using high resolution accurate mass (HRAM) mass spectrometry coupled to ultra-high performance liquid chromatography. The results of the global metabolomics revealed significant changes based on both age and diet within all three strains. Principal component analysis revealed that the influence of diet caused a greater variation in the significantly changing metabolites ($p < 0.05$) than that of age for the BN and F344 strains, while the SD strain showed a large influence from diet at the 4 week time point. As expected, metabolites involved in lipid metabolism and bile acid formation were upregulated in the animals maintained on a HF diet compared to the regular diet. There were also significant changes observed in acetyl-coA concentrations between the two diets at all of the time points for all strains. A targeted LC-MS/MS quantification of TCA cycle intermediates was performed on liver metabolite extracts. The results of this targeted analysis revealed significant increases in α-ketoglutarate, succinyl-coA, and fumarate for the HF diet across all three strains. The BN strain showed a significant increase in malate in the HF diet compared to the regular diet, whereas the SD strain had a significantly higher concentration of citrate in the liver of the HF diet than the other two strains. The results of this study show that Sprague-Dawley showed greater metabolic variability to diet changes than Fisher 344 or Brown Norway strains. It also shows that age is an important experimental variable to consider when performing metabolic studies.

3069 Physicochemical Characterization and *In Vitro* Toxicity of Emissions from a 3D Printer

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Three dimensional (3D) printers are widely used for prototyping and building small physical objects in schools, home and businesses. Feedstock material used in 3D printing is polymer thermoplastic filament that may contain additives such as metals, ceramics, wood fiber, carbon fiber, graphene, or silica to impart aesthetic or functional properties. The use of 3D printers with polymer thermoplastics is of concern for workers and consumers because they emit a mixture of ultrafine particles and volatile organic compounds (VOCs) that are associated with respiratory and cardiovascular diseases. The scope of this study was to characterize aerosolized emissions from 3D printers and evaluate their toxic effects in human small airway epithelial cells (SAEC). Emissions were generated from a commercially available 3D printer while operating for 1.5 h with acrylonitrile butadiene styrene (ABS) or polycarbonate (PC) filaments. Both particles and VOCs were collected using an impinger sampler. Samples were characterized for their physicochemical properties, cellular cytotoxicity, oxidative stress response, apoptotic effects, and cytokine production. Results showed that printers with PC filaments generated two-fold more particles/ml than ABS. Mean sizes of PC and ABS-emitted particles in cell culture media were 201 ± 8 nm and 198 ± 10 nm, respectively. Bisphenol A and styrene were the predominant VOCs collected in the media for the PC and ABS emissions, respectively. At 24 h post exposure, both PC and ABS emissions elicited significantly increased cytotoxicity, with PC being more toxic than ABS. Moreover, PC induced higher production of reactive oxygen species, and decreased in total antioxidant capacity and glutathione peroxidase activity than ABS. Furthermore, both PC and ABS emissions induced apoptosis in SAEC with the PC emissions induced four-fold more apoptotic cells than the ABS emission. Cytokine and chemokine profiling showed that PC emissions induced higher production of seven proinflammatory cytokines and chemokines than ABS. Taken together, the results indicate that the emissions generated by PC and ABS filaments induce toxicity in SAEC, and the exposure to the PC emission induces more toxicity than that of the ABS emission.

