mice were anesthetized and lungs and systemic tissues were preserved by whole body vascular perfusion of paraformaldehyde while inflated with air. A separate, clean-air control group was studied. Sirius Red stained sections from lung, diaphragm, chest wall, heart, kidney and liver were analyzed. Enhanced darkfield microscopy and morphometric methods were used to detect and count MWCNT in tissue sections. Counts in tissue sections were expressed as number of MWCNT per cm^2 of tissue (mean±SE, N=8 mice per group). Although agglomerates account for approximately 60% of lung burden, only singlet MWCNT were observed in diaphragm, chest wall and systemic tissues. At one day post exposure, the average length of singlet MWCNT in diaphragm was comparable to that of singlet MWCNT in the lungs 5.6 ± 0.6 versus 5.1 ± 0.6 um, respectively. There were 26 ± 13 and 134 ± 25 per cm^2 in tissue sections of diaphragm at 1 day and 48 weeks post exposure, respectively. On average, there were 18 ± 5 and 50 ± 20 per cm^2 singlet MWCNT observed in systemic organ tissue sections at 1 day and 48 weeks, respectively. The burden of singlet MWCNT in parietal pleura, respiratory musculature and systemic organs at 48 weeks post exposure was significantly higher than at 1 day post exposure. Results demonstrate that inhaled MWCNT, which deposit in the lungs, are transported to the parietal pleura, the respiratory musculature and the systemic organs in a singlet form and accumulate with time following exposure.

PS 453 Genotoxicity of Long, Tangled Carbon Nanotubes in Mice.

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Long, needle-like multiwalled carbon nanotubes (MWCNTs) have been described to induce inflammation, genotoxic effects and mesothelioma in the respiratory system of mice, but the mechanisms behind these adverse effects are not well understood. The stiffness of the CNTs has been suggested to play a crucial role in their clearance from the lungs, affecting their toxicity. We have earlier observed that long, needle-like MWCNTs increase DNA damage in murine lungs. To find out whether the shape of the CNTs could affect their genotoxical properties, we examined here whether also long, but tangled MWCNTs (outer diameter 8-15 nm; Cheaptubes Inc), administrated either by pharyngeal aspiration or inhalation, could be genotoxic in C57BL/6J mice locally in the lungs or systematically in peripheral leukocytes and bone marrow erythrocytes. Cell samples were collected 24-h after a single pharyngeal aspiration (0.02-4 mg/ml) or a 4-day inhalation exposure (4 h/day; 17.5 mg/m3) to the MWCNTs. DNA damage was assessed by the comet assay in bronchoalveolar lavage (BAL) cells and lung cells. DNA double strand breaks were assessed by the γ-H2AX assay in peripheral leukocytes and (after pharyngeal aspiration) in lung cells. Micronuclei, a biomarker of chromosome damage, were analyzed in bone marrow polychromatic erythrocytes sampled 24 h after the end of the inhalation exposure. No significant dose-dependent increase in DNA damage (comet assay) was seen in the BAL or lung cells of mice treated by pharyngeal aspiration or by inhalation exposure. The long, tangled MWCNTs neither induced systemic genotoxic effects in peripheral leukocytes or bone marrow. Our findings suggest that the stiffness of long MWCNTs is a central characteristic with respect to their genotoxicity in vivo, with thinner and flexible tangled MWCNTs, which tend to form agglomerates, showing no genotoxic effects. (Funded by the Finnish Work Environment Fund)

PS 454 High-Fat Diet Leads to Increased Lung Inflammation and Airway Resistance following Multiwalled Carbon Nanotubes Exposure.

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Obesity has become a worldwide epidemic responsible in large part for the rising costs of health care. Obesity leads to systemic low-grade inflammation increasing risk for the development of diseases such as diabetes, but the link for respiratory disease is less clear. We investigated the effect of a high fat diet on lung inflammation and lung physiology when exposed to multi-walled carbon nanotubes (MWCNT). Nanomaterials, including MWCNT, are used in an increasing number of consumer products. Given their small dimensions with large surface area and often very unique properties with high deposition efficiency they can induce significant immune responses in the lung. In this study, C57Bl/6 mice were kept on a high fat diet for 6 weeks and then exposed to MWCNT, via oropharyngeal instillation. Measurements were taken 24 hr later to determine changes in inflammation and respiratory physiology, specifically lung resistance. Mice given particle on the high fat diet had significantly increased levels of IL-1β, a pro-inflammatory cytokine produced by the inflammatory complex the inflammasome, as well as increased lung resistance compared to mice on the control diet given particle. In order to further investigate inflammatory changes due to the high fat diet additional studies examined the influx of inflammatory cells in response to MWCNT exposure. Mice on the high fat diet exposed to MWCNT had a greater influx of neutrophils and eosinophils compared to control diet mice exposed to particle. These results indicate that a high fat diet leads to an increase inflammatory response with measurable physiological alterations in the lungs when exposed to MWCNT. This work was supported by NIH grants RC2 ES018742 and P20 RR017670.

