PS 457 Multiwalled Carbon Nanotube-Induced Lung Tumors.

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Carbon nanotubes have many promising applications. Although the low density and small size of carbon nanotubes makes respiratory exposures to workers likely during the production or use of commercial products, there is limited data on carcinogenicity of inhaled multi-walled carbon nanotubes (MWCNTs). We have therefore utilized a two stage initiation/promotion protocol to determine whether inhaled MWCNTs act as a complete carcinogen and/or promote the growth of cells with existing DNA damage. Six week old, male, B6C3F1 mice received a single dose of either methylcholanthrene (MC, 10 μg/g BW, i.p.) or vehicle (corn oil). One week after i.p. injections, mice were exposed by inhalation to MWCNTs (5 mg/m3, 5 hours/day, 5 days/week) or filtered air (controls) for a total of 15 days. The B6C3F1 mouse used in this study has intermediate susceptibility to lung carcinogenesis, and data obtained will have relevancy to existing human lung tumor data because lung tumors in this mouse strain exhibit many molecular and morphological similarities to human pulmonary tumors. At 17 months post-exposure, mice were euthanized and examined for lung tumor formation. Twenty percent of the filtered air controls, 33% of the MWCNT-exposed, and 50% of the MC followed by air-exposure, had a mean of one tumor per mouse. By contrast, 100% of the mice which received MC followed by MWCNTs had tumors with an average of 3.6 tumors per mouse. Additionally, mice exposed to MWCNTs or MC followed by MWCNTs had larger tumor volumes than their corresponding air-exposed control groups. Our preliminary data suggests that MWCNT exposure promotes the growth of spontaneously and chemically initiated lung cells, resulting in the development of lung tumors. In this study, mouse MWCNT lung burden approximates feasible human occupational exposures. Therefore, the results of this ongoing study indicate that caution should be used to limit human exposures to MWCNTs.

PS 458 Toxicological Evaluation of Pulmonary Exposure to Graphenes of Different Sizes.

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Research on the uses and manufacturing of nano graphene has increased dramatically in the past decade. Thus, worker inhalation of graphene nanopowders is likely. The goal of this study was to evaluate the lung toxicity of three non-oxidized graphene (Gr) samples of different sizes [20 μm lateral x 7-10 nm thick (Gr20), 5 μm lateral x 7-10 nm thick (Gr5), and <2 μm lateral x 1-2 nm thick (Gr1)]. Gr samples were diluted in physiological dispersion medium (DM) and characterized for size, surface reactivity, and free radical generation *in vitro*. Male C57BL/6J mice received 4 or 40 μg of Gr1, Gr5, or Gr20, or 40 μg of carbon black (CB; particle control), or DM (vehicle control) by aspiration. Mice were sacrificed at 4 hr (day 0), 1, 7, and 28 days post-exposure. Lung lavage was performed, the fluid and cells were retained, and indices of lung injury and inflammation were examined. Particle/aggregate size ranged from ~ 5-300 μm, 0.5-60 μm, and 0.2-5μm for Gr20, Gr5, and Gr1, respectively, with CB being similar to Gr1. Electron spin resonance (ESR) indicated that all Gr samