dostratified epithelium under air-liquid interface (ALI) conditions to mimic lung tissue in vitro. Differentiation was validated using hematoxylin and eosin (H&E) staining for general cell morphology and periodic acid-Schiff (PAS) staining to confirm mucin production, which is characteristic of mature bronchial epithelium. Following differentiation validation, HIF-1α was silenced in NHBE cells using siRNA transfection with Lipofectamine RNAiMAX, and we also validated transfection efficiency using Western blot analysis. The differentiated and transfected cells were then exposed to various concentrations of acrolein(20μM, 40μM, 80μM) under normoxic (21% O_2) and hypoxic (1% O_2) conditions. Apoptosis was quantified via Caspase 3 activity using a fluorescence-based assay, antioxidant capacity was assessed by measuring glutathione (GSH) levels with an enzymatic method, and oxidative stress was evaluated through a reactive oxygen species (ROS) assay. These assays were conducted to analyze cellular responses to acrolein exposure, comparing outcomes across oxygen conditions and HIF-1α modulation. **Results:** The initial phase of the study involved the successful differentiation and validation of the cells using H&E and PAS staining. After that, a dose-response study was done with acrolein under both normoxic and hypoxic conditions, identifying an IC_{50} of 80 μM in each environment. Following dose optimization, HIF-1α was effectively silenced in NHBE cells through transfection with 50 nM HIF-1α siRNA and transfection was confirmed by significant HIF-1α knockdown in Western blot analysis. NHBE cells were then treated under different treatment groups in normoxic and hypoxic conditions. Upon acrolein exposure, cells exhibited elevated reactive oxygen species (ROS) levels, with a notably higher increase in HIF-1α-silenced cells exposed to acrolein. Glutathione (GSH) levels decreased significantly in HIF-1α-silenced cells under acrolein exposure, suggesting reduced antioxidant defenses, particularly under hypoxia. Caspase 3 activity also showed increased apoptosis in silenced cells exposed to acrolein. These findings underscore the crucial role of HIF-1α in protecting cells against acrolein-induced oxidative stress and cell death, especially under hypoxic stress. **Conclusions:** This study highlights the critical protective role of HIF-1α in mitigating acrolein-induced oxidative stress and cell death, especially under hypoxic conditions. The findings emphasize that silencing HIF-1α exacerbates oxidative damage and apoptosis in bronchial epithelial cells exposed to acrolein and also demonstrates HIF-1α's importance in cellular defense mechanisms against environmental toxins. These insights contribute to toxicology by deepening our understanding of how cells adapt to pollutants and support future strategies to enhance antioxidant defenses and reduce respiratory damage from toxins in vulnerable populations.

3431 Investigating the Effects of Wildfire Smoke on Extracellular Vesicle Release and Cargo as Key Mediators of Wildfire Smoke Toxicity Using an Equine *In Vivo* Approach

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Background and Purpose: Wildfires continue to adversely affect the well-being of people across the globe, with impacts predicted to increase due to climate change. Evidence demonstrates that inhaling smoke conditions relevant to wildfires is associated with altered immune responses, decreased lung function, and increased risk of hospitalization due to asthma exacerbation; however, the mechanisms underlying wildfire smoke-induced pulmonary responses remain to be established. An emerging mechanism of toxicity incorporates extracellular vesicles (EVs). EVs are membrane-bound small particles released by cells constitutively and in response to perturbation, transferring molecular signals between cells and across tissues. Numerous studies implicate EVs as critical mediators of human disease, including exposure-induced health outcomes. However, no studies to-date have assessed proteomic cargo of respiratory EVs in association with wildfire smoke exposure *in vivo*. **Methods:** Here, we leveraged bronchoalveolar lavage fluid (BALF) from horses (n = 6) exposed to ambient smoke from the 2023 Canadian wildfires and matched samples from the same horses during periods with good air quality to understand wildfire-associated changes in EV characteristics and proteomic cargo. Notably, horses represent a useful model organism for studies of ambient air pollution because they develop spontaneous respiratory disease, such as asthma (unlike rodents) with similar pathophysiology to human disease, and horse BALF samples can be collected at high volumes, increasing feasibility of measuring EV cargo. Furthermore, horses are constantly grazing outdoors, where they are exposed to ambient air pollution, including smoke during wildfire events, and can be sampled repeatedly over time. Horse BALF was processed into cellular and cell-free fractions, with the cellular fraction used for cell differential analysis and the cell-free fraction used for EV isolation via size-exclusion chromatography. EV size and concentration were determined via nanoparticle tracking analysis, and EV proteomic cargo were assessed using liquid chromatography mass spectrometry. **Results:** There were no significant differences in BALF cell differentials or EV particle characteristics (size, concentration) in wildfire vs. control samples. However, of the 2,411 proteins detected in horse BALF EVs, 83 were significantly differentially expressed, with 33 upregulated and 50 downregulated proteins in wildfire vs. control samples. For example, progranulin and glutathione synthetase, which play roles in the inflammatory response and response to oxidant stress, respectively, were significantly increased in expression. In contrast, CD47, CD151, and connexin 43, which play roles in the immune system and tissue structure, were significantly decreased in expression. In agreement with differential expression analysis, pathway analysis predicted decreased activation of pathways such as neutrophil degranulation, nervous and immune system cell signaling, and phagosome formation following wildfire smoke exposure. **Conclusions:** Taken together, these findings demonstrate that wildfire smoke exposure impacts respiratory EV homeostasis, including processes related to immune system suppression. Importantly, disrupted respiratory immune homeostasis in the form of immune suppression could increase susceptibility to lung disease and infection. Future studies are needed to validate findings in humans, to further investigate the mechanistic role EVs play in respiratory response to wildfire smoke, to evaluate changes in other cargo contained within EVs (e.g., miRNA, lipids), and to understand cell-of-origin-specific changes in EV cargo.

