3454 ALI Benzene Exposure Induces Oxidative Stress in the Bronchial Epithelium and Promotes Immune Cell Chemotaxis *In Vitro*

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Background and Purpose: Volatile organic compounds (VOCs), ubiquitous outdoor and indoor air pollutants, are associated with the exacerbation of existing respiratory disease. Exposure to VOCs is also associated with the development of respiratory diseases, yet the molecular mechanisms of VOC-induced respiratory disease development (RDD) are unclear. **Methods:** To investigate VOC-induced RDD, we employed an *in vitro* model of the bronchial epithelium, a cell type exposed to inhaled VOCs *in vivo* and involved in the development of respiratory diseases. We hypothesized that VOC exposure of the bronchial epithelium would alter the expression profile of genes and proteins involved in oxidative stress and inflammation, common pathways involved in RDD. To test this, we exposed confluent monolayers of the bronchial epithelial cell line, 16HBE cells, cultured at air-liquid interface (ALI) to benzene, a common VOC associated with the onset of respiratory disease. The 16HBE monolayer was exposed to benzene concentrations spanning ambient and occupational/disaster-related levels (10, 100, 1000, and 10,000 parts per billion, ppb) to investigate the relationship between exposure and oxidative stress and pro-inflammatory endpoints. **Results:** In the absence of cytotoxicity, benzene exposure increased gene expression of the antioxidant, heme oxygenase 1 (HMOX-1). ALI benzene exposure also increased the gene expression of multiple targets related to chemotaxis and chemokine signaling, such as C-C Motif Chemokine Receptor 3 (CCR3), C-X-C Motif Chemokine Receptor 3 (CXCR3), and the C-X3-C Motif Chemokine Receptor 1 (CX3CR1). Concurrently, we observed decreased gene expression for the pro-inflammatory cytokines interleukin (IL)-6, IL-8, IL-13 and tumor necrosis factor alpha (TNF-α). Regardless of this dampened cytokine gene expression, neutrophils migrated towards benzene-exposed 16HBE conditioned medium, suggesting benzene exposure promotes the epithelial release of neutrophil chemotactic factors. Interestingly, all endpoints were observed in the absence of significantly increased gene expression of cytochrome P450-2E1 (CYP2E1) the main metabolizer of benzene associated with initiating the genotoxic effects of benzene. **Conclusions:** Collectively, these data suggest that benzene exposure may perturb antioxidant signaling and promote immune cell chemotaxis and binding to epithelial receptors. Oxidative stress and inflammation are common precursors of RDD. Thus, this *in vitro*, ALI model may be an viable tool to investigate the molecular mechanisms elicited following epithelial VOC exposure. Ultimately, these data will help fill critical gaps in our understanding of the relationship between VOC exposure and RDD, and will help inform preventive interventions, including policy and regulatory changes, and identify new therapeutic targets.

3455 Aryl hydrocarbon Receptor (AhR) as a sensor of environmental pollutantsmodulating the expression of inflammatory and atherogenic biomarkers

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Background and Purpose: The AhR is involved in inflammation and the expression of genes regulating immune responses and that the AhR is involved in other signaling pathways such as Toll-like receptors (TLR) and NF-κB. More recently, studies have shown that the AhR responds to the exposure of ambient particulate matter (PM) air pollution and mediates at least partially the toxicity of PM. PM from various sources including traffic, engine emissions, industrial activities, and wildfire smoke contain Polycyclic Aromatic Hydrocarbons (PAHs) and dioxin-like chemicals which bind to and activate AhR. Exposure to PM generated via traffic related air pollution (TRAP) or wildfires involves complex mixtures of chemicals activating AhR signaling and other signaling proteins of the non-canonical AhR pathways. **Methods:** Exposure to PM is well known to be associated with chronic inflammatory diseases including asthma, atherogenesis and cardiovascular disease as well as an increased risk to develop certain types of cancer. Although, the AhR is involved in developmental and many biological pathways, the chronic and sustained activation of AhR by environmental pollutants and chemical mixtures can lead to various pathological endpoints and adverse health effects. The impact of exposure to PM from various sources on the expression and dysregulation of critical factors involved in atherogenesis and carcinogenesis have been tested in macrophages. A pathway-focused gene expression analysis shows differential expression patterns of inflammatory and atherogenic biomarkers induced by PM from traffic related air pollution (TRAP) and PM derived from wildfire smoke. **Results:** The results demonstrate a marked activation of AhR accompanied by the induction of cytochrome P450 1A1 (CYP1A1), following exposure to PM samples collected from both wildfires and TRAP. Additionally, cytokines and immunomodulatory enzymes were upregulated in response to PM. **Conclusions:** Identifying biomarkers of PM exposure that are relevant to human chronic diseases will be crucial for translating findings from in vitro screening assays into in vivo animal studies and, ultimately, understanding their impact on human health.