3070 Capture Compound Mass Spectrometry: Elucidating Off-Target Binding to Deconvolute Drug Toxicity

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Understanding both the on- and off-target protein binding interactions of small molecules is an essential part of the drug discovery process. A large proportion of toxicity findings in non-clinical or clinical studies are precipitated by the parent drug or a metabolite binding an off-target protein target, such as an enzyme or receptor, modifying its function resulting in cellular dysfunction and toxicity. Capture Compound® Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific-binding protein targets for small molecules and peptides. The technology combines medicinal chemistry and *in vitro* pharmacology, coupled to high resolution proteomics mass spectrometry to isolate and identify target proteins that are responsible for an observed biological response. Thus, through *in vitro* investigation in target tissues of interest, the candidate proteins and pathways causing *in vivo* toxicity can be elucidated. An overview of CCMS technology and its application in identification of off-target compound activity is presented. The CCMS technology has been used to determine on- and off-target interactions of the catechol-O-methyl transferase (COMT) inhibitor tolcapone in a human liver cancer cell line (HepG2). A comprehensive interaction profile was generated, revealing both on- and off-target binding proteins. Differential profiles of tolcapone which causes liver toxicity and entacapone that does not were elucidated and highlighted 3-hydroxyisobutyrly-CoA hydrolase (HIBCH) as a candidate target mediating toxicity. Medicinal chemistry was then initiated focusing on molecules without HIBCH activity resulting in 'tolcapone-like' molecules with reduced toxicity profiles that could lead the way to the development of improved COMT inhibitors.

3071 A Practical High-Throughput Co-Culture Plate to Screen Paracrine and Endocrine Interactions

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Traditional HTS in microtiter plates is limited to one cell type, excluding important paracrine and endocrine cell:cell interactions, while Transwell® inserts are not compatible with 384 and 1536 well robotic HTS. We have developed a microtiter plate based co-culture system dubbed MICRO-MT as a solution to fulfill the HTS co-culture and multi-culture needs of the drug discovery and toxicity testing market. In the MICRO-MT, cells are monocultured in individual wells of a microtiter plate with standardized dimensions as usual, but by simply increasing fluid volume, the media from monocultures in adjacent wells are bridged through the integrated microchannels allowing co-culture through diffusion of metabolites between wells. Because the MICRO-MT operates by simple diffusion and in a traditional microtiter plate footprint, it doesn't require additional equipment beyond that already in use and is compatible with existing infrastructure. To illustrate the utility of this approach we developed a conferred steroid metabolism assay using an estrogen receptor driven luminescent reporter cell line, MVLNs alone or in co-culture with HepG2 cells. HepG2 cells contain high levels of aromatase activity which converts testosterone to estrogen, while MVLN cells do not express aromatase. MVLN cells in monoculture treated with 17B-estradiol ranging 100pM to 10nM showed high luminescent activity, whereas testosterone treatment showed no luminescent activity as predicted. In contrast, co-culture of MVLN and HepG2 cells in the MICRO-MT showed dose-responsive increases in MVLN luminescent activity in response to testosterone treatment (EC50 = 4.1nM) indicating HepG2 cells converted testosterone to estrogen which then diffused in the reporter well containing MVLN cells activating the luminescent reporter. We have also found that this platform recapitulates a paracrine Sonic Hedgehog (SHH) signaling response, whereby SHH ligand produced from GMSM-K SHH transfected epithelium generates robust SHH pathway activation in co-cultured Shh Light II cells that is antagonized receptor antagonists vismodegib and cyclopamine, but also secretory antagonists Ruski-43 and U18666A. These studies show that the MICRO-MT plate is a viable solution to support paracrine and endocrine interactions in a technically simple format that is amenable to HTS.

3072 Evaluation of Transdermal Drug Delivery and Toxicity in a Microphysiological Body-on-a-Chip System

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Body-on-a-chip (BoaC) *in vitro* systems are a promising technology for increasing the predictive power of drug efficacy and toxicity in humans compared to traditional animal models. These interconnected multi-organ systems can be improved by expanding drug delivery methodologies to include oral and transdermal applications. Towards that goal, we have developed a heart-liver-"skin" BoaC system to assess the toxicity of topically administered drugs dosed acutely and chronically. To validate the topical delivery system, the moderate permeation drug diclofenac (1.5 and 3% solutions), and the low permeation compounds ketoconazole (0.11%), hydrocortisone (1%), and acetaminophen (1.5%) were applied to a synthetic skin surrogate (Strat-M membrane) and toxic effects on liver and cardiac physiology were compared to data generated from an acute drug exposure applied systemically. Medium concentration of each drug after topical application was monitored over time

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