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3471 Subchronic effects of different nickel-based thermal spray coating aerosols on lung toxicity using an animal inhalation model

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Background and Purpose: Thermal spray coating is an emerging industrial process that applies molten metal under pressure onto a surface as a protective coating. The aerosols that are formed during this process may contain metals, such as nickel (Ni), chromium (Cr), manganese (Mn), and iron (Fe). Exposure to these potentially toxic metals may pose an adverse health risk to the manual operator, who often performs the process without proper respiratory protection, as well as to other workers in the vicinity of the operation who commonly are not using any respiratory protection at all. Information about the possible health effects and the physical and chemical properties for nickel-based thermal spray coating aerosols is lacking. **Methods:** A thermal spray coating aerosol generator and inhalation exposure system was developed to perform animal studies to simulate workplace exposures. Male Sprague-Dawley rats were exposed by whole body inhalation to 10 mg/m^3 x 4 hr/d x 4 d with aerosols generated from electric arc wire- thermal spray coating using two different nickel (Ni)-based consumable wires-PMET885 and PMET876. Control animals were exposed to filtered air. At 45 and 90 d after the last exposure, bronchoalveolar lavage (BAL) was performed on the right lung and a histopathological analysis was performed on the left lung to assess lung toxicity. Animal body weights were measured throughout the 90-d post-exposure period to assess general health status of the exposed animals. **Results:** The metal composition of each aerosol was determined by inductively coupled plasma- atomic emission spectroscopy (ICP-AES): PMET885 (97% Ni, 2% Al) and PMET876 (56% Ni, 17% Cr, 17% Mo, 5% Fe, 3% Mn). The generated particles were complex metal oxides arranged as chain-like agglomerates with similar mass median diameters (MMADs) of 316 (PMET885) and 367 nm (PMET876). At 45-d post-exposure, BAL fluid lactate dehydrogenase (LDH; lung cell injury marker) and the number of total BAL cells recovered (index of inflammation) were significantly elevated for the PMET885 group compared to air control. This increase in lung toxicity persisted for 90 d and was still significantly different from air control, however, LDH and total number of BAL cells recovered were significantly less than both values for the PMET885 group at 45-d post-exposure. Histopathologic pulmonary changes observed at 45-d and 90-d postexposure for the PMET885 group included bronchiolo-alveolar chronic inflammation and associated alveolar fibrin and alveolar epithelial hyperplasia. Chronic inflammation and the associated pulmonary changes were similar at both timepoints, although the chronic inflammation was more focused on terminal bronchioles and slightly increased in severity in the 90-d post-exposure group. In contrast, LDH and the number of BAL cells recovered for the PMET876 group were slightly but significantly elevated at 45-d post-exposure but not at 90-d post-exposure when compared to air control. Both parameters of toxicity were significantly elevated in the PMET885 group when compared to the PMET876 group at both 45- and 90-d post-exposure timepoints. Similar histopathologic changes were observed in the lungs of some rats exposed to the PMET876 aerosol compared with the PMET885 aerosol at 45-d post-exposure, but they were less prevalent and severe. There were some animals in the PMET876 group that were similar in histopathologic response to air controls at 45-d and 90-d post-exposure. This may suggest no lung response to the PMET876 aerosol and/or resolution of associated lung toxicity at the timepoints examined in the study. In the assessment of general health status, animal body weights were significantly less at each timepoint measured throughout the 90-d post-exposure regimen for the PMET885 aerosol compared to air control beginning after the second day of exposure. There was no significant difference in body weight at any timepoint during or after exposure to the PMET876 aerosol compared to air control. **Conclusions:** The PMET885 aerosol was more pneumotoxic at both timepoints compared to the PMET876 aerosol. In addition, lung toxicity persisted for 90-d after exposure to the PMET885 aerosol, although the lungs appeared to be recovering as injury and inflammation were both subsiding compared to the response at 45-d post-exposure.Results of this inhalation toxicology study indicated that different Ni-based thermal spray coating aerosols were toxic to the lungs, and the degree of pulmonary injury, inflammation and pathological alteration appeared to be associated with the Ni content in the thermal spray consumable.

