outcomes of some specific pollutants. A discussion will follow by presenting various tools for exposure assessments, including *in vivo* internal dose assessments and a novel aggregate modeling approach. This session aims to address the significance and challenges of assessing indoor air quality due to the diverse nature of indoor pollutants. It will illustrate how the exposure data are incorporated into assessment tools to identify data gaps and inform decisions for regulation, mitigation, and prevention of adverse health outcomes.

1128 Air Pollution Exposure and Health: Filling in the Blanks

J. Thornburg, and R. Chartier. *RTI International, Research Triangle Park, NC.* Sponsor: E. Mutlu

Globally, exposure to high levels of air pollution is responsible for an estimated 7 million premature deaths annually with ~ 4 million directly attributable to exposure to household air pollution (HAP). Lack of access to affordable clean fuels makes exposure to HAP a much more pervasive problem in low- and middle-income countries where some of the most at-risk populations (pregnant women, small children) may be disproportionately exposed to high levels of HAP. In addition to increased mortality, air pollution has been linked to a myriad of adverse health outcomes, both acute (e.g. respiratory infections, cardiac) and chronic (e.g. COPD, cancer). While numerous adverse health outcomes have been associated with air pollution exposure ($PM_{2.5}$ in particular), little is known about the mechanisms by which personal PM exposures affect health risk. Personal PM exposure measurements have become more commonplace over the past decade and now provide the measurement accuracy needed to assess dose-response relationships. Here we will discuss findings from three projects that use state-of-the-art tools and methods to examine air quality, exposure, and potential health impacts: 1) Malawian infant $PM_{2.5}$ exposures measured by the RTI MicroPEM personal exposure monitor and the relationship with nasopharyngeal carriage of streptococcus pneumoniae, 2) a toxicological study of red oak and cow dung combustion PM emissions using filter extractions and a cellular model (A549 and THP-1), and 3) cytotoxicity and gene expression changes of lung epithelial cells exposed to wood smoke at the air-liquid interface (ALI) using a novel ALI exposure chamber. The cumulative findings of these studies contribute to our overall goal of better understanding the impacts of biomass burning-derived emissions, PM toxicity, personal exposure burden, and potential health effects. While these were disparate projects, a combination of these methods, and the measurement tools used therein, in a single field study could help bridge the gaps between the fundamental physical and chemical properties of PM that contribute to exposure toxicity (size distribution and composition), the personal exposure measurements used to collect real-world exposure data, and the potential mechanisms that drive the health outcomes of the exposed.

1129 Investigation of Systemic Exposure of Volatile Organic Compounds (VOCs) following Inhalation Exposure: A Case Study with Mixed Xylene Isomers

E. Mutlu. *NIEHS/NTP, Research Triangle Park, NC.*

The alkylbenzene class of compounds, specifically mixed xylene isomers, were nominated to the National Toxicology Program (NTP) for testing due to its widespread potential for inhalation exposure. Xylene (as a mixture of isomers) is one of the four VOCs in BTEX (benzene, toluene, ethylbenzene (EB), and xylene) and is found in crude oil. Primary route of exposure to xylenes is via inhalation from consumer products (e.g. paint), occupational exposures (solvents), industrial emissions, and automobile exhaust. Benzene and EB which are either carcinogenic and possibly carcinogenic, respectively, in humans are structurally similar to xylenes. However, potential adverse effects of xylene are unclear at the present time. Commercially available xylenes contain up to ~20% of EB. In order to evaluate the toxicity of xylenes in the absence of EB, a test material was generated by blending individual isomers at a ratio of 20:56:24 for ortho: meta-: para-xylene, which is the ratio found in commercial xylene mixtures. As a part of a larger study investigating the toxicity of the xylene mixture following whole body inhalation exposure in male and female HSD:Sprague Dawley SD rats and B6C3F1/N mice, blood samples were collected from animals exposed to 0 (control), 150, and 600 ppm (6 h, 5 days/wk, 4 wks) at 2 and 24 h (rats only) after the last exposure to understand systemic exposure. Each individual isomer increased with the exposure concentration in both rats (~210-4400 ng/mL) and mice (~90-7100 ng/mL) when samples in blood collected 2 h after the last exposure. Concentrations in rat blood at 24 h were at or near control levels for 150 ppm group (<1 ng/mL) and considerably lower for 600 ppm group (~1.5-34 ng/mL) compared to 2 h samples (~1400-4400 ng/mL) for all the isomers. The blood ratios were similar to that in the test material suggesting that the disposition was similar between isomers. In general, assessment of systemic exposure of VOCs is challenging due to analyte volatility and hence stringent measures for sample collection and storage are critical to ensure accurate exposure data are generated. An estimation of systemic exposure is essential to aid interpretation of toxicology studies. This talk is focusing on one example for the feasibility of generating systemic exposure data in a larger program evaluating the toxicity of inhalation chemicals at the NTP.

