37th Annual Meeting of the Allegheny-Erie Society of Toxicology Regional Chapter

Community-Based Health and Safety Initiatives

University Club, University of Pittsburgh
Pittsburgh, PA
May 6–7, 2024

University of Pittsburgh | Center for Health, Environment, and Engaged Research

SOT | Society of Toxicology
Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

Pitt Public Health
Environmental and Occupational Health

The theme for the 2024 Allegheny-Erie Society of Toxicology (A-E SOT) annual meeting will focus on local research focusing on furthering health and safety initiatives in our region.

Meeting highlights:

1) Graduate and undergraduate student "*Lunch with an Expert*" meeting.
2) Poster sessions on all toxicological subjects.
3) Awards for best presentation, best methodology, best overall poster, and best undergraduate poster.
4) Sponsors & Exhibitors will be present to exhibit state-of-the-art services and scientific equipment.

Undergraduate Researchers and Mentors: Attend, present your current research, network, and benefit from unique programming for students.

For additional information, please contact Elaine Freeman, MS, DABT, A-E SOT President (efreeman@exponent.com).

A-E SOT Website: http://www.toxicology.org/groups/rc/allegheny/index.asp

PRELIMINARY SCHEDULE

Monday, May 6

9:00 AM–9:45 AM	Registration
10:00 AM–10:15 AM	Welcome and Announcements
10:15 AM–12:30 PM	***SYMPOSIUM 1:*** **Hazard Characterization and Control**
10:15 AM	Introduction/Symposium Overview–Elaine Freeman
10:30 AM	**Keynote Speaker: Dr. James Fabisiak, University of Pittsburgh, Center for Health Environments and Communities, Environmental and Occupational Health**
11:30 AM	Emily Nicholls, University of Pittsburgh Environmental and Occupational Health
11:50 AM	Emily Rice, Department of Microbiology, Immunology & Cell Biology, West Virginia University Cancer Institute, School of Medicine, West Virginia University
12:10 PM	William Mandler, National Institute for Occupational Safety and Health
12:30 PM–1:15 PM	BREAK

1:15 PM–3:30 PM	***SYMPOSIUM 2:*** **Understanding Exposures and Risks to Public Safety**
1:15 PM	Introduction/Symposium Overview
1:30 PM	**Keynote Speaker: Dr. John Stolz, Duquesne University, Director, Center for Environmental Research and Education, Professor, Environmental Microbiology**
2:30 PM	Yuchen Sun, Environmental and Occupational Health, University of Pittsburgh
2:50 PM	Meghan McGraw, Department of Physical Medicine and Rehabilitation, University of Pittsburgh
3:10 PM	Hunter Russell, Department of Physiology, Pharmacology & Toxicology, West Virginia University
3:30 PM–3:45 PM	Break
3:45 PM–6:30 PM	Poster Session – Networking – Refreshments
6:30 PM–8:30 PM	Group Social

Tuesday, May 7

8:00 AM–9:30 AM	Registration and Breakfast
9:30 AM–11:30 AM	***SYMPOSIUM 3*****: Application of Hazard and Exposure Assessment in Risk Characterization**
9:30 AM	Introduction/Symposium Overview
9:45 AM	**Keynote Speaker: Dr. Danelia Stricklin, Branch Chief of the Pathology and Physiology Research Branch, NIOSH**
10:45 AM	A. Kiyanda, Department of Environmental and Occupational Health, University of Pittsburgh School of Public Health
11:05 AM	CM Vanderpure, Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh
11:25 AM	Rob Gresser, Environmental and Occupational Health, University of Pittsburgh
11:45 AM–12:45 PM	“Lunch with an Expert” & Networking
12:45 PM–1:00 PM	Awards
1:00 PM	Closing Comments and Adjourn

Key Note Speakers

Dr. James P Fabisiak is an Associate Professor of Environmental & Occupational Health, Vice-Chair of Practice, and Director of Center of Healthy Environments and Communities (CHEC), at the University of Pittsburgh. He attended Syracuse University (BS) and Cornell University (MS) prior to receiving his PhD in Pharmacology at the Pennsylvania State University School of Medicine at the Milton S. Hershey Medical Center. He went on to postdoctoral training at the University of Vermont and was the recipient of a Parker B. Francis Fellowship in Pulmonary Biology and Respiratory Medicine. He then went to the University of Pittsburgh and spent some time in the Division of Pulmonary & Critical Care Medicine and the Department of Pharmacology before joining the Department of Environmental & Occupational Health. He considers himself a mechanistic toxicologist studying the cellular and molecular responses of injury, inflammation, repair, and pathologic remodeling in the lung following environmental insult. He has received funding from the National Institutes of Health, US Environmental Protection Agency, and other sources and has contributed to over 50 peer-reviewed publications. More recently he has "expanded his portfolio" to include community-based activities such as studying public health risks associated with point source air pollution, unconventional natural gas drilling and marine oil spills. He serves as an author of several reports and peer-review manuscripts examining community air quality and health in Southwest PA. Current funded projects include surveillance of environmental and health impacts of the East Palestine derailment and feasibility assessment of indoor air quality testing for people seeking services to age-in-place.

Title: ***Pitt Public Health's Center for Healthy Environments and Communities (CHEC): A Lens for Academic Research into Regional Environmental Health Concerns***

Academic and scholarly activities in environmental health often pursue questions on global or general scales while neglecting their relevance at a regional level. Southwest PA (SWPA) has a legacy of environmental degradation via industrial development and natural resource extraction. In addition, current and emerging problems of poor air quality, unconventional natural gas development, and petrochemical industrial expansion, among others, pose potential health hazards as well. The regional perspective takes on additional priority for environmental justice concerns with the need to highlight those communities most exposed and impacted. The CHEC was founded nearly 20 years ago to serve as an interface between academic environmental health research and communities in SWPA. The overall mission of CHEC is to apply a data-driven and fact-based approach to understand and explore potential environmental health issues in SWPA. CHEC seeks to advance this broad mission primarily from the perspective of informing and engaging multiple community stakeholders beyond traditional academic peers and channels. This includes environmental advocacy groups, regulatory agencies, community policy makers, and average citizens. This presentation will highlight some specific examples of how CHEC works to advance its mission via 4 platforms: 1) Utilize existing large data sets in a manner that highlights regional environmental health issues and make them accessible to concerned stakeholders, 2) Gather and analyze scientific data in both investigator-initiated and community-based participatory research settings, 3) Provide academic and educational support to individuals and groups who advocate for a healthier environment, and 4) Provide a platform that organizes and supports collaborative efforts between environmental and other scientists in these efforts.

Dr. John Stolz is a Professor in the Department of Biological Sciences and Director of the Center for Environmental Research and Education at Duquesne University. He has a BS degree in biology from Fordham University and a PhD from Boston University in microbial ecology and evolution. He was a National Research Council Postdoctoral Fellow at the NASA Jet Propulsion Laboratory and the Department of Geology and Planetary Sciences, California Institute of Technology, and held a National Science Foundation Postdoctoral Fellowship in Plant Biology in the Biochemistry Department at the University of Massachusetts, Amherst. His main research interests are in the community structure of microbial mats and stromatolites; the microbial metabolism of metals and metalloids including arsenic, selenium, and nitrate; and water quality impacts of unconventional shale gas extraction. He is an American Association for the Advancement of Science Fellow and recipient of the Dewey Award from Clean Water Action. Dr. Stolz has published 105 journal articles and 46 book chapters and author-edited three books.

Title: ***Community-Based Outreach for Drinking Water Quality***

The goal of the Water Quality Project at Duquesne University is to protect water resources, both surface and groundwater, that provide drinking water where extractive activities are occurring, particularly the development of unconventional oil and gas (UOG). This work has included sampling and analysis of surface (i.e., creeks, streams, and rivers) and groundwater (i.e., private water wells) for contaminants such as salts, metals, light hydrocarbons, and radionuclides. The results have helped establish parameters that allow determination of the sources of contamination, and whether this contamination is related to UOG activities (i.e., drilling, fracking, pipeline construction, waste disposal, injection wells). We also determined that UOG waste taken to sanitary landfills is impacting the composition of the landfill leachate with both toxic compounds and radioactivity. This harmful leachate is often sent to public wastewater treatment plants and then discharged into surface waters in the environment. Our results have shown a concerning increase in radioactivity in sediment downstream of these discharges, and in some cases, negative impacts to downstream public drinking water facilities. The primary radionuclide is radium-226, which decays to radon-222. We have also demonstrated that deicer products made from oil and gas waste, such as AquaSalina, contain significant levels of radium-226 (and Ra-228), in addition to being radon generators. The most important part of this project, however, has been providing free water testing and educational outreach to individuals and communities. Working with local community groups such as ProtectPT and the Center for Coal Field Justice, the public outreach has helped inform communities about water quality (i.e., how to interpret a water analysis report) and how they can protect their water resources.

Dr. Daniela Stricklin is the Branch Chief for the Pathology and Physiology Research Branch (PPRB) at the Health Effects Laboratory Division (HELD) of the National Institute for Occupational Safety and Health (NIOSH) within the Centers for Disease Control and Prevention (CDC). PPRB conducts health effects research to better understand the impact of a wide array of occupational exposures on workers. Dr. Stricklin recently joined NIOSH after moving over from the US Food and Drug Administration Office of *In Vitro* Diagnostics reviewing companion diagnostics. She previously served as a subject matter expert for the Department of Energy in pandemic response planning and a program manager in the Office of Domestic and International Health Studies. Dr. Stricklin spent several years as a principal scientist at Applied Research Associates, coordinating research and modeling to support chemical, biological, radiological, and nuclear (CBRN) preparedness and response. She coordinated the radiological interagency Public Health and Medical Working Group for FEMA, served on the Oak Ridge Associated Universities Distinguished Scientists Advisory Board, the Medical Technology Enterprise Consortium Board of Directors, and the US NATO Research Task Group for Radiation Bioeffects. During a tenure at the National Academy of Sciences, she served as the PI for the Radiation Effects Research Foundation (RERF) program and a subject matter expert on radiation health issues. Dr. Stricklin began her career as a research scientist at the Swedish Defence Research Institute where she developed methods for the assessment of exposures to CBRN agents, established a biodosimetry laboratory, initiated a research and development program for early diagnostics, led health risk assessments in peacekeeping missions, and initiated a European Union (EU) project to coordinate and harmonize emergency response protocols across EU states. Dr. Stricklin has a BS in Chemistry from Livingston University, MPH in Molecular Epidemiology from University of Pittsburgh, and PhD in Environmental Health from Johns Hopkins University.

Title: ***The Promise of Adverse Outcome Pathway Analysis in Translational Research***

Abstract: An adverse outcome pathway (AOP) describes the stepwise process by which an exposure leads to a deleterious health effect. AOP provides a structure for integrating biological observations associated with adverse events into a construct relevant to risk assessment, and therefore, may also help guide research needed to characterize key processes in the AOP. Since an exposure can lead to a wide array of biological responses, the AOP can help focus attention on the specific key events that lead to adverse health outcomes out of the vast number of possible biological observations. It thereby helps to sort the myriad system responses to different or even multiple exposures to focus on those that dictate overall outcome. An AOP can involve multiple molecular initiating events (MIEs) and key events (KEs) that lead to an overall observation of a health effect such as cancer or fibrosis. AOP frameworks may include multiple MIEs and KEs that can be used for different exposures that lead to common outcomes. This can facilitate our understanding of the impact of multiple hazards, how combined exposures impact health outcomes, and support risk predictions. This talk will provide a brief description of AOP, its foundations, and its utility. A short illustration of the AOP process through a case example will be provided. How the AOP framework can be used to integrate biological data, guide future research, and develop computational models that help predict health risks will be presented. Finally, the need for validation of such models and applications through molecular epidemiological studies will be discussed.

MEETING REGISTRATION

Please contact Elaine Freeman, A-E SOT President (efreeman@exponent.com) if you plan to register late (on-site) so we can plan accordingly for programs.