PS 455 Multiwalled Carbon Nanotubes Cause Mild Inflammation in the Aorta without Pulmonary Toxicity in a Rapidly Aging Mouse Model.

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Exposure to ambient particulates has been shown to cause co-morbidity in elderly. Brain and muscle ARNT-like protein-1 (Bmal1) clock gene–deficient mice, with an accelerated aging and prothrombotic phenotype, were used to study the pulmonary and cardiovascular toxicity of multiwalled carbon nanotubes (CNTs). At the age of 8 weeks, wildtype and knockout Bmal1 mice were oropharyngeally aspirated once weekly during 5 consecutive weeks with 6.4 μg (32 μg in total), 25.6 μg (128 μg in total) of CNTs or the vehicle as control.
Cell counts in the bronchoalveolar lavage fluid indicated no inflammatory response 24 hours or 2 months after the last aspiration despite the presence of particle-laden macrophages. Cytokine measurements in lung homogenates showed trends for IL-1β, IL-6 and KC increases only in the wildtype mice aspirated with 128 μg CNTs but this response disappeared after 2 months.
In wildtype mice, aspiration of 128 μg CNTs caused a non-significant decrease in platelet and red blood cell counts, no significant differences for the aPTT and PT clotting tests were found and clotting factor FVIII was (non-significantly) decreased 24 hours after the last aspiration and increased 2 months later. In the BMAL1 knockout mice, FVIII was increased after 24 hours but decreased after 2 months.
A macrophage staining (MAC-3) on sections of the aorta showed endothelial activation and vascular inflammation in 60% of the 128 μg dosed knockout animals. There were no changes observed in the aortas of the wildtype mice.
In this study we showed that multiple dosing (5 weekly doses) of CNTs induced a mild vascular inflammation in the high dosed Bmal1 knockout mice in the absence of pulmonary toxicity.
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PS 456 Investigation of the Pulmonary Bioactivity of Double-Walled Carbon Nanotubes.

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Nanotechnology is one of the world's most promising new technologies. In turn, carbon nanotube production is estimated to reach into the millions of tons within the decade. Our laboratory has previously established that exposure to multi-walled carbon nanotubes (MWCNT) causes lung inflammation and fibrosis in mice after pharyngeal exposure. However, the bioactivity of double-walled carbon nanotubes (DWCNT) has not been determined. In this study we explored the hypothesis that DWCNT would promote pulmonary toxicity by analyzing the pulmonary bioactivity of the DWCNT. To test this hypothesis, male mice (C57BL/6J) were given a single dose of one of the following by pharyngeal aspiration: 1) 0.9% saline with 0.3% (w/v) carboxymethyl cellulose (CMC; vehicle control), or 2) DWCNT (0-40 μg/mouse) suspended in vehicle [0.9% saline with 0.3% (w/v) CMC]. Whole lung lavage (WLL) was conducted at 1 and 7 days post-exposure. Lungs of non-lavaged animals were also collected and processed for histopathologic analysis at 7 and 56 days post-exposure. The results show the DWCNT exposure caused a dose-dependent increase in WLL polymorphonuclear leukocytes, indicating that DWCNT exposure initiates pulmonary inflammation. DWCNT exposure also caused a dose-dependent increase in LDH activity as well as albumin levels in WLL fluid, indicating that DWCNT exposure promotes cytotoxicity as well as decreases in the integrity of the blood-gas barrier in the lung. Also, at 56 days post-exposure, the presence of fibrosis was noted in the highest dose exposure group (40 μg/mouse). In conclusion, this study provides insight into the previously uninvestigated pulmonary bioactivity of DWCNT exposure. The results confirm that DWCNT exposure does promote inflammation and fibrosis in the lung. The results also indicate that DWCNT have a similar pulmonary bioactivity as the previously studied MWCNT.

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