toxicology to track the bio-distribution of the ^{14}C-labeled metabolite/catabolite through the GBA when exposed to drugs or neurotoxicants. Radiocarbon tracing of the metabolite or catabolite of interest is commonly detected using accelerator mass spectrometry (AMS), an analytical technique that is highly reproducible and sensitive, with detection limits as low as zeptomoles of ^{14}C. However, AMS provides no structural information of its target analytes, and therefore cannot be used to distinguish between radiolabeled molecules. Parallel accelerator and molecular mass spectrometry (PAMMS) is a technology developed at Lawrence Livermore National Laboratory that uses a flow splitter to simultaneously perform traditional liquid chromatography-mass spectrometry (LC-MS) and liquid-sample AMS from a single sample injection. This approach enables identification of metabolites labeled with ^{14}C by combining the extreme sensitivity of AMS with the separation and detection capability of LC-MS. In the present study, we used a simple monoculture of *Lactobacillus brevis*, a bacterial strain known to produce high amounts of GABA via the enzyme glutamate decarboxylase. The monoculture was incubated with and without ^{14}C-labeled L-glutamic acid, and the supernatant collected at 2 and 24 hours, and 4, 7 and 14 days *in vitro*. Preliminary results demonstrate chromatographic separation of GABA and glutamic acid derived from cultured cells. ^{14}C signal of glutamic acid was detected with AMS, as well as other radiolabeled metabolites that have yet to be identified. Experiments are ongoing to identify the metabolites and characterize their changes in concentration. This will demonstrate that ^{14}C-labeled L-glutamic acid from *Lactobacillus brevis* results in (1) microbial byproducts labeled with ^{14}C, (2) labeling of the metabolite of interest, and (3) tracking the production/lifespan of ^{14}C-labeled microbial catabolites over 2 weeks. In summary, we will develop a ^{14}C labelling approach as a tool to study the GBA, and elucidate how toxins modulate this complex interaction. *This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 through LDRD award 22-FS-002. (LLNL-ABS-842478).*

4436 Issue of Seroconversion of AAV NAb Antibodies in Prescreened NHPs Undergoing Gene Therapy Studies

R. K. Dhawan, C. Balzer, L. Campbell, B. Egan, and D. Nicholson. *Charles River Laboratories, Wilmington, MA.*

Following FDA approval of new AAV[CF1] based drugs, a huge increase in the number of gene therapy trials employing AAV- based viral vectors has been observed over the past few years. Efficacy of these AAV drugs can be compromised by the presence of neutralizing antibodies (NAb) against AAV. AAV NAbs are common in both humans and NHPs as a result of immune responses to previous exposures. Therefore, it is very important to prescreen NHPs prior to selection for gene therapy studies as NAbs may interfere with the biodistribution of the AAV drugs, potentially impacting the outcome of clinical trials. Prescreening of NHPs is routinely performed at the vendor site or during quarantine at the study site; however, determining how far in advance to test these NHPs to avoid seroconversion between testing and the start of a study remains an important question. To begin to answer this question, we performed two separate studies tracking seroconversion in NHPs. In the first study, 100 cynomolgus macaques were sourced from a single Asian supplier and housed in separate rooms. These NHPs were not always kept with the same roommates and occasionally were grouped with cohorts from other suppliers. Sera was collected from NHPs at three time points starting with all 100 animals at the start of the study (0 months). Prior to the next collections, some animals were released for studies so sera from only the remaining 87 at 4 months and 65 at 7 months post arrival were collected. Of the 65 NHPs remaining at 7 months, the sera samples from 20 NHPs, 12 males and 8 females, were tested by AAV2, AAV8, AAV9 and AAVrh74 NAb screening assays at sample dilutions of 1/10, 1/20 and 1/40. While the majority of NHPs showed no significant change in AAV NAb titer over the course of the study, a small number of animals showed an increase in NAb titer greater than 4-fold in 4-7 months. In a second study, 300 male and 300 female cynos from a single supplier were screened on arrival (week 0) with 76 of them negative for AAV8 NAbs. Another round of AAV8 NAb screening was performed at week 8 with 13/76 sera showing seroconversion. Additionally, these 13 seroconverted NHPs (week 0 vs 8) were screened by AAV2 and AAV9 NAb assays showing a reduced rate of seroconversion for these two serotypes indicating seroconversion for different serotypes was not similar in individual NHPs. Both studies confirmed seroconversion occurs in NHPs with time. This suggests that, in addition to prescreening, a second round of confirmatory screening is highly recommended, preferably within a month prior to beginning gene therapy studies.