3472 Using computational fluid dynamics to estimate the efficiency of dosing by changing the position of the rat snout and airflow received using an inhalation directed flow chamber

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Background and Purpose: Inhalation administration is a common dose route used in nonclinical development for delivering drugs to the lungs. The standard approach is to evaluate drug safety in both a rodent and non-rodent species. With the continued use of both New Chemical Entities (NCEs) and biopharmaceuticals, rats continue to be the rodent species of choice. The standard method for delivering the aerosol to rats is using a snout-only inhalation chamber called a direct flow chamber. During the exposure period, the rats are restrained in plastic clear restraint tubes. Consistent dosing is pivotal in ensuring effective study conduct and data interpretation to draw accurate conclusions about the safety of the test article, however, it is commonly observed that the animal and animal snout frequently move during the dosing period and require adjustment where possible. Furthermore, there is anecdotal evidence that the animal-to-animal variation with the dose received is greater for the inhalation route of administration compared to other routes such as oral and intravenous. In this study, Labcorp wanted to establish whether the position of the snout relative to the end of the aerosol chamber delivery tube contributed towards this perceived animal-to-animal dose variability. **Methods:** Labcorp designed a computational fluid dynamics (CFD) model of a rat head including the snout using 3d scanning software. Details of the 3d scanning methodology have been presented previously (Ford T and Moore S, Development of using 3D scanning, 3D printing and computer modelling techniques to improve inhalation systems, Association of Inhalation Toxicologist, 2024). The dimensions of the inhalation chamber and standard restraint tube were measured and incorporated into the model. Three positions were evaluated - 1 mm from the end of the tube, 10mm from the end of the tube and 5mm to the side. The airflow per chamber port was also varied between 0.33 and 1.25 L/min.The respiration rate (1.75 mL) and tidal volume (150 breathes/min) was kept constant throughout this assessment to ensure a direct comparison could be established. **Results:** The highest % delivery for the CFD model across all airflow was for the snout position only being only 1 mm from the end of the delivery tube. The values varied between 44% and 48% across the airflow range with the maximum absorption being achieved at 0.42 L/min per port. The lowest % delivery was observed at airflows of between 0.625 and 1 L/min 10 mm from the end of the delivery tube with values of ~34%. The % delivery increased steadily to 43% at 0.33 L/min per port. 5mm to the side varied between 43% at 0.42 L/min per port and 35.5% at 1 L/min per port. Further analysis was performed where the snout position was offset by 5 mm upwards and 5 mm downwards. The results where the snout position was offset by 5 mm downwards gave a delivery efficiency of only 32% at 1 L/min per port, decreasing from 40% at 0.33 L/min per port. The results where the snout position was offset by 5 mm upwards gave comparable results to the 5 mm side offset. **Conclusions:** In conclusion, the CFD data suggests that delivery rate is reduced as the animal's snout moves further away from the delivery tube, either directly or to the side or downwards. The optimum airflow per port to maximize the delivery is at 0.42 L/min per port.This research is important in assessing the study integrity and data interpretation and to help in designing more effective inhalation snout-only restraint tubes and using the most efficient airflow per port.