3456 CO_2-Induced Metabolic and Inflammatory Dysregulation in Neutrophils: Implications for Cognitive Dysfunction

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Background and Purpose: Carbon dioxide (CO_2) exposure up to 5000 ppm is considered non-toxic; however, acute inhalation of CO_2 has been linked to Polymorphonuclear cell (PMN) activation and cognitive decline. We have shown that CO_2 exposure in humans increases neutrophil superoxide production and inhibits PMA induced-oxidative burst. We hypothesize that CO_2 exposure modulates neutrophil metabolism, causing the generation of ROS and stimulating inflammatory response, contributing to cognitive dysfunction. This study is to determine the pathways impacted by CO_2 in CO_2-exposed subjects and investigate the effect of CO_2 on neutrophil oxidative burst and metabolic functions. **Methods:** Human exposure and RNA sequencing, the subjects were exposed to 600 ppm (control) or 2500 ppm CO_2 in chambers for 2hrs. Peripheral blood was obtained from the subjects 4 h post-exposure. Total RNAs from peripheral blood mononuclear cells (PBMCs) were isolated and subjected to RNA sequencing analysis. Retinoic acid differentiation of HL60 cells were carried out to measure effect of CO_2 on neutrophil metabolisms such as glycolysis, mitostress test and oxidative burst. HL-60 were differentiated in culture for 5 days with 1 μM all-trans retinoic acid and differentiation was confirmed using Giemsa staining to visualize multi-lobed nuclei. Metabolism in the differentiated HL-60 was assayed using the XF96 seahorse efflux analyzer. Immunocytochemistry (ICC) was performed for phospho-p47phox (Ser370) and membrane separation was also performed and western blot was performed for p47 phox to assess NADPH oxidase activation. Statistical analysis was performed using one-tailed Student's t-tests with $p< 0.05$. In addition, RNA sequencing was conducted on PBMCs collected from human participants exposed to 2500 ppm CO_2 versus 600 ppm (control) [Z score, $p<0.05$)]. **Results:** PBMC RNA sequencing data demonstrate that CO_2 exposure in humans regulates multiple pathways; stimulating neutrophil degranulation genes (ADAM10 and ATP11A), inhibiting ROS detoxification (SOD2), upregulating inflammatory signals (TNF and CCL22), enhancing kinases (MAP2K7, PIK3C2B), and mitochondrial electron transport chain (ETC) enzymes (CoQ10A) while inhibiting phosphofructokinases (PFKBP4). CO_2-exposed HL60 demonstrated a significant increase in basal OCR (Air: 2.3 ± 0.7 pmol/min, CO_2: 3.6 ± 1.2 pmol/min, $p < 0.05$), indicative of increased non-mitochondrial respiration. Furthermore, oxidative burst (Area under curve) in response to PMA was significantly reduced by CO_2-exposure (Air : 5283 ± 626 pmol/min, CO_2 : 3969 ± 701 pmol/min, $p < 0.05$). ICC showed increased p-p47phox in the membrane of CO_2-exposed HL60 compared to Non-CO_2 group. Membrane separation through ultracentrifugation was performed on HL60 and there was increase in presence of p47 phox in the membranous portion. These data are consistent with NADPH oxidase activation. **Conclusions:** CO_2 exposure in humans results in significant alterations in both inflammatory and metabolic gene expression. In particular, reduction in glycolytic (PFKBP4) and increased ETC expression (CoQ10A). Interestingly, the neutrophil cell model HL-60 upon direct exposure to CO_2, where there was an apparent shift to non-mitochondrial metabolism. In conjunction, with these metabolic changes we observed an increase in membrane association and phosphorylation of p47 phox, indicating that CO_2 exposure activates NADPH oxidase in the absence of stimulation. We think these bioenergetic changes are mirrored in humans upon CO_2 exposure and may be a component of the loss of cognitive function.