4437 Pulmonary Evaluation of Whole-Body Inhalation Exposure of Polycarbonate (PC) Filament 3D Printer Emissions in Rats

M. T. Farcas[1], W. McKinney[1], W. Mandler[1], A. Knepp[1], S. Service[1], L. Battelli[1], S. A. Friend[1], R. Leboul[1], T. A. Thomas[2], J. A. Matheson[2], and Y. Qian[1]. *[1]NIOSH, Morgantown, WV; and [2]US Consumer Product Safety Commission, Rockville, MD.*

Three-dimensional (3-D) printing is an emerging technology, and increasing evidence shows that fused filament fabrication (FFF) 3-D printing using polycarbonate (PC) filaments often releases a significant amount of ultrafine particulates (UFPs) and volatile organic compounds (VOCs). Toxicological implications of these emissions on human health still have not been fully determined. This study sought to determine if 3D printing emissions induced pulmonary and systemic toxicity in rats. A real-time whole body inhalation exposure system was applied in this study. Three commercially available desktop polycarbonate 3D printers were concurrently printing to generate aerosol with a mixture of particles and VOCs. Male Sprague Dawley rats were exposed to a single concentration of PC emissions or filtered air for 4 hours/day, 4 days/week for 1, 4, 8, 15, and 30 days. The PC average emissions delivered to the exposure chamber had average mass concentration of 0.5 mg/m^3 with a count-median electric mobility diameter of 70 nm. The average count concentration was 460,760 particles/cm^3. At 24 hours after the last exposure, the rats were examined for pulmonary injury, inflammation, and fibrotic responses via bronchoalveolar lavage fluid (BALF) analysis. In addition, several blood biomarkers of muscle, renal, hepatic and metabolic function were assessed. Our results showed that PC particles were deposited in the alveolar region and were engulfed by macrophages. The morphological analysis demonstrated that the particle uptake had not induced changes in alveolar macrophage cell morphology. Furthermore, there were no significant changes in LDH activity, the number of total cells and the cell differential in BALF of exposed rats. In addition, no significant changes were observed in selective cytokines and chemokines in BALF or in biomarkers of muscle, metabolic, renal, and hepatic function in blood. Taken together, exposure to PC emissions at 0.5 mg/m^3 did not produce significant changes in bronchoalveolar markers of lung damage or blood markers of altered muscle, renal, hepatic and metabolic function at 1-day post-exposure.

4438 Analysis of the Human Nasal Airway Epithelium Architecture and Physiology by Volume EM and Artificial Intelligence for Analyzing Molecular and Cellular Responses to Inhaled Toxicants

A. Vijakumaran[1], C. Godbehere[1], M. Dalbay[1], A. Raja[2], A. Abuammar[1], A. James[3], J. Llodra[1], N. Morone[1], P. Goggin[3], K. Czymmek[4], J. Zelikoff[2], K. Narayan[5], and V. Mennella[1]. *[1]University of Cambridge, Cambridge, United Kingdom; [2]NYU School of Medicine, New York City, NY; [3]University of Southampton, Southampton, United Kingdom; [4]Danforth Center for Plant Research, St. Louis, MO; and [5]Frederick National Laboratory for Cancer Research, Frederick, MD.*

To analyze the effect of toxicants *in situ* at the tissue level, the application of methodologies capable of assessing tissue-level 3D organization and cellular content are required. Volumetric Electron Microscopy (vEM) is rapidly emerging as a promising tool to characterize tissues at the nanometer scale with an unprecedented level of detail. However, this technique has been limited by the requirement of time-consuming manual segmentation. Here, we present the first *in situ*, isotropic reconstruction by Focused-Ion-Scanning Electron Microscopy (FIB-SEM) of a tissue well established in the use of toxicological studies surrounding particulate matter (PM): the human nasal airway epithelium. By using Artificial Intelligence-driven volumetric segmentation and quantitative analysis of FIB-SEM data we observe extensive reorganisation of intracellular organelle quantity, morphology, and topology (e.g., mitochondria, lysosome and lipid vesicles), and rearrangements in cytoskeletal architecture, across differentiation. This approach generates a model that can be extremely useful for toxicological assessments and hazard identification. Analysis of lung and other tissue volumes obtained after *in vivo* and *in vitro* exposure are being analyzed to detect and quantify toxicant-induced changes at the global scale. Altogether, our study demonstrates how vEM techniques can be applied to study human airway cells response to environmental pollutants, inhaled toxicants and drugs targeting the airway tissue. *Work supported by grants received by the MRC TU unit, University of Cambridge and NYU School of Medicine, Division of Environmental Medicine. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.*

4439 Achieving Physiological Wall Shear Stress during *In Vitro* Inhalation Toxicology Studies

S. E. Sarles, E. C. Hensel, and R. J. Robinson. *Rochester Institute of Technology, Rochester, NY.* Sponsor: D. Conklin.

Current *in vitro* systems for airway exposure studies do not consider presenting flow conditions to cell cultures that mimic the human airway, limiting investigation of disease mechanisms, pathogenesis, and signaling pathways. *In vitro* exposure techniques that fail to expose airway cultures to transverse fluid flow may lack physiological relevance. An exposure system with an integrated mesoscale fluidic culture system capable of puffing and subsequent ambient air inhalation enables accurate dose and mechanical cues to be delivered to cultures. Fluid shear must be sufficient to evoke a response in cells, while not detaching cells from their culture surface or damaging cell membranes. Direct measurements of fluid shear at cultures may interrupt flow and exert non-physiological stress on cells. Computational Fluid Dynamics (CFD) can estimate wall shear stress; however it is costly in labor, time, and computational power. The iterative nature of experiment

62nd Annual Meeting & ToxExpo
Nashville, TN • March 19–23, 2023

The Toxicologist

Supplement to *Toxicological Sciences*

SOT | Society of Toxicology

Toxicological Sciences

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080 Volume 192, Issue S1 March 2023
www.academic.oup.com/toxsci

Publication Date: March 14, 2023