susceptibility into *in vitro* testing approaches. Fundamental challenges facing the interpretation of toxicity data from these models are 1) a lack of consensus on the normal range of variation in commonly used endpoints and 2) a poor definition of the magnitude of an exposure-induced change required for an effect to be considered pathologic. We sought to characterize the pre- and post-exposure ranges of variability for mature culture morphology after single- and repeated-acute exposures using trans-epithelial electrical resistance (TEER), ciliary beat frequency (CBF), and oxidative stress-associated gene expression in organotypic *in vitro* bronchial tissues constructed from donor-matched, co-cultured pHBECs and human lung fibroblasts. Preliminary experiments revealed that co-cultures eliminated the formation of cyst-like structures in the epithelium and promoted functional and molecular divergence that epithelial mono-culture does not recapitulate. pHBECs also exhibit similar cell-type diversity, CBF, and viability characteristics; however, donors exhibit disparate mature TEER values and oxidative stress endpoints, most notably a significant decrease in *HMOX1* gene expression after repeated exposure to a household concentration of ambient acrolein, which supports an adaptation response that requires future research. Future work will characterize and identify applications of this model that advance our ability to recapitulate and detect inter-individual variability *in vitro,* determine the implications for environmental justice research, and assess the potential to replace aspects of current animal models. *Does not reflect US EPA policy.*

4204 Identification of a Gastrointestinal (GI) Toxicity Gene Signature from Common Chemotherapeutic Agents using Organoids and GI-Tissue

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Many compounds commonly used as chemotherapeutic agents have dose-limiting complications due to toxicity. GI toxicity is one of the most common adverse effects. Symptoms include diarrhoea, dehydration and ulceration which increases the susceptibility to infection partly due to epithelial damage causing impaired barrier function. The evaluation of GI toxicity in novel therapeutics is crucial for future development pipelines. Human and mouse organoids were exposed to chemotherapeutic agents known to cause GI toxicity (CPT11 and Iressa for 8 hours at a range of concentrations). The organoids were lysed, RNA extracted and an RNA-Seq library was generated. Samples were sequenced using 2 x 75bp PE reads. For *in vivo* assays mice were treated with 50 or 100mg/kg CPT11 for 4 or 8 hours before small intestine (SI) tissue was removed for RNA-Seq analysis. Mice were also treated with 100, 200 or 400mg/kg Iressa for 8 or 24 hours and SI collected for RNA-Seq analysis. Sequences were aligned to the relevant genomes using BWA and normalised using DESeq2. Normalised gene counts were analysed using Partek Genomics Suite and Ingenuity Pathway Analysis (IPA) was used for gene set enrichment (GSEA) and pathway analysis of differentially expressed genes. For all treatments and time points the mechanism of action (MOA) of each agent was evident from the GSEA and pathway analysis. CPT11 induced DNA damage and cell cycle arrest in organoids and mouse tissue, whereas Iressa demonstrated downregulation of cell cycle and chromosomal replication and mitotic genes. Analysis of toxicity pathways in IPA identified a number of differentially expressed genes that were consistent with increased toxicity. A gene signature was identified and a scoring system used to demonstrate signature engagement. This signature was activated in all treated samples in both organoid and SI tissue treated with CPT11 or Iressa, irrespective of the MOA of both agents. Furthermore, this signature could be engaged in organoids and could be an alternative method for GI toxicity testing. Further analysis of this signature in gene expression studies from GI toxicity inducing agents submitted to the NCBI Gene expression omnibus (GEO) demonstrated that the signature was activated with a variety of agents with different MOA's such as NSAIDs, gamma-secretase inhibitors, CDK8/19 inhibitors and CAR-T-cells.

4205 Metabolic Competency of an Airway Organotypic Culture Model

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Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion processes from cigarette smoke, diesel exhaust, and wood burning, and some have been associated with several forms of cancer, including lung. PAHs are bioactivated into their reactive metabolites by metabolizing enzymes in the body to cause mutations and altered gene signaling leading to tumor growth. The airway epithelium is a primary route of exposure for inhaled toxicants and 3D organotypic culture models represent an important advancement for toxicity testing compared to traditional *in vitro* models that lack metabolic capability and multicellular structure/communication associated with the bronchial epithelium *in vivo*. However, limited data exists regarding the metabolic capacity of these cells, which limits their use in quantitative studies for assessment of dosimetry or predictive modeling of toxicity compared to *in vivo* studies. Therefore, primary human bronchial epithelial cells (HBEC) cultured in 3D at the air-liquid interface were utilized as a model for PAH inhalation toxicity. A number of comparative approaches have been utilized to assess the metabolic competency of HBEC after treatment with benzo[a]pyrene (BaP, 10-50 µg/mL). Benchmark modeling was used to analyze global gene expression data for identification of dose-response sensitive genes and pathways. BaP treatment had a significant effect on DNA damage, xenobiotic response, and oxidative stress pathways, and qPCR confirmed dose-dependent changes for several Phase I and II enzymes. Preliminary UPLC and P450-glo activity data show the formation of BaP metabolites present in cells and media as well as increased CYP1A1 activity after BaP treatment, respectively. Future studies will apply the CometChip assay to evaluate PAH metabolite-induced DNA damage and activity-based protein proteomics methods to study metabolic enzyme activity and evaluate the correlation with gene expression and metabolite data. Overall, this study will help determine the relevance of *in vitro* 3D primary culture models for chemical toxicity evaluation in the lung.