3457 Characterization of PFAS-Containing Aqueous Film-Forming Foams and Their Thermal Degradation Products: Implication for Human and Environmental Health

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Background and Purpose: Per- and poly-fluoroalkyl substances (PFAS) have emerged as a major environmental health problem of our time. Aqueous Film-Forming Foams (AFFF) are traditional firefighter foams based on PFAS and a major source of PFAS environmental pollution. The main focus of this study was to analyze the chemical composition and thermal degradation behavior of AFFFs that can influence the fundamental physicochemical properties relevant to human exposures: phase distribution and mobility of contaminants (gas, vapor, nano aerosol, PFAS size distribution); chemical composition (generation of free radicals, chain reactions leading to new species, including fluorinated hydrocarbons, inter-species conversions, generation of HF gas and/or acid), toxic gases (CO, SO_2, NOx). These thermal degradation byproducts are biologically relevant to human exposure because they alter lung dosimetry, mode of action, and lung toxicology. **Methods:** For this study, we constructed an environmental chamber testing platform suitable for characterizing thermal degradation behavior and physico-chemical transformation of common AFFFs under controlled experimental conditions. Six representative AFFF foam samples out of 16 characterized foams were burned using a standardized temperature ramp (25 ^{0}C to 800 ^{0}C, 10 ^{0}C/min) under sufficient O_2 conditions (21%). We characterized foam emissions with a suite of instruments that measure nano aerosol properties (size and mass distribution and number concentration, HF gas and aerosols, toxic gases (CO, SO_2, NO_x), VOCs, ROS (by EPR), and aldehydes, and collected samples for subsequent

chemical analysis by LC-MS/MS, NMR, SERs, total F by CIC, and other techniques. Extensive physicochemical characterization of the raw foams (LC-MS/MS, LC- Q-TOF MS, Raman, NMR, ICP-MS, establish a baseline for their input chemical composition, including PFAS content and foam matrix. Both targeted (50 species, LC-ESI-MS/MS) and untargeted (HR-QTOF-MS/MS) PFAS analysis (against a database of >6k compounds) was completed in raw AFFF foam samples, nano aerosols collected in water impingers, as well as 1hr aged aerosols, and other collection media. Matrix components were quantified using both targeted (20 compounds) and untargeted analysis. **Results:** Thermal degradation of AFFF foams resulted in exclusive nano aerosol generation with the median particle size of 30-40 nm. Twelve to 26 species/AFFF were quantified in targeted analyses and 18 to 54 species/AFFF were identified in untargeted analyses of foams and TDPs. PFAS concentrations in foams ranged from 23.03 to 40481.3 µg/g. PFOS, PFOSA, and PFNA were the most abandoned PFAS species in AFFF, while PFBA, PFBS, PFHxA, and PFHxS were also present in significant amounts. Untargeted analysis revealed 18 to 54 different PFAS species in the same AFFFs. New PFAS species were formed due to thermal degradation, with an average of four new species detected in the breakdown products. Additionally, high amounts of ROS were found by EPR in the fresh nano aerosols and the aged one suggesting persistent ROS. Toxic gases such as CO, SO_2, and NO_x were also generated during the burning of AFFFs in the 1-10 ppm range. High amounts of formaldehyde, acetaldehyde, propionaldehyde, acetone and smaller amounts of higher aldehydes were also formed across all AFFFs. No HF was detected under these TD conditions. F mass balance revealed that targeted analysis could account for 0.4% to 76% of total F, with a median value of 5%. At least 16 matrix components were quantified in AFFF foams and TDPs. **Conclusions:** Thermal degradation of AFFF containing PFAS results in the formation of nano aerosols, new PFAS species, significant amounts of reactive oxygen species, formaldehyde, acetaldehyde, and toxic gases, which have important implications for environmental and human health. This study provides critical insights into the chemical transformations and emissions resulting from the thermal degradation of PFAS-containing firefighting foams, highlighting the need to expand the safety evaluation of AFFFs and their replacements beyond raw foams and to incorporate additional metrics for exposure and risk assessment of firefighting foams - old and new-to mitigate environmental and health risks.

