Aerosol-related particle dosimetry: an experimental in vitro inhalation approachfor studying biological effects as a result of defined modification of particle number-andmass-based dose-metrics

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Defining the dose using a relevant dose-metric is of fundamental importance for evaluation and interpretation of toxicological data. Selection of the most appropriate dose-metric for inhalation exposure to aerosols is under discussion regarding specific physico-chemical properties of the test material such as particle size, but also types of the aerosols(e.g. poorly soluble, combustion, cigarette smoke).To enable experimental investigations on aerosol and dosimetry characteristics, an experimental in vitro inhalation model was established. It allowed for studying the otherwise unchanged toxicological properties of an aerosol in defined states regarding particle size, mass-and number concentration. Thus, the unique opportunity to investig atethe most relevant dose-metricfor aerosol exposure was realized.Afirst applicationused a highly concentrated aerosol of ultrafine particles from 2-stroke engine exhaust. An ageing unit based on Brownian coagulation allowedfor variation of number and mass concentration independently from each other. Acute local biological effects were investigated by exposure of air-lifted interface (ALI) human lung cultures (A549) under optimized exposure conditions (P.R.I.T.® ExpoCube®). Viability (WST-1), mitochondrial membrane potential (JC-1) and interleukin-8 release were analyzed.Dose relationships were studied after 30 or 60 minuteexposuresto four different aerosol conditions, each representing different number-and mean particle sizes atcomparable mass concentrations. Analytical determinationof particle depositionand filtered aerosol exposures validated discrimination between particle-and vapor-phase related effects. Strong correlations were found between particle mass dose and biological effects, but not between particle number dose and biological effects. It is expected that these characteristics are a consequence of the physico-chemical aerosol characteristics (oily) and therefore are in good agreement with classification of the relevant dose metrics which are under discussion for other aerosol types.Asignificant characterization ofcomplex relationships regarding dosimetry in inhalation by application of the in vitro inhalation modelis indicated by the results. Hence, it may be possible to get more insight and experimental evidence to find most appropriate dose metrics also for other types of aerosols.

Examination of the exposome in an animal model: the impact of high fat diet and rat strain on local and systemic immune markers following occupational welding fume exposure

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In this study, an experimental model was designed to investigate the impact of multiple exposomal factors on susceptibility to acute lung inflammation following an occupationally-relevant pulmonary exposure, as well as immune responses involved in the subsequent resolution of inflammation. Genetic influence was addressed by using two different strains of rats (Sprague-Dawley [SD] and Brown Norway [BN]). To assess the potential impact of behavioral/lifestyle factors on the immune response, a high fat (HF) "Western diet" was also incorporated into the study. Male SD and BN rats were maintained on HF or regular (Reg) diets for 24wk, wherein inhalation exposure to stainless steel welding fume (WF) occurred between 7 and 12wk. A set of animals was euthanized at 7, 12, and 24wk to evaluate local and systemic immune markers corresponding to the baseline, exposure, and recovery phases of the study, respectively. At 7wk, animals maintained on the HF diet exhibited several notable alterations in immune markers independent of WF exposure—the effects of which were strain-dependent and more pronounced overall in SD rats. Rats fed HF exhibited increases in circulating leukocyte number and blood neutrophil proportionality, as well as elevated percentages of lymph node (LN) B-cells. At 12wk, indices of acute lung inflammation (LN cellularity, bronchoalveolar lavage [BAL] neutrophil number, alveolar macrophage activation) were elevated in all WF-exposed animals. Diet appeared to preferentially impact the SD strain at this time point again, as several inflammatory markers were further increased in HF animals over the Reg diet group directly following WF exposure. Comparatively, diet-associated effects in the BN strain were most evident at 24wk. Overall, the BN strain was slower to recover from WF exposure than SD rats, and this process was compromised further in BN rats fed the HF diet, as both local and systemic immune alterations were still evident in HF/WF BN rats at 24wk. Collectively, HF diet appeared to have a greater impact on immune status modulation and acute inflammatory responses in SD rats, but a more pronounced effect on inflammation resolution in BN rats. These results emphasize the importance of genetic, behavioral, and environmental factors in the modulation of immunological responsivity and highlight the often overlooked role of the exposome in shaping biological responses.

All animals used in this study were housed in the NIOSH Morgantown AAALAC-accredited animal facility. All experiments were performed in accordance with an ACUC-approved animal protocol (18-004) and all relevant guidelines and regulations set forth by CDC/NIOSH and AAALAC.