1130 Exposure, Health Risks, and Control of Volatile Organic Compounds in Nail Salons

L. D. Montoya. *University of Colorado Boulder, Boulder, CO.* Sponsor: E. Mutlu

Nail salon technicians face chronic exposure to volatile organic compounds (VOCs), which can lead to a range of adverse health outcomes including skin, eye, and respiratory irritation as well as headaches, neurological issues, reproductive complications, and cancer. Research studies have examined various aspects of nail salon environments including VOC exposure, Occupational Exposure Limits compliance, and ventilation. Our research team measured indoor levels of formaldehyde and the aromatic compounds benzene, toluene, ethylbenzene, and xylenes (BTEX) in 6 Colorado nail salons. We also measured personal exposure concentrations for 9 VOCs (BTEX, acetone, ethyl acetate, n-butyl acetate, methyl methacrylate and 2-butanone). The study determined that the concentrations of some of these compounds were comparable to those measured in studies of oil refinery and auto garage workers. Cancer risk models determined that a 20-yr exposure to formaldehyde and benzene concentrations measured in our study will significantly increase worker's risk of developing cancer in their lifetime. Our team also characterized VOC emissions from typical nail care products and conducted control chamber studies to investigate the removal of VOCs using low-cost, sorbent sinks (i.e., coco coir, biochar, and activated carbon) and active flows provided by synthetic jets. Additional optimization studies were conducted using a novel, low-cost plaster matrix treated with activated carbon (AC) using acetone as the model VOC. These controlled studies determined that VOC removal by sorbent sinks increased with external surface area and with thickness to a lesser extent. Active flow conditions also enhanced VOC removal from the air. Experimental data were then used in nth-order general rate models with VOC-concentration-in-air and VOC-mass-adsorbed as fit parameters. The model results indicated that sorbent sinks provided significant VOC removal in a 1400 m^3 nail salon but required approximately 25 m2 of surface area. Estimated effective ventilation rates from the model ranged 3.32-9.03 mg m-2 h-1, similar to values reported in a previous study. These low-cost sorbent sinks were then adapted into 'air-cleaning' art configurations that were informed by nail salon owners and technicians.

1131 Fungal Exposures within the Indoor Environment

T. Croston. *NIOSH, Morgantown, WV.* Sponsor: E. Mutlu

Fungal contamination found within damp indoor environments negatively affects indoor air quality, and exposure to fungal bioaerosols and secondary metabolites has become an area of great public health concern. No exposure limits exist to protect occupants and workers exposed to these contaminated environments. Assessments of these environments have identified prominent fungal species proposed to contribute to health ailments, such as "Sick Building Syndrome". Associations between adverse respiratory health effects and exposure to indoor fungal contamination have been identified; however, the physiological and toxicological effects following repeated fungal inhalation exposure have not been fully characterized. An acoustical generator system delivered dry, unmodified aerosolized fungal spores to mice housed in nose-only chambers to mimic the natural route of human inhalation exposure. We used occupationally relevant doses of NTP-nominated fungal species, *Aspergillus fumigatus*, *Stachybotrys chartarum*, and *Aspergillus versicolor*, inhaled by the mice in order to investigate the mechanisms influencing the pulmonary immune responses following exposure. These subchronic exposure studies demonstrated that the pulmonary immune responses are characterized by differing T-cell phenotypes, as well as variable RNA expression and proteomic profiles. While *A. fumigatus* exposure elicited a mixed T-cell response, *S. chartarum* and *A. versicolor* elicited more of a Th2-dominate response following 13 twice-weekly exposures. Histological assessment identified pulmonary inflammation, collagen deposition and tissue remodeling following exposure to all three fungal species independently. Results suggest that the fungal component inducing immune and physiological responses vary between fungal species, such as viability or fragmentation of the fungal test article or the influence of secondary metabolites. Additional studies are warranted, including mixed fungal exposures and utilization of *in vitro* systems, to advance the understanding of the mechanisms that contribute to the

