period by boosting it at the beginning of the study followed by intermittent maintenance exposure to shape a rectangular AUC. As a first step, we performed a 4-week intermittent exposure 2-year inhalation study without initial boost, mimicking the increment of lung burden of conventional inhalation study by referring a rat study by Kasai et al., 2016. Male C57BL/6 mice were exposed to Mitsui MWNT-7 aerosol generated by a Taquann system (J. Toxicol. Sci. 2013) using 53 micrometer mesh-filter, 6 hours per day, once per every 4 weeks, for a total of 26 times for 2 years. Mass concentrations were 2.7 ± 0.1 mg/m^3 for the low concentration group (group L) and 5.2 ± 0.2 mg/m^3 for the high concentration group (group H). MMAD was ca. 500 nm. No difference in mortality was observed between the groups. Grossly, the lungs were grayish-white to gray and "voluminous" along with an increase in weight in a dose-dependent manner, i.e. control, 165.6±8.8 mg, group L, 336.2±25.2 mg, and group H, 369.4±25.5 mg. The lung burden of MWNT-7 at 2 years was 61.1±2.2 microgram/lung in group L and 91.6±21.5 microgram/lung in group H. Histologically, chronic granulomatous foreign body responses against MWNT-7 and fibrosis in a form of respiratory bronchiolitis was observed along with a proliferation of terminal bronchial epithelium and proliferation of type II cells of the adjacent alveoli. Septal and pleural inflammatory/fibrotic lesions were also observed. Nodular lesions, diagnoses as adenocarcinoma, were identified in two cases of group L. Adenocarcinoma cells were positive for TTF-1 and negative for CC10, indicating type II alveolar epithelial origin. Further details will be presented. *Health and Labour Sciences Research Grant, Japan.*

PS 4716 Effects of Subacute Inhalation Exposure to Multiwalled Carbon Nanotubes in B6C3F1/N Mice and Sprague Dawley Rats

K. A. Thongphanh, K. L. Zmich, and K. E. Pinkerton. *University of California Davis, Davis, CA.*

Multiwalled carbon nanotubes (MWCNT) are a subset of carbon nanotubes ranging from 10nm to 200nm long. MWCNT are applied in construction, engineering, and electronic applications, and have several physiochemical properties as well as a high aspect ratio. Materials with high aspect ratios can be easily inhaled, and this quality has been a concern in the safety of inhaled carbon nanotubes. To date, MWCNT studies have utilized intratracheal or intrapharyngeal aspiration routes of administration, and studies using inhaled MWCNT have focused on chronic rather than subacute effects on pulmonary health. The goal of this study was to elucidate the subacute effects of inhaled MWCNT in mice and rats, and their ability to clear MWCNTs from the lung. It is hypothesized that subacute inhalation of MWCNT results in increased lung inflammation, total cell counts in bronchoalveolar lavage (BAL), retention of MWCNT in alveolar macrophages, and increased inflammatory cytokine expression 1-week post-exposure which resolves by 5 weeks post-exposure in B6C3F1/N mice and Sprague Dawley rats. Adult, male, pathogen-free B6C3F1/N mice and Sprague Dawley rats were assigned to one of four doses for MWCNT exposure (control, 0.06, 0.2, and 0.6mg/m^3; n=5 per group). Animals were whole-body exposed to assigned doses of L-MWCNT-1020 via a particle attrition chamber and single jet disperser/aerosolizer. Exposures were conducted for 6 hours/day, 5 days/week for 5 weeks total, followed by a 1-week or 5-week recovery period. Animals were then anesthetized via an intraperitoneal injection of Beuthanasia and tracheotomized. Bronchoalveolar lavage (BAL) was harvested, and right lung lobes were collected for downstream RNA and protein extraction and analysis via RT-qPCR and ELISA respectively. BAL samples were cytospun onto slides for neutrophil counts and cell differentials. Left lung lobes were inflated and fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned onto slides for H&E staining. BAL from mice in the 1-week recovery group conveyed significantly higher number of total cells when exposed to 0.6mg/m^3 MWCNT compared to those exposed to lower concentrations. Neutrophil counts from BAL of rats in the 0.2 and 0.6mg/m^3 MWCNT exposure groups with 1-week recovery exhibited a dose-dependent increase compared to the control group. In both models, the 5-week recovery period groups exhibited an attenuation of these effects. For both mice and rats, the highest dose treatment group exhibited significantly higher levels of neutrophil chemokine protein CXCL1 compared to control after 1-week recovery. MWCNT were found to be retained in BAL and lung tissue of both 1 and 5-week recovery groups for both species. In conclusion, acute inflammation is observed 1-week post-exposure but resolves at the 5-week recovery time point. However, the continued retention of MWCNT inclusions in lung macrophages without the presence of overt inflammation or injury raises concerns about the potential long-term effects of MWCNT inhalation exposure. *Funding: National Toxicology Program in MWCNT Research, NIEHS U01 ES020127.*

PS 4717 Characterization and Toxicity Assessment of Aerosolized Particles Generated during Cutting of Carbon Nanotubes–Embedded Concrete

V. Kodali, J. R. Roberts, K. Fraser, R. Gill, T. Eye, W. McKinney, A. Afshari, A. Erdely, and E. Lee. *NIOSH, Morgantown, WV.*