__ $110 - full/associate member
__ $150 - non-member
__ $60 - member: postdoctoral fellow or graduate student
__ $80 - non-member: postdoctoral fellow or graduate student
__ $50 - retired
__ $150 - late (i.e., on-site registration)
__ $0 - high school or undergraduate student
__ $0 - high school or undergraduate chaperone

Credit Card Payment/Registration (SOT login required - member & non-member options exist):
online registration

DIRECTIONS TO THE UNIVERSITY CLUB

Soldiers and Sailors parking garage (4390 Bigelow Blvd) directly adjacent

From Pittsburgh International Airport (From the West)

- Take I-279 toward Pittsburgh. Pass through the Fort Pitt Tunnel, stay to the right, and bear right at the first ramp over the bridge onto I-376
- Exit I-376 at the Oakland/Forbes Avenue Exit (2A)
- Merge onto Forbes Avenue toward Oakland
- Continue on Forbes Avenue and make a left onto Bigelow Blvd. Cross over 5th Ave and Soldiers and Sailors Garage is on your left.

From the Pennsylvania Turnpike (From the East)

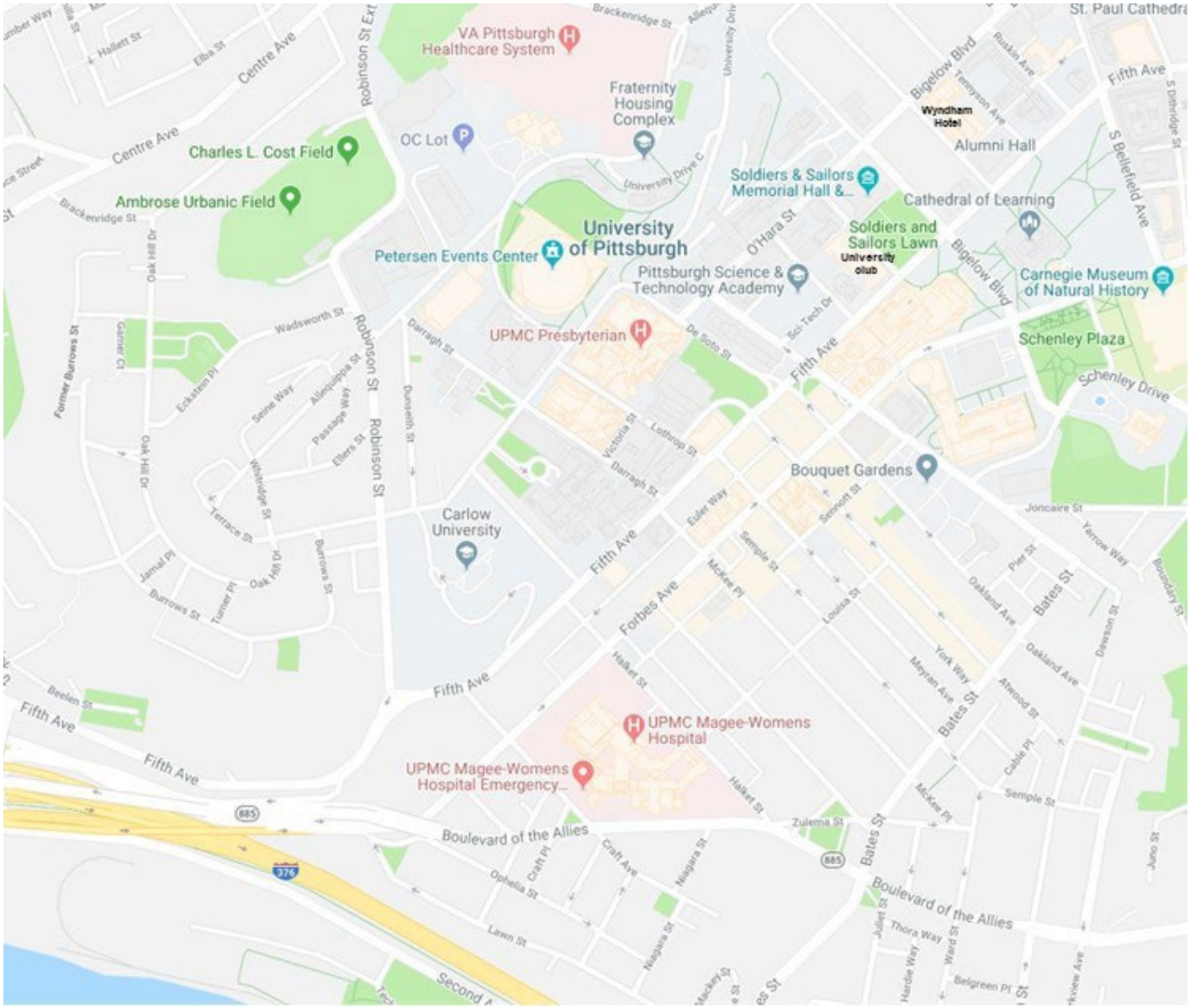
- Take the Pittsburgh/Monroeville Exit (Exit 57). Follow I-376 toward Pittsburgh
- Take the Oakland Exit (3B). Proceed up the hill through the first traffic light
- Continue on Bates Street where you can reach Forbes Avenue by turning left on ANY of the following roads off of Bates: (in order of which you would drive by) McKee Place, Semple St., Atwood St., or S. Bouquet St.
- Continue on road of your choice until you reach the intersection with Forbes Avenue
- Continue on Forbes Avenue and make a left onto Bigelow Blvd. Cross over 5th Ave and Soldiers and Sailors Garage is on your left.

From the North

- Take I-79 South to I-279 South to I-579 (Veterans Bridge – Exit 8A)
- Follow Signs to Oakland/376 East onto the Blvd. of the Allies exit.
- Follow the Boulevard of the Allies, staying in the left lane. After the Monroeville split, look for an immediate right on to the Forbes Avenue exit.
- Continue on Forbes Avenue and make a left onto Bigelow Blvd. Cross over 5th Ave and Soldiers and Sailors Garage is on your left.

From the South

- Take I-79 North to I-279 North towards Pittsburgh
- Pass through the Fort Pitt Tunnel, stay to the right, and bear right at the first ramp over the bridge onto I-376
- Exit I-376 at the Oakland/Forbes Avenue Exit (2A)
- Merge onto Forbes Avenue toward Oakland
- Continue on Forbes Avenue and make a left onto Bigelow Blvd. Cross over 5th Ave and Soldiers and Sailors Garage is on your left.


VA Pittsburgh Healthcare System
Fraternity Housing Complex
OC Lot
Charles L. Cost Field
Ambrose Urbanic Field
University of Pittsburgh
Petersen Events Center
Pittsburgh Science & Technology Academy
UPMC Presbyterian
Soldiers & Sailors Memorial Hall &...
Cathedral of Learning
Soldiers and Sailors Lawn
University club
Carnegie Museum of Natural History
Schenley Plaza
Bouquet Gardens
Carlow University
UPMC Magee-Womens Hospital
UPMC Magee-Womens Hospital Emergency...
Wyndham Hotel
Alumni Hall
Centre Ave
Fifth Ave
Forbes Ave
Boulevard of the Allies
Robinson St
Bates St
Bigelow Blvd
Schenley Drive

LODGING

A block of rooms has been reserved under the “University of Pittsburgh AESOT” at the Wyndham Pittsburgh University Center hotel that is within two blocks of the meeting venue. Reservations made before May 1, 2024, received the meeting rate of $136.00/night (plus taxes and incidentals). Parking is available at the hotel.

Call the Hotel directly and ask for the A-E SOT room block to see if any availability remains. To stay outside of the booking window, please contact the hotel directly. The best number M–F 8:00 AM–4:30 PM is Earl Eggleton at 412-682-6251.

Wyndham Pittsburgh University Center
100 Lytton Avenue
Pittsburgh, PA 15213
Phone: 412 682-6200

MONDAY AM SESSION

AN ANALYSIS OF BIRTH OUTCOMES IN RELATION TO $PM_{2.5}$ EXPOSURE AMONG ENVIRONMENTAL JUSTICE COMMUNITIES IN SOUTHWESTERN PENNSYLVANIA

ER NICHOLLS[1], JP FABISIAK[1], JM BUCHANICH[2], N BORTEY-SAM[1], SE WENZEL[1]

[1]University of Pittsburgh Environmental and Occupational Health, Pittsburgh, PA [2]University of Pittsburgh Biostatistics, Pittsburgh, PA

$PM_{2.5}$ refers to atmospheric particulate matter ≤ 2.5 microns in diameter, exposure to which may contribute to premature birth. Environmental justice (EJ) communities may be exposed to greater levels of air pollution than their non-EJ counterparts, resulting in a greater burden of health-related outcomes. This analysis explored whether difference in exposure to ambient $PM_{2.5}$ contributed to premature birth among those living in EJ communities. Using satellite-derived monthly averages at a resolution of 1 km^2, 1-year average pre-birth exposure values for $PM_{2.5}$ and its constituents were calculated for a cohort of 151,812 births from SWPA, excluding the City of Pittsburgh, from 2010-2018. Relative risks (RRs) were calculated to compare low (< 9 $\mu g/m^3$) to medium (9-12 $\mu g/m^3$) and high (>12 $\mu g/m^3$) average 1-year pre-birth exposures and by EJ status (based on the mother's census tract of residence). 15% (n = 23,609) of mothers resided in an EJ location and, overall, exposure to total $PM_{2.5}$ was 4% higher ($p < 0.01$) in this group. The RR of premature birth was 1.40 (95% CI: 1.33-1.46, $p < 0.01$) for EJ compared to non-EJ tract mothers. Compared to mothers in the low $PM_{2.5}$ category, RRs in the medium and high categories were 1.06 (95% CI: 1.01-1.11, $p = 0.022$) and 1.24 (95% CI: 1.16-1.33, $p < 0.0001$), respectively. In the high $PM_{2.5}$ category, the RR was higher among those living in an EJ tract (RR: 1.27, 95% CI: 1.12-1.45, $p = 0.0006$) compared to those in living in a non-EJ census tract (RR: 1.16, 95% CI: 1.08-1.28, $p = 0.0002$). Continued work will include developing a model to look at the relationship between $PM_{2.5}$, EJ status, and premature birth. This project will help identify areas most affected by air pollution and the extent to which air pollution contributes to adverse birth outcomes.

THE ROLE OF LINC-RAINY IN LUNG CANCER RADIATION RESISTANCE

ES WESTEMEIER-RICE, MT WINTERS, TW RAWSON, I MARTINEZ

Department of Microbiology, Immunology & Cell Biology, West Virginia University Cancer Institute, School of Medicine, West Virginia University, 1 Medical Center Drive, 1818 MBRCC, Morgantown, West Virginia 26506.

Lung cancer is the leading cause of cancer-related deaths worldwide. A major issue facing clinicians and patients alike is inherent resistance to radiation therapy. Recently, certain long non-coding RNAs (lnc-RNAs) have been shown to regulate pathways involved in resistances to lung cancer treatments. Our group recently published a group of long non-coding RNAs, named the linc-SPRY3 RNAs, play an important role in radiation therapy sensitivity. These long non-coding RNAs, originating from the Y-Chromosome, have been shown *in vitro* and *in vitro* to decrease tumor burden after radiation. Some male Non-Small Cell Lung Cancer (NSCLC) cell lines have been shown to have complete loss of the Y-chromosome (LOY). In turn, they act more radiation resistant. After adding the three linc-SPRY3 RNAs artificially into cells, they were more sensitive to radiation and had increased apoptosis. Based on preliminary data, we hypothesize the linc-SPRY3 RNAs interacts with critical pathways to increase sensitivity to DNA double strand breaks. Through RNA-Sequencing and other preliminary experiments, we found the linc-SPRY3 RNAs regulate the expression of Cell Division Cycle 6 (CDC6) and Cell Division Cycle 25A (CDC25A) genes. By over

expressing the individual linc-SPRY3 RNAs, we have seen an increase in cell cycle progression and senescence induced after radiation. Additional preliminary data suggests these lncRNAs may act as radiosensitizers in *in vitro* PDX models, delivered via nanoparticles. We are currently characterizing this lncRNA by S9.6 Immunoprecipitations, *in vitro* metastasis models, and fluorescent in situ hybridization. We will characterize the mechanism of the linc-SPRY3 RNAs in radiation resistance through genetic modifications and further downstream analysis via the over-expression and knockdown of these linc-RNAs. The characterization of these linc-RNAs will be critical for the development of potential diagnostic or therapeutic therapies.