3432 Role of xanthine oxidase in ultrafine particle and ozone inhalation induced pulmonary dysfunction in obese mice

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Background and Purpose: Epidemiological and experimental evidence indicate that the lungs of obese individuals are among the most susceptible organs to air pollution-induced adverse outcomes. However, mechanisms implicated in this phenomenon, especially the role played by xanthine oxidoreductase (XOR) in the lungs of obese individuals in the context of air pollution exposures is largely unknown. We aimed at evaluating the role of xanthine oxidase (XO) by genetic and pharmacological manipulation hypothesizing that its inhibition would be beneficial. **Methods:** Obesity/metabolic syndrome was induced in C57BL/6 mice by feeding high-fat (60% calories from fat) or control diets for 18 weeks. Ultrafine carbon black (CB) and ozone (O_3) aerosols were generated by mixing 2.5 mg/m^3 CB and 1 ppm O_3 and animals were exposed by whole-body inhalation for 3 hours per day for four days and euthanized 24 hours after last exposure. Lung inflammation was quantified by broncho-alveolar lavage and real-time PCR while lung function was assessed by FlexiVent. Oxidant production was evaluated by electron paramagnetic resonance spectroscopy. XO enzyme activity was assessed by an HPLC based assay with electrochemical detection. **Results:** Aerosol characterization by scanning and transmission electron microscopy demonstrated ultrafine size agglomerates. Real-time exposure chamber aerosol concentration monitoring confirmed stable aerosol exposures. Obese mice demonstrated a decrease in lung function that worsened after exposure to co-exposure aerosols. Similarly, inhalation exposure resulted in augmented neutrophils (p>0.01) in the lavage of obese mice compared to lean mice. A significantly greater gene and protein expression of multiple inflammatory cytokines/chemokines IL-6, KC, IL-33, TNF-α, TSLP and IL1-β were observed in exposed obese mice compared to lean exposed mice. EPR an immuno spin-trapping demonstrated significantly greater lavage, lung tissue, plasma and liver XO activity in obese mice compared to lean mice. Administration of XO -specific inhibitor febuxostat (50 mg/L in drinking water) significantly prevented inhalation exposure-induced exacerbation phenotype. **Conclusions:** In conclusion, we demonstrate that XO contributes to the exacerbated lung and systemic inflammatory phenotype in diet-induced obesity and its inhibition can be used as an intervention to counter air pollution-induced adverse outcomes in obese subjects.

3433 Pulmonary Toxicity of Combustion Byproducts from Lithium-Ion Battery Fires

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Background and Purpose: The frequency of lithium-ion (Li-ion) battery-related fires is increasing globally, creating new challenges for firefighters and growing public safety concerns because they produce copious amounts of smoke and intense heat. Li-ion battery fires can quickly spread and are hard to extinguish with traditional methods. However, the effects of combustion byproducts from Li-ion battery fires on the respiratory system are not well understood. **Methods:** We collected airborne smoke particulate matter (PM) during fires and post-fire soot samples resulting from Li-ion battery thermal runaway events (18650 cylindrical cells). Non-respirable particles with a diameter greater than 10 microns were removed through a filtration process. The soot and PM samples were chemically analyzed and assessed for lung toxicity and pulmonary function in CD-1 mice after oropharyngeal aspiration (100 μg of the smoke sample) at 4 and 24 h post-exposure. Lung toxicity outcomes were compared with those of a variety of air pollutant particles that were published in our previous papers. Chemical and biological results were further analyzed using a computational approach (R programing software) to identify hazardous chemical components driving toxicity outcomes. **Results:** The soot sample contained more carbon species than the PM sample (37% vs. 17%) whereas more anions were measured in the PM sample (12% vs. 6%). Concentrations of inorganic elements (mostly

