1159 $PM_{2.5}$ from a Marcellus Shale Drilling Operation Induces Cardiovascular Toxicity

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Unconventional natural gas well development (UNGD) of the Marcellus Shale geological formation has continued to be a burgeoning energy driver in the US. Thus, rural communities and regions are experiencing increased industrial activities and air pollutant exposures. Our laboratory has identified increased concentrations of particulate matter (PM) in the fine (<2.5 μm, $PM_{2.5}$) and ultrafine (<0.1 μm, $PM_{0.1}$) size ranges near UNGD areas. Furthermore, negative consequences of gas well emissions on health outcomes have been reported in the epidemiological literature. We collected high-volume $PM_{2.5}$ samples onto PTFE filters over 1 week during fracture stimulation at a Marcellus Shale gas well site. Additional samples were taken upwind, and downwind from the well pad during the same time-frame. The samples were liberated from the filters into ultrapure water and dried via lyophilization. Previous *in vitro* data has shown significant cytotoxicity based on distance downwind from the well pad. Young Sprague Dawley rats were exposed to 100 or 300 μg/rat $PM_{2.5}$ from the drill site via intratracheal instillation. $PM_{2.5}$ significantly increased heart rate (HR, Sham 317±8 BPM vs. $PM_{2.5}$ 342±8 BPM). However, separately 100 or 300 μg/rat did not significantly alter HR. *In vivo*, arteriolar responses to metabolic vasodilation, endogenous neurotransmitter vasoconstriction, and endothelial vasodilators were negative. In isolated mesenteric arterioles, there was significant enhancement of phenylephrine-induced vasoconstriction 1 nM (% Max Constriction Sham 2.5±1.0%, $PM_{2.5}$ 8.8±2.6%). Taken together, these data suggest that exposure can significantly increase heart rate, and induce arteriolar vasoconstriction, though the mechanisms are unknown. *Funding: NIEHS R15ES028005.*

1160 Temporal Differences in Oxidative Potential and Chemical Composition of $PM_{2.5}$

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Exposure to fine particulate matter ($PM_{2.5}$) has well-established systemic human health effects. Oxidative stress is a hypothesized mechanism for the health effects associated with $PM_{2.5}$ exposures. Oxidative potential (OP) is the measure of a substance's capacity to oxidize a target molecule. The OP of $PM_{2.5}$ has recently been suggested as a measure that is more indicative of human health effects than the routinely measured $PM_{2.5}$ concentration. The purpose of this experiment is to analyze the OP of $PM_{2.5}$ collected on air filters and determine if there differences in the OP of $PM_{2.5}$ collected from the same location on different days. $PM_{2.5}$ was collected onto PTFE-coated filters from a monitor placed in a public park in Eugene, OR on different days in the Winter. $PM_{2.5}$ will be extracted from each filter via sonication in methanol. An aliquot of the extraction solution will be used to measure OP using the dithiothreitol (DTT) assay. An additional aliquot will undergo analysis via inductively coupled plasma - mass spectrometry (ICP-MS) to quantify elements (n=30). Correlations between OP, $PM_{2.5}$ mass, and chemical composition will be made. Initial testing of a subset of the filters shows significant differences in elements based on the day that $PM_{2.5}$ was collected, including: Cd, Ce, and Pb ($p \leq 0.05$, one-way ANOVA). The DTT assay has been optimized and calibration curves for the assay are reproducible with no significant difference observed between replicates and with an r^2 value consistently above 0.99. $PM_{2.5}$ from the park location collected on different days has shown up to a 2-fold difference in OP for a subset of samples (range of 8.3 to 4.2 nM DTT consumed/μg $PM_{2.5}$ for three filters). We anticipate to see differences in OP for $PM_{2.5}$ collected on different days due to the chemical composition, particularly if redox active elements are in higher concentrations. Correlations will help us identify components of $PM_{2.5}$ that may be impacting the OP more so than the total mass. This research will add to the growing evidence and justification for investigating the OP of $PM_{2.5}$.