PULMONARY EXPOSURE TO DUSTS FROM ENGINEERED STONE INDUCES LUNG INFLAMMATION AND FIBROSIS

W. K. MANDLER[1], C. QI[1], D. THOMPSON[1], A. F. HUBBS[1], T. A. CRABBS[2], A. K. KNEPP[1], S. KEELEY[1], L. A. BATTELLI[1], S. A. FRIEND[1], J. M. ANTONINI[1], Y. QIAN[1]

[1]National Institute for Occupational Safety and Health, Morgantown, WV [2]Experimental Pathology Laboratories, Inc. Durham, NC

Adult male Sprague-Dawley rats (n=8) were intratracheally instilled with dusts derived from three ES products with varying crystalline silica (CS) levels (high (CS90), medium (CS50), low (CS0.2)), granite dust (positive control), or saline (control). All groups received a single 10 mg/rat dose. Animals were euthanized at 1-, 21-, and 84-days post-exposure for evaluation of inflammatory responses and fibrosis.

Bronchoalveolar lavage fluid (BALF) lactate dehydrogenase (LDH), a marker of cell injury, was elevated in all groups at day 1, indicating initial lung damage. However, by day 21, only the high and medium CS ES groups exhibited persistent LDH elevation. At day 84, LDH levels remained elevated in these two groups and were even higher compared to earlier time points. Similarly, total cell count in BALF displayed a pattern of early increase followed by resolution in most groups, except for the high and medium CS ES groups, which maintained elevated cell counts throughout the study. Histological examination revealed persistent lesions on the lung surface and enlarged tracheobronchial lymph nodes in the high and medium CS ES groups at day 84. Additionally, these groups displayed greater degrees of alveolar lipoproteinosis, granulomatous inflammation, and both epithelial cell hypertrophy and hyperplasia compared to other exposure groups. Furthermore, they exhibited granulomatous inflammation in the bronchus-associated lymphoid tissue and the tracheobronchial lymph nodes, along with associated fibrosis in the latter.

These findings suggest that exposure to ES dust, particularly those with high CS content, may induce persistent lung injury and inflammation in rats, potentially leading to long-term health consequences. Further research is warranted to elucidate the underlying mechanisms and explore potential mitigation strategies in occupational settings.

MONDAY PM SESSION

MATERNAL BLOOD MICRORNA SIGNATURES AND ASSOCIATIONS WITH MATERNAL SMOKING STATUS DURING PREGNANCY IN WESTERN PENNSYLVANIA

YK SUN, Z ZIMMERMAN, J CATOV, A BARCHOWSKY, A SANDERS, N BORTEY-SAM, AND W-Y TANG

Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA

Maternal tobacco smoke (TS) exposure is implicated in adverse pregnancy and birth outcomes, including preterm birth, low birth weight, and fetal growth restriction, which in turn may elevate the risk of disease susceptibility later in life. These long-term effects are thought to be modulated by epigenetic mechanisms operative during pregnancy. In this study, we explore the influence of maternal TS exposure on epigenetic modifications, focusing on the regulation of microRNAs (miRNAs).

We examined the miRNA expression profiles in maternal blood samples from 46 pregnant women during 2nd trimester, enrolled in Magee Obstetric Maternal and Infant (MOMI) cohort in Western Pennsylvania. Smoking status was ascertained via maternal self-report and confirmed through mass spectrometry analysis of urinary cotinine concentration. RNA isolated from maternal blood was subject to miRNA cDNA synthesis, miRNA profiling and validated by qPCR.

We identified 65 significantly upregulated and 10 significantly downregulated miRNAs in smokers, enriched for pathways associated with organismal injury, cellular development, and inflammatory responses. Validation of miRNA profiling data via qPCR confirmed the downregulation of *miR-192-5p*, *miR-217-5p*, *miR-423-5p*, and *miR-449b-5p* in TS-exposed group, previously linked to smoking status or adverse birth outcomes. MiRNA target analysis also highlighted *miR-499b-5p* as a potential mediator of *CYP1A1* (a crucial enzyme in phase I metabolism) transcription. We observed a significant positive correlation between cord blood *CYP1A1* mRNA levels and maternal blood *miR-499b-5p* levels in smoking mother-infant pairs, implicating *miR-499b-5p* in *CYP1A1* regulation and fetal developmental toxicity.

Our findings underscore the potential impact of maternal TS exposure on maternal and fetal health via miRNA-mediated regulatory pathways. Such assessments could serve as valuable tools for early detection of later-life health outcomes in vulnerable populations.

RESTORATION OF BRAIN STRUCTURAL CONNECTIVITY AFTER CHRONIC ARSENIC EXPOSURE USING NEUROMUSCULAR ELECTRICAL STIMULATION

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Departments of [1]Physical Medicine and Rehabilitation, [2]Environmental and Occupational Health, University of Pittsburgh, PA

Epidemiological studies have demonstrated a strong inverse relationship between arsenic levels in the urine and brain function. Although regenerative medicine-based therapeutics and environmental enrichment partially reversed cognitive declines in arsenic exposed animals, there still exists a dearth of optimal treatments. The overarching goal

of this study is to bridge the gap between the fields of rehabilitation sciences and environmental toxicology by investigating whether clinically known musculoskeletal rehabilitation interventions, such as neuromuscular electrical stimulation (NMES) for declining brain health, could reverse arsenic-impaired neural connectivity in mice exposed for 5 weeks to 100 µg/L arsenite. Using a mouse model where we have reported arsenic-impaired muscle metabolism and function, we found exposure disrupted brain connections, specifically to the left corpus callosum, as well as decreased whole brain fractional anisotropy (FA). NMES for two weeks after terminating arsenic exposure restored neural connections and diffusivity metrics to a healthy state, as determined by PCA analysis based on whole brain characteristics. These data implicated neural tract organization as a main driver for differentiating between arsenic exposed brains with and without NMES. Arsenic exposure resulted in lateralized reduction of connectivity in the left hemisphere, specifically in regions associated with sensorimotor relay and decision making. Conversely, connectivity changes following NMES in arsenic-exposed mice were predominant in the right hemisphere in similar functioning brain regions. These findings suggest that NMES could be used as a potential rehabilitation strategy to reverse the pathogenic effects of arsenic exposure, and possibly other environmental-derived muscle impairments, on brain health. *Supported by UPitt Pepper Center and the Pittsburgh Foundation Grant (AS).*

UTILIZING MATERNAL NANO-TITANIUM DIOXIDE PARTICLE INHALATION TO EVALUATE GESTATIONAL INSULT ACROSS SUBSEQUENT PREGNANCIES

RUSSELL HUNTER, TERESA GLUTH, ELIZABETH BOWDRIDGE

West Virginia University, Morgantown WV

Complications of pregnancy such as intrauterine growth restriction, gestational hypertension, and preeclampsia present prevalent and serious risks to fetomaternal health. Epidemiological data suggests that the development of pregnancy complications in a previous pregnancy is major risk factor for predicting the onset of gestational complications in subsequent pregnancies. A growing body of bench side and epidemiological research is demonstrating the contributions of particulate matter (PM) inhalation exposure on the development of such gestational complications. The current study will utilize nano-TiO2 inhalation as a means to produce modest but well-characterized insult to gestational health. Our laboratory has also shown numerous health effects of maternal nano-TiO_2 exposure, including alterations in placental architecture and alterations in birthweight. During each gestation, dams were exposed to either nano-TiO_2 (12 mg/m3) or HEPA-filtered air (25 mL/min) for 6 hours per day for 6 non-consecutive days between gestational day (GD) 10 and 19. Dams were then allowed to deliver their pups naturally for their first parity, and sacrificed at GD 20 of the second to evaluate gestational, fetal, and placental outcomes. Additionally, maternal echocardiographic assessment was conducted via ultrasound prior to conception and on GD 18 in the second parity. Our findings indicate that nano-TiO_2 dams had lowered ejection fraction at 4 weeks post delivery from the first pregnancy, and dams exposed to nano-TiO_2 during both pregnancies had decreased cardiac output at GD 18 of the secondary pregnancy. Placental weight was lowered in dams with subsequent TiO2 exposure, this response was sexually dimorphic with placentas of male fetuses being significantly decreased with exposure, while female placentas were not statistically different across exposures. Overall, the current study found evidence of differential cardiac remodeling during pregnancy following a previous gestational insult, and alterations in placental development in male offspring.

TUESDAY AM SESSION

MATERNAL LEVELS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AND METALS DURING THE 2nd TRIMESTER OF PREGNANCY IN WESTERN PENNSYLVANIA

A KIYANDA[1], N PRICE[2], W TANG[1], Z ZIMMERMAN[1], J CATOV[2], A BARCHOWSKY[1], N BORTEY-SAM[1*] AND A SANDERS[1,2*]

[1]Department of Environmental and Occupational Health, [2]Department of Epidemiology University of Pittsburgh School of Public Health, Pittsburgh, PA, *Contributed equally

The legacy of industrialization may contribute to a higher risk of exposure to metals and/or PFAS among populations in Western Pennsylvania (WPA). There is a higher prevalence of tobacco use, a known source of metal exposure, during pregnancy in WPA in comparison to other regions of the US.

38 metals/metalloids and 11 PFAS compounds were quantified in samples collected from 46 women in their 2nd trimester of pregnancy participating in a nested pilot study of the Magee Obstetric Maternal and Infant (MOMI) cohort in WPA counties between 2016-2021. Geometric mean (GM) concentrations of highly detected (>60%) toxicants were calculated and stratified by smoking status (current vs. never). GM of urinary metals and serum PFAS from MOMI participants were compared to a 2018 nationally representative study of women (NHANES). Linear regression was performed to examine the role of sociodemographic characteristics on toxicant levels.

18 metals and 2 PFAS (PFOS and PFOA) were highly detected (>60%). Compared to the NHANES data, higher concentrations of five metals were observed in the subset of non-smoking MOMI participants. MOMI participants who actively smoked had higher concentrations of all detected metals in comparison to the NHANES participants. Regardless of smoking status, MOMI participants had lower concentrations of PFOA and PFOS. Compared to Allegheny County, a higher concentration of uranium was associated with residence in neighboring counties after adjustment for sociodemographic covariates.

Biomonitoring environmental chemicals during pregnancy is paramount to prevent prenatal exposures of potential harm to mother and fetus. Further research is necessary to determine the sources of exposure in WPA and potential health effects to better inform population-level policies towards exposure reduction or prevention that are protective of maternal and child health.

VINYL CHLORIDE EXACERBATES ALCOHOL-INDUCED LIVER DAMAGE IN MICE

CM VANDERPUYE[1], PR MUDDASANI [1], J LI[1], G ARTEEL[1,2] AND JI BEIER[1,2,3]

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Background. Vinyl chloride, an industrial chemical, causes liver injury at high concentrations, but low-level exposure (<1 ppm) is currently considered safe. The consequences of sub-OSHA exposure on human health are not well understood, especially in the context of VC as a risk-modifying agent. Previous work from this group confirms the role of VC in exacerbating experimental nonalcoholic fatty liver disease (NAFLD) caused by a Western diet. Both NAFLD and alcohol-associated liver disease (ALD) have similar liver pathologies, and the impact of VC exposure in ALD has not been investigated. Moreover, the prevalence of ALD worldwide raises concerns about how VC exposure can

enhance disease progression to more severe stages of injury. The purpose of this study was to determine if VC exposure worsens liver damage caused by chronic alcohol (EtOH) consumption. **Methods.** C57Bl/6J mice, pair-fed or fed a Lieber-DeCarli EtOH diet, were exposed to VC (<1 ppm), or room air for 6 hrs/d, 5 d/wk for 5 weeks. Plasma and liver samples were collected for determination of injury. **Results.** VC exposure exacerbated EtOH-induced liver damage and oxidative stress, and moderately increased inflammation. Although the increase in lipids caused by ethanol was not impacted by VC exposure, the pattern of steatosis shifted to more micro-vesicular fat, which is often indicative of mitochondrial dysfunction. In line with that VC also changed mRNA expression of genes regulating metabolism. **Conclusion.** Various environmental toxicants are known to modify or enhance liver injury in NAFLD models. Findings from the present study suggest that VC interacts also with other lifestyle factors such as alcohol consumption resulting in exacerbated alcohol-associated liver damage. This emphasizes that environmental chemical exposure, such as to VC, potentially drives interindividual risk for developing or enhancing disease.

