so, significantly decreases the risk of mutations, because it is not 3-NBA that is particularly harmful, but the process the human body takes once it enters lung tissue. This metabolism pathway could be replicated in the exhaust systems of diesel-powered automobiles to abate the production of this compound, similar to how urea is sprayed into the exhaust system to limit the production of NOx compounds. Further experimentation would investigate the practicality of this application.

2015 Single Cell Transcriptomics Identifies Key Cellular Players in an Animal Model of Asbestos-Induced Pulmonary Fibrosis

N. Joshi, A. Misharin, and S. Budinger. *Northwestern University, Chicago, IL.*

Occupational and indoor exposure to asbestos can lead to the development of pulmonary fibrosis years after exposure has ceased, leading to significant morbidity and mortality. Asbestos fibers can lodge within the bronchoalveolar duct junctions and small airways of humans and mice respectively, persisting for years. Although multiple cell types have been implicated as important participants in the development and progression of asbestos-induced lung fibrosis, the specific mechanisms and key cellular players involved are not known. Using a comprehensive combination of unbiased single cell transcriptomic profiling (scRNA-Seq), genetic lineage-tracing, flow cytometry and *in situ* RNA hybridization, we tested the hypothesis that monocyte-derived alveolar macrophages are key drivers of asbestos-induced pulmonary fibrosis via epithelial cell injury and fibroblast proliferation. C57Bl6 mice were exposed to TiO_2 (control) or asbestos fibers intratracheally. Lungs were harvested 14 days later to capture the early stages of pulmonary fibrosis and scRNA-Seq libraries were prepared from cell suspensions using the 10X Chromium platform. Profiling 24,060 cells identified 24 known cellular populations represented in all experimental conditions. All populations exhibited transcriptional changes during the development of fibrosis. Importantly, the emergence of a new distinct subpopulation of alveolar macrophages was observed in asbestos-exposed animals. This subpopulation was characterized by an immature phenotype and elevated expression of genes known to be causally associated with fibrosis such as *Mmp12*, *Retnla, Chia1* and *Pdgfa* (involved in fibroblast proliferation). Furthermore, these cells expressed *Itgam* and *Cx3cr1*, suggesting a monocyte origin. Remarkably, this new subpopulation was represented only by cells from asbestos-exposed mice and was absent in control conditions. Flow cytometry, lineage-tracing analyses and immunohistochemistry confirmed this subpopulation to be monocyte-derived alveolar macrophages. Immunofluorescent microscopy confirmed that *Pdgfa*-expressing cells were specifically recruited to the areas of fibrosis and were located in the proximity of *Pdgfra*-expressing fibroblasts. Cre/lox-mediated genetic deletion of this population by targeting Casp8 prevented the development of pulmonary fibrosis. Collectively, these studies are the first to show a causal association between asbestos-induced epithelial lung injury, localized recruitment of monocyte-derived alveolar macrophages and subsequent development of spatially restricted lung fibrosis.

2016 Lack of Lung Tumor Promotion after Inhalation of a Copper-Nickel Welding Fume in A/J Mice

P. Zeidler-Erdely[1], A. Erdely[1], R. Salmen[1], L. Battelli[1], T. Dodd[1], M. Keane[1], W. McKinney[1], S. Stone[1], M. Donlin[1], H. Leonard[1], J. Cumpston[1], J. Cumpston[1], R. Mercer[1], B. Chen[1], R. Andrews[2], M. Kashon[1], J. Antonini[1], and L. Falcone[3]. *[1]NIOSH, Morgantown, WV; [2]NIOSH, Cincinnati, OH; and [3]West Virginia University, Morgantown, WV.*