1161 Effect of a High-Fat Diet and Occupational Exposure in Different Rat Strains on Lung and Systemic Responses: Development of an Animal Model to Examine the Exposome

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The exposome is the measure of all exposures of an individual in a lifetime and how those exposures relate to health. Important components of the exposome include lifestyle (diet), environmental and occupational exposures, and individual genetic predisposition. Mapping of the exposome could improve the understanding of disease and aid in prevention strategies and possible cures of many diseases. The goal was to develop an experimental model of the exposome by collecting biological samples during critical life stages of an exposed animal that are applicable to worker populations. Genetic contributions were assessed using strains of male rats with different genetic backgrounds [Fischer-344 (F344), Sprague-Dawley (SD), Brown-Norway (BN)] maintained on a regular (REG) or high fat (HF) diet for 24 wk. At wk 7 during diet maintenance, groups of rats from each strain were exposed to welding fume (WF; 20 mg/m^3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air until wk 12, at which time some animals were euthanized. A separate set of rats from each strain were allowed to recover from WF exposure until the end of the 24 wk period. Bronchoalveolar lavage fluid and serum were collected at 7, 12, and 24 wk to assess general health indices. Exposure to WF during maintenance on a HF diet caused specific adverse health outcomes directly after exposure as well as after a 12-wk recovery phase. Depending on the animal strain, there was evidence that WF exposure and HF diet together worsened lung toxicity and kidney function as well as altered different serum enzymes and proteins. The exposomal factors of diet, exposure, and strain were all important, depending on the health outcome measured. Exposure had the most significant influence on the pulmonary responses, whereas strain and diet were the most significant contributors regarding parameters related to extrapulmonary responses. Principal component analysis further confirmed the influence of strain on the responses measured, indicating the importance of genetic predisposition as an exposomal factor. In summary, this study showed that an animal model can be useful in the assessment of the exposome as external lifetime exposures can be easily controlled and adverse health outcomes measured.

1162 Differential Responses of Murine Alveolar Macrophages to Elongated Mineral Particles of Asbestiform versus Non-Asbestiform Varieties

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Occupational exposures to asbestiform elongated mineral particles (EMP) may lead to diffuse fibrosis, lung cancer, malignant mesothelioma and autoimmune diseases. Cleavage fragments (CF) are chemically identical to asbestiform varieties of mineral, but there is no consensus on whether to treat them as asbestos from the toxicological and regulatory standpoint. Alveolar macrophages (AM) are the first-responders to inhaled particulates, participating in clearance and activating the other resident and recruited immunocompetent cells, which has an impact on the long-term outcomes. In the current study we are addressing the question of how differences in EMP crystal growth habit (asbestiform vs. non-asbestiform) affect AM responses. MPI cells, a non-transformed line that closely mimics AM phenotype, were treated with mass-, surface area- (s.a.), and particle number- (p.n.) equivalent doses of respirable asbestiform and non-asbestiform riebeckite/tremolite EMP (median lengths 4.5-5.5 μm) for 24 h with or without LPS (5 ng/ml). We assessed viability and apoptotic response, lactate dehydrogenase (LDH) and cytokines in cell supernatants. Riebeckite/tremolite asbestos and CF were taken up and induced similar LDH leakage and decrease in viability at the s.a. equivalent doses. At the equal mass, asbestiform EMPs were clearly more cytotoxic. When treated with equal p.n., CF had more pronounced cytotoxic effects. Apoptosis induction was more pronounced in asbestos-treated cells, compared to CF in all comparisons (mass/s.a./p.n.). There was an increase in chemokines and elevated pro-inflammatory cytokine secretion compared to control. Principal component analysis of the cytokine/chemokine secretion showed close clustering for the s.a. and p.n. equivalent treatments. LPS stimulation shifted the cytokine profiles towards inflammation compared to non-LPS-stimulated cells, with more IL-1β and TNF-alpha secretion in asbestos-treated cells compared to CF. In conclusion, murine AM initial responses to respirable EMP of similar lengths, but different growth habit depend on the s.a. metric rather than the mass or the p.n. The study also confirms that asbestiform habit itself is an important determinant of some signaling pathways, i.e. apoptosis. Finding out what metric is critical for the mineral fiber toxicity is a complex task and the *in vivo* study with the same EMP is underway to further address the issue.

