2845 Development of an *In Vitro* Approach to Point-of-Contact Inhalation Toxicity Testing of Volatile Compounds, Using Organotypic Culture and Air-Liquid Interface Exposure

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In vitro chemical risk assessment using human cells is emerging as an alternative to *in vivo* animal testing with reduced costs, fewer animal welfare concerns, and the possibility of greater human health relevance. *In vitro* inhalation toxicity testing of volatile compounds poses particular challenges. Here we report our efforts to establish a testing protocol in our own lab using the EpiAirway bronchial epithelium cell culture model and the Vitrocell 12/12 system for air-liquid interface (ALI) exposures. For purposes of method development, we used methyl iodide (MeI) as a test compound. We examined viability, cytotoxicity, and epithelial integrity responses. Dose-dependent, reproducible responses were observed with all assays. EpiAirway and BEAS-2B cytotoxicity responses to acute exposure were roughly similar, but EpiAirway was more resistant than BEAS-2B by the viability measurement, suggesting a proliferative response at low MeI concentrations. If wells were sealed to prevent evaporation, in-solution MeI concentration-response could be used to predict the response to MeI vapor within 2-fold by converting from the media- to the air-concentration at equilibrium using the blood:air partition coefficient for MeI. The long-term stability of EpiAirway cultures enabled repeated exposures over a 5-d period, which produced responses at lower concentrations than did acute exposure. We are now using these *in vitro* methods to expose multiple cell culture models (e.g., MucilAir, SmallAir, EpiAlveolar) to 1,3-dichloropropene vapor, in order to determine tissue-specific local points of departure (PoDs). Airway dosimetry modeling will be used to predict *in vivo* equivalent external concentrations for these PoDs, which can be directly compared to empirically determined values from *in vivo* studies.

2846 Inhalation Exposure of Acrylonitrile Butadiene Styrene Filament 3D Printer Emissions Induces Pulmonary and Systemic Toxicity in Rats

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Fused filament fabrication (FFF) 3-D printing is an emerging technology that has recently gained wide popularity among both consumers and manufacturers due to their increased product efficiency, reduced waste, and greater design flexibility. Numerous studies demonstrate that during thermal decomposition of the filaments, incidental ultrafine particles (UFP) and volatile organic compounds (VOCs) with potential adverse respiratory health effects are released into the air. This study sought to evaluate the respiratory and systemic toxicity of emissions from printing with acrylonitrile-butadiene-styrene (ABS), the most common thermoplastic filament on the market. A real-time generation system was designed to allow for concurrent printing of three commercially available desktop 3-D printers and delivery of an aerosol comprised of a mixture of particles and VOCs to the animal exposure chamber. A time-course exposure study was conducted via whole-body inhalation exposure. Male Sprague-Dawley rats were exposed to a single concentration for 4 h/d throughout five exposure durations: 1, 4, 8, 15, and 30 d (4 d/wk). At 24 h after the last exposure, pulmonary injury, inflammation, and fibrotic responses, as well as systemic toxicity blood markers, were assessed. 3-D printing generated particulates with average particle mass concentration of 240 ± 90 μg/m^3, and an average geometric mean particle mobility diameter of 85 nm (geometric standard deviation 1.6). The number of macrophages in bronchoalveolar lavage increased significantly at day 15. IFN-γ and IL-10 were significantly increased at days 1 and 4. Neither pulmonary oxidative stress responses nor histopathological changes of the lungs and nasal passages were found among the treatments. There was an increase in platelets and monocytes in the circulation at day 15. Several serum biomarkers of hepatic and kidney functions were significantly higher at day 1. Under the current conditions of this experiment, it was concluded that the emissions from ABS filament caused minimal and transient pulmonary and systemic toxicity. This work is critical to fill the knowledge gap regarding the potential toxicological effects of exposure to the FFF 3-D emissions, which would help to establish effective control strategies and exposure limits to prevent adverse health effects from 3-D printing emission exposure.

2847 Is TGF-β1/SMAD3 Signaling Differentially Modulated in Obesity?

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Obesity is associated with severe asthma and poor response to conventional asthma treatments. Human airway smooth muscle (HASM) cells from obese human donors exhibited increased agonist-induced force generation with elevated cytosolic Ca^{2+} flux and myosin light chain (MLC) phosphorylation. Transforming growth factor beta (TGF-β) is a pleiotropic cytokine with pivotal roles in asthma, airway hyperresponsiveness (AhR) and obesity-associated metabolic diseases. Whether TGF-β1 signaling is differentially modulated in HASM in obesity is unknown. To investigate, we hypothesized that TGF-β1/SMAD3 signaling is differentially modulated in obese donors in HASM cells. HASM cells from lean and obese donors pre-treated with vehicle or TGF-β1 (10ng/mL 18 h) were treated with contractile agonist carbachol (CCh 10 uM) and lysates were collected. MLC, SMAD3 and protein kinase B (Akt) phosphorylation were determined by immunoblotting. In parallel, Interleukin-8 (IL-8) levels were determined in supernatants. Collagen (COL1A1) expression in HASM cells was determined by qRT-PCR. TGF-β1-induced SMAD3 phosphorylation was not significantly different between obese and lean donor derived HASM cells, though obese donors trended towards lower levels compared to lean (Mean±SEM: lean 11.54±8.86 obese 6.45±3.57 n=6 donors/group; p=0.606). Similarly, TGF-β1-induced MLC phosphorylation was comparable between obese and lean donors (lean 0.11±0.04 obese 0.07±0.019 n=4 donors/group; p=0.438). TGF-β1-induced IL-8 release and Akt phosphorylation were comparable between obese and lean donors (IL-8: lean 1199.7±251.3 pg/mg obese 1530.9±740.5 pg/mg n=2 donors/group; pAkt: lean 0.107±0.019 obese 0.078±0.014 n=5 donors/group). Preliminary findings showed that baseline COL1A1 expression was higher in obese donor HASM cells compared to lean by two-fold (fold-change 2.2±0.17 n=1 donor/group), though TGF-β1-induced levels were lower in the obese donor cells compared to lean (lean 3.14±0.26 obese 2.33±0.13 n=1 donor/group). TGF-β1-induced SMAD3 and MLC phosphorylation showed decreasing trends in obese donor HASM cells compared to lean donors, although the differences were not statistically significant. This suggests that TGF-β1/SMAD3 signaling has little effect on defining the obesity phenotype in HASM cells.

2848 Derivation of an Occupational Exposure Limit for β-glucans

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β-Glucans are abundant bacterial, yeast, and fungal cell wall polysaccharides that have been shown to activate the immune system. Detectable concentrations of β-glucans have been identified in common occupational inhalation exposure scenarios associated with industries such as agriculture, food processing, and waste management. No exposure threshold values for inhalation of β-glucans have been set to date either within or outside of the United States. Therefore, establishment of an occupational exposure limit OEL for β-glucan exposure is critical to the protection of worker health, as these exposures have been linked to immunosuppressive and inflammatory reactions and possibly the development of respiratory diseases. Thus, we sought to derive a protective OEL for inhalation exposure of β-glucans based on consideration of human and non-human health effect data for this class of compounds. The body of literature demonstrates that inhalation β-glucans affects the respiratory tract and modulate immune responses, leading to symptoms such as nasal congestion, irritation, airway hyperreactivity, flu-like symptoms, inflammation, and decreased lung function. However, the available data in humans showed severe methodological limitations due to lack of a representative study size, appropriate control populations, and clear dose-response relationship. As such, an OEL of 150 ng/m^3 was derived for β-glucan based on the most relevant nonclinical study identified. This OEL provides a valuable input to the occupational risk assessment process and can guide risk management and exposure control decisions. Future work includes use of this OEL derivation framework for setting a protective inhalation limit for the general population, which is applicable for exposure to β-glucans in nicotine-based products including traditional and electronic cigarettes, for example.

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