The International Agency for Research on Cancer classified welding fumes as a Group 1 carcinogen (*carcinogenic to humans*) in 2017. The process of stainless steel welding creates fumes rich in carcinogenic metals such as chromium (Cr). Our lab has previously demonstrated that stainless steel welding fumes promote lung tumors in tumor-susceptible A/J mice. Consumables devoid of Cr are being produced in an attempt to limit worker exposures to potentially carcinogenic metals. The aim of this study was to characterize a new copper-nickel (Cu-Ni) fume and then investigate if inhalation of this fume would promote lung tumors in mice using a two-stage (initiation-promotion) model. To determine particle mass size distribution, a Micro-Orifice Uniform Deposit Impactor (MOUDI, model 110; MSP corp., Shoreview, Minn.) with additional Nano-MOUDI stages (MSP model 115) was used. Characterization of the fume indicated that most of the particles were between 0.1 and 1 µm in diameter, with a mass median aerodynamic diameter of 0.43 µm. Male A/J mice (4 - 5 weeks old) were initiated with 3-methylcholanthrene (MCA; 10 µg/g IP) or corn oil and, beginning 1 week later, were exposed to air or Cu-Ni welding fumes for 4 hours/day, 4 days/week, for 9 weeks. At 30 weeks, mice were sacrificed and lung tumor multiplicity and incidence were evaluated. MCA/Cu-Ni welding fume exposure significantly decreased tumor number and tumor size compared to MCA/air controls (7.11 ± 0.93 tumors *vs.* 15.57 ± 0.75 tumors and 0.57 ± 0.01 mm in diameter vs. 1.15 ± 0.02 mm in diameter, respectively). Future studies are planned to investigate the pneumotoxicity of Cu-Ni fume in A/J mice.

2017 Functional Significance of the SLC26A4 Gene in Silica-Induced Pulmonary Toxicity

T. Sager. *NIOSH, Morgantown, WV.*

Occupational exposure to silica may result in potentially fatal diseases such as silicosis and cancer. Understanding molecular mechanisms responsible for silica-induced pulmonary toxicity is of great importance in preventing silicosis and other effects associated with occupational silica exposure. Previous studies in our laboratory identified a correlation between silica-induced pulmonary toxicity and SLC26A4 gene overexpression in the lungs of rats. However, the functional significance of this gene in silica induced pulmonary toxicity is not understood. To determine the role of the SLC26A4 gene in silica-induced pulmonary toxicity, SLC26A4 wild type (WT) and knockout (KO) mice were employed. All mice were exposed to either air or crystalline silica (15 mg/m^3, 6 hours/day, 4 days) and pulmonary toxicity was assessed at 1 day, 3 months, 6 months, and 9 months post-exposure. Pulmonary response parameters including, lactate dehydrogenase (LDH) activity, oxidant production, cell counts (including infiltrating neutrophils and alveolar macrophages), and gene expression changes were assessed. Silica exposure resulted in the induction of pulmonary toxicity and inflammation in both the WT and KO mouse strains, compared to corresponding air exposed controls. However, there were significant differences ($p<0.05$) in the measured pulmonary toxicity parameters between silica exposed WT and KO groups. For example, induction of pulmonary inflammation in the silica exposed mice was hallmarked by a significant increase in infiltration of neutrophils in the lung. This infiltration was vastly different between the WT and KO groups. Specifically, at 3 months post-exposure neutrophil infiltration in the WT mice was 480 fold higher compared to air exposed controls while being 205 fold higher in the KO mice. At 6 months post-exposure, neutrophil infiltration in the WT mice was 192 times higher than air controls while the KO mice had, a significantly lower, 54 fold increase in PMN number, compared to air controls. At 9 months post-exposure neutrophil number was 45 fold higher in WT mice and only 9 fold higher in KO mice compared to air controls. In conclusion, both the WT and KO mice presented with an enhancement in pulmonary toxicity parameters measured, however, the severity of silica induced pulmonary toxicity was more in the WT mice compared to the KO mice. These findings support the hypothesis that the SLC26A4 gene does, in fact, play a role in silica induced pulmonary toxicity.

