

W 2602 **Understanding the Impact on the Immune System of Occupationally Relevant Exposures to Multiwalled Carbon Nanotubes**

N. Walker, *NIEHS, Research Triangle Park, NC.*

Extensive efforts in research and development have led to extraordinary advances in nanoscience, nanotechnology, and the utility of nanomaterials. Uses of nanomaterials range from structural improvements, to building materials, to improvement in medical devices and drug delivery. As a result, there has been an increase in the potential for human exposure to nanomaterials not only inadvertently in occupational settings, but also in consumers and patients that utilize nanomaterials. Multiwalled carbon nanotubes (MWCNT) are a class of nanomaterials used for a variety of applications including structural enhancement of building materials and sports equipment, and for improvement of electrical and thermal conductivity for electronics. Animal studies have largely focused on the health effects following pulmonary exposure in rodent models. Many of these studies have demonstrated the occurrence of pulmonary inflammation associated with fibrotic changes in the airways. However, the majority of these studies investigated pulmonary exposure at dose levels that are significantly higher than occupational exposure levels. Recent studies have also demonstrated that pulmonary exposure to carbon nanotubes can result in systemic effects (cardiovascular changes and immunotoxicity), including increased expression of inflammatory cytokines genes in splenic lymphocytes in rats. These findings suggest that carbon nanotubes could target the immune system and raise the concern that low-level exposure may impact host resistance to infections or development of neoplastic diseases or increase susceptibility to allergy or autoimmune diseases. To address this issue, a consortium of academic and federal scientists initiated an integrated approach to investigate the toxicity of low-level occupationally relevant exposures to a representative high-purity "long and thin" aggregated MWCNT. Characterization of this MWCNT shows that it aggregates into micron-sized "cotton ball" structures that morphologically appear similar to MWCNTs sampled in the workplace. Key features of the approach were the well-characterized, GLP-compliant inhalation exposure system used for treatment of the rats and the partnership between the federal and academic laboratories assessing different models of impacts of the exposure on the immune system. Importantly, all of the immunotoxicity studies that will be discussed in this session were obtained from the same exposure cohorts, thereby enhancing the ability to integrate results across all studies and participating laboratories. The session will identify the occupational exposures to MWCNT, put the experimental work into a human-relevant context, and then highlight the complementary approaches undertaken by the different groups to characterize the effects of MWCNT on innate and adaptive immune responses following low-dose pulmonary inhalation exposures.

W 2603 **Perspectives from the Field: Occupational Exposures to Carbon Nanotubes in the US**

M. Dahm, *NIOSH, Cincinnati, OH.* Sponsor: V. Johnson

Early exposure assessment studies for carbon nanotubes (CNT), which reported personal breathing zone samples collected within workplaces, focused on the collection of samples for the total gravimetric mass of all particles or indirectly estimated mass specific to CNT. However, the interpretation of gravimetric sampling for CNT has proven difficult due to the unique characteristics of CNT which includes low densities and mass. More recently, the measurement of elemental carbon from personal breathing zone collections has provided more realistic mass-based workplace exposure estimates. Plausible exposure metrics and results from 12 workplace exposure assessment site visits conducted within facilities producing or using some form of CNT within the United States will be presented and contrasted with similar studies being performed internationally. These interpretations include realistic occupational exposures to forms of CNT commonly found in industry and examination of trends seen in exposure by type of industry (electronics, composites, plastics, producers). In addition, specific metrics of exposure, such as the mass of elemental carbon at both the respirable and inhalable size fractions as well as air, dermal, and sputum samples analyzed by microscopy, will be discussed.

W 2604 **Pulmonary and Systemic Immunotoxicity following Inhalation of Multiwalled Carbon Nanotubes**