3473 INHALATION OF 3D PRINTING EXHAUST USING FLUORESCENT ABS FILAMENT MODULATES RESPIRATORY IMMUNE RESPONSE

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Background and Purpose: 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 and its effect on health is unknown. **Methods:** We examined the emissions of extruding fABS filament from 5 printer heads, and subsequently exposed mice to the emissions for up to 24 days (4 days/week, 4hrs/day). To directly examine particles produced during the exposure, we collected particulate on filters, and imaged particles using scanning electron microscopy. We fractioned the particle size using a MOUDI™ Impactor to determine the overall aerodynamic particle size distribution. We collected volatile organic compounds (VOCs) using the C1-C10 canister method to measure levels of potentially toxic volatiles. The day after the last exposure for each group, animals were weighed, euthanized, and lung and brain tissues were collected. To measure levels of gene expression, we used quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to measure changes in the mRNA levels of different immune system & inflammation biomarkers in the lungs. To examine changes in protein levels, we used quantitative western blotting. **Results:** Mass Median Aerodynamic Diameter (MMAD) particle size was 255 nm, and the mean particle concentration inside the exposure chamber was 41 mg/m^3; over 20 times higher than when standard ABS filament was used in the same exposure system. VOC analysis determined that toluene was present in concentrations as high as 32 ppm, a level of extended exposure in which central nervous/behavioral, reproductive/developmental, color vision and ototoxic effects have been documented. Gene expression in lung tissue (qRT-PCR) demonstrated clear reductions in gene transcripts that code for pro-inflammatory cytokines suggestive of an immunoregulatory effect. Notably, TNF-α was significantly lower at 1 & 8 days than 24 days in exposed animals, and Interleukin-12 (IL12) & Interferon-gamma (IFNγ) were reduced in exposed animals across all exposure durations. Quantitative western blot experiments also demonstrate significant reductions in interleukin-6 (IL6) expression in the hypothalamus, as well as significant changes in androgen receptor (AR) expression. **Conclusions:** Based on these results, we suspect that either higher concentrations of particulate generated by fABS or the increased levels of volatile toluene underly these effects. Reductions in IL12 in lung tissues may be responsible for the reduction in the other cytokines reported; IL12 is known to stimulate the production of IFNγ, TNF-α, and

NK cells. These data are consistent with research demonstrating IFN-γ reduction and changes in TNF-α in both microplastic and toluene exposure studies (Fujimaki et. al., 2007, Bishop et. al., 2024). In combination with a growing body of research, this data suggests that workers who are routinely exposed to high levels of 3D printing emissions could potentially be at increased risk of respiratory infections or disease. References: Bishop CR, Yan K, Nguyen W, Rawle DJ, Tang B, Larcher T, Suhrbier A. Microplastics dysregulate innate immunity in the SARS-CoV-2 infected lung. Front Immunol. 2024 May 13;15:1382655. doi: 10.3389/fimmu.2024.1382655. PMID: 38803494; PMCID: PMC11128561. Fujimaki, H., Yamamoto, S., Tin Tin Win, S., Hojo, R., Sato, F., Kunugita, N., & Arashidani, K. (2007). Effect of long-term exposure to low-level toluene on airway inflammatory response in mice. *Toxicology Letters*, *168*(2), 132-139. https://doi.org/https://doi.org/10.1016/j.toxlet.2006.11.008

