances later in life. However, the cardiovascular consequences of this gestational O3 exposure remain largely under-characterized. Considering evidence linking early life

exposures to particulate matter and other AAPs to postnatal cardiovascular dysfunction, herein, we sought to explore whether similar phenotypes were present in offspring from O3 exposed dams. We hypothesized that offspring born to O3 exposed dams would have worsened cardiovascular function as they age into adulthood, with a differential response across sexes. To evaluate the cardiovascular and metabolic consequences of gestational O3 exposure, 12-week-old timed-pregnant Long-Evans rats were exposed to either filtered air or 0.8 ppm O3 for 4 hours/day on gestation days (GDs) 5 and 6. Following weaning, male and female offspring were monitored for cardiometabolic health until ~5 months of age. Maternal O3 exposure did not result in changes to most of the metabolic measurements collected before 4 months of age (body weight, food intake, body composition, glucose tolerance, blood pressure). However, indirect calorimetry testing at ~4 months old, revealed that offspring born to O3 exposed dams had altered respiratory exchange ratio. This effect differed by sex - increased in females and decreased in males. Furthermore, histological assessment of adipose tissue revealed that female offspring experienced adipocyte hyperplasia in the retroperitoneal depot. M-mode dobutamine stress echocardiography at ~5 months, as well as cardiac histology, revealed male offspring born to O3 exposed dams had altered cardiac structure and function, including thickening of the left ventricular wall, increased ejection fraction, and increased fractional shortening. For female offspring born to O3 exposed dams, pulsed-wave doppler assessment of transmitral valve flow revealed an increased myocardial performance index due to decreased aortic ejection time. Based on these findings, we performed RNAseq on archived hearts from GD 21 fetuses. This analysis revealed sexually dimorphic effects of gestational O3 exposure on cardiac gene expression, supporting the hypothesis that this exposure may disrupt cardiac development. Collectively, these results demonstrate maternal exposure to O3 is detrimental to offspring cardiovascular development, extending our previous findings, demonstration that peri-implantation O3 exposure results in multi-organ dysfunction in adult offspring. [This abstract does not reflect US EPA policy.]