## 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

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