compared to the untreated control. Similarly, for NR8383, DQ12 quartz caused an increase in extracellular LDH at concentrations ≥ 45 µg/mL. A significant increase *(p < 0.05)* in beta-glucuronidase activity was observed at the highest concentration tested (180 µg/mL). CeO_2 and PA-6 did not cause a significant increase in extracellular LDH concentration ($p > 0.05$) for ImmuPHAGE™ cells suggesting that the cell membrane was intact, there was a reduction in metabolic activity noted ($p < 0.001$) at the highest concentration (630 µg/mL) of CeO_2 and PA-6. This contrasted with the response from NR8383 cells where membrane integrity was compromised and a statistically significant increase in the extracellular LDH concentration was observed for concentrations ≥ 90 µg/mL for both CeO_2 and PA-6 ($p < 0.05$). For ImmuPHAGE™, beta glucuronidase activity was significantly increased ($p < 0.01$) after exposure to PA-6 at the two highest concentrations of 315 µg/mL and 630 µg/mL but not CeO_2 while rat macrophages responded in similar manner but at lower magnitude.HCIA analysis methodology was successfully applied to characterize the morphology and cell health of rat and human cells *in vitro*. By combining key cell morphological features with cell health responses, a phenotype profiling approach was adopted to identify a 'fingerprint' of immune cell responses within both models to different compounds. This study revealed interspecies differences in response to the same stimuli. Rat macrophages were more sensitive than human cells and showed more adverse responses associated mainly with decreased mitochondrial activity. **Conclusions:** Although there were similarities between the response for corundum for both ImmuPHAGE™ and NR8383, there were also differences. While both cell types responded to DQ12 in a dose dependent manner, rat cells appeared to be more sensitive, showing increase in LDH and GLU at lower doses. Moreover, NR8383 responses were seen for PA6 and CeO_2 which were not present in the human ImmuPHAGE™ cells. These disparities suggest that rat models may overestimate particle toxicity for humans. Furthermore, the phenotyping approach demonstrates the potential of the multi-parameter phenotyping methodology as an early non-clinical screening tool for predicting the safety of particles. Our study is a first step in evaluating the applicability of ImmuPHAGE™ cells for particle toxicity studies, demonstrating promising results while also highlighting interspecies differences that require further investigation. The outcomes of this study emphasise the need for human-relevant models and advanced techniques to improve inhalation toxicology predictions.

3489 Characterization of Particles Generated by a Novel Powder Flame Thermal Spray Coating System

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Background and Purpose: Flame Thermal spray coating (FTSC) is a process where a mixture of metal and ceramic coating powder is melted at high temperatures and sprayed onto a surface using air pressure. This creates a complex aerosol containing various elements, such as copper, chromium, nickel, zinc, and aluminum. However, there is limited information available about the health effects associated with exposure to these aerosols. Additionally, the chemical and physical properties of these aerosols are not well understood. To address this gap in knowledge, our goal was to develop an automated FTSC aerosol generator and inhalation exposure system that could simulate workplace exposures. **Methods:** The system we developed was divided into two areas: (A) an enclosed room with a closed spray booth for the coating process, and (B) an exposure chamber equipped with particle characterization devices. We examined the physicochemical properties of aerosols generated during FTSC using four popular coating powders: F-316HCR-PL10, N-610-FS1, F-S420-PL2, and N-C276-FS2. The coating process was carried out continuously for 2 hours within the spray booth, with the metal feedstock pipe rotating in a circular and up-and-down motion. The spray gun was controlled by computer software and fired at programmed 1-sec intervals for up 12 to 16 sprays during the testing period. **Results:** During the 2-hour testing period, we were able to achieve and maintain a targeted concentration of 8 mg/m^3 in the exposure chamber. We also ensured minimal fluctuations in relative humidity, temperature, and CO_2 levels. This allowed us to generate continuous powder spray coating aerosols at a desired concentration for extended periods without interruption. Generated particles were arranged as chain-like agglomerates similar in metal profile to the composition of the consumable coating powder. **Conclusions:** This system will be valuable for future animal exposure studies aimed at understanding the potential health risks associated with FTSC aerosols.