2018 Understanding the Lung-Gut Axis by Modeling the Influence of Welding Fume Inhalation Exposure and Lifestyle on the Profile of Gut Microbiome and Systemic Immune Cells

V. Kodali, K. Roach, M. Kashon, T. Boots, M. Shoeb, G. Boyce, T. Meighan, T. Eye, P. Zeidler-Erdely, J. Roberts, J. Antonini, and A. Erdely. *National Institute for Occupational Safety & Health, Morgantown, WV.*

The gut microbiome has a regulatory influence on various systemic organs, and altered microbiome diversity correlates with various diseases and pathological conditions. The goal of the current work was to profile and correlate the influence of occupational pulmonary exposure (welding fume), lifestyle (high fat diet) and age on the gut microbiome and immune cell phenotype populations in blood, lung lymph nodes and spleen. Male Sprague-Dawley rats were maintained on a regular chow (RG) or high fat (HF) diet for 24 wk. At wk 7, groups of rats maintained on each diet were exposed by inhalation to stainless steel welding fume (WF; 20 mg/m3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air until wk 12, at which time some animals from each group were euthanized. A separate set of rats from each group were allowed to recover from WF exposure until wk 24. At these three time points, immune cells from various systemic locations were profiled using flow cytometry. The DNA from the lower gut feces was extracted and sequenced for 16s. The ratio of firmicutes to bacteroidetes consistently decreased in RG-fed rats and increased for HF-fed rats over the 24 wk period. This was further exacerbated in WF-exposed animals. Random forest classifiers were used to distinctly identify specific alterations at genus and species level for the various treatments. There was no change in total leukocyte number but there was a significant increase in neutrophils recovered from the blood of rats fed the HF vs the RG diet. In the lungs, there was no change in the leukocyte profile between rats with various diets after WF exposure; however, following a recovery period, lung neutrophil and lymphocyte numbers, in addition to percent of pulmonary macrophages, remained significantly elevated in rats maintained on

the HF diet. In the spleen and lymph nodes, like the lung, WF exposure did not change the response with various diets. However, as the animals aged, the HF diet caused a significantly elevated B:T lymphocyte ratio in both the spleen and mesenteric lymph nodes compared to the RG diet. The percent of CD8+ T-lymphocytes remained elevated in the lymph nodes of the HF but not RG-fed rats. Taken together, the data suggests that diet by itself causes a dysbiosis in the gut microbiome and immune populations. This effect is irrecoverably exacerbated over time when exposed to a secondary pulmonary insult, such as welding fume.

PS 2019 Reducing the Respiration Rate and Improving Animal Welfare in Inhalation Large Animal Studies by Using a 3D Printer and Designing Specific Masks

S. Moore, and E. Moore. *Envigo, Huntingdon, United Kingdom.*

Inhalation large animal dosing requires the use of a facemask to deliver the aerosol to *in vivo* species. Exposure dosing exposure period can be up to 240 min depending on the target dose. However, the historical masks that have been used by the industry are modified from existing off the shelf clinical or veterinary masks and generic in size and as such are a compromise for their application. Envigo designed and manufactured new masks to improve animal welfare by making them specific for inhalation dosing masks by using 3d printing. Casts were made of various sizes of large animals and photographed at 30° divisions using a 3d-scanner. The individual photos from each cast were then merged into a single 3d image by the AutoCAD/CAM software. Prototype masks and seals were then produced using 3d printer software that could be adhered to the animals face with the use of Velcro straps. Validation consisted of delivering 5% CO2 introduced from the back of the mask to simulate a large animal attached to the exposure system to ensure that EtCO2 (end-tidal CO2) values returned to baseline between breathes over a wide range of operating parameters. Upon introduction of the new masks, decreased the mean *in vivo* dog respiration rates from a mean of 24.9 and 20.0 breathes/min in the males and females to 16.9 in both sexes. Decreases in respiration rates in mature primates were also observed from 42.4 and 43.3 breathes/min in the male and females to 34.6 and 40.8. The mean primate values decreased from 71.4 breathes/min in the males and 77.5 in the females to 64.7 and 64.1 respectively. These decreases in the respiration rate was also accompanied by anecdotal visual dosing observations that the animals appear noticeably calmer especially primates and thus improving animal welfare. This personalised approach to mask fitting and design has been adopted for all future studies.

