rats were divided equally into 2 groups, intraperitoneally injected vehicle or MWNT-7 at 1 mg/kg body weight (BW) (a MWCNT dose inducing peritoneal mesothelioma in all treated rats within a year) and euthanized to collect blood samples at the end of week 32. Serum samples were then prepared and processed for the proteomic analysis. A 2-dimensional gel electrophoresis revealed a total of 259 protein spots with significantly altered intensities, 13 of which were identified by a nano LC-MS/MS. Among such proteins, the declined level of apolipoprotein A-IV was validated in 3 different experiments; i) a time-course study in which the rats were dosed with 1 mg/kg BW of MWNT-7 and sequentially necropsied for 32 weeks, ii) a 2-year carcinogenicity test for MWNT-7 with doses of 0.05 or 1 mg/kg BW, and iii) a 1-year comparative analysis for 2 different types of MWCNTs, SD-1, a thick and long fiber (carcinogenic), and SD-2, a thin and tangled fiber (noncarcinogenic) with doses of 1 mg/kg BW. As for all animals, serosal tissues of the coelomic organs were histopathologically evaluated and serum levels of a set of lipoprotein-related molecules were biochemically analyzed. As a result, serum levels of apolipoproteins A-I and -IV, and a ratio of HDL-cholesterol to total-cholesterol were time-dependently decreased during the development of mesothelioma, and they were inversely correlated with the severity of the tumor. This study provides potential biomarkers in association with the induction of mesothelioma by MWCNT and a new indication of the dysregulation of the lipid homeostasis in mesotheliomagensis. *Funded by Health and Labor Sciences Research Grant (H30-Kagaku-Shitei-004) from the MHLW, Japan.*

PS 2189 Pulmonary Effects of Fe_3O_4-PEG-PLGA Nanoparticles in Human Bronchial Epithelial Cells and in Wild-Type and Nrf2 Knockout Mice following Pharyngeal Aspiration

H. Sato[1], C. Zong[1], S. Devineau[2], C. McCord[2], S. Ichihara[3], O. Brookes[2], K. Itoh[4], M. Yamamoto[5], S. Boland[2], A. Baeza-Squiban[2], and G. Ichihara[1]. *[1]Tokyo University of Science, Noda, Japan; [2]Université de Paris, Paris, France; [3]Jichi Medical University, Shimotsuke, Japan; [4]Hirosaki University, Hirosaki, Japan; and [5]Tohoku University, Sendai, Japan.*

Fe_3O_4-PEG-PLGA nanoparticles (NPs) are promising bionanomaterials due to its potential application in medical areas. The present study investigated pulmonary effects of exposure to Fe_3O_4-PEG-PLGA NPs and role of Nrf2 in them. Male C57BL/6JJcl wild-type (WT) and Nrf2 KO mice were given a single dose of 0, 10, or 30μg Fe_3O_4-PEG-PLGA NPs by pharyngeal aspiration. To confirm effects on human cells, air-liquid interface cultures of the bronchial epithelial cell line Calu-3 were treated with equivalent doses (20 and 60μg/cm^2). 14days after exposure, mice were euthanized and bronchoalveolar lavage fluid (BALF) was collected. Total protein (TP), total and differential cell counts in BALF were examined. Lungs were sectioned and stained with Prussian Blue. Gene expression of selected proinflammatory cytokines in mice lung and human cell cultures were checked by qPCR. Cytotoxicity, barrier function and cytokine release were also evaluated in Calu-3 cultures. Exposure to 30μg Fe_3O_4-PEG-PLGA NPs increased total cells and macrophages in WT mice, TP, lymphocytes, neutrophils and eosinophils in Nrf2 KO mice, and basophils in both genotypes. Prussian Blue staining of the lung showed internalization of Fe_3O_4-PEG-PLGA NPs by macrophages in both genotypes exposed to 10 or 30μg of Fe_3O_4-PEG-PLGA NPs. Exposure to Fe_3O_4-PEG-PLGA NPs increased dose-dependently pulmonary expression of TNF-α, KC and MIP-2 only in Nrf2 KO mice, SOD-1, GcLc, GcLm, MMP2, and TGF-β only in WT mice. No alteration of cell viability or barrier function were observed in human epithelial cell cultures and analysis of human cytokine release and gene expression are in progress. The results suggest that exposure to Fe_3O_4-PEG-PLGA NPs induces infiltration of inflammatory cells in the lung of mice, and Nrf2 plays a role in regulation on leucocyte migration and inflammatory response induced by exposure to Fe_3O_4-PEG-PLGA NPs. *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 760928 (BIORIMA). We thank in particular ISTEC-CNR, leading work package on materials, and COLOROBBIA, the industrial provider of Fe3O4-PEG-PLGA nanoparticles.*

PS 2190 Pulmonary Inflammation Response Comparison of Nano-Clay to Machined Dusts of Nano-Clay-Enabled Composite

T. A. Stueckle[1], J. Coyle[1], H. Shane[1], J. Jensen[1], K. Roach[1], C. Johnson[1], R. Derk[1], L. Battelli[1], S. Friend[1], M. Orandle[1], S. Agarwal[2], R. K. Gupta[2], C. Z. Dinu[2], E. G. Lee[1], and L. Rojanasakul[1]. *[1]NIOSH, Morgantown, WV; and [2]West Virginia University, Morgantown, WV.*