FIRE DEPARTMENT DECONTAMINATION PROTOCOLS: DO THEY REDUCE TOXICANT EXPOSURES FOR ALL FIRST RESPONDERS?

R. GRESSER

Firefighters have a dangerous job. They signed up knowing and accepting the risks such as burns, building collapses, explosions, and hazardous materials. However, the one thing they didn't agree to is the one that is most dangerous to them: their bunker gear being full of toxic chemicals. The talk will show a 90 second trailer to a new video that talks about the problem of PFAS in firefighting gear, a short history of the problems with firefighting gear and toxic materials, and an update of what is happening with PFAS litigation at present. It will also be noted that this problem is not limited to firefighters, but also includes other first responders, miners, steelworkers, and pipeline workers.

POSTER ABSTRACTS

PRIOR EXPOSURE TO CORTICOSTERONE AND ORGANOPHOSPHATE IMPACT THE NEUROINFLAMMATORY RESPONSE TO MILD TRAUMATIC BRAIN INJURY IN A RAT MODEL OF GULF WAR ILLNESS.

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Gulf War Illness (GWI) is a multi-symptom disorder affecting veterans of the 1990-91 Gulf War. While the underlying cause of GWI has been elusive, growing evidence supports a role for neuroimmune dysfunction in long-term pathophysiology. Several GW-related exposures have been suggested to have a role in the development of GWI, including pesticides, medications, nerve agent, stress, and oil well smoke. Previously, we developed a model of GWI combining exposure to exogenous stress hormone, corticosterone (CORT), to mimic high physiological stress, with the sarin surrogate diisopropyl fluorophosphate (DFP). We found that CORT DFP exposure results in significantly exacerbated neuroinflammation. In addition to chemical exposures, veterans with GWI were also at risk for mild traumatic brain injuries (mTBI). Recent studies found a correlation between the number of mTBIs experienced during deployment and GWI symptom severity. Thus, we aimed to understand how mTBI may have interacted with exposures during deployment to influence long-term symptom severity. We combined our CORT DFP rat model with one or two mTBI using the projectile concussive impact model to assess neuroinflammation. Cytokine expression was measured

in the brain and serum using qPCR or multiplex protein assays, respectively. Damage to the brain was evaluated histologically by FluoroJadeB staining of injured neurons. We found that prior exposure to CORT DFP increased the neuroinflammatory response to mTBI exposure and repeated mTBI exposure resulted in increased serum cytokines. While one mTBI produced neuroinflammatory effects near the site of impact, multiple mTBIs expanded the number of affected brain areas; all in the absence of neuronal damage. These observations correlate with reports of increased GWI symptom severity in ill veterans with increasing numbers of mTBI during deployment and support a role for neuroinflammation in the development of these worsened symptoms.

IN VITRO TOXICITY EVALUATION OF A WELDING FUME COMPOSED OF COPPER AND NICKEL IN EPITHELIAL AND MACROPHAGE CELLS

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Welding fumes were classified as a Group 1 carcinogen (*carcinogenic to humans*) in 2017 by the International Agency for Research on Cancer based on sufficient epidemiological evidence and limited evidence in experimental animals. Toxic metals commonly found in the fumes are chromium (Cr), iron (Fe), and nickel (Ni). Copper (Cu)-based welding consumables are currently being investigated as a less toxic alternative. The objective of this study was to characterize the toxicological potency and evaluate the mechanism of toxicity of a Cu-Ni welding fume. Toxicity was evaluated at a dose range of (0-100 µg/ml) in human bronchial epithelial (BEAS-2B) and mouse macrophage (RAW 264.7) cells. The relative potency of the welding fume was compared with the major constituents including nickel (II) oxide (NiO; <10 µm and <50 nm sizes), copper (II) oxide (CuO; <10 µm and <50 nm sizes), and iron (III) oxide (Fe_2O_3; <5 µm). Physicochemical characterization including size, charge, dissolution, and acellular reactivity was done. Toxicity and relative potency were measured by evaluating membrane damage and cell viability in both cell types. Genotoxicity was evaluated at 0-3.2 µg/ml by cytokinesis-block micronucleus (CBMN) assay at doses less than IC70 in accordance with OECD TG487. γ-H2AX, a cellular response to repair double-strand DNA breaks, was assessed as a complimentary measure to the micronuclei to determine genotoxicity. Cu-Ni fume was found to be acutely toxic and the rank order in toxicity was Cu-Nano ≥ Cu-Ni > Cu-Micro > Ni-Nano > Ni-Micro > Fe. Acute toxicity was primarily driven by the Cu component in both cell types. At equal mass, the nanocomponents were more toxic compared to their micron-sized components. Cellular and acellular oxidative stress followed a similar trend, suggesting the mechanism of toxicity was due to reactivity of the particulate. Cu-Ni fume was found to be genotoxic, induced micronuclei formation, and caused DNA damage. In vitro, Cu-Ni fume causes acute toxicity and genotoxicity. *In vitro* studies are needed to further evaluate the carcinogenicity of this welding fume.

GENERATION AND CHARACTERIZATION OF COMBUSTION EMISSIONS THAT SIMULATE WILDLAND URBAN INTERFACE FIRES

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The frequency, size and severity of wildfires are projected to increase as climate change continues. The proximity of wildfires to human development, the Wildland Urban Interface (WUI), presents unique health risks. The objective of this research was to simulate these fires in a laboratory setting to identify aerosol and chemical emission profiles from mixed synthetic and biomass sources. Sawdust from four timber types were collected and mixed with up to three different synthetic building materials that were ground into small sizes. Pellets were formulated from these mixtures and fed into a customized pellet stove with the emissions sampled from the exhaust flue. Measurements included: 1) particle counts, 2) particle size by count, 3) combustion gas (CO, CO_2, SO_2, NO, CH_4) levels, 4) total volatile organic compounds (VOCs) levels, 5) humidity, and 6) temperatures at various points in the combustion / sampling chain. Off-line measurements included: 1) gravimetric measurements of the total aerosol mass concentration, 2) particle size by mass, 3) transmission and scanning electron microscopy for visualization of particles, 4) inductively coupled plasma mass spectrometry (ICP-MS) for metals analysis, 5) gas chromatography mass spectrometry (GC-MS) for speciation of the specific gasses, and 6) aldehyde chemicals and levels. Custom software and hardware were developed to control the combustion process and perform automated sampling. The addition of the synthetic materials, such as foam board insulation, produced higher levels of total VOCs (+142%), Formaldehyde (+100%) and Acrolein (from undetectable to 51 µg/sample). A system with custom pellets to simulate WUI fires that controlled the combustion process with emissions measurements was successfully developed. Future tests will include animal studies to determine the health effects from the inhalation of these emissions. Support: NIH U54 GM104942 (TRN), R01 ES031253 (SH), funded in part by UL-CIRI, P20 GM103434

MITOCHONDRIAL TAGGED PEPTIDE SS-31 PROTECTS FROM LIVER DAMAGE CAUSED BY VC AND WESTERN DIET.

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Background. Vinyl chloride (VC) directly causes liver injury at high exposure levels. While lower concentrations do not overtly damage the liver, they can exacerbate injury due to overnutrition. Impaired mitochondrial function, dysregulated mitochondrial membrane potential and impaired respiration are key pathological features. Here, SS-31, which has been shown to reduce mtROS production while also increasing respiratory function, and preventing mitochondrial permeability transition, was tested for therapeutic intervention. **Methods.** C57Bl/6J mice were exposed to VC (<1 ppm), or air for 6 hrs./d, 5 d/wk. for up to 12 wks. Mice were fed Western (WD), or control diet (CD). A subset of mice was injected with SS-31 (3 mg/kg/d i.p.), three times a week for the last 9 weeks of exposure. Plasma and liver samples were collected for determination of injury and mitochondria were isolated for respirometry. AML-12 cells were exposed to chloroacetaldehyde (CAA, 0-5 µM) ± SS-31 for mitochondrial reconstruction analyses. **Results.** VC exposure enhanced liver injury caused by WD feeding, as previously observed. The primary effect appeared to impact hepatic metabolic function, coupled with oxidative damage and mitochondrial dysfunction. Respirometry indicated that mitochondria from VC-exposed mice exhibited impaired electron transport chain function. Intervention with SS-31 significantly decreased indices of liver injury (ALT). Despite not protecting against lipid peroxidation (MDA), SS-31 decreased hepatic free fatty acids. Moreover, SS-31 protects mitochondria from CAA-induced swelling in AML-12 cells. **Conclusions.** These data support our previous findings that mitochondria have a central role in VC+WD-

induced liver injury. Moreover, SS-31, in part, protected against damage and mitochondrial swelling caused by VC. Additional experiments are needed to elucidate the underlying mechanisms.

3XTG MICE HAVE WORSE CEREBROVASCULAR FUNCTION WITH ELECTRONIC CIGARETTE EXPOSURE

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The use of electronic cigarettes (Ecigs) has increased drastically due to advertising to youth and non-smokers. Exposure to cigarettes has long been connected to Alzheimer's pathology, with about 14% of cases attributable to smoking. In the limited investigations into the harms of Ecigs, most vascular data reveals vaping is just as harmful as smoking. Further, the vessel impairment is nicotine-independent and either base e-liquid component leads to blunted cardio- and cerebro-vascular outcomes. Therefore, we wanted to test the hypothesis that vaping would have worse outcomes on cerebrovascular and behavioral function in Alzheimer's disease using a triple transgenic mouse model. Male/female 3xTg-AD (APPSWE, PS1M146V, and tauP301L, n=6) and WT-AD (C57BL6/129S, n=6) were randomly assigned into Air and Ecig exposed groups. The Ecig groups were vaped for 8-weeks, 1.5hours/day, 5days/week at 17.5watts, 50:50 vegetable glycerin, and propylene glycol, no nicotine/flavoring. After 8-weeks, middle cerebral artery (MCA) function was assessed using pressure myography. Compared to WT Air, the 3xTg Air had a 55±4% decrease in MCA reactivity to ACh ($p<0.05$). The 3xTg Ecig group had a 75±3% reduction in vessel function compared to WT Air, and a 25±6% blunted reactivity compared to the 3xTg Air group ($p<0.05$). Behavioral data with open field (evaluating locomotor activity) showed the 3xTg Ecig had a 23±10% decrease in total activity compared to 3xTg Air group, and a 44±9% decrease compared to WT Air ($p<0.05$). While Y-maze (evaluating working memory) showed only 22±9% successful alternation in the Ecig 3xTg compared to 51±8% in the 3xTg Air, and 59±5% in the WT Air ($p<0.05$). These data suggest the cerebrovascular impairments induced by vaping may exacerbate Alzheimer's-like symptoms in mice. They also highlight the need for further investigation into the role Ecigs might have with Alzheimer's and potentially other brain-related pathologies in humans.

