molecule, AE001, was well tolerated and there were no adverse clinical observations or systemic effects detected by assessment of clinical pathology parameters or following histopathological examination of all major organs. The respiratory tracts were examined histologically with no indications of local toxicity. The stability of the absorption-enhancer in dose formulation solutions up to 50 mg/ml was determined *in vitro* by assessing the biological activity on epithelial cell-cell adhesion. The absorption enhancer, AE001, was capable of disrupting adhesion of confluent epithelial cells.

2040 Carbon Black and Ozone Co-exposure Present Novel Prospects of Disease Susceptibility

S. Xie, W. T. Goldsmith, A. B. Abukabda, K. L. Garner, E. C. Bowdridge, T. P. Batchelor, J. Mustafa, V. Castranova, T. R. Nurkiewicz, and S. Hussain. *West Virginia University School of Medicine, Morgantown, WV.*

Environmental exposures are inherently mixed in nature; however, all the current exposure limits are based on single toxicant exposures. Even with reduced mass based exposures, the severity or incidence of environmental and occupational diseases has not necessarily been reduced. We hypothesize that environmental mixtures/co-exposures can have differential consequences as compared to individual/single toxicant exposures as ultrafine particles can carry gaseous components of environmental pollution to the deeper lung. We developed a whole-body inhalation system for co-exposure to ozone (O_3) and ultrafine carbon black (CB). Mice were exposed to 2.0±0.01ppm O_3 and/or 10±1.4mg/cm^3 CB for 3 hours. Particle mobility diameter was 140 nm as measured by scanning mobility particle analyzer (SMPS 3938). Aerosol aerodynamic diameter of 84 nm was measured by an electrical low pressure impactor (ELPI+). Co-exposure aerosols demonstrated a 31% increase in abiotic oxidative potentials as demonstrated by ferric reducing ability of serum (FRAS) assay and a 6% increase in surface oxygen contents. Ultra deep RNA-seq analysis revealed differentially activated pathways related to development, immune and inflammatory processes. A significant modulation of oxidative free radical formation was measured using immune-spin trapping. A 3-4 fold higher and more persistent lung inflammation was induced by co-exposures. We observed alterations in FEV0.1, airway hyperresponsiveness, tissue elastance and tissue damping indicating more severe lung function decline in co-exposure groups. In conclusion, our results signify the urgent need to evaluate environmental co-exposures and to revisit current permissible exposure levels. *Funding: NIH/NIGMS U54GM104942-03 (SH), NIH RO1ES015022 (TRN), NIH HL027339 (SJM).*

2041 Effects of Subacute Inhalation Exposure to Multiwalled Carbon Nanotubes in Mice and Rats

E. A. Laing[1], C. P. Bautista[1], S. A. Tiscione[1], J. M. Bustamante[1], J. Singh[1], J. Zhang[2], A. Gupta[3], J. Richey[3], G. L. Baker[3], M. Stout[4], L. S. Van Winkle[1], and K. E. Pinkerton[1]. *[1]University of California Davis, Davis, CA; [2]Sichuan University, Chengdu, China; [3]Battelle, West Jefferson, OH; and [4]NIEHS/NTP, Research Triangle Park, NC.*