3458 *In Vitro* Synergistic Toxicity of Heavy Metal-Contaminated Airborne Micro- and Nanoplastics (MNPs) on Human Lung Cells

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Background and Purpose: Micro- (<5 mm) and nanoplastics (<100 nm) (MNPs) are increasingly present in the atmosphere, raising concerns about their potential health impacts via inhalation. Moreover, these airborne MNPs can carry hazardous substances like heavy metals (e.g., lead, cadmium, manganese) on their surfaces, which may exacerbate their toxic effects. However, the mechanisms by which metal-bound MNPs affect health, particularly through inhalation, remain poorly understood. Understanding these mechanisms is crucial for assessing the risks associated with airborne MNPs and establishing risk mitigation strategies. Thus, this study aimed to elucidate the biological interactions of inhaled MNPs contaminated with metals, focusing on their potential adverse effect to cause respiratory toxicity. **Methods:** This study examined the toxicity of airborne polyamide (PA) MNPs, both unbound and metal-bound, using an air-liquid interface (ALI) system to assess their impact on human lung cells (A549). Metal adsorption onto PA MNPs was quantified using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and examined by X-ray Photoelectron Spectroscopy (XPS) for the chemical bonding states of elements on the sample surface. The physicochemical properties of PA MNPs and metal-bound PA MNPs were characterized using various methods: Brunauer-Emmett-Teller (BET) method-surface area, Transmission Electron Microscopy (TEM)-size and morphology, Zetasizer-zeta potential. PA MNP and metal-bound PA MNP aerosols were generated by Wright Dust Feeder II and characterized by SMPS and GRIMM for size distribution. Exposure to PA MNPs or metal-bound PA MNPs was conducted using the ALI system. We evaluated cytotoxicity responses, oxidative stress, and cytokine levels. **Results:** PA MNPs exhibited a bimodal size distribution [0.36 µm (GSD=1.61) and 3.51 µm (GSD=1.50)] in an ALI system, with Pb showing the highest adsorption capacity (9.7 mg/g), followed by Cd (4.5 mg/g), with Mn (2.0 mg/g). The effects of PA MNP and Pb-bound PA MNP exposure on cell viability, oxidative stress and cytokine production were evaluated after 4 hours of exposure in A549 cells at 0 h and 4 h post-exposures. Cells exposed to Pb-bound PA MNPs exhibited significant cytotoxicity, lipid peroxidation, and reactive oxygen species (ROS) production. Additionally, elevated cytokine levels (IL-8) were observed, indicating an inflammatory response. In contrast, no significant effects were observed for unbound PA MNPs. We demonstrated that Pb-bound PA MNP induce a toxicity response through ROS production, as shown by the relationship analysis between inflammation factors (cytotoxicity response, lipid peroxidation, and cytokine levels) and ROS production. **Conclusions:** These findings suggest that airborne MNPs can adsorb metals, producing enhanced toxic effects on respiratory health through synergistic ROS production. This synergistic effect may represent a significant risk in environments with high levels of plastic and heavy metal contamination.