EFFECTS OF HIGH-FAT DIET ON CARDIAC OUTPUT IN THE OFFSPRING OF NANO-TITANIUM DIOXIDE EXPOSED DAMS

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Maternal nano-TiO_2 exposure in Sprague-Dawley (SD) rats during gestation can result in lower birth weight and negative adult health complications in their offspring. A potential solution to this is the addition of a high-fat diet (HFD; 60% fat calories) for the exposed offspring to have compensatory weight gain. However, diet modification can cause obesity that results in a multitude of poor health effects. The aim of this study was to determine the consequences that a HFD induces in the offspring of nano-TiO_2 or air dams-exposed during gestation. Dams were exposed to nano-TiO_2 (12 mg/m^3) or HEPA-filtered air (FA; 25 mL/min) for 6 hours per day for 6 non-consecutive days between gestational day 10 and 19. The dams then were allowed to deliver pups naturally. Pups were weaned at 3 weeks and

randomly assigned to standard chow (SC) and HFD groups for both FA and nano-TiO_2 exposed dams. Baseline echocardiographic data was collected via ultrasound for each group at 8 weeks of age prior to the start of diets. The HFD groups then received HFD chow for 12 weeks while the SC groups continued standard diet for the same period. Our findings indicate that male cardiac output and stroke volume increases for the nano-TiO_2 exposed HFD (+ 12.05 mL/min; + 40.74 µL) and SC rats (+ 11.84 mL/min; + 37.04 µL) and FA SC rats (+ 1.24 mL/min; + 13.36 µL), but significantly decreased in the FA HFD rats (- 8.31 mL/min; - 11.32 µL). The FA HFD rats also had significant increases in weight gain than the other groups (281.0 g ± 31.03 g). In females, cardiac output and stroke volume remained relatively constant except for a decrease in nano-TiO_2 HFD rats (- 27.75 mL/min; - 67.11 µL). Both the FA and nano-TiO_2 exposed HFD rats had statistically significant increased weight gain over controls (147.7±16.28; 164.4±17.91, respectively). This study indicates that *in utero* exposure to nano-TiO_2 results in cardiac dysfunction, potentially through weight gain, in adult SD rats when placed on a HFD.

PFAS ORGAN BURDEN AND PROTEOME CHANGES FOLLOWING DERMAL EXPOSURE

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Purpose: Occupations prone to repeated dermal perfluoroalkyl substance (PFAS) contact include construction, manufacture, and firefighting. Despite regular detection of PFAS in human samples and dermally encountered products; their dermal toxicokinetic profiles are overlooked. To address this gap, we measured the organ distribution of 10 occupationally relevant PFAS after acute dermal exposure. The mechanisms by which these PFAS elicit dermal and immune changes are also poorly characterized; to probe this for a common carboxylic acid PFAS, perfluorobutanoic acid (PFBA), proteome changes following exposure were identified. **Methods:** Mice were dermally exposed, 25µl to each ear, for 10 days to 0.5% perfluorooctanoic acid or perfluorooctane sulfonate, 5% perfluoroheptanoic acid, perfluorohexanoic acid, perfluoropentanoic acid, PFBA, perfluoropentane sulfonate, or perfluorobutane sulfonate, or 2.5% perfluoroheptane sulfonate or perfluorohexane sulfonate; controls received vehicle. PFAS per gram of wet liver, spleen, lung, and ear tissue was measured in samples collected 24 hours after the final dose via LC-MS/MS. Ear, spleen, and bone marrow proteomes were collected via LC-MS/MS. Proteome Discoverer identified and quantitated peptides. Proteins with significantly altered expression were analyzed with DAVID (NIH). **Results:** All exposed mice had tissue PFAS burdens above background levels, with ear>liver>spleen & lung per tissue weight for the PFAS species tested. Sulfonic acid PFAS (S-PFAS) exhibited a positive trend between tissue burden and carbon chain length. Dissimilarly, a parabolic trend was observed for carboxylic PFAS (C-PFAS). Proteomic analysis of the ear, spleen, and bone marrow identified 709, 340, and 176 differentially expressed proteins respectively between PFBA exposed and controls. GO terms for these proteins included DNA packaging and myeloid cell differentiation. **Conclusion**: Unequal distribution of S-PFAS and C-PFAS after dermal exposure suggests functional groups may contribute to differing absorption, distribution, or excretion rates, but further study is required to identify the contribution of each to these observations. Protein-level changes in secondary and primary immune tissues supports the need for further work into identifying dermal and immunological outcomes of PFBA exposure.

SUB-CHRONIC EXPOSURE TO E-CIGARETTE VEHICLE CONSTITUENTS ALTERS GENE AND PROTEIN EXPRESSION IN LUNG EPITHELIAL CELLS

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E-cigarette (e-cig) use is increasing in popularity across the US. Our previously published data indicate that pulmonary injury biomarkers related to EMT, oxidative stress, and cell survival/apoptosis were changed following exposure to e-cig aerosols containing nicotine. However, the contribution of the base constituents and changes related to time-dependent effects of e-cigs have not been reported. This study aims to address the significant gap regarding the toxic contributions of e-cig vehicles and their time-dependent effects on lung epithelial cells. BEAS-2B cells were cultured and exposed to e-cig aerosols at an air-liquid interface (ALI). Using the CORESTA recommended method Nº 81, cells were exposed to PG/VG (100%). A forced-air exposure group acted as an ALI control. Cells were exposed to an aerosol of 1:3 (e-cig:air volume) for a total of 60 puffs/day, five consecutive days per week, for two consecutive weeks. Cell proliferation was inhibited in both exposure groups. Compared to corresponding air controls: 1) BCL-2 was upregulated in both PG and VG in the first week of exposure, and in the second week, these upregulations were 1.6-fold in PG and 1.7-fold in VG, 2) BAX decreased in both PG and VG in the first week of exposure, with further decreased by 1.1-fold in PG and 1.2-fold in VG in the second week, 3) E-cadherin decreased in both exposure groups in the first week and decreased by 2.8-fold in PG and 7.0-fold in VG in the second week and 4) Twist1 expression was upregulated in both exposure groups in the first week and further increased by 1.4-fold in PG and 1.1 in VG in the second week. Changes in E-cadherin were confirmed by Western blot. Altogether, our findings suggest e-cig vehicle constituents PG/VG deteriorate normal lung epithelial cell function through different mechanisms, and can initiate the pathogenesis of pulmonary disease.

IN VITRO **TOXICITY ASSESSMENT OF SPINEL FERRITE NANOPARTICLES AND UVB CO-EXPOSURE IN HUMAN EPIDERMAL KERATINOCYTES.**

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Spinel ferrite nanoparticles (SFNPs) have attracted significant attention due to their unique characteristics that make them promising candidates for diverse applications. However, before these materials can be considered for potential uses, investigation of their toxicity is prudent. It is also of importance to address the combined effect of SFNPs and UVB as UVB-induced skin inflammation contributes to a number of cutaneous diseases. This study was carried out to assess the ability of two SFNPs, $NiFe_2O_4$ and $CoFe_2O_4$, alone or combined with UVB to induce cell cytotoxicity, oxidative stress, inflammation, and DNA damage in human epidermal keratinocytes (HEK). Further, modes of cell death (apoptosis, necroptosis, ferroptosis) were investigated. Exposure to SFNPs induced dose-dependent cytotoxicity, ROS accumulation, release of inflammatory mediators, increases in oxidative stress markers and DNA damage. Pre-exposure to UVB caused significant increase in observed responses. Based on the hierarchical clustering analysis of the inflammatory cytokine responses, cells exposed to SFNPs and UVB were segregated from the rest of the exposure groups. Moreover, inhibitors of apoptosis, ferroptosis and necroptosis prevented cells damage induced by SFNPs exposure. However, zVad-fmk, pan-caspase inhibitor, demonstrated the strongest preventive effect when cells were pre-treated to UVB. Ferrostatin-1 or necrostatin-1, ferroptosis or necroptosis inhibitors, also rescued a significant percentage of HEK. Accumulation of oxidative stress markers was inhibited by Ferrostatin-1 and zVad-fmk but not by Necrostatin-1. Altogether, these data indicate that SFNPs alone or combined with UVB were associated with induction of oxidative stress, release of inflammatory mediators and DNA damage. Majority of damaged HEK undergo either caspase- or lipid peroxidation-dependent regulated cell death, suggesting apoptosis

and ferroptosis mechanisms. Additional studies are required to better understand the precise molecular mechanisms of programmed cell death induced by SFNPs alone or co-exposed with UVB.

EFFECT OF INHALATION EXPOSURE TO CELLULOSE NANOCRYSTALS ON REPRODUCTIVE OUTCOMES OF MALE MICE

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Crystalline nanocelluloses (CNC) have good electrical, optical, and mechanical properties which make them desirable for industrial applications. We investigated adverse reproductive outcomes due to inhalation exposure to CNC aerosol, generated from a bulk supply of wood pulp derived cellulose nanocrystals. C57BL6/J male mice were exposed to precise concentrations of airborne CNC (5 mg/m^3, 5 h/day, 5 days/week for 1st and 2nd week and 4 days/week for 3rd week). Cauda epididymal sperm samples, testes, and serum were collected to evaluate sperm alterations, oxidative stress, changes in the hormonal levels, and inflammatory cytokine responses and perform testes histopathology at 24 h, 2-, 6- and 12 months post exposure. CNC inhalation significantly elevated abnormality in sperm heads and tail/mid-piece as well as reducing the number of motile sperm at all time points of recovery. Sperm DNA integrity was assessed as DNA fragmentation index was significantly elevated only 24h post CNC inhalation and then reduced to the air-control level. Interstitial edema and occasional dystrophic seminiferous tubules with arrested spermatogenesis and degenerating spermatocytes were found in testes 6- and 12 months post inhalation while no changes were seen at the early time points. CNC inhalation produced a significant imbalance in the levels of testosterone (the recovery time of 2-12 months) and luteinizing hormone (12 months post exposure). Assessment of testicular oxidative damage showed significantly higher amounts of protein carbonyls at all time points of recovery. A hierarchical cluster analysis of 23 cytokines/chemokines/growth factors of the testes separated inflammatory cytokines (G-CSF, IL-6, IL-12p70, and MIP-1α) and revealed patterns that differentiate early responses from later time points. Overall, these results demonstrate that CNC inhalation exposure induces sustained male reproductive toxicity observed up to 12 months of recovery.

A NOVEL AEROSOL GENERATION SYSTEM FOR DIVERSE COMBUSION PROCESSES: VALIDATION AND CHARACTERIZATION OF EMISSIONS

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Open-air burning (e.g., wildland-urban interface (WUI) fires and waste disposal at military-burn-pits) creates complex aerosols. Inhalation exposure to these complex aerosols is linked with risks to Community-based Health and Safety, the theme for 2024 Allegheny-Erie Society of Toxicology Meeting. Using a modified pellet stove, our objectives were to: 1) determine operating parameters that simulate real-world inhalation exposures to emissions from burning diverse materials and 2) characterize the particles and chemicals emitted from these conditions. Commercial wood pellets

and custom formulations containing commonly used materials in military-burn-pits (plastics, rubber, and wood) were combusted with jet fuel with additives (JAA). Emissions were characterized for particle size distributions (PSD), total concentration, morphology, and gases (total and speciation of volatiles) using gas chromatography mass spectrometry (GCMS). Combustion of commercial pellets with JAA emitted fewer total particles (#/cc3: 7.0e+6 + 1.1e+6 vs 4.5e+7 + 8.3e+6) and volatiles (parts per millions: 0.63 + 0.26 vs 0.97 + 0.41) vs without JAA. PSD with count or mass median diameter was < 200 nm (geometric standard deviation >1.5) regardless of JAA. Electron microscopy confirmed ~ 100 nm particle agglomerates with diverse morphology. Total gases measured by GCMS revealed > 40-fold higher in emissions without JAA (170 + 15 mg/m3) vs with JAA (4 + 2 mg/m3) containing potential human carcinogens (formaldehyde and acetaldehyde). Combusting plastic and rubber containing pellets produced 4 % of methyl-methacrylate and 10 % of methyl-isobutyl-ketones out of total gases yield, respectively. The successful validation of our combustion generator demonstrated reliable, repeatable, and multi-disciplinary ability to characterize combustion emissions from diverse materials. In the future, more diverse wood varieties and building materials will be studied to better address domestic or WUI fires, and perform whole-body inhalation exposures to these emissions. Support: NIH U54 GM104942 (TRN), R01 ES031253 (SH), P20GM103434

CHRONIC HIGH PHYSIOLOGICAL STRESSOR MIMIC, CORTICOSTERONE, PRIMES THE NEUROINFLAMMATORY RESPONSE TO DERMAL SULFUR MUSTARD EXPOSURE: A POTENTIAL CONTRIBUTION TO INITIATION OF GULF WAR ILLNESS.