V. J. Johnson, *Burleson Research Technologies, Inc., Morrisville, NC.*

Animal models have been used to investigate the toxicity of inhalation exposure to multiwalled carbon nanotubes. Recent studies have demonstrated alterations in inflammatory and fibrotic responses in the lungs of rats and mice. Importantly, studies have shown that pulmonary exposure to MWCNT can

alter systemic immune responses. Therefore, inhalation exposure to MWCNT has the potential to increase susceptibility to immune and inflammatory diseases both in the lung and in distal target tissues/organs. The purpose of the present work was to investigate the immunotoxicity of occupationally relevant exposures to MWCNT using a comprehensive approach of examining effects on innate, humoral, and cell mediated immunity. Three models were used to investigate pulmonary exposure to 0, 0.06, 0.2, and 0.6 mg/m³ MWCNT; 1) 30-day inhalation exposure in female SD rats, 2) 30-day inhalation exposure in female B6C3F1/N mice, and 3) 90-day inhalation exposure in female B6C3F1/N mice. Assessment of innate immunity included pulmonary mononuclear cell phagocytosis and natural killer cell activity. Humoral immunity was determined through quantification of antibody responses following immunization with sheep red blood cells (SRBC) and keyhole limpet hemocyanin (KLH). Cell mediated immunity was assessed by measuring T-cell proliferation and cytotoxic T-cell activity in response to influenza infection. MWCNT exposure increased the number of mononuclear cells in the lung with a corresponding increase in phagocytosis of bacteria that was significant in MWCNT exposed mice. Systemically, there was a decrease in the humoral immune response to SRBC immunization in both species. Minimal effects were observed on the other immune parameters investigated. These data support that pulmonary exposure to occupationally relevant levels of MWCNT can influence local and systemic immune responses.

W 2605 **Consequences of Inhalation Pre-Exposure to Multiwalled Carbon Nanotubes on Airway Inflammation and Fibrosis Induced by House Dust Mite Allergen**

J. Bonner, *North Carolina State University, Raleigh, NC.*

Inhalation exposure to MWCNT has been shown to exacerbate lung inflammation in rodent models of pre-existing allergic lung disease. However, to date no studies have addressed the effect of allergen post-exposure after inhalation of occupationally relevant doses of MWCNT. Male B6C3F1/N mice were exposed by whole body inhalation for 6 hours a day, 5 days a week, for 4 weeks to 0.06, 0.2 and 0.6 mg/m³ of MWCNTs. Mice then received 25 µg of HDM by intranasal aspiration 6 times over 3 weeks. Mice were necropsied at 3 and 30 days after the final HDM dose to evaluate changes in cell differential counts and cytokines in bronchoalveolar lavage fluid (BALF), IgE in serum and lung pathology. MWCNT exposure reduced TH2 immune responses 3 days after HDM post-exposure as evidenced by reduced eosinophils and IL-13 in BALF, reduced airway mucous cell metaplasia and reduced serum IgE. However, inflammatory lesions around small airway and vessels were only observed 3 days after HDM allergen in mice exposed to MWCNTs. Moreover, pro-fibrotic cytokines (IL-1β and TGF-β1) and airway fibrosis were increased at 30 days by MWCNT exposure in a dose-dependent manner with or without HDM allergen post-exposure. In conclusion, inhaled MWCNTs reduced TH2 pulmonary immune responses to allergen in mice but caused pro-fibrotic events regardless of allergen post-exposure.

W 2606 **Characterization of Inflammatory Responses and Redistribution of MWCNT following Aerosol Exposure in B6C3F1/N Mice**

A. Holian, *University of Montana, Missoula, MT.*

Little is known regarding systemic effects of inhaled MWCNT. Therefore, we examined lung and systemic effects in B6C3F1/N mice that were exposed to a relatively pure MWCNT (L-MWCNT-1020, Sun Innovation) by inhalation at exposure concentrations of 0.06, 0.2, 0.6 mg/m³ with humidified air. B6C3F1/N mice, housed in Hazelton 2000 exposure chambers, were exposed for a total of 22 days over a period of one month. The mice (10 mice per group along with a filtered air exposed group) were received 2 weeks post exposure and tissues collected within 24 hours of arrival. Cytokines in lung lavage fluid and plasma were analyzed using Meso Scale Discovery. Laser Scanning microscopy, Stimulated Raman Scattering and CytoViva (Spectral Feature Fitting) was used to analyze MWCNT in macrophages and distribution in tissues. Flow Cytometry was used to examine changes in various immune cells in the lung and spleen. We observed dose-dependent increase in IL-1β, TNF-α, and IL-6 in lavage fluid. In contrast, we noted dose dependent decreases in TNF-α, IL-6, IL-10 and IL-33 in plasma. AM content of MWCNT increased dose-dependently. Stimulated Raman scattering and CytoViva techniques demonstrated that MWCNT redistributed from the lung through the lymph nodes to the spleen where there were changes in lymphoid cell populations.



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