The field of nanotechnology is growing exponentially, with investments totaling more than $25 billion. Multi-Walled Carbon Nanotubes (MWCNTs), a type of engineered nanoparticle, possess superior conductive and mechanical properties, resulting in an increase in their production and use. MWCNT toxicity is affected by a variety of factors—length, diameter, purity—and current research shows variable outcomes to MWCNT exposure. The primary objectives of this study were to determine the fate and transport of MWCNTs in the lungs, their ability to induce inflammation, and their relative retention in the lungs. Mice and rats were separately exposed by inhalation to target concentrations of MWCNT (0, 0.06, 0.2, or 0.6 mg/m^3; n=5/group) for 6 hours/day, 5 days/week, over a period of 30 days. Lungs were analyzed 1 and 5 weeks post-exposure (PE). Bronchoalveolar lavage (BAL), cell differentials of BAL, protein concentration, and lung histopathology were assessed. There was a significant increase in the total number of cells in the BAL of mice at 1 week PE in the 0.6 mg/m^3 group compared to control. By 5 weeks PE, total number of cells returned to within control values. In rats, there was a significant dose-dependent increase in BAL neutrophils at 1 week PE in the 0.6 mg/m^3 treatment group compared to all other groups. By 5 weeks PE, the neutrophilic inflammatory response was attenuated, but a minimal dose response was still observed. Although inflammation subsided by 5 weeks PE, there remained a high retention rate of MWCNTs in BAL at this time point in both species. There was a significant increase in concentration of CXCL1 protein for the 0.6 mg/m^3 treatment group compared to control in both species at 1-week PE. No lung histopathology was noted in either species for any doses or time points compared to control. Mice and rats display a dose-dependent inflammatory response to MWCNTs in total number of cells and neutrophils. These values return to control values within 5 weeks PE. Continued retention of MWCNTs in the lungs at 5 weeks PE raises concerns regarding possible lasting effects, such as pulmonary fibrosis or cancer.

2042 Critical Analysis of Diacetyl and Bronchiolitis Obliterans

N. Moore, B. Bennett, S. P. Barnes, C. A. Robbins, and B. J. Kelman. *Veritox, Inc., Redmond, WA.*

Diacetyl, a natural component of fermented foods and a butter-flavoring agent, was linked to cluster of purported bronchiolitis obliterans (BO) in a group of workers from a single microwave popcorn plant in 2002. Not all animal toxicity, occupational health, and exposure studies agree that diacetyl can cause BO. We conducted a weight of evidence analysis to assess whether the animal studies predict BO in humans generally and among popcorn consumers. A comprehensive PubMed literature search was performed to identify animal studies with relevant routes of exposure (inhalation, intratracheal instillation, or oropharyngeal aspiration). Identified studies were critically reviewed and organized in a systemic manner (by type of respiratory tract lesions). Results indicated rodent pulmonary lesions occurred mainly in nasal passages, less frequently in the upper respiratory system, and rarely in lower airways; the depth of respiratory tract lesions correlated directly with exposure concentrations, which was consistent with diacetyl's hydrophilic nature. There is a fundamental disparity between the predominantly nasal/upper respiratory tract lesions caused by diacetyl inhalation in rodents and the deep-lung injury observed in cases of BO. The hypothesis for the pathology discrepancy lies in differences between rodent and human breathing patterns and nasal morphometries, causing diacetyl to be more efficiently absorbed by the nasal cavities of rats than humans. In rats breathing 1 ppm, modeling predicts the nasal region absorbs ~80%, trachea/bronchial regions ~18%, and <2% enters the bronchiolar region. In nasal breathing humans, the nasal region absorbs ~30%, trachea/bronchial regions ~62%, and ~8% enters the bronchiolar region. Bronchiolar penetration may approach 24% in humans upon coupling light exercise with exclusive mouth breathing, but our review indicated the modeling assumptions may not be valid. The recent NTP inhalation studies exposed rats to relatively high diacetyl concentrations and observed no lung lesions in rats exposed up to 25 ppm over 3 months or 12.5 ppm over 2 years (i.e., lung lesions were observed only when rats were exposed to 50 ppm for 3 months or 25 ppm for 2 years; NTP, 2018). We found the animal toxicity datasets, together with occupational and consumer diacetyl exposure concentrations, do not support a link between diacetyl and BO.