PS 2020 Comparison of Deposition Efficiency and Uniformity of Monodisperse Solid Particle Deposition in Two Air Liquid Interface (VITROCELL 24/48 and AMES 48) *In Vitro* Exposure Systems with Computational Fluid Dynamic (CFD) Predictions

M. Oldham[1], J. Zhang[1], G. Gilman[2], P. Kosachevshy[1], F. Lucci[3], A. K. Kuczaj[4], N. D. Castro[1], A. A. Rostami[1], Y. B. Pithawalla[1], J. Hoeng[4], W. J. McKinney Jr.[1], and K. M. Lee[1]. *[1]Altria Client Services LLC, Richmond, VA; [2]Enthalpy Analytical, Richmond, VA; [3]Philip Morris Products, S.A., Neuchatel, Switzerland; and [4]Philip Morris Products S.A., Neuchatel, Switzerland.*

For toxicological assessment of inhalable chemicals, *in vitro* exposure systems that enable aerosols to be delivered directly to the apical surface of respiratory cells (air liquid interface; ALI) provide a more realistic exposure method than traditional submerged *in vitro* cultures. Quantitative aerosol dosimetry (delivered dose) is critical for interpretation of biological results generated from these ALI *in vitro* exposure systems and potential extrapolation to human exposures. Using two commercially available ALI *in vitro* exposure systems (VITROCELL® 24/48 and AMES 48) particle deposition efficiency and uniformity of deposition across the cell culture inserts and petri dishes were experimentally quantified and compared with CFD predictions. Four diameters of monodisperse fluorescent particles (0.51, 1.1, 2.1, and 3.2 µm mass median aerodynamic diameter) were used in the experimental measurements. For the VITROCELL® 24/48 exposure system, experimentally measured particle deposition efficiency ranged from a mean (N= 3 runs) of 0.013% to 0.86% as a function of particle diameter. Variability in the uniformity of particle deposition across the cell culture inserts was observed and ranged from 40% to 150% of the mean number of particles. There was good agreement between experimentally measured and CFD predicted particle deposition efficiency and uniformity of particle deposition for the VITROCELL® 24/48 exposure system. For the VITROCELL® AMES 48 exposure system, three different sampling flowrates (5, 10, and 20 cc/min) were evaluated. The 10 cc/min sampling flowrate provided the most consistent number (65 - 135% of mean number of depositing particles), regardless of particle size. Experimentally measured deposition efficiency (10 cc/min flowrate) ranged from a mean (N=3 runs) of 0.07% to 0.43% as a function of particle diameter. Quantitative aerosol dosimetry in these two ALI exposure systems enables improved experimental design and extrapolation to human exposures.

PS 2021 HIF-1α and IL-1β Are Two Key Events of the Lung Inflammation and Fibrosis Induced by Particles Used in Li-Ion Batteries

V. Sironval, M. Palmai-Pallag, Y. Yakoub, D. Lison, and S. van den Brule. *Université Catholique de Louvain, Brussels, Belgium.*