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Nearly 1/3 of veterans from the 1991 Gulf War returned with a multi-symptom disorder characterized by debilitating fatigue, cognitive dysfunction, chronic widespread pain, gastrointestinal distress, respiratory problems, skin abnormalities, etc. now known as Gulf War Illness (GWI). Symptoms of GWI are consistent with features of chronic sickness behavior, the underlying basis of which is neuroinflammation. Many wartime exposures have been proposed to serve as the instigating event for GWI including pesticides, nerve agent prophylactic, oil well fires, vaccinations, sand and dust particles, and chemical and biological weapons. Similar symptomology after sulfur mustard exposures includes acute toxicity to the nervous, respiratory, cardiac, dermal, and digestive systems with chronic conditions lasting many years after exposure. Here, our established chronic CORT priming regimen (7 days of 200 mg/L 0.6% EtOH in the drinking water) was used prior to an acute (4-8 min) dermal sulfur mustard exposure in adult male C57BL/6J mice. Results revealed significant increases in TNFα, CCL2, and IL-1β in the cortex and subcortical areas of CORT + sulfur mustard mice at 6 hours post-exposure, as well as a significant increase in the astrocyte marker, glial fibrillary acidic protein (GFAP), mRNA 6 hours after CORT+sulfur mustard exposure in the cortex; these changes resolved by 24 hours. These initial results suggest that dermal sulfur mustard exposure is capable of producing CORT-primed neuroinflammatory responses similar to what has been previously observed in our organophosphate-based GWI model. Thus, exposure to non-organophosphate chemical warfare agents like sulfur mustard, particularly in combination with stress, may have the potential to develop the underlying neuroimmune dysfunction that has been associated with GWI pathology and warrants further study.

ANALYSIS OF SERUM METABOLOME OF RATS FOLLOWING INTRATRACHEAL INSTILLATION OF MULTI-WALLED CARBON NANOTUBES

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Background and Purpose: While there has been increasing application of nanotechnology in variety of industrial applications, there have been relatively few health effects studies in workers. One of the primary routes of worker exposure to nanomaterials is inhalation. The aim of this study is to identify metabolic phenotypes which can be used to establish biomarkers for adverse outcomes and better understand adverse outcome pathways related to disease following exposure to multi-walled carbon nanotubes (MWCNT). **Methods:** Male Sprague Dawley rats (~10 weeks old, ~300 g) were administered a single intratracheal (IT)-instillation to a low effect (10 mg) and high effect (500 mg) dose of a well-characterized MWCNT, Mitsui-7, or dispersion media as vehicle control (DM). Following exposure, rats were humanely euthanized at 7 d, 1 m and 3 m. Prior to euthanization at each time point, rats were fasted overnight. Serum was collected, and bronchoalveolar lavage was performed on the right lung and histopathology was performed on the left lung of rats. A battery of cytokines and proteins were evaluated in lavage fluid. For the metabolomics data, the serum samples were prepared for high-performance liquid chromatography with tandem mass spectrometry (LC-MS). Hydrophilic interaction liquid chromatography (HILIC) was performed in negative mode. Following spectra acquisition, the raw data was analyzed using Compound Discover version 2.3 software (ThermoFisher Scientific) for small molecule identification which also provides metabolites matched to metabolic pathways in the Metabolika database within Compound Discover. The small molecules with associated names and chemical formula were mapped to the Kyoto encyclopedia of genes and genomes (KEGG) database. The positively identified metabolites obtained were then subject to pathway analysis using Ingenuity Pathway Analysis (IPA) to provide a biological context to the metabolomics data and connect metabolic changes and disease-relevant pathways, as well as to generate a list of potential metabolomic biomarkers for further studies. Four comparisons were performed in IPA as follows: (i) vehicle control (DM), 7 d low-dose, 7 d high dose, (ii) DM, 1 m low-dose, 1 m high dose, (iii) DM, 3 m low-dose, 3 m high-dose and (iv) low and high doses for all 3 time-points. **Results:** Pathology analysis showed early onset granulomatous fibrosis in the high dose only scored as moderate on d 7 and 1 m and mild at 3 m. Cytokine evaluation in the lung showed persistent increases in proteins in the high dose group that have been positively associated with inflammatory disease, including IL-1b, IL-18, IP-10, TNF-a, MIP-2, and RANTES. Only MIP-2 and TNF-a were elevated at multiple time points in the low dose. Approximately 3000-3500 small molecules were identified for each of the three time-points. Metabolika analysis mapped approximately half of the small molecules to 230-270 metabolic pathways depending on time and treatment groups. In this analysis, the top mapped pathway for the high dose at 7 d differed from that of DM and the low dose. This was the super pathway of lipoxygenase which is associated with inflammatory disease. For analysis in IPA, of the small molecules with associated names and chemical formula, 178 (7 d), 173 (1 m) and 161 (3 m) were positively identified in the KEGG database. The greatest difference in the activation/inhibition of upstream regulators in the disease and function categories in IPA analysis occurred when comparing the high and low doses to control at 7d, with the high and low dose following a similar pattern to each other for many of the pathways. At 1 m, pathways related to disease and function categories of cell viability and uptake of 2-deoxyglucose were activated at both doses compared to control and lipid peroxidation pathway was activated at high dose when compared to control. At month 3, pathways related to cellular infiltration by macrophages and cell movement of antigen presenting cells were activated at high dose in comparison to control. When comparing high and low dose to each other at 7 d, there were also a greater number of differences than that observed for high versus low dose comparisons at 1 or 3 m. In this comparison, the greatest differences were in activated pathways related the disease and function categories of apoptosis, necrosis, immune cell activation, lipid metabolism, production of

reactive oxygen species, and inflammatory cell activation among others. **Conclusions:** The metabolomics analysis showed a consistent pattern with activation/inhibition trends for majority of the diseases or canonical pathways following MWCNT exposure are opposite to those of control. Metabolites common to both doses over time may serve as candidates for biomarkers of exposure. The greatest differences in upstream regulators in serum and cytokines in lavage between the low and high dose occurred at 7 d, the time point where inflammation begins to resolve for the low dose but progresses toward disease in the high dose, suggesting this time point may be best suited for development of biomarkers of disease. It is important to note that many statistically significant metabolites identified by compound discoverer could not be incorporated into pathway analyses databases resulting in potentially critical loss of data. Further analyses of the metabolome are necessary to better delineate the metabolic pathways involved in adverse outcome pathways.

INHALATION OF 3D PRINTING EXHAUST USING FLUORESCENT ABS FILAMENT INDUCES CHANGES IN ENDOCRINE RECEPTOR AND CYTOKINE EXPRESSION

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Additive manufacturing (3D printing) using thermally extruded plastics has enabled industries to innovate the process of fabrication to produce consumer products. 3D printers use a variety of plastics, colors, and dyes and has become increasingly popular in workplaces, schools and private residences over the past decade. Recent evidence suggests that fumes from 3D printers can contribute to reproductive, and neurological dysfunction, and that workers using unventilated 3D printing machines are at the greatest risk. Previous research has reported the presence of plastic nanoparticles in the emissions of 3D printers, and these particles may pose a health hazard. Fluorescent acrylonitrile butadiene styrene (fABS) is a recently developed filament for 3D printing that fluoresces in response to UV light. We examined the emissions of extruding fABS filament from 5 printer heads with an average airflow of 22 L/min, and subsequently exposed mice to the emissions for up to 24 days (4 days/week, 4hrs/day). Using scanning electron microscopy, we verified the presence of plastic particles of various shapes and sizes on collection filters. Additionally, we fractioned the particle size using a MOUDI™ Impactor to determine the overall aerodynamic particle size distribution. Mass Median Aerodynamic Diameter (MMAD) particle size was 255nm, and the mean particle concentration inside the exposure chamber was 41 mg/m^3 over 50 times higher than when standard ABS filament was used in the same exposure system. Quantitative western blot experiments demonstrate significant changes in endocrine receptor (androgen) and cytokine (interleukin 6) expression in the hypothalamus. No significant changes were detected in the hippocampus. Based on these results, we suggest that the significantly higher concentration of particulate generated by fABS pose an even greater health hazard than traditional filaments for workers routinely using 3D printers, and that exposure to these emissions could potentially affect reproductive-endocrine regulation.

PROBABILISTIC EXPOSURE MODELING TO EVALUATE CONTRIBUTION VARIABILITY OF CHOCOLATE BAR INTAKE AND OTHER LEAD SOURCES TO BLOOD LEAD LEVELS

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Detectable lead (Pb) in chocolate candy bars has been a recent topic of interest, but it is important to consider aggregate exposures to sources of environmental Pb. In this study, a probabilistic exposure assessment was paired with a biokinetic model to evaluate age-specific variability in the contribution of Pb in soil, dust, water, chocolate, and other dietary sources to blood lead levels (BLLs).

Concentrations of Pb in chocolate bars were measured by an accredited laboratory. Daily bar consumption amounts were based on nationally representative surveys, and model-recommended intakes were used for soil, dust, water, and air. Nationally representative distributions were prepared for soil, dust, and water Pb concentrations as well as dietary Pb intake and chocolate consumption amounts. Probabilistic (Monte Carlo) algorithm iterations were prepared using Argo (v4.1.3) Excel Add-In. Exposure factors were drawn from the lognormal input distributions and defined correlations between concentrations or intakes. R (v4.3.0) was used to set-up and execute simulations in EPA's All Ages Lead Model (AALM) v2.0 to estimate 10,000 lifetime BLL profiles. Contributions were determined from descriptive summary statistics of the sensitivity analysis simulations.

Soil and dust exposures were appreciably correlated with BLL, but BLLs were not sensitive to changes in water, chocolate bar, or other food Pb exposures. Dust and soil concentrations had the highest contribution to variance for intake and BLLs, while food and chocolate had the lowest. The central tendency and high-end modeled BLLs were greater than the corresponding NHANES (National Health and Nutrition Examination Survey) BLLs for all age groups, indicating the predictions were precautionary relative to current national data. Average BLLs were below the CDC blood lead reference value (BLRV) for all modeled ages, and exceedances were associated with upper-bound soil and/or dust concentrations. Overall, the results suggest that Pb in chocolate bars contributes minimally to Pb exposure compared to soil and dust.

THE INFLUENCE OF E-CIGARETTE WATTAGE ON AEROSOL MASS CONCENTRATION AND FORMALDEHYDE EMISSIONS ASSOCIATED WITH VASCULAR DYSFUNCTION

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E-cigarette (Ecig) devices aerosolize e-liquid and emit particles, including formaldehyde. We used 5(W)atts and 30W, and e-liquid (50:50 PG:VG) with or without nicotine (50 mg/mL and 0 mg/mL, respectively). Puff topography was as follows: 83 mL puff volume, 5s of puff duration, and 3 min of interval between puffs. A cascade impactor was used to assess particle size. Data from 5 puffs with a 2 LPM flow rate was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using probit model. Real-time aerosol mass concertation was assessed using a Casella micro dust monitor (CEL-712). Formaldehyde concentration was evaluated using head-space technique with gas chromatography mass spectrometry. We correlated these data to ex vivo vascular reactivity (measured via pressure myography) in the middle cerebral artery (MCA) of Sprague Dawley rats. Particle size distribution was bimodal with MMAD of 0.72 um (GSD 1.6) at 5W and 0.62 um (GSD 1.5) at 30W. Average aerosol concentration at 5W was (no nicotine=163±126 mg/m^3; nicotine=231±125 mg/m^3, p=ns) and at 30 W (no nicotine=917 ±382 mg/m^3; nicotine=1346±369 mg/m^3, p=ns)(5W vs 30W $p<0.05$). Formaldehyde concentration at 5 W was (no nicotine=1417±33 PPM; nicotine=1441±33 PPM, p=ns) and at 30 W was (no nicotine=2426±8 PPM; nicotine=2426±33 PPM, p=ns)(5W vs 30W $p<0.05$). Ex vivo vascular reactivity of MCA resulted in vascular impairment by 19.0% and 19.6% (with 5W no nicotine and 5W nicotine rats, respectively, p=ns) and by 48.1% and 46.8% (with 30W no nicotine and 30W nicotine rats, respectively, p=ns)(5W vs 30W $p<0.05$). Emission from 30W

exposure resulted in greater aerosol mass and formaldehyde concentrations and more vascular dysfunction than 5W exposure. Thermal degradative products from the Ecig base solution emit particles and chemicals that induce cardiovascular dysfunction.