2043 Moderate *Aspergillus versicolor* Inhalation Exposure Triggers Neuroinflammation

T. B. Ladd[1], M. A. Barnes[2], C. L. Mumaw[1], B. J. Green[2], D. H. Beezhold[2], and M. L. Block[1]. *[1]Indiana University School of Medicine, Indianapolis, IN; and [2]NIOSH, Morgantown, WV.*

Increasing evidence implicates indoor mold exposure in cognitive deficits in children, a process hypothesized to also occur in adults. However, the mechanisms by which inhaled mold and the associated allergic pulmonary response might impact the brain are unknown. Though neuroinflammation is associated with memory deficits and impaired cognition, the environmental triggers of neuroinflammation are poorly understood and the central nervous system (CNS) pro-inflammatory consequences of inhaling mold spores is largely unknown. To address whether a moderate exposure to a common mold found in damp indoor environments, *Aspergillus versicolor* (AV), could cause neuroinflammation, 8 week old female B6C3F1/N mice were exposed to filtered air, 1x10^5 heat inactivated AV spores, or 1x10^5 live AV spores 2 times per week for 1, 2, or 4 weeks. At 48 H after the final exposure, the neuroinflammation marker profile was assessed by RT-qPCR. Analysis of brain tissue from the 4 week exposure revealed significantly elevated pro-inflammatory markers in response to only the live AV exposure in several brain regions: the olfactory bulb (TNFα), frontal lobe (TNFα, IL-1β, and CX3CR1), midbrain, and cerebellum (TNFα and CX3CR1). To discern how early neuroinflammation began in response to AV inhalation, 1 and 2 week exposure samples were tested for TNFα mRNA expression. Interestingly, results demonstrate that heat inactivated AV significantly increased TNFα mRNA levels in the olfactory bulb in the 1 week exposure, but not in the 2 or 4 week exposures. In response to inhalation of live AV, TNFα mRNA levels were significantly elevated in brain tissue from 1 week exposure (olfactory bulb and midbrain) and 2 week exposure (frontal lobe, midbrain, and cerebellum). Taken together, these results demonstrate that inhalation of live AV spores triggers neuroinflammation in several brain regions with 1, 2, and 4 week exposures, suggesting a generalized CNS response. However, heat inactivated spores were shown to exert a CNS pro-inflammatory response in only the initial, early 1 week exposure.

These findings provide much needed insight into the underlying mechanisms of how the allergic pulmonary response and mold inhalation exposure affects the brain.

2044 Role of FFAR1/FFAR4 in Excitation Contraction Coupling in Human Airway Smooth Muscle (HASM) Cells

S. Xu, A. Schwab, N. Karmacharya, J. Jude, and R. Panettieri. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Asthma is an airway disease characterized by airway hyperresponsiveness (AhR), inflammation and remodeling. Enhanced contractile phenotype of airway smooth muscle (ASM) cells in mediates bronchoconstriction and AhR in asthma. Free fatty acids are emerging as signaling molecules with importance in metabolic and inflammatory diseases. Studies showed that long-chain free fatty acids (FFA) acting through G protein-coupled receptors (GPCR), elicit bronchoconstriction in guinea pig models. Hypothesis: We hypothesized that the free fatty acid receptors FFAR1 and FFAR4 modulate excitation contraction (EC) coupling in human ASM cells to regulate AhR. HASM cells were treated with vehicle (ethanol) or GW9508, a synthetic FFAR1/4 agonist, (1-10uM) for short duration (10 min) or 24 h. Myosin light chain (MLC) phosphorylation was determined in the presence or absence of the contractile agonist carbachol (Cch, 10 uM). In parallel, GW9508-treated HASM cells were loaded with fluo-8 Ca2+-binding dye and carbachol-induced cellular Ca2+ mobilization was measured using fluorescent microscopy. In short duration treatment, GW9508 significantly attenuated basal and Cch-induced MLC phosphorylation (n=3 donors) and significantly induced basal and Cch-induced Akt phosphorylation (n=3 donors). Following 24 h exposure, GW9508 significantly attenuated baseline and agonist-induced MLC phosphorylation (n=3 donors). The short duration and 24 h exposure to GW9508 have little effect on Cch-induced Ca2+ mobilization in HASM cells. Our findings show that GW9508 attenuates MLC phosphorylation, a biochemical marker of ASM cell shortening in HASM cells, therefore has the potential to broncho-protect human airways from hyper-reactivity.