Advanced manufacturing techniques increasingly rely on engineered nanomaterials (ENMs) as filler material for nanocomposites (NCs). Organomodified nanoclays (ONCs) represent a significant proportion of ENM fillers used in NCs to enhance strength, barrier function, and flammability. One class of organic coatings, quaternary ammonium compounds, possess known inflammatory, sensitization, and cytotoxic properties following dermal and inhalation exposure. At present, a need exists to evaluate how repeated inhalation exposure to ONCs and machining dust particles released from nanoclay-enabled NCs impacts pulmonary inflammation responses. In the present study female Balb/C mice were exposed via six repeated aspirations to Cloisite 93A (an ONC; 16.7 or 41.7 μg), virgin polypropylene (VPP) NC machining dust, and 1% Cloisite 93A PP NC dust (50 or 150 μg) particles. Bronchioalveolar lavage (BAL) and lung tissue samples were collected up to 28 days post-exposure to evaluate inflammatory markers. Collected BAL fluid showed transient infiltrates of neutrophils, eosinophils, B cells, CD4+ T cells, and CD8+ T cells dose-dependently in particle-exposed mice. Neutrophil infiltrates persisted up to day 28 in Clois93A-exposed mice. Clois93A caused elevated interstitial CD4+ T cells (day 1) while NP exposed animals showed elevated interstitial B cells. Particle exposure caused transient increases in cDC2s, pDCs, interstitial macrophages, and activated (CD86+) monocytes. Clois93A induced elevated CD11b+ DCs (day 1 and 7) and CD86+ CD103+ DCs (day 1 and 28). Irrespective of ONC or particle, hyper-acute inflammatory cytokine induction was observed, characterized by acute inflammatory (MIP1α, MDC, IL1β, TNFα, IL6, CXCL1, CXCL2), Th2 (IL5, IL13), and tissue remodeling (TIMP1, MMP9) cytokines. High dose Clois93A and 1% Clois93A PP also elicited MIP1α, MDC, MMP9, CXCL2, IL1α release on day 7 and elevated MIP1α, IL5, and IL6 on day 28 compared to other treatments. In summary, ONC presence and particle source along the ONC lifecycle influenced leukocyte airway, interstitial, and inflammatory marker responses.

PS 2191 Toxicokinetics of a Be-7 Tagged Carbon Black Sample following Intratracheal Instillation into Rat Lungs

O. H. Creutzenberg, V. Hammann, and S. Wolf. *Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany.* Sponsor: A. Bitsch

One of the concerns with inhalation of nanomaterials is the possibility that due to their small size, nanomaterials may translocate to, and cause adverse effects in tissues and organs beyond the lungs. Carbon black is an industrially produced particulate form of elemental carbon, and is often used in particle toxicology investigations as a reference nanomaterial. Although the primary particles of carbon black may be in the nano size range, carbon black invariably exists outside the reactor in which they are formed only in the form of aggregates which are collections of fused primary particles, and agglomerates which are collections of weakly bound aggregates. To understand the fate of carbon black in the body, a high surface area form of carbon black was radiolabeled with the gamma tracer beryllium-7 by proton beam irradiation. A purification sequence was performed to remove any soluble Be-7 by successive washing with ethanolic solution, slight hydrochloric acid and artificial lung fluid. The final Be-7 labeled test item was collected on a filter. Approx. 0.3 mg of carbon black was intratracheally instilled into rat lungs, an amount which was not expected to cause rat lung overload. The urine/feces excretion, blood concentration and organ concentrations were analysed up to a month following instillation to elucidate the fate of the carbon black after deposition in lungs. Excretion amounted to 18.2% in feces and 7.4% in urine in the first 2 days; from day 3 post-treatment the carbon black was no longer detectable. In blood the measured values were within the background range. Sacrifice of animals and analysis of organs and tissues on day 20 post-treatment revealed that the administered carbon black still was retained in lungs (approx. 74.2% of total amount). A very small amount of 0.4% was detected in lung-associated lymph nodes (LALN). - A separate analysis of the agglomerate size distribution of carbon black in lung simulation fluids (laser diffraction) showed the following results: Under concentration conditions of the instillation treatment the 50% percentiles were 12.1 µm, 9.2 µm and 8.7 µm in saline, artificial alveolar (AAF) and lysosomal fluid (ALF), respectively. Therefore, there is a strong agglomeration tendency of carbon black in lung fluids, and a lack of absorption and systemic translocation.

PS 2192 Pulmonary Response in Sprague Dawley Rats following Single Exposure to Aerosolized Graphene Oxide

P. Upadhyay[1], L. S. VanWinkle[1], S. M. Thomasy[1], D. Bitounis[2], P. Demokritou[2], and K. E. Pinkerton[1]. *[1]University of California, Davis, Davis, CA; and [2]Harvard T.H. Chan School of Public Health, Boston, MA.*

Graphene oxide (GO) is a two-dimensional (2-D) nanomaterial which is widely used in drug delivery systems, biosensors, medical imaging, electronics, and energy storage. As a result of increased commercial use, there is a potential risk of inhalation of aerosolized materials including planar 2D nanomateri-

Annual Meeting & ToxExpo

VIRTUAL EVENT • MARCH 2021

The Toxicologist

Supplement to *Toxicological Sciences*

Toxicological Sciences

ISSN 1096-6080
Volume 180, Issue S1
March 2021

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

SOT | Society of Toxicology

www.academic.oup.com/toxsci

Publication Date:
March 12, 2020