1163 Multimodal Mass Spectromtry Analysis following Repeated Intratracheal Instillation of Dispersed Silver Nanoparticles in Rats

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Silver nanoparticles are among the most widely manufactured nanomaterials and have been incorporated into a wide variety of consumer products such as textiles, detergents, medical devices, drug delivery products, anti-microbial

sprays, personal care products, paints/coatings, and water purification. This has led to the potential increase in risk of worker exposure to these particles. We have previously conducted an *in vivo* study to characterize pulmonary and systemic effects following repeated exposure where rats were intratracheally instilled once a week for eight weeks with 9.35 µg or 112 µg of dispersed silver nanoparticles (Nano-Ag) or dispersion medium (DM) as a vehicle control. Lung histopathology, and analyses of bronchoalveolar lavage fluid (BALF) and serum were performed at 7, 28, and 84 days after the last exposure. Lung injury was characterized by alveolar and interstitial inflammation, as well as oxidative stress. In the current study, a metabolomic approach was employed to characterized changes in the BALF and serum to further examine mechanisms of toxicity and establish a potential panel of biomarkers of exposure and effect. Metabolite characterization was conducted with matrix assisted laser desorption ionization (MALDI) and liquid chromatography mass spectrometry (LC-MS). The metabolomics analysis revealed a significant increase in 44 metabolites in BALF, including 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) and cholesterol in the rats exposed to 112 µg at the 7- and 28-day time points. Rats exposed to the high dose had a significant increase in ~60 serum metabolites, with the greatest degree of change in the alanine biosynthesis pathway intermediates in the serum, which have been shown to be indicators of liver toxicity. There was a >2.0-fold increase in alanine, valine, and glutamate in the 112 µg exposed animals at the 7- and 28-day time points. The results of this study show that pulmonary exposure to nanosilver particles leads to changes in the BALF lipidome, which may be related to the pathway of lung injury and oxidative stress. In addition, increased levels of circulating metabolites indicative of liver toxicity agree with numerous *in vivo* studies by other investigators. Further analysis of these molecules and lipids will be conducted to establish a potential biomarker panel for nanosilver exposure and effect.

1164 Single Cell RNA Sequencing and Mouse Transcriptome Analysis Show Amelioration of SEB-Induced Acute Lung Injury via Altering Metabolic Profile of THC-Treated Mice

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Inhalation of Staphylococcal Enterotoxin B (SEB) is known to induce acute lung injury (ALI) and studies from our laboratory have shown that THC, a psychoactive ingredient found in *Cannabis sativa,* can induce anti-inflammatory cells such as T regs. Lysine is an essential amino acid that plays several important roles by decreasing IFNg cytokine, inhibition of ERK1/2 and NF-κB signaling pathway, antimicrobial activity against Gram positive bacteria and production of carnitine, which is key in fatty acid metabolism and used as nutritional supplement. In the current study, we investigated the metabolic profile in ALI with or without THC treatment. Lung microbiota was collected and 16S rRNA sequencing was performed. 16S rRNA metagenomic data were generated and functional profiles predicted using PICRUST. Methionine and lysine biosynthesis were increased significantly in THC treated group while lysine degradation was upregulated significantly in vehicle group. Furthermore, we analyzed the metabolomic profile in serum by mass-spectrometry analysis and we found that lysine and carnitine concentrations were increased significantly in THC treated group. Moreover, we found by single cell RNA sequencing of whole lung tissue that solute carrier (Slc25a3 and Slc25a39) genes, which are carnitine transporters, were increased statistically in THC group in CD4+ and CD8+ T cells as well as MDSCs. In addition, carnitine palmitoyltransferase 1 (CPT1), a mitochondrial enzyme responsible for the formation of acyl carnitine which is transported from cytosol to mitochondrial matrix was also increased in CD4+ T cells and alveolar macrophages following THC treatment. Furthermore, we confirmed the results by transcriptome analysis that the solute carrier family was increased significantly in lung infiltrating mononuclear cells of THC treated group. Moreover, our studies on fuel source use showed that SEB-activated T cells treated with THC are glucose-independent while vehicle group is glucose-dependent. Together, THC modulates metabolic functions of lung microbiota and T cells which may affect their signaling, differentiation and toxicity which led to improve homeostasis in lungs. *Supported by NIH grants P01AT003961, R01AI123947, R01AI129788, R01ES019313, and P20GM103641.*