EXPOSURE AND MACROPHAGE HEALTH ASSESSMENT OF MICROPLASTIC DUSTS RELEASED FROM MACHINING OF BORON NITRIDE NANOTUBE-ENABLED COMPOSITES ALONG ITS LIFECYCLE

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Boron nitride nanotubes possess unique electrical insulation and radiation shielding abilities for use in light weight ceramics and flame-retardant insulation applications. Enabling plastic composites with nanomaterials can affect technological performance and particle release characteristics during use scenarios, including nano- and microplastic release. The current study aimed to characterize airborne particle release during controlled machining of BNNT-enabled epoxy composites, evaluate the effect of weathering on airborne particle release, and conduct an initial hazard characterization of collected respirable particulate using macrophages. Three different BNNT-enabled epoxy composites (0%, 1%, and 4% BNNT by weight) were sanded in a controlled laboratory apparatus using 2 different zirconium aluminum sandpaper grits (P100 and P180). Direct reading instruments were used to quantify released particle number and size distributions while offline filter samples assessed particle concentration, morphology, elemental composition, and BNNT protrusions. Next, composites were weathered under UV light and water for 2,016 hours, followed by sanding described above. Differentiated macrophages were exposed to respirable BNNT composite particulate (0 – 20 μg/cm^2) for 24 hours. Cells were assayed for cytotoxicity, reactive oxygen species, and mitochondrial membrane polarization. 4% BNNT epoxy released significantly more particulate (41,000 particles/cm^3) than 0% and 1% composites (30,000 particles/cm^3) with P100 grit sandpaper. Weathering increased particle release of 4% composite during sanding (61,000 particles/cm^3) compared to non-weathered composite. Collected respirable BNNT epoxy sanding dust caused minimal acute cytotoxicity to human macrophages. Dusts caused a dose-dependent increase in intracellular reactive oxygen species with no change on mitochondrial membrane polarization. No clear difference between % loading and macrophage response was observed. Our current findings indicate that BNNT % loading and weathering affects particle number release during machining processes in part due to the number and strength of BNNT surface bonding with the epoxy matrix. *Funded by NIOSH Nanotechnology Research Center grant 9390DT6*.

EFFECTS OF POLYCARBONATE EMISSIONS GENERATED BY 3-DIMENSIONAL PRINTING ON CIRCULATING HORMONE CONCENTRATIONS

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3D- printing is used to manufacture plastic products. Heating plastic feedstock generates emissions containing particulate matter and toxic chemicals that can be inhaled by people using the printers. Manufacturers have controls in place to prevent workers from inhaling emissions. Less expensive printers in schools and homes often don't have these protective controls. Exposure to emissions generated during printing has been associated with negative health effects. For example, inhalation of polycarbonate (PC) emissions results in deposition of bisphenol A (BPA) in the respiratory system and reproductive dysfunction. Therefore, we hypothesized inhalation of 3D printer emissions (3DE) would result in changes in endocrine function. Male Sprague Dawley rats (n = 48) were exposed to filtered air or emissions generated using PC filament with 5-3D-printers (exposure 4 h/d). The 4 h average particle concentration in the breathing space was 2.15mg/m^3. Animals were exposed for 1d or 4 d/week, or until they had been exposed for 4, 8, 15 or 30d. Using BPA measured during a single exposure, a deposition model estimated BPA deposition in the respiratory system was 0.115 µg/day. Progesterone and thyroid stimulating hormone (TSH) concentrations were reduced after exposure to 3DE. Follicle stimulating hormone (FSH) and estradiol concentrations were higher in animals exposed to 3DE. In conclusion, inhalation of 3DE resulted in changes in concentrations of reproductive hormones and TSH. These changes may be due to BPA in the PC stock; The effects of 3DE inhalation are similar to those seen with ingestion of BPA. Changes in hormone levels may have significant health effects on exposed workers. Disclaimer. *The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the NIOSH, Centers for Disease Control and Prevention.*

A COMPARISON OF PRENATALLY BIOMONITORED NEPHROTOXIC METALS MIXTURE (NMM) COMPOSITIONS: ARSENIC, CADMIUM, AND LEAD IN PA AND US POPULATIONS

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Epidemiological data can inform toxicological studies of environmental mixtures to assess real-world population-based risk assessment for kidney health outcomes. We explored NMM composition profiles of women from two US-based studies to determine possible reference mixtures for future kidney toxicological studies and population risk assessment. Urinary concentrations of arsenite (As(3)), cadmium (Cd), and lead (Pb) were measured in the (1) Magee Obstetric Maternal and Infant (MOMI) database (N= 46) of non-smoking and smoking pregnant women in western PA, and (2) NHANES 2017-2020, a nationally representative sample of US women ages 20-44 (N=51,458,561; n=550). Mixing ratios were calculated from the proportion each metal contributed to an individual's total molar concentration of three metals. Participants were assigned to clusters based on similar mixing ratio profiles using k-means clustering. For comparison, the overall mixing ratios for As(3), Cd and Pb respectively were: NHANES: 36% - 36% - 28%, MOMI-smokers: 20% - 47% - 33%, MOMI-nonsmokers: 26% - 42% - 32%. Three mixing ratio clusters (C) were identified for both NHANES and MOMI (n=23,706,124; n=20,196,933; n=7,555,504 and n=8; n=20; n=18). NHANES: (C1) 56% - 26% - 18%; (C2) 17% - 53% - 30%; (C3) 32% - 17% - 51%. MOMI: (C1) 61% - 23% - 16%; (C2) 20% - 55% - 25%; (C3) 14% - 36% - 50%. Smoking status was not associated with cluster assignment. Three mixture composition clusters were identified for each study population. While the overall mixing ratios were relatively evenly split into thirds, the three respective clusters differed by higher proportions (>50%) of either As, Cd, or Pb. Future toxicologic work will consider multiple NMM reference mixtures in dose-response curves examining kidney endpoints.

EPIGENETIC EFFECTS OF AIR POLLUTION ON INFLAMMATORY GENE TRANSCRIPTION IN ASTHMATIC PATIENTS LIVING IN WESTERN PENNSYLVANIA

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The impacts of air pollution are believed to be influenced by epigenetic changes, exacerbating chronic respiratory conditions. Our hypothesis posits that individuals residing near established industrial air pollution sources exhibit distinct blood DNA methylation patterns in immune pathway genes, particularly interleukin (IL)-1B and -6, compared to those living farther away. We anticipate these differences to correlate with more severe asthma outcomes. Using geocoded mapping, we categorized well-characterized patients from AELHI into two groups: those within 10 miles of a known pollution source in Pittsburgh (n=29) (exposure group) and those living beyond 10 miles (n=32) (control group). DNA methylation levels of *IL1B* and *IL6* in blood samples were measured by methylation-specific PCR. Furthermore, we evaluated the effect of benzo(a)pyrene (BaP), a significant component of most air pollution, on DNA methylation of *IL1B* and *IL6 in vitro* using peripheral blood mononuclear cells (PBMCs). The two groups exhibited differences in race, BMI, socioeconomic status, PM2.5 levels and FEV1. After adjustment for BMI and age, we observed decreased methylation at the *IL1B* proximal promoter region ($p=0.002$) in the exposure group compared to controls. Similarly, there was a significant reduction in methylation levels ($p<0.001$) at the *IL6* proximal promoter region in the exposure group as compared to controls. PBMCs treated with BaP for six days showed lower methylation levels of *IL1B* ($p<0.001$) and *IL6* ($p=0.01$). In conclusion, asthma patients residing in areas with high industrial pollution showed significant differences in DNA methylation patterns in cytokines *IL1B* and *IL6*. *In vitro* studies revealed that BaP induced DNA methylation changes in these cytokines. Our findings suggest that air pollution may epigenetically regulate inflammatory responses in asthma, potentially leading to poorer outcomes.

CYTOTOXIC EFFECTS OF ANTHRACYCLINE COCKTAILS IN E. COLI

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Over the last half century, anthracyclines (doxorubicin and epirubicin) have represented one of the most commonly used classes of anti-breast cancer drugs. It is known that anthracyclines interact with DNA in a very complex manner. The major anthracycline anti-tumor function is thought to be accompanied by an inhibition of DNA Topoisomerase II activity, which will cause double stranded breaks at a target site. Studies in our lab have shown that anthracyclines are mutagenic and cytotoxic in bacteria (Salmonella typhimurium and Escherichia coli) and can induce intrachromosomal recombination events in the yeast Saccharomyces cerevisiae. The objective of this study is to accurately assess the vital role of an anthracycline as an anti-cancer drug. Wild type E. coli cells were exposed to either of two anthracycline based chemotherapeutic cocktails: (doxorubicin, cyclophosphamide, and cisplatin) and (epirubicin, cyclophosphamide, and cisplatin). Cell viabilities were determined for each set of chemical mixtures. These studies will provide information about the effectiveness of these cocktails.

REPRODUCTIVE OUTCOMES AFTER EXOGENOUS ESTRADIOL TREATMENT IN NANOTIO2 EXPOSED DAMS

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Maternal engineered nanomaterial (ENM) inhalation during gestation is associated with endocrine disruption and poor reproductive outcomes that affect fetal development. Our laboratory has shown that maternal ENM inhalation reduced circulating estradiol (E2), decreased fetal weight, and increased fetal reabsorptions. Additionally, placental tissue analyzed for *3βHSD*, *17βHSD*, *StAR*, and *SRY* gene detection indicated disruption of the steroidogenic pathway in exposed dams. The aim of the present study was to determine if administration of E2 to dams could ameliorate the impaired reproductive outcomes seen in response to maternal ENM inhalation exposure during gestation. Pregnant Sprague-Dawley (SD) rats were exposed to nano-titanium dioxide (nano-TiO2) aerosols (12.35 ± 0.13 mg/m3/6h/6d) on gestational days (GD) 10-19. Exogenous E2 (EE) was administered via a subcutaneous silastic implant (0.5 mm) prior to the first day of exposure on GD 10 to mimic levels of E2 comparable to sham-control rats during pregnancy. Dams were euthanized on GD 20 for assessment of litter size, resorption number, along with fetal and placental weight. Litter size was not reduced in nano-TiO2 + EE dams (8.67 ± 3.51 pups) compared to dams exposed to nano-TiO2 alone (5.33 ± 5.05 pups). Nano-TiO2 + EE dams did not display an increased number of pup resorptions (11.33 ± 4.73 sites vs. 8.67 ± 3.51 pups) compared to exposed to nano-TiO2 dams (12.83 ± 2.86 sites vs. 5.33 ± 5.05 pups). However, pup weight was significantly reduced in dams exposed to nano-TiO2 + EE (4.22 ± 1.20) compared to dams exposed to nano-TiO2 (5.92 ± 0.48). Placental efficiency was also significantly reduced in nano-TiO2 + EE dams (8.25 ± 1.71) compared to nano-TiO2 dams (10.57 ±1.45). Interestingly, circulating estrogen was not significantly different at GD 20 in dams exposed to nano-TiO2 + EE (45.90 pg/ml ± 34.00) compared to dams exposed to nano-TiO2 (43.77 pg/ml ± 31.53). Administration of EE did not result in decrease in endocrine disruption and poor reproductive outcomes. However, this could be due to excessive amounts of EE administered by the implants or the timing of the administration of the EE. Further research is currently pursuing both these factors. This data will enable future research to focus on estrogen signaling in offspring and determine downstream signaling pathways.