Physicochemical Drivers of Primary Toxicologic Outcomesinduced by Carbon Nanotubes and Nanofibers from U.S. Facilities

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Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F) is known to induce inflammation, cytotoxicity, or tumorigenesis, and is a concern in the occupational setting. We have established toxicity profiles from male C57BL6/J mice aged8-10 weeks exposed to either 4 or 40 µg of one of nine different CNT/F via oropharyngeal aspiration as well as human epithelial BEAS-2B cells (0-24 µg/ml), differentiated THP-1cells (0-120 µg/ml), and human fibroblasts (0-2 µg/ml)for four primary outcomes: genotoxicity, inflammation, histopathology, and translocation. An overarching goal of our expansive study was to determine the relationship between particle physicochemical characteristics and those four major outcomes. The nine materials had a wide range of characteristics including diameter (6-397 nm), length (0.1-50 µm), surface area (18-238 m2/g), aspect ratio (2-1396),

residual metal catalyst (0.3-6.2 %), density (0.007-0.220 g/cm3). While all materials induced some extent of adverse outcomes, not all materials induced the same specific outcomes, or the same severity or persistence of quantified toxicity endpoints. The distinguishing physicochemical characteristics were noted as particle physical dimensions and agglomeration size and shape. As a general trend, materials of longer length and diameter, as well as particles with larger or less spherical agglomerations, were more likely to induce greater genotoxicity and more severe and persistent inflammation. Furthermore, the particle size and agglomeration characteristics were determinants of the severity and general location for histopathological changes, specifically the bronchial/bronchiolar or alveolar regions. Extrapulmonary translocation did not follow these same trends with a narrower range of peak liver accumulation at 84 days post-exposure which correlated with one day singlet lung burden. Importantly, physical dimension profiling indicated only a small population of individual CNT/F in the sample need to have the larger length and diameter to confer greater toxicity. The study identified physicochemical drivers of CNT/F toxicity, which was supported by an integrated approach, combining experimental evidence with computational modeling, and may have potential for broad application.

Comparison of Biological Response between Submerged, Pseudo-Air-Liquid Interface, and Air-Liquid Interface Exposure of A549 and Differentiated THP-1 Co-cultures to Combustion-Derived Particles

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Air liquid interface (ALI) exposure systems are gaining interest, and studies suggest enhanced response of lung cells exposed to particles at ALI as compared to submerged exposure, although the results have been somewhat inconsistent. The majority of ALI studies have used monocultures and measured particle deposition using assumptions including consistent particle deposition, particle density, and shape. This study exposed co-cultures of A549 and differentiated THP-1 cells to flame-generated particles using three exposure methods: ALI, pseudo-ALI, and submerged. The dose at ALI was measured directly, reducing the need for assumptions about particle properties and deposition. For all exposure methods an enhanced pro-inflammatory response (TNFα) and Cytochrome P450 (CYP1A1) gene expression, compared to their corresponding negative controls, was observed. ALI exposure induced a significantly greater TNFα response compared to submerged exposure. The submerged exposures exhibited greater induction of CYP1A1 than other exposure methods, although not statistically significant. Some of the factors behind the observed difference in responses for the three exposure methods include differences in physicochemical properties of particles in suspending media, delivered dose, and potential contribution of gas-phase species to cellular response in ALI exposure. However, given the difficulty and expense of ALI exposures, submerged exposure may still provide relevant information for particulate exposures.

New Approach Methodologies for the hazard assessment of nanocellulose (NC): Tier 1 Testing – High Content Analysis Based Evaluation of the Toxicity of NC Materials in Human Intestinal and Macrophage Models

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Nanocellulose (NC) is an emerging material in the food sector with foreseen applications in food packaging, novel food, as well as food additives. To date, the potential hazards associated with oral exposure to NC are not well characterized. The nanoscale features of NC require a nano-specific assessment, which should focus on whether cellulose nanofibers may display local toxicity and if they are able to cross the intestinal epithelia, leading to systemic exposure. A tiered approach was implemented for the hazard evaluation of a panel of NC samples including nanofibrillated cellulose (NFC), cellulose nanocrystals (CNC) and bacterial nanocellulose (BNC) materials.

In the first tier, a high content analysis-based approach was used to obtain a maximum amount of information on the cellular responses in intestinal and macrophage cell models following exposure to a panel NFC, CNC and BNC materials. A series of endpoints including cytotoxicity, DNA damage response, oxidative stress, and the pro-inflammatory response was quantified following a 24h treatment of Caco-2 and THP-1 cells with NC. No cytotoxic effects on the panel of endpoints were observed in the intestinal Caco-2 model. However, in differentiated THP-1 cells, while only slight cytotoxic effects were observed on cell counts and nuclear morphology, significant increases in pro-inflammatory responses (IL-8 secretion) were observed.

A selection of NC materials demonstrating significant cytotoxic effects in Tier 1 studies will be further investigated in Tier 2 studies where the uptake and crossing of the intestinal barrier will be assessed. Tier 2 will also involve the assessment of local effects, including inflammation and genotoxicity, of NC on complex models of the gastrointestinal epithelium. As well, effects of digestion and modification by the human microbiome will be studied. Tier 3 testing will involve repeated dose toxicity assessment in complex models of the intestinal epithelium.

Inflammation and transcriptome changes induced by asbestiform fibers in mice are ameliorated by a small molecule synthetic

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Asbestos exposure leads to the development of chronic, life-threatening diseases like malignant mesothelioma, pleural disease, and asbestosis. Due to the long disease latency, asbestos-related disorders are often not detected until they reach a chronic, often fatal stage, thus limiting effective therapeutic options. It is thought that unresolved inflammation following asbestos exposure contributes to disease development. As such, early intervention with anti-inflammatory molecules may prevent disease progression to a chronic state. The flaxseed lignan secoisolariciresinol diglucoside (SDG) is a small molecule antioxidant and free radical scavenger, with anti-inflammatory effects in various disease models. Here, we describe the in vivo immune and gene regulating activity of a synthetic SDG during

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