1165 Anti-TNFα Antibody Mitigates Sulfur Mustard-Induced Lung Injury in Rats

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Sulfur mustard (SM) is a vesicating chemical warfare agent that causes severe lung injury when inhaled. Acute sulfur mustard-induced toxicity is due, in part, to persistent accumulation of macrophages in the lung and the release of inflammatory mediators including cytokines, chemokines, eicosanoids and growth factors. The proinflammatory cytokine, tumor necrosis alpha (TNFα), is released from activated macrophages; it has been shown to contribute to lung injury by promoting inflammatory cell accumulation in tissues and stimulating the release of other inflammatory mediators. This leads to oxidative stress, airway hyperresponsiveness, and tissue remodeling. In these studies, we tested the hypothesis that anti-TNFα antibody treatment would mitigate mustard induced acute lung inflammation and injury. Male Wistar rats were exposed to SM vapors (0.4 mg/kg) or air control and treated with either monoclonal anti-TNFα antibody (15 mg/kg) or vehicle 15-30 min later. Animals were euthanized 3 days after exposure, bronchoalveolar lavage fluid (BAL) and lung tissue collected. Treatment of rats with SM resulted in lung injury and inflammation as measured by increases in bronchoalveolar lavage fluid (BAL) cell and protein content. SM exposure also resulted in increased numbers of lung macrophages expressing tumor necrosis factor (TNF) α and heme oxygenase (HO)-1 indicating inflammation and oxidative stress. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v.) 15-30 min after SM inhalation reduced lung injury and inflammation, SM-induced levels of HO-1 and TNFα were also suppressed by anti-TNFα antibody treatment. These data demonstrate that inhibiting TNFα may be an effective approach to mitigating acute lung injury induced by vesicants. *Supported by NIH Grants U54AR055073, R01ES004738, and P30ES005022.*

1166 Comparison of Overlooked yet Prevalent Polycyclic Aromatic Hydrocarbon Mixtures on Adverse Effects in Lung Cells

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Polycyclic aromatic hydrocarbon (PAH)s are major components of firsthand and secondhand smoke (cigarette and marijuana) and air pollution, among other sources, and thus far, research has almost exclusively focused on the higher molecular weight (HMW; >5 rings) PAHs for all health effects (e.g., asthma, inflammation and cancer). However, the low molecular weight PAHs (2-4 rings) are in higher prevalence than HMW PAHs in all exposure settings and our previous studies implicate these LMW PAHs in altering critical signaling pathways in lung cells. Specifically, we previously demonstrated that two of these LMW PAHs, 1-methylanthracene (1-MeA) and fluoranthene (Flthn), inhibit gap junctions, decrease connexin 43 (Cx43; primary connexin in lung) expression, activate MAP kinases, and induce inflammatory mediators, such as tumor necrosis factor alpha (TNF). Gap junctional intercellular communication (GJIC) is involved in lung tissue homeostasis and is reduced in early stage lung carcinogenesis. Our hypothesis is that adverse effects of PAH mixtures will differ depending on the PAHs tested. We used MTS assays for cytotoxicity, scalpel-loaded/dye transfer assays to measure GJIC, connexin (Cx)43 immunoblots, as well as quantitative RT-PCR in a mouse alveolar type II pneumocyte cell line (C10 cells) in the absence or presence of equimolar ratios of these mixtures (1-MeA:Flthn) or (1-MeA:Flthn:Phenanthrene (Phe)) at several time points. Additional studies validated findings in a human lung cell line (BEAS2B). Cytotoxicity was observed at doses >60 µM in C10 cells with both 2 and 3PAH mixtures, however, was higher in 3PAH mixtures. The 30 min dose response for GJIC showed that both PAH mixtures had more GJIC dysregulation than the individual PAHs, however, the dose response for the 3PAHs was shifted left compared to 2PAHs; similar findings were observed in the BEAS2B cells. Cx43 expression was reduced in response to either PAH mixture after 24 h. Lastly, we observed significant differences between pro-inflammatory cytokines in response to 3PAHs versus 2 PAHs. Collectively we provide evidence that a relevant environmental exposure to LMW PAHs can elicit adverse effects in lung cells and are dependent on the specific PAHs. Future studies will investigate the combination of both HMW and LMW PAHs in these responses. *Funded by R15ES024893-01 (AKB)/FAMRI CIA (AKB).*

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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