Preconception Exposure to Arsenic and Its Impact on Maternal and Fetal Health: Placental Transcriptome Analysis

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Chronic exposure to inorganic arsenic (iAs) in drinking water above the EPA's standard (10 ppb) can pose risks to human health. Regional and sociodemographic disparities likely contribute to inequalities in drinking water iAs concentration and related health issues. Maternal exposure to iAs is related to pregnancy complications and adverse health outcomes where the placenta plays a critical role. This study employs a mouse model to test the hypothesis that exposure to iAs may affect placental function and subsequent offspring development. Female virgin C57BL/6J mice were exposed to 0 or 10 ppb iAs in drinking water from 2 weeks before conception until gestational day 16. Subsequently, placental tissues were collected for RNA-seq. RNA-seq data identified 994 and 362 differentially

expressed genes (DEGs) in male and female placentae, with 25 DEGs in common. Additionally, GSEA revealed distinct transcriptional patterns. Exposure to iAs in the male placentae induces transcription of genes that activate mitochondrial respiration and inhibit T cell differentiation and immune responses. In the female placentae, DEGs were overrepresented in metabolite transport activation and immune response suppression. Of note, placental growth factor (*Pgf*) levels were reduced in both male and female placentae. In conclusion, iAs exposure may disrupt placental function and affect fetal development through transcriptional changes. Furthermore, sex-specific transcriptional changes may contribute to sexual dimorphism in offspring health and adult disease risk. Importantly, our study lays the groundwork for developing effective public health strategies and interventions to screen and safeguard women and fetuses in elevated sociodemographic and socioeconomic risk levels for iAs-induced pregnancy complications and adverse health outcomes.

METABOLIC RESPONSES TO *IN UTERO* NANO-TITANIUM DIOXIDE EXPOSURE AND HIGH FAT DIET INTO ADULTHOOD

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Exposure to nano titanium dioxide (nano-TiO2) *in utero* of Sprague Dawley dams results in decreased litter and pup size. Additionally, negative health outcomes into adulthood have been observed in these animals exposed during gestation. The current study explored the adult health impacts of *in utero* exposure and its effects on metabolism. Sprague Dawley dams were exposed to HEPA filtered air (FA) or nano-TiO2 for 6 days during gestational days 10-19. Dams were then allowed to birth their pups naturally and were weaned at 3 weeks of age. The resulting animals were placed on a standard chow diet (SC) or a high fat diet (HFD) at 12 weeks of age. Groups of male and females were separated into FA SC (n=16), nano-TiO2 SC (n=20), FA HFD (n=11), and nano-TiO2 HFD (n=23). Animals were weighed weekly, and at 22 weeks of age an intraperitoneal glucose tolerance test (GTT) was performed to test for glucose intolerance. At 25 weeks old animals were euthanized and organs were harvested and weighed. Organ weights were scaled to the total body weight of the animals. FA HFD males were significantly heavier (461.8 g ± 37.29 g) than the FA SC (399.7g ± 27.26 g), nano-TiO2 SC (371.2 g ± 28.02 g) and nano-TiO2 HFD males (389.8 g ± 31.03 g). Cumulative weight gain in nano-TiO2 HFD males (281.0 g ± 31.03 g) was shown to be significantly higher than FA SC (264.6 g ± 27.26 g) and nano-TiO2 SC males (267.7 g ± 28.02 g). FA HFD (267.4 g ± 16.29 g) and nano-TiO2 HFD females (262.2 g ± 17.90 g) were both significantly heavier than SC females. FA SC females (245.4±12.68) were significantly heavier than nano-TiO2 SC females (223.4±12.20). Cumulative weight gain for FA HFD females (147.7±16.28) and nano-TiO2 HFD females (164.4±17.91) was significantly higher than FA SC (131.4±12.68) and nano-TiO2 SC females (134.0±12.20). Both HFD males had a higher glucose intolerance than FA SC and nano-TiO2 SC males. Nano-TiO2 HFD males had significantly higher glucose intolerance compared to FA HFD males. Nano-TiO2 HFD females had a significantly higher glucose intolerance than the other experimental groups. Heart weight of nano-TiO2 exposed animals was significantly smaller compared to air control animals. Epididymal and renal fat weights of exposed animals are significantly larger compared to control air animals. Liver index of exposed animals was shown to be significantly smaller than control. Testicular weights of exposed males were significantly smaller than FA males. No significant differences were found in ovarian weight between all female experimental groups. No significant effect of diet was seen in heart, epididymal fat, renal fat, liver, or testes weights. Overall, the current study indicates that *in utero* exposure results in metabolic dysregulation through perturbations in weight gain, glucose

intolerance, and increased fat deposition in adult Sprague Dawley rats when placed on a HFD. Therefore, when a mother is exposed to nano-TiO2 during pregnancy her child may experience metabolism dysregulation as adults.

CIGARETTE SMOKE PROTECTS FROM *HELICOBACTER*-INDUCED GASTRIC INFLAMMATION: IMPLICATIONS IN GASTRIC CARCINOGENESIS

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Gastric cancer is the fifth most common cause of cancer worldwide and the fourth leading cause of cancer-related deaths. 90% of gastric cancer cases are associated with chronic inflammation induced by *Helicobacter pylori* infection. Approximately half of the world's population is infected with this bacterium, but only a small percentage develops cancer. While *Helicobacter* infection is the best-known factor of cancer initiation, there are a variety of risk factors associated with gastric cancer, including a high salt diet, alcohol intake, and tobacco use. Several studies have shown a correlation between cigarette smoking and increased gastric cancer development. Cigarette use is highly prevalent in the United States, with the CDC reporting an estimated 28.3 million people smoking cigarettes in 2021. We hypothesize that cigarette usage synergizes with *H. pylori* infection, increasing mutational burden by the bacteria and increasing the risk of cancer development by overt immune suppression. Male C57BL/6 mice were infected with *H. pylori* and began smoking within one week of infection. The mice were exposed to 10 cigarettes daily, five days a week, for 8 weeks. H&E staining of the gastric corpus revealed no changes in gastric morphologies between the Mock+Air and Mock+Smoke mice. However, Infected+Air mice had worse pathologies than the Infected+Smoke. Flow cytometry analysis showed an increase in macrophages, B cells, and T cells in the Infected+Air group compared to the Infected+Smoke. This result was further demonstrated by immunofluorescence staining of the gastric corpus. These results indicate that cigarette use reduces the severity of *H. pylori*-induced gastric inflammation. We suspect that suppression of the immune response inhibits proper control of the bacteria, which can directly damage the gastric epithelium, leading to an increased mutational burden.

GENERATIONAL REPRODUCTIVE OUTCOMES FOLLOWING INHIBITION OF XANTHINE OXIDASE BY FEBUXOSTAT IN NANO-TIO_2 EXPOSED DAMS

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Gestational inhalation of nano titanium dioxide (nano-TiO_2) in Sprague Dawley dams negatively impacts litter and pup size, placental efficiency, endocrine and oxidant species balance, and microvascular function. Additionally, the Developmental Origins of Health and Disease theory proposes that an adverse *in utero* environment, such as that caused by toxicant exposure, can negatively affect the offspring's health throughout their life. We have previously demonstrated that maternal inhalation of nano-TiO_2 negatively impacted the reproductive outcomes in their F1 females, which had smaller litters, smaller pups and decreased estrogen when compared with females born to control dams. Gestational exposure to nano-TiO_2 was also correlated with systemic redox dysfunction and increased inflammatory markers in the adult offspring. Recently, we found that treating the rats with Febuxostat (Uloric), a

Xanthine oxidoreductase (XOR) inhibitor, prior to and throughout pregnancy restored litter size, pup size, and circulating estrogen of F0 dams exposed to nano-TiO_2. Therefore, we hypothesize that Febuxostat is protective against toxic insult during gestation and may recover some reproductive, endocrine, and redox endpoints in F1 offspring. Female Sprague-Dawley rats were given Febuxostat dissolved in water (50 mg/L) or distilled water *ad libitum* starting one week prior to mating until parturition. There was no difference in the average daily water consumption between the water and febuxostat groups prior to pregnancy (29.5±1.1 mL/day, n=3 vs 30.9±2.3 mL/day, n=6) or during pregnancy (46.7±3.8 mL/day, n=3 vs 41.4±3.7 mL/day, n=5). During gestation, dams were exposed to either nano-TiO_2 (12 mg/m3) or HEPA-filtered air (25 mL/min) for 6 hours per day for 6 non-consecutive days between GD10 and 19. Dams were then allowed to deliver their pups naturally, and pups were weaned at 21 days old. At weaning, dams were sacrificed for tissue collection and uterine inspection. The Water-TiO_2 dams had a trending increase in resorption rate when compared to all other groups. Developmental milestones and weight gain of the pups were also tracked from weeks 1-10. There were no significant differences between the pup weights between 1-3 weeks old. There were also no significant differences between the groups for time to fur-budding, upper incisor eruption, ear canal opening, or eye opening. At 10 weeks old, the F1 males were sacrificed to evaluate their body composition, XOR activity, redox function, and inflammatory profile. The F1 males of Water-TiO_2 exposed dams has significantly smaller testes by body mass than those of Water-Air dams (1.23±0.06%, n=8 vs 1.42±0.02%, n=4), which was restored when febuxostat was administered to exposed dams during gestation (Febux-TiO_2: 1.41±0.03%, n=12). However, there were no differences in body weight, renal fat, epididymal fat, or circulating testosterone of the 10-week-old F1 males. F1 females were mated with control males after puberty was achieved (~8 weeks old) and will be sacrificed on GD20 to evaluate litter size, fetal weight, placental weight, resorption sites, circulating estrogen and cytokines, XOR activity, and oxidative stress levels. Taken together, these observations indicate that XOR inhibition in pregnant dams protects the F1 generation, at least in part, from nano-TiO_2 inhalation exposure induced adult health dysfunctions.

ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY STUDIES OF OXIDATIVE STRESS IN THE PLASMA OF ELECTRONIC CIGARETTES EXPOSED RATS: A TIME COURSE STUDY

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Electronic cigarettes (Ecig) have been distributed on the global market as both safe alternative to combustion cigarettes and potential approach to aid smoking cessation. Recently we have shown that Ecig exposure impairs middle cerebral artery (MCA) reactivity, and that it takes 3 days for MCA reactivity to return to normal (*Mills et al., Exp Physiol 2022*). We tested the hypothesis that reactive oxidative species (ROS) are linked to the impaired MCA reactivity we studied blood samples for Air and Ecig exposed rats using electron paramagnetic resonance (EPR) spectroscopy. Sprague-Dawley rats were 4 months old at sacrifice and were exposed to either Aair (n = 6) or Ecig (single exposure 60-puffs, n = 19). The blood samples were collected postexposure on day 0 (D0, 1-4 h postexposure), day 1 (D1, 24-28 h postexposure), day 2 (D2, 48-52 h postexposure), and day 3 (D3, 72-76 h postexposure). EPR was performed using the redox sensitive hydroxylamine spin probe, 1-hydroxy-3-carboxymethyl-2,2,5,5-tetramethyl-pyrrolidine (CMH), which is EPR silent, but when oxidized by ROS forms EPR active compound 3-carboxymethyl-2,2,5,5- tetramethyl-pyrrolidinyloxy radical ($CM^{\bullet}$). We find that the level of ROS is significantly increased in the D0, D1, and D2 plasma samples compared to air exposed rats ($p<0.05$), and that ROS level in the

D3 plasma samples return to air control levels. The ROS levels match/correlate with our previous findings of impaired MCA reactivity from D0 to D2 and restored function by D3. Our EPR study show that ROS in plasma is a principal contributor to the cerebrovascular dysfunction induced by Ecig exposure. EPR spectroscopy technique can be used to non-invasively evaluate the harm potential from ROS in humans.