Carbon nanotube (CNT) as a reinforcement enhances durability and performance of concrete composites. Exposure to some forms of CNT is known to cause adverse pathological outcomes. To understand potential toxicity arising from use of CNT-enabled concrete composites as it goes through various occupational life cycle stages, we evaluated 1) the physicochemical characteristics and toxicity of the as-produced CNT and 2) how particulate release during concrete manipulation (e.g., cutting) is altered by CNT incorporation. Physical dimensional profiling indicated the as-produced CNT had a geometric mean length of 0.72 µm and 32 nm in diameter. Pulmonary injury and inflammation exerted by the CNT was assessed by exposing C57BL/J mice by oropharyngeal aspiration to a bolus dose of 4 and 40 µg. Animals were sacrificed on 1, 7 and 28 days to access toxicity. The toxicity of the as-produced CNT was similar to an agglomerated CNT studied in our previous research, lacking the more severe toxicity associated with longer length and diameter CNT. Mechanism-based screening of the initiating events *in vitro* in differentiated THP-1 macrophages were predictive of the *in vivo* outcome observed. For evaluating the second aim, three types of concrete blocks, 0% (reference), low%, and high% CNT, were tested in a custom designed enclosure housing an apparatus for the cutting of a block with an automated computer-controlled process. The highest particle number concentration (163,821 particles/cm^3) was measured for the reference cylinder, while others showed similar concentrations (131,689 particles/cm^3 and 140,954 particles/cm^3 for the low% and high% blocks, respectively). There was no shift in the size distribution of the released aerosols from addition(s) of CNT. The released particulate was predominantly respirable particulate consisting of quartz, feldspar, etc. and often appeared to be agglomerated materials that included both paste and aggregate minerals with an aerodynamic diameter ~5µm. No free CNT were observed by electron microscopy from low% and high% samples. Toxicity screening of the released particulate is still ongoing but based on the size distribution profiles of the released aerosols, not a remarkable alteration in toxicity is expected from the particulate released due to addition of CNT to concrete.

PS 4718 The Acute and Short-Term Inhalation of Carbon Nanofiber (CNFs) in Sprague Dawley Rats

M. Jo[1], B. Kim[2], Y. Kim[3], J. Kim[4], H. Kim[4], J. Shin[5], G. Lee[6], K. Ahn[7], M. Gulumian[8], and I. Yu[9]. *[1]HCTm, Icheon-si, Korea, Republic of; [2]Seoul National University, Seoul, Korea, Republic of; [3]Chung-Ang University, Seoul, Korea, Republic of; [4]H&H Bio, Asan, Korea, Republic of; [5]KCOMWEL, Incheon, Korea, Republic of; [6]PINE Ltd., Ansan, Korea, Republic of; [7]Hanyang University, Ansan, Korea, Republic of; [8]North-West University, Johannesburg, South Africa; and [9]HCT, Seattle, WA.*

The inhalation toxicity of CNFs is not clearly known due to relatively few related studies reported. An acute inhalation study and short-term inhalation study (5 days) were therefore conducted using Sprague-Dawley rats. In the acute inhalation study, the rats were grouped and exposed to a fresh air control or to low (0.238 ± 0.197), moderate (1.935 ± 0.159), or high (24.696 ± 6.336 mg/m3) CNF concentrations for 6 h and thereafter sacrificed at 14 days. For the short- term inhalation study, the rats were grouped and exposed to a fresh air control or low (0.593 ± 0.019), moderate (2.487 ± 0.213), or high (10.345 ± 0.541 mg/m3) CNF concentrations for 6 h/day for 5 days and sacrificed at 1, 3, and 21 days post-exposure. No mortality was observed in the acute inhalation study. Thus, the CNF LC50 was higher than 25 mg/m3. No significant body or organ weight changes were noted during the 5 days short-term inhalation study or during the post-exposure period. No significant effects of toxicological importance were observed in the hematological, blood biochemical, and coagulation tests. In addition, the bronchoalveolar lavage (BAL) fluid cell differential counts and BAL inflammatory markers showed no CNF-exposure-relevant changes. The histopathological examination also found no CNF-exposure-relevant histopathological lesions. Thus, neither acute nor 5 days inhalation exposure to CNFs induced any noticeable toxicological responses.

PS 4719 Effects of Carbon Nanodots on the Expression of Inflammatory Genes in Mouse Liver Tissue Induced by TNF-Alpha

S. Ahmed[1], H. Zhu[2], Y. Li[2], and Z. Jia[1]. *[1]University of North Carolina at Greensboro, Greensboro, NC; and [2]Campbell University, Buies Creek, NC.*

Atherosclerosis is known to be the main cause of cardiovascular disease and is regulated by pro-inflammatory molecules such as macrophage chemoattractant protein-1 (MCP-1), interleukin 1 beta (IL- 1 beta), interleukin 6 (IL-6). A new class of nanoparticles, Carbon Nanodots (CNDs), have been expressed as potential candidates for bioimaging, biosensing, and drug delivery. The liver is known to be the main metabolic organ that is involved in metabolizing abnormal lipid metabolism and complex inflammatory disease. However, the effect of CNDs on the liver has not been investigated. In this study, the impact of CNDs on TNF-a-mediated expressions of pro-inflammatory genes in mouse liver tissues was examined. C57BL/6 mice were treated with TNF-a (25mg/kg bw), CNDs (2.5 mg/kg CNDs), both TNF-a and CNDs, or neither to serve as the control. Spleen tissue was collected and homogenized, and the isolated RNA was used to make cDNA for the expression of various genes associated with the inflammatory response using real-time PCR. Our results showed that TNF-α increased the expression of pro-inflammatory genes, including MCP-1, IL-1β, and IL-6, and decreased the expression of anti-inflammatory genes, including glutathione s-transferase and heme oxygenase-1. CNDs treatment improved the expression of these genes in the liver of TNF-α-treated mice. This study would contribute to a better understanding of the role of CNDs on inflammation *in vivo*.

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