Rechargeable Li-ion batteries (LIB) offer undeniable advantages compared to other technologies and are increasingly used worldwide. They contain micrometric and low solubility particles, consisting of toxicologically relevant elements, implying a potential for inhalation exposure in occupational settings. New proposed applications such as printable or spray-paintable batteries might also expose consumers. As the health hazard of these materials is not documented, we performed the first study on the respiratory hazard of 3 leading LIB components ($LiFePO_4$ or LFP, $Li_4Ti_5O_{12}$ or LTO, and $LiCoO_2$ or LCO) and investigated their mechanisms of action. Lung responses were assessed in mice after oro-pharyngeal aspiration of LIB particles or crystalline silica used as reference. Acute inflammatory lung responses and oxidative stress were recorded with the 3 LIB particles and silica, LCO being the most potent. Inflammation persisted 2 m after LFP, LCO and silica, in association with fibrosis in LCO and silica lungs. Only LCO stabilized hypoxic-like factor (HIF)-1α, a pro-inflammatory and carcinogenic transcriptional factor stabilized by Co ions, after 3 d [1]. In view of the large variety of existing and in development LIB particles, their increasing production, use and disposal, a predictive *in vitro* assay of their lung toxicity appears essential to better control health risks. By inhibiting HIF-1α or IL-1β responses, we identify these 2 markers as key events of LCO lung toxicity. The *in vitro* and *in vivo* study of a large range of LIB particles with different % of cobalt allows us to confirm the predictive value of the *in vitro* HIF-1α and IL-1β induction for the lung toxicity of all LIB particles. We conclude here that particles used in LIB represent a respiratory hazard. Exposure to LIB particles should, therefore, be strictly controlled in occupational settings. LCO was more potent than crystalline silica to induce inflammatory and fibrotic responses. IL-1β and HIF-1α stabilization represent key events in the lung toxicity of LIB particles and appear useful biomarkers to compare the large number of LIB particles in development or on the market. *[1] V. Sironval, et al.,* Archives of Toxicology *92, 1673-1684 (2018).*

PS 2022 Timing of Rat Gestational High-Fat Diet and Sex Determine Increased Susceptibility to Allergic Responses in Offspring

S. Gavett[1], M. Hargrove[2], S. Snow[1], P. Phillips[1], C. Gordon[1], and U. Kodavanti[1]. *[1]US EPA, Research Triangle Park, NC; and [2]ORISE, Research Triangle Park, NC.*

We previously showed offspring from Long-Evans rat dams given a high-fat diet (HFD) before, during, and after pregnancy had increased pulmonary and metabolic responses to acute ozone exposure. Increased susceptibility to allergy and asthma may also result from maternal HFD consumption. To determine if there is a window of susceptibility to allergic responses in offspring due to HFD consumption during gestation, dams were fed a control diet (CD) throughout gestation, HFD on gestation days (GD) 1-11 followed by CD on GD 12-22 (HFD/CD), CD from GD 1-11 followed by HFD on GD 12-22 (CD/HFD), or HFD throughout gestation (HFD). Male and female offspring (average 16 weeks old on day 0) were sensitized intranasally (i.n.) with 10 µg house dust mite (HDM) antigen on days 0 and 7 and challenged i.n. on day 21. Non-allergic rats received saline vehicle only on sensitization days. Respiratory responses to methacholine aerosol (0, 10, 20, and 40 mg/ml saline) were assessed by whole body plethysmography 2 days after challenge. Enhanced pause (PenH), an indicator of labored breathing, was significantly increased in female HDM-allergic HFD and CD/HFD groups compared with the female allergic CD group during 40 mg/ml MCh exposure. There were no differences among male groups, and other indices of respiratory function were not affected by maternal diet in non-allergic or HDM-allergic groups. Alveolar macrophages constituted ~99% of bronchoalveolar lavage fluid (BALF) cells in all non-allergic groups. BALF neutrophils, eosinophils, and lymphocytes together comprised ~6% (females) to ~12% (males) of total cells in HDM-allergic groups, indicating mild allergic inflammation, but there were no effects of maternal diet on inflammatory responses. Maternal diet also had no influence on BALF protein and albumin, which were increased in HDM-allergic groups compared with non-allergic groups. In contrast, BALF γ-glutamyl transferase, a marker of lung injury, was significantly increased (1.8-1.9-fold) in both

58TH ANNUAL MEETING & ToxExpo · MARCH 10–14, 2019

The Toxicologist

Supplement to *Toxicological Sciences*

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 168, Issue 1
March 2019

www.academic.oup.com/toxsci

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

www.toxicology.org

Publication Date: February 18, 2019