physiological and toxicological responses that follow fungal exposure. Such studies may lead to improvements in biomarker identification, as well as the contribute to establishment of recommended exposure limits.

1132 Prediction of Population Exposures to Chemicals in the Indoor Residential Environment

K. Isaacs. *US EPA, Research Triangle Park, NC.* Sponsor: E. Mutlu

The emissions of chemicals from indoor, or "near-field", sources such building materials and household articles contribute significantly to human exposure. These chemicals may include plasticizers (e.g., phthalates), flame retardants, synthetic fragrances, environmental phenols, and other volatile or semivolatile compounds. Many of these chemicals have been investigated for association with various health endpoints including endocrine disruption and asthma. Unfortunately, existing sources of hazard and exposure data do not directly address many thousands of chemicals that may be present in the indoor environment or used in commerce. This talk will focus on high-throughput methods being developed by the EPA Office of Research and Development to characterize human near-field exposures to these chemicals. Models and analytical approaches are being developed to address critical gaps in relevant information required to predict chemical occurrence, emission, fate, and ultimately human exposure in indoor environments. Informatic methods are being used to extract reported chemical ingredient information from thousands of public documents (e.g., SDS sheets, ingredient list, manufacturer disclosures) while new non-targeted analytical methods are being used to screen for thousands of unreported chemicals in consumer products. These data are being integrated with product sales information for U.S. households to identify critical co-exposures having common endpoints (e.g., endocrine disruption) that may ultimately increase risk. In addition, new structure-based models are being developed to predict critical parameters for estimating chemical emission from consumer articles used indoors. Finally, the talk will cover the development of population-based screening-level and mid-tier models for predicting indoor fate and transport of chemicals to air and dust and the exposures that result from human contact with these media. The high-throughput exposure estimates generated via these methods can be integrated with *in vitro* hazard information (assay results or models for bioactivity) to develop screen-level metrics of risk. Overall, these high-throughput methods will allow the identification and further testing of those chemicals which are more likely to pose a risk to humans.

1133 Mind the Gap: Finding Practical Ways to Fast-Track the Future of Animal-Free Toxicology Testing

A. Lowit. *US EPA, Washington, DC.*

In recent years, the march toward an animal-free future for safety assessment has accelerated and now seems within reach. However, in the agricultural, chemical, and pharmaceutical sectors, animal studies are still quite heavily relied upon to characterize the hazard and risk profile of a new chemical or product. Where regulations currently demand the generation of animal data, performing parallel assessments using nonanimal methods will help to bridge the gap and fast-track the animal-free future of toxicology testing. This Symposium aims to be a practical session to provide guidance and shared examples that can bridge the current gap, culminating in a panel session where recommendations can be discussed and explored. For example, good-quality, integrated kinetic data and predictions could provide a wealth of information that could be used for IVIVE, setting up *in vitro* assays at relevant concentrations, and increase confidence in the safety assessment. Integrated mode-of-action investigations could provide better information on the mechanism of effect seen in animals and their potential human relevance. This will require a concerted effort between different stakeholders and the adoption of modern and common practices to testing and assessment. This Symposium also will consider what the agricultural, chemical, and pharmaceutical sectors can learn from industries that are already operating in an animal-free environment, and how future technologies can help to usher in a truly animal- free toxicity testing future. In presentation 1, Dr. Lowit will present the perspective of a regulatory agency, the US EPA, who have set an ambitious target for reducing animal use and explain options open for waiving animal studies and using new approach methodologies (NAMs). In presentation 2, Dr. Terry will present an overview of a modern agricultural product's mammalian toxicology program, where animal studies are still heavily relied upon but where avenues exist to fully embrace and implement the 3Rs (replacement, reduction, and Refinement) and NAM approaches wherever possible. Dr. Dent will then present experience and learnings from the cosmetic industry, which has been "animal-free" for many years and leads the field in application of NAMs. Finally. Dr. Boekelheide will summarize the learnings and present a vision for "bridging the gap" between a reliance on animal data and the animal-free future of toxicology testing. After each of these 30-minute presentations (with five minutes each for questions), there will be a 25-minute panel discussion that will be moderated by Dr. Sewell and will be a practical session to discuss examples of real use presented in the previous talks. Key examples will be identified by speakers ahead of time for the panel discussion and preagreed "charge questions" will be designed to draw out discussion from the audience and panel on the pros/cons/barriers to application of each example to different sectors. For example, perhaps methods in integrated toxicokinetics could be better or differently applied in different sectors. How could this help to speed the adoption of animal-free approaches, and what are the benefits, costs, and challenges associated with the different approaches outlined? An important feature of the discussion also will be to further explore what the different sectors can learn from each other in the application of nonanimal approaches. The output from this panel session will form a publication in the peer-reviewed literature to widen the audience and discussion even further.