3490 Transcriptomic changes in response to polyamide-6 inhalation: a comparative study between rats and *in vitro* human models

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Background and Purpose: Inhalation is one of the routes of exposure to micro- and nanoplastics in humans. Inhalation hazards are usually assessed by in vivo inhalation toxicity testing in rats. However, the anatomy and physiology of the respiratory tracts of rats and humans (e.g., breathing mode, branching pattern, clearance velocity) differ, which limits the relevance of findings from rat studies to human health. In addition, there are substantial and growing efforts to reduce and replace animal testing by new approach methodologies (NAMs) capable of predicting human effects with confidence. According to that, a transcriptomics approach was used to compare pathways affected by inhalation exposure to polyamide-6 (PA-6) nanoparticles in rat and in vitro human models of both the bronchial and the alveolar regions. This study aimed to identify toxicity pathways associated with nanoplastics exposure and assess the human relevance of the rat in vivo model. **Methods:** Nano-sized PA-6 was synthesized in-house by solvent precipitation of PA-6 plastics granules, as a part of the Microplastics and Human Health Consortium (MOMENTUM). The particle size ranged from 50 to 200 nm. A repeated dose inhalation study according to OECD Test Guideline 412 was conducted. Animal experiments were performed in an AAALAC-approved laboratory in accordance with the German Animal Welfare Act, and were approved by the local authorizing agency. Female Wistar rats were exposed to PA-6 (2, 10, and 50 mg/m^3) for 28 days (5 days/week, 6 hours/day). Lung burden was determined using Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), and the internal doses were used to derive surface area doses based on rat lung surface (4000 cm^2). In vitro human models were exposed to three concentrations of PA-6 in PBS [Bronchial: MucilAir™ (10.3, 25.4 and 64.9 µg/cm^2), Alveolar: AlveolAir™ (5.2, 10.6 and 34.3 µg/cm^2) and A549 cells (2.3, 12.9 and 28.4 µg/cm^2)] for 24 hours using VITROCELL® Cloud. Tissues were mechanically disrupted using CK14 bead-beating tubes and total RNA was extracted using the Maxwell® RSC simplyRNA Tissue Kit on a Maxwell® RSC 48 Instrument. Stranded NGS Libraries were generated using the NEBNext Poly(A) mRNA Magnetic Isolation Module and NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina®. Sequencing was performed on an Illumina NextSeq2000 platform. Rat-human orthologs were mapped using orthogene R package. Raw counts were analyzed using DESeq2 R package to obtain differentially expressed genes (p-value adjusted < 0.05, fold-change ≥ 1.5 or ≤ 0.67), and pathway enrichment analysis were performed using ClusterProfiler R package. For dose-response determination, log2 transformed counts in were analyzed in BMDExpress 3 software. **Results:** The rat was more sensitive when comparing mass-doses based on surface area (µg/cm^2), with 0.005 µg/cm^2 as the lowest benchmark dose median for pathway changes. In contrast, the human in vitro models showed a weaker response to the exposure of PA-6. Pathway enrichment analysis using differentially expressed genes revealed a lower number of enriched pathways for A549 and AlveolAir™ compared to the rat model, while no pathways were altered for MucilAir™. Besides interspecies differences, the stronger response in the rat could also reflect the longer exposure (repeated exposure in rats and single exposure in vitro). Analysis of gene expression with a dose-response relationship revealed further changes, and similarities between the in vitro alveolar models (AlveolAir™ and A549 cells) and the rat were observed. Ontology terms enriched in the rat (e.g., GO:0030593 - neutrophil chemotaxis) and linked with adverse effects observed in those animals (e.g., infiltration of neutrophils in bronchoalveolar lavage fluid) were also enriched in AlveolAir™ and A549 cells. Reactome pathways related to cytokines secretion (i.e., interleukin (IL)-4, IL-13 and IL-10) were identified in both human alveolar models and the rat lung. In AlveolAir™, an even greater overlap with rat pathways was identified, including platelet degranulation, cholesterol biosynthesis, and iron uptake and transport. For the bronchial model, MucilAir™, a high variability between donors (n = 4) and limited gene expression changes were observed for the tested doses; thus, no pathway was identified as enriched using the BMD approach. Higher doses could not be achieved due to technical limitations. **Conclusions:** In this study, we employed transcriptomics to compare pathways in the rat lung and in three models of the human respiratory tissue that are affected by PA nanoplastics. While the rat lung generally reacted at lower concentrations and with more pathways, further investigations are needed to confirm this finding. Three human in vitro lung models were compared and AlveolAir™ and A549 models showed reactions similar to each other and the rat lung. This study identifies genes and pathways affected by PA-6 exposure. It also highlights the common and different reactions of rat lungs in vivo and human in vitro models. Further studies will need to investigate whether differences are reflecting interspecies differences or differences due to limited complexity of the in vitro models. This will ultimately pave the way to experimental models with human relevance which are truly alternatives to animal testing.

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