4206 Acute Exposures to Methyl Ethyl Ketone, Health Effects, and Safety Guidance for Occupational Settings

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Methyl ethyl ketone (MEK), also known as 2- butanone, is a colorless industrial solvent with a sharp odor that is used in surface coating, synthetic resins, artificial leathers, and glues. MEK is often used in mixtures with acetone, toluene, or alcohols. Acute exposure to MEK in humans has been reported to cause irritation to eyes, nose, and throat in addition to neurological effects such as depression, nausea, and headache. Acute exposures, defined as a single exposure or an exposure for a short duration of time might sometimes be lethal or pose immediate danger to health. The National Institute for Occupational Safety and Health (NIOSH) develops immediately dangerous to life or health (IDLH) values to identify air concentration levels that are lethal and may cause adverse health effects and at/above which workers must escape from the contaminated environment. Findings from a series of NIOSH-sponsored human experimental studies report that at exposure concentrations of MEK ranging from 200-400 ppm for 2-4 hours duration, there were no significant neurobehavioral effects based on psychomotor tests for choice reaction time, visual vigilance, and self-reported mood states. There was no report of irritation to throat, tearing of eyes, or headache and nausea except for the report of a strong odor. However, animal studies have shown that MEK exposures to 10,000-30,000 ppm for 10- 30 minutes are associated with a 50% decrease in respiration (RD_{50}) and lack of response to stimulus. At higher concentrations of 65,000 ppm for 45 minutes, 50% of the animals are reported dead (LD_{50}). Based on evaluation of toxicological evidence, including dose-response information from human and animal studies, we have assessed the toxicological endpoints for acute exposure to MEK as respiratory irritation, neurobehavioral effects, and lethality at very high doses. After conducting a risk assessment using LD_{50} data from animal studies, adjusting exposure time to 30 minutes, and applying appropriate uncertainty factors, the current IDLH value is set at 2650 ppm. For acute sensory irritants like MEK, RD_{50}s might be appropriate to rank the potency and establish exposure limits. Hence, our future goal is to assess the applicability of RD_{50} and evaluating appropriate uncertainty factors to assess an IDLH for MEK that is more protective to workers in occupational settings.

4207 A Novel, Human-Relevant Replacement Screening Alternative to Animal Testing for Environmental Chemicals

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Reports indicate a whopping 5% of birth defects are affecting the musculoskeletal system. Exposure to chemicals released from everyday sources are largely overlooked as a cause of birth defects. Ideally, evaluation of new toxicants would be tested on a multitude of species, however human embryonic exposure is clearly not ethical. Current assessments extrapolated to human exposure consists of *in vivo* rodent model systems, *in vitro* embryotoxicity assays, and *in silico* prediction programs. Yet, these methods remain in need of a higher predictivity rate on humans. In addition, while the above-mentioned methods are considered standard for screenings, they remain labor- and cost-intensive and require sacrificing pregnant animals to recover embryos. With the advent of human pluripotent stem cell models and their application to derive bone cells, it has now become possible to leverage these cells for risk assessment associated with environmental exposure. Here we present results from screening a bank of 18 training chemicals with known effects to the skeleton. These chemicals, including 4 reference chemicals, were selected from the ToxCast I library to establish reliable parameters that would allow us to statistically group toxicants based on their embryotoxicity. Cells were concomitantly exposed to the selected chemicals during differentiation to osteoblasts to obtain half-maximal inhibitory concentrations based on two endpoints: cytotoxicity (MTT assay) and matrix calcification. The latter was used to indirectly quantify the amount of mature, functional bone-forming osteoblasts from a calcium assay, normalized to protein content of the cultures. All half-maximal inhibitory concentrations were then related to a half-maximal inhibitory concentration obtained from exposing fully differentiated fibroblasts. This was done using three Fisher functions developed for mouse cells. Using this biostatistical model, we were able to correctly categorize 11 chemicals based on their known *in vivo*

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