3474 TLR2 and TLR3 Exhibit Distinct Roles in the Inflammatory Response of Alveolar Macrophages to Organic Dust Exposure

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Background and Purpose: Occupational dust exposure is a significant health concern, ranking among the leading causes of common disease progressions like Chronic Obstructive Pulmonary Disease (COPD) and asthma. Agricultural workers are frequently exposed to organic dust from sources such as animal feed, droppings, and contaminated soil, which can contain endotoxins and other particles, leading to an increased risk of developing these lung diseases when inhaled. This risk extends not only to the workers but also to individuals living in the surrounding communities, including children and other adults. Toll-like receptors (TLRs) belong to a large family of pattern recognition receptors and are regarded as the ancient 'gatekeepers' of the immune system. Organic dust extracts (ODE) have been found to contain numerous TLR ligands. The role of TLRs in mediating inflammatory responses in the lung, particularly in alveolar macrophages, is crucial for understanding and managing lung inflammation caused by organic dust exposure. Our laboratory has discovered that alveolar macrophages are also producers of IL-22, challenging the traditional paradigm that this unique anti-inflammatory cytokine is exclusively produced by lymphocytes. However, the mechanisms underlying IL-22 induction in alveolar macrophages remain unknown, which forms the basis of our study to identify the role of TLR activation in alveolar macrophage activation and IL-22 production. **Methods:** To investigate, we treated MH-S cells (Immortalized murine alveolar macrophages) with known agonists for TLR2, TLR3, TLR4 and TLR9 for 6 and 24 hours, followed by the quantification of IL-6, IL-10, and IL-22 using sandwich ELISA. Additionally, we conducted inhibition studies by treating alveolar macrophages with known respective antagonists for 1 hour, followed by exposure to 1% ODE for 6 and 24 hours, with subsequent ELISA quantification of the cytokines. **Results:** Our results indicate that TLR2 agonist treatment led to the highest induction of IL-22 in supernates after 6 hours, suggesting a transient, rapid role for TLR2 in instigating inflammation resolution processes. Conversely, TLR3 agonism exhibited the least induction of IL-22. Despite the low induction of IL-6 and IL-10 following TLR3 activation in our inhibition studies, treatment with the TLR3 inhibitor CU-CPT and 1% ODE significantly restricted both IL-6 and IL-10 production, with cytokine levels comparable to baseline controls treated with DMSO. These findings suggest that a major component of ODE may act as a TLR3 ligand or that TLR3 exhibits slower kinetics. **Conclusions:** The significance of these studies lies in their potential to enhance our understanding of the distinct roles TLR2 and TLR3 play in lung inflammation and resolution. Future research directions include replicating these findings in human models, exploring the roles of other human TLRs, and conducting gene assays to further investigate the transcription kinetics and downstream events associated with TLR2 and TLR3. The resulting insights could help contribute to the development of targeted therapeutic strategies for lung inflammation induced by organic dust exposure.

3475 Elucidating the role of IL-5 in the pulmonary response to inhalation of *Aspergillus versicolor* and *Stachybotrys chartarum* using a murine IL-5 knockout model

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Background and Purpose: A changing climate has resulted in an increased frequency and severity of hurricanes and flooding that devastate communities and leave structures inundated with water. Water damage in buildings promotes growth of fungal species that can be harmful to human health. This is of concern given that humans spend over 90% of their time indoors - whether that be at home, work, or school. *Aspergillus versicolor* and *Stachybotrys chartarum* are two fungal species commonly found in damp indoor environments. Inhalation of fungal spores can lead to lung inflammation and development of allergic disease. Interleukin-5 (IL-5) is a mediator of the inflammatory allergic response and is a clinical target for treatment; however, further research is needed to fully understand the role of IL-5 in response to fungal exposure, how sex as a biological variable influences this process, and to ultimately develop tools to assess exposure and treatments to mitigate respiratory ailments. **Methods:** Male and female C57BL/6J or IL-5 genetic knockout mice were nose-only exposed to HEPA-filtered air, $3x10^5$ viable *A. versicolor* spores, or $1x10^4$ viable *S. chartarum* spores for up to one hour, twice weekly, for 13 weeks using an acoustical aerosol generator system. The dose for each fungal species was the estimated pulmonary deposition per exposure. Functional respiratory assessment was performed using whole body plethysmography at week 0, week 4, week 8, and week 13 allowing for repeat measures of individual mice over time. Mice were euthanized 24 hours following the final exposure and immunologically relevant tissues were collected to assess local and systemic immune responses. Pulmonary responses were further assessed by lung histopathology, quantification of immunoglobulins in sera, flow cytometry analysis of immune cell populations in murine tissues, and measurement of Th1 and Th2 associated genes using quantitative polymerase chain reaction. **Results:** Preliminary data suggests that sex is an important factor to consider in the response to the inhalation of *A. versicolor* and *S. chartarum* in this mouse model. Whole body plethysmography indicated that overall, females have lower respiratory function than males in wild-type mice exposed to air only at baseline. Exposure to *S. chartarum* led to a decrease in respiratory function as measured by tidal volume in IL-5 -/- female mice compared to male mice after 13 weeks of exposures. Histology and flow cytometry showed increases in immune cell infiltration into the lung and the development of adaptive immune responses in mice exposed to fungal spores compared to air only control mice. Preliminary analysis exhibited that the mechanisms by which these pulmonary responses develop differed following *A. versicolor* and *S. chartarum* exposure. **Conclusions:** IL-5 is a clinical target for treatment in allergic airway disease due to its role in the inflammatory response and differentiation of eosinophils. It is imperative to investigate the role of IL-5 in the response to fungal exposure given that anti-IL-5 therapies often inhibit the function of IL-5 in humans. The goal was to understand how the immune response differs following exposure to two common fungal contaminants, focusing specifically on the role of IL-5 and the influence of sex as a biological variable. Together, these data provided insights on the mechanisms of the immune response to fungal exposure that can be applied to the assessment of fungal-related respiratory disease in humans.