2045 Preservation of Xenobiotic Metabolizing Capacity in Airway Cells *In Vitro*: A Species Comparative Approach Using Cells from Mice, Monkeys and Humans

J. Kelty[1], N. Kovalchuk[2], X. Ding[2], and L. Van Winkle[1]. *[1]University of California Davis, Davis, CA; and [2]University of Arizona, Tucson, AZ.*

In vitro culture of primary airway cells at the air-liquid interface (ALI) preserves ciliary function and mucin production, creating an epithelial monolayer similar to *in vivo* airways. The preservation of xenobiotic metabolic enzyme activity is not documented in existing *in vitro* airway cell culture literature beyond gene expression. Naphthalene, which requires bioactivation by cytochrome P450 enzymes (P450s) to cause toxicity, was used to determine the impact of *in vitro* cellular metabolic capacity on toxicity testing. Human small airway cells were purchased, primary trachea cells from C57Bl6 mice and rhesus macaques were isolated, and HBE1 cells were used for baseline analysis. Primary cells were also maintained at ALI for 1 month *in vitro*. Phase I metabolic potential of cells was defined using gene-expression measured by digital-PCR, enzyme activity using Promega P450-1A2-Glo, and metabolism of naphthalene measured by mass-spectrometry. Phase II metabolic potential was defined by analysis of gene-expression of glutathione synthesis and metabolic enzymes, measurement of baseline reduced and oxidized glutathione using HPLC, and measurement of the glutathione response of cells to naphthoquinones. For P450s and microsomal epoxide hydrolase, *in vitro* gene-expression was the highest in mouse ALI cells and the lowest in HBE1 cells. Mouse ALI cells expressed the highest amount of GcLc (~5X higher than HBE1), GcLm (~50X higher), and GSTM1 (~100,000X higher). GSTP1 expression was 10X lower in mouse ALI cells versus HBE1 cells. Assays of mouse ALI cells for metabolism of 1A2-substrate and naphthalene confirmed maintenance of P450 activity *in vitro*, while the HBE1 cells were found to lack P450 activity. Relative to the freshly isolated mouse trachea cells, the mouse ALI cells preserved ~30% of the P450-1A2 activity. About half of the naphthalene in the mouse ALI exposure was metabolized to form glutathione-conjugates, while HBE1 cells did not generate any metabolites. The source species of airway cells impacts the resulting xenobiotic metabolic capacity *in vitro*. Direct measurement of enzyme activity and glutathione content in addition to gene-expression is recommended to detect metabolically-derived toxicity in airway cell culture systems as activity is not well preserved in a cell line, even when differentiated. *Funding: R01 ES020867, P30 ES023513 and T32 HL007013.*

2046 Comparative Study of Multiwalled Carbon Nanotubes and Pro-inflammatory IL-1 Beta Production: The Role of Purification and Surface Functionalization

H. Lee[1], A. Taylor-Just[1], D. You[1], D. Lison[2], S. Brûle[2], C. Ziemann[3], O. Creutzenberg[3], S. Simon[4], A. Vulpoi[4], F. Turcu[4], and J. C. Bonner[1]. *[1]North Carolina State University, Raleigh, NC; [2]Université Catholique de Louvain, Louvain, Belgium; [3]Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; and [4]Babes-Bolyai University, Cluj-Napoca, Romania.*