Li and Ni) were similar (3%) on a mass basis between two smoke samples. Notably, fluoride which can cause various adverse health effects was found to be up to 5% of each sample. At 4 and 24 h post-exposure, both smoke samples caused significant increases in neutrophils, proinflammatory cytokines (IL-6 and MIP-2), and vascular injury biomarkers (protein and albumin). Cellular injury biomarkers (LDH and NAG) were also increased but not significantly. A significant increase in airflow obstruction (as measured by Penh) was observed at 4 and 24 h post-exposure to the soot and PM sample. The soot sample also significantly increased peak expiratory flow and decreased relaxation time. Comparing neutrophil data from this study to data published in our previous papers, the smoke soot and PM exhibited similar lung inflammatory outcomes caused by air pollutant samples from wildfires, diesel exhaust and coal-powered plant emissions. The data-driven computational analysis showed that F, Br, Co, Ni, and Li from both samples were key chemical contributors to the lung toxicity responses. **Conclusions:** The findings show that (1) Li-ion battery fires can produce smoke and residual soot that contain hazardous heavy metals and fluoride, and (2) acute exposures to the smoke PM and soot can lead to airway constriction and lung inflammation in a similar fashion to other combustion-related air pollutants. Knowledge of the toxicity of combustion byproducts from Li-ion battery fires can inform communication strategies to advise first responders and the public to protect their health during these fire events. [This abstract does not represent the views or policies of the Environmental Protection Agency nor the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention].

3435 Leveraging *In Vitro* Inhalation Toxicology to Assess the Impact of Technological Advances on Traffic Emission Toxicity

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Background and Purpose: Traffic is a significant contributor to urban air pollution and despite strict emission regulations, several unregulated emission components remain. These include particulates with a diameter smaller than 23 nm, semi-volatile organic carbon (SVOC) emissions, and black carbon (BC). Therefore, it is crucial to study the potential toxicity of these aerosol components. Likewise, how the technological advantages, such as cleaner fuels and after-treatment systems affect the exhaust emissions toxicity should be estimated with toxicological research. To achieve this, in vitro methods, such as the air-liquid interface (ALI) system, can be utilized to conduct whole aerosol exposures. Evaluating if the ALI exposure system provides appropriate results can be achieved by comparing it to different in vitro and in vivo methods. **Methods:** In the present study, the toxicity of the exhaust emissions from Euro 6 compliant passenger cars operated with different fuels, was assessed with two different in vitro methods; whole aerosol exposures with thermophoresis-based in vitro air-liquid interface (ALI) exposure system and filter-collected particulate matter (PM) with submerged in vitro exposure. In addition, these results are compared to the in vivo mouse exposures from similar exhaust sources. The study aimed to evaluate the potential effect factors such as fuel composition, after-treatment systems, and operating temperature on the emission toxicity. The second aim was to compare the in vitro thermophoresis-based ALI, in vitro submerged, and in vivo exposure methods. Toxicological endpoints such as cytotoxicity, inflammatory markers, and genotoxicity were included. **Results:** The present study's results indicate that the higher aromatic content of diesel and gasoline fuels, the lack of after-treatment systems, and the cold operating temperature of the vehicles increase the toxicity of exhaust emissions. This was observed in inflammatory responses and an increase in genotoxicity. From the after-treatment systems, the high effectiveness of the particulate filter in decreasing the exhaust PM emissions was emphasised also in the toxicological results. It was noted that cold operating temperatures led to increased emissions from all tested vehicles, resulting in higher levels of genotoxicity. Comparing the two different in vitro methods displays generally similar results in toxicity of emissions. However, the PM doses in the ALI exposures were multi-fold lower, implying the role of the gaseous compounds in exhaust toxicity. Moreover, the in vitro and in vivo results revealed similar trends in genotoxicity. **Conclusions:** The present study introduces the thermophoresis-based ALI method to study the toxicity of unregulated traffic emissions in vitro. It also underlines the potential effect of technologies such as after-treatment systems and cleaner fuels on exhaust toxicity. These results underscore the significance of toxicological research in validating technological advancements while pinpointing specific aerosol components to target for further reducing the adverse health effects associated with air pollution.