1134 Recent Progress toward Reducing Animal Use and Adopting New Approach Methods

A. Lowit. *US EPA, Washington, DC.*

In a September, 2019 directive, EPA's Administrator Andrew Wheeler calls for the agency to pursue a reduction in animal testing. The memo states, EPA will reduce its requests for, and funding of, mammal studies by 30% by 2025 and eliminate all mammal study requests and funding by 2035. EPA's Office of Chemical Safety and Pollution Prevention recently has made progress towards reducing animal use and adopting new approach methods (NAMs). This presentation will provide examples related to granting waivers for mammalian studies for a variety of study types such as inhalation and carcinogenicity studies and for ecotoxicology studies in fish and birds, including a recent waiver guidance published in 2020 for some bird studies. Advances in NAMs and use of pharmacokinetic information in dose setting for use in cancer and chronic toxicity testing will be discussed. EPA has also recently announced implementation of QSARs (e.g., https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/comptox/ct-opera/opera.html) and computational approaches for endocrine disruption of pesticide active ingredients and inerts. In addition, EPA's Office of Pesticide Programs released the first ever human health risk assessment using *in vitro* studies used as points of departure for quantitative risk assessment (https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0159-0008). Specifically, in collaboration with the National Toxicology Program, EPA has used skin sensitization *in vitro* studies coupled with artificial neural network-based defined approach (DA) to determine points of departure used in the isothiazolinone draft risk assessments instead of using laboratory animal data to evaluate risks for dermal sensitization.

1135 Advancing Safety Assessment in the Crop Protection Sector: Building the Bridge to an Animal-Free Future

C. Terry. *Corteva Agriscience, Indianapolis, IN.*

A paradigm shift is underway in chemical safety assessment with the potential to greatly reduce reliance on animal testing in favor of *in vitro* methods and other New Approach Methodologies (NAM). While the science and technology supporting use of such methods has advanced tremendously in recent years, animal testing is still considered the 'gold standard' for regulatory decision-making, particularly in the crop protection sector. Even so, there are multiple innovative strategies that can be employed to reduce animal use without compromising the integrity or utility of the information needed for safety determinations. First, toxicological endpoints should be integrated to the extent possible to reduce animal testing. For example, neurotoxicity, immuntoxicity and genotoxicity endpoints can be readily assessed in a single 90-day rodent toxicity study, thus eliminating the need for stand-alone studies to address these endpoints. In addition, generation of toxicokinetic data (without use of satellite animals) provides important internal exposure information to contextualize organ toxicity as well as key information for selection of relevant dose-levels. Further, when toxicological findings are observed *in vivo*, mechanistic *in vitro* assays can be employed to investigate the mode of action and human relevance of the effects, thus reducing the need for follow-up testing and providing key risk management information. Finally, *in vitro* methods—often in combination (e.g. defined approaches) - are increasingly being implemented as replacements for acute toxicity endpoints, currently conducted *in vivo*. Further adoption of NAMs for regulatory decision making will require continued cooperation with multiple stakeholders working together to bring forward and implement relevant and health-protective approaches.

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