3459 Inhaled Vitamin D as a Protectant Against Vitamin D Deficiency-Induced Air Pollution Sensitivity

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Background and Purpose: People of color are disproportionately affected by environmental lung diseases and respiratory infections. This has been hypothesized to be due to increased prevalence of vitamin D deficiency (VDD) among people of color. In addition to health and nutritional disparities caused by socioeconomic and structural inequities, higher levels of melanin in people of color leads to decreased subcutaneous vitamin D synthesis, the primary route of vitamin D generation. VDD has been linked to increased severity and occurrence of environmental lung diseases, such as asthma and COPD, and increased risk of influenza and COVID infection, indicating that the disproportionate impact of environmental lung diseases and respiratory infections on people of color may be due in part to increased prevalence of VDD. Our lab has also recently shown that in humans, lower vitamin D levels may lead to worse outcomes after acute exposure to ozone, a prevalent global oxidant air pollutant. Interestingly, while vitamin D has been proposed as a treatment for the pulmonary effects of VDD, studies of oral vitamin D supplementation for the treatment of lung disease have produced mixed results. We therefore investigated the role of vitamin D inhalation in modifying responses of human airway cells exposed to air pollutants and evaluated potential antioxidant mechanisms. **Methods:** We exposed well-differentiated, primary human bronchial epithelial cells (HBECs) at an air-liquid interface to the ubiquitous photochemical oxidant pollutant, ozone (0.4 ppm for 4 hours), following administration of vitamin D to the basolateral or apical compartment. Apical treatment was delivered via aerosol produced by vibrating mesh nebulizers using a novel *in vitro* aerosol delivery system, called the **O**n-**P**late **A**erosol **D**elivery **A**rray **(OPADA),** while basolateral treatment was added in the media. Protective effects of vitamin D were evaluated using RNA-sequencing, RT-qPCR, and ELISA assays for IL-8. For mechanistic investigation, 16-HBE cells expressing the glutathione redox potential sensor roGFP were pre-treated with vitamin D 24 hours before exposure to a model oxidant, hydrogen peroxide. **Results:** We found that basolateral 30-minute pre-treatment and apical 24-hour pretreatment with vitamin D reduced the ozone-induced secretion of the pro-inflammatory cytokine, IL-8. RNA-sequencing of the 30-minute pretreatment samples showed that ozone-induced upregulation of inflammation- and oxidative stress-related genes such as IL-8, FFAR2, COX-2, and NFKB2 were downregulated with basolateral vitamin D treatment. Similarly, gene set enrichment analysis (GSEA) revealed that basolateral vitamin D treatment reversed ozone-induced increases in inflammation, oxidative stress, and immune dysfunction pathways. In our roGFP-16HBE model, vitamin D pre-treatment was found to attenuate glutathione oxidation induced by hydrogen peroxide, indicating that vitamin D may act as a membrane antioxidant. **Conclusions:** Despite the established links between circulating vitamin D and respiratory health, no studies have investigated the use of vitamin D inhalation as a protectant against air pollutant-induced pathological responses. Here, we demonstrate that: 1) inhaled vitamin D attenuates oxidative stress and inflammatory gene expression induced by oxidant pollutants such as ozone in HBECs, and 2) vitamin D may act as a membrane antioxidant through attenuation of oxidative stress. Our data suggest inhaled vitamin D may be a feasible therapeutic strategy for treating the pulmonary effects of VDD and may have utility in reducing and preventing health disparities associated with lung diseases, such as the increased prevalence of asthma and severity of respiratory virus infections in people of color. Ongoing studies aim to further elucidate mechanisms by which inhaled vitamin D attenuates oxidant-induced inflammation and explore the utility of vitamin D inhalation.

3460 Wildfire Smoke Chemicals Accumulate in the Brain and Olfactory Bulb of Rats Following Intranasal Instillation

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Background and Purpose: Frequency and size of wildfires are increasing with rapid climate change, and wildfire smoke produces mixture of air pollutant, including particulate matter and toxic chemicals, threatening public health. Epidemiological studies have shown that wildfire smoke exposure not only is associated with cardiovascular or respiratory diseases, but also associated with increased risk for neurodegenerative diseases. However, it remains unknown how and which chemicals or particulates in wildfire smoke can enter the brain to results in molecular and cellular changes. Our study aims to determine the specific chemicals of wildfire smoke that enters the brain and potential route of entrance for these chemicals. We will use wildfire smoke condensate labeled with 14C chemicals to trace these compounds using accelerator mass spectrometry (AMS) and understand the pharmaco-toxicology of the chemicals in wildfire smoke *in vivo*. **Methods:** Male Sprague Dawley rats (n=7-10) were intranasally instilled with 50 µg/kg of 14C-labeled palmitic acid, catechol, or benzo(a) pyrene spiked into 1 mg/mL of smoldering eucalyptus smoke condensate or vehicle (0.9% NaCl). Serum samples were collected before dosing and at 5m, 15m, 30m, 1h, 2h, 4h, 8h, 24h, and 2 weeks after dosing for PK/PD analysis. Lung, brain, olfactory bulb, esophagus, liver, kidney, spleen, stomach, and heart tissues were collected at 30m, 2h, 4h, 24h, and 2 weeks after dosing for AMS analysis. Bronchoalveolar lavage

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