3436 A Cardiopulmonary Investigation of RAGE Signaling Following a Sub-chronic $PM_{2.5}$ Exposure

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Background and Purpose: Repeat exposure to fine particulate matter ($PM_{2.5}$), a complex mixture of particles and chemical components, is hypothesized to induce both inflammation and oxidative stress, however a specific driving mechanism of action has yet to be revealed. The receptor for advanced glycation end-products (RAGE) is a self-promoting receptor with multiple initiating ligands that is involved with chronic inflammation and oxidative stress. $PM_{2.5}$ exposure has been found to increase RAGE activation in human bronchial epithelial cells, and has been found to induce cardiac fibrosis in female C57BL6 mice. Specific signaling pathway influence in the cardiopulmonary response to repeat exposures to urban $PM_{2.5}$ is needed to further understand the mechanism of damage driving adverse health effects from urban $PM_{2.5}$ exposure.This study explores the role of RAGE signaling in cardiopulmonary responses following sub-chronic exposures to urban $PM_{2.5}$. **Methods:** C57BL6 wildtype and RAGE knockout (RKO) male mice (n=10/group) were dosed intranasally once per week for 11 weeks to saline, 10µg, or 100µg of an urban air standard reference material (SRM 1649b). Echocardiograms were used to monitor left and right ventricle function pre- and post- weekly dosing to determine heart function across time. After 11 weeks, bronchoalveolar lavage fluid (BALF) was collected to investigate pulmonary inflammation represented by total cell counts. Genes involved with inflammation (i.e. IL-6, TNF-α) and oxidative stress (i.e. SOD-1,2) were analyzed via qPCR in lung tissue samples. **Results:** The total cell counts in BALF were significantly different between wildtype and RKO mice exposed to 100µg of $PM_{2.5}$ ($p \leq 0.05$, 2-way ANOVA) with a non-significant but similar trend observed in the 10µg group. Gene expression data indicated there were not significant changes between genotype or treatment for IL-6, MCP-1, SOD-1, or SOD-2, however TNF-α and CYP1A1 were significantly different between genotypes in the groups exposed to 100 µg of SRM ($p \leq 0.05$, 2-way ANOVA). The data analysis of the pre- (n=11/animal) and post- (n=11/animal) dose echocardiograms are being finalized in order to understand the subchronic effect of treatment throughout the duration of the study. **Conclusions:** The future directions of this work are to analyze fibrotic markers and investigate other tissues to further understand the systemic effect of a repeat exposure to $PM_{2.5}$. In conclusion, changes in the cellular recruitment into the lung BALF were observed in wildtype mice. Completion of echocardiograms will help determine the role of RAGE signaling in cardiovascular function and the role of urban $PM_{2.5}$ dose in a repeat dosing scenario. The completion of this research project will determine the influence of RAGE signaling and dose concentration on the pulmonary inflammation and cardiovascular function following repeat exposure to urban $PM_{2.5}$.

3437 Particulate Matter and Melatonin Alter Autism Spectrum Disorder-like Behaviors in Developing Zebrafish

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Background and Purpose: Autism Spectrum Disorder (ASD) is a group of neurodevelopmental disorders that can impact communication and social behaviors. Although the full causes of ASD are unknown, it is considered to be a product of a combination of genetic and environmental factors, including environmental contaminants, like air pollution. Particulate matter (PM) is the solid and liquid portion of air pollution and has been associated with various human health impacts, including ASD. However, due to the complex chemical composition of particulate matter, little is known about how exposure to air pollution can influence the development of ASD-like behaviors during development. Therefore, various measures of sociality and anxiety were assessed in developing zebrafish exposed to particulate matter compared to those induced with the ASD phenotype. **Methods:** Developing zebrafish (n=33/group) were exposed to a particulate matter standard reference material individually and in combination with either valproic acid (VPA), an inducer of ASD, or melatonin, a natural product hypothesized as a treatment of ASD-related symptoms. Mortality, hatching, and morphology were monitored for the duration of the study. Behavioral assays designed to test for general developmental toxicity and ASD-related behaviors (e.g., sociality, aggression, anxiety), including larval photomotor response (LPR), light/dark preference, shoaling activity, mirror attack, and social contact were performed on subsequent days during and after exposure. **Results:** During the LPR, which measures movement during light and dark conditions, zebrafish exposed to PM or VPA experienced a 50% reduction in swim distance, while combination PM and VPA treatments appeared to elicit a potentially additive or synergistic effect, decreasing swim distance by 110%. In the social assay, exposure to VPA alone (but not PM) elicited a decrease in exploratory behavior and PM alone elicited an increase of nearly 275% in social behavior compared to the control. Surprisingly, melatonin supplementation appeared to ameliorate the effect of PM on social behavior, but exacerbate the effect of PM on the LPR. Video and statistical analyses of additional behavioral assays are currently in progress. **Conclusions:** This research is working to elucidate the impact of PM and melatonin on the development and progression of behaviors related to autism spectrum disorder in developing larval zebrafish to ultimately expand knowledge of PM related health impacts beyond the cardiovascular and respiratory systems.

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