With advancements in nanotechnology, the application of multi-walled carbon nanotubes (MWCNTs) in commercial products has been increasingly expanding. Consequently, increase in exposure of humans to MWCNTs raised questions about potential risks of such materials. The efforts to characterize specific features of MWCNTs that may potentially be associated with adverse health effects have been challenging as variations in the purification process and surface functionalization alter physicochemical properties. As part of the ERA-NET SIINN project ICONS ("International Collaboration On Nanotube Safety"), we examined the consequence of purification, followed by surface functionalization, on MWCNT-induced production of interleukin 1 beta (IL-1 beta) by human macrophages *in vitro* and in the lungs of mice *in vivo*. A library of eight differently purified (chemically or thermally) and functionalized (-COOH or -NH_2) MWCNT samples were prepared from a single batch of industrially relevant Nanocyl NC7000. THP-1 monocytes were PMA-differentiated to macrophages (40 ng/mL, 48 hrs) and exposed to 1, 10, 100 µg/cm^2 MWCNTs. Cell supernatants were analyzed for IL-1 beta via ELISA. Mice (C57BL6 strain, N=4 per group) were exposed via oropharyngeal aspiration to the same library of MWCNTs at doses of 1.6 and 4 mg/kg. After 3 days, BALF was collected and IL-1 beta analyzed via ELISA. THP1 cells exposed to thermally-purified MWCNTs functionalized with -COOH or -NH_2 produced more IL-1 beta than when exposed to NC7000. Thermally-purified MWCNTs functionalized with either -COOH or -NH_2 produced greater IL-1 beta in the BALF from mice compared to NC7000, whereas chemically purified MWCNTs functionalized with either -COOH or -NH_2 produced less IL-1 beta in BALF compared to NC7000. These data suggest that the purification method used prior to surface functionalization is an important determinant in mediating inflammasome activation and IL-1 beta release as part of the innate immune response to MWCNTs. *Funding: Supported by NSF Grant 15-022 and NIEHS Training Grant T32ES007046.*

2047 Toxicity Screening of Volatile Chemicals Using a Novel Air-Liquid Interface *In Vitro* Exposure System

J. Zavala[1], J. Gause[2], L. A. Dailey[3], N. M. Hanley[2], Q. Krantz[2], D. Davies[2], P. Evansky[2], J. Harrill[2], and M. Higuchi[2]. *[1]US EPA/ORISE, Research Triangle Park, NC; [2]US EPA, Research Triangle Park, NC; and [3]US EPA, Chapel Hill, NC.*

Traditional *in vitro* dosing methods require, for example, the addition of particulate matter (PM), PM extracts, or chemicals in dimethyl sulfoxide (DMSO) or water into cell culture medium. However, about 10% of chemicals nominated for study in the US Environmental Protection Agency's (US EPA) Toxic Substances Control Act (TSCA) chemical substance inventory are insoluble in DMSO, water, or are volatile, thus their toxicity cannot be adequately tested using traditional *in vitro* dosing methods. To circumvent the difficulties in screening for volatile/insoluble chemicals, we developed a new cell culture exposure system (CCES) that permits cells to be exposed at an air-liquid interface (ALI). The ALI method permits a direct pollutant-to-cell interaction in which the test substance is in its natural state, thus providing a more realistic exposure scenario. This novel system is capable of testing 6 different chemical concentrations simultaneously to generate concentration-response curves. In our on-going study, we use the BEAS-2B cell line and primary normal human bronchial epithelial (NHBE) cells to assess the toxicity of volatile chemicals in the TSCA work plan. We exposed cells for 2 h to six concentrations in half-log dilutions, plus an air (vehicle) control to generate concentration-response curves; 1,3-butadiene, 1-bromopropane, acetaldehyde, acrolein, carbon tetrachloride, dichloromethane, formaldehyde, and trichloroethylene have been tested to date. We assessed cell viability 4 h post-exposure via the CellTiter-Glo Assay while we assessed cytotoxicity by measuring lactate dehydrogenase (LDH) in the basolateral medium. Cell lysates were collected 4 h post-exposure for whole transcriptome targeted RNA-Sequencing (i.e., BioSpyder TempO-Seq™). The objective of this study is to evaluate the capability of the transcriptomic data to identify concentration-dependent changes in mechanism/mode-of-action for volatile chemicals and evaluate the ability of the transcriptomic data to group chemicals by similar bioactivity profiles for potential grouping and read across applications. Our highest doses per chemical induced <20% cytotoxicity, while our lowest doses were targeted to not observe adverse effects. *Abstract does not reflect views or policies of the US EPA.*

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