3476 Woodfire Smoke Induced Mucociliary Airway Injury is Preventable with MEK Inhibition

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Background and Purpose: From the warmth of woodburning stoves or campfires to the devastation of wildfires, woodsmoke permeates both our ambient and household environment, but the health risks from these exposures are not known. Despite improvement in United States air quality through recent decades, wildfire emissions have now worsened the air we breathe. Furthermore, the frequency of wildfires is projected to increase due to climate change in the next several decades. The emissions from wildfires are highly variable and rely on differences in fuel, burning conditions, and environmental factors. In recent epidemiological studies, componentsof WFS have been shown to exacerbate respiratory and cardiovascular issues, although the mechanisms for this airway injury remain poorly understood. Mucociliary clearance (MCC) is the primary innate defense mechanism of the lung, and failure of this system results in a variety of respiratory diseases. Mucus secretion traps pathogens, particles and pollutants, and unilateral beating by cilia propels the mucus layer up and out of the airway to be expectorated or swallowed. Although the relationship between lung disease and ciliary dysfunction has been established, very little work has been done to identify the specific effects of environmental exposures on ciliary proteins, ciliary structure, and ciliary function. Some studies have reported environmental exposures to be associated with an activation of several cell signaling pathways. Post-translational modifications (PTM) regulate nearly all biological processes. Of note, protein phosphorylation is a regulatory mechanism that is important in nearly all cellular processes, such as protein synthesis, cell division, growth, or development. The Ras-Raf-MEK-ERK signaling pathway cross-talks with several other cellular signaling pathways and has been shown to be associated with ciliary motor behavior, ciliary gating, and cytoskeletal rearrangement. The MAPK pathways are well-known regulators of the cell cycle but have also been shown to control ciliary length in a wide variety of organisms. To date, several drugs have emerged as a rational therapeutic for treatment of Ras related cancers. Although these drugs, such as Pimasertib (AS703026), have only been studied in the context of cancer, the involvement of the Raf/MEK/ERK in regulating cell survival, cell cycle progression, and differentiation has been well established and could have other therapeutic potential. Therefore, this proposal aims to understand the mechanisms of ciliary injury following WFS exposure and identify potential therapeutic agents to mitigate this injury. **Methods:** Utilizing healthy human bronchial samples, our specialized air liquid interface (ALI) primary human airway culture approach allowed for mucociliary cells to be exposed to Woodfire Smoke (WFS) in a highly reproducible manor, relevant to ambient biomass burning. Pine wood was partially combusted and human mucociliary cells at air liquid interface were exposed to WFS for 3 minutes a day for 5 days. Extensive characterization of the particulate matter in our exposure system was assessed through SMPS, APS, mass quantification and excitation emission matrix. Airway Basal Cell injury following WFS exposure was assessed through bulk RNA sequencing, Proteomic and Phosphoproteomic analysis, and functional live beating ciliary video analysis. ERK inhibition was performed through addition of Pimasertib (100uM) 30 minutes prior to WFS exposure each day. Protection of ciliary injury through ERK inhibition was analyzed through live-beating ciliary video analysis and transmission electron microscopy and compared to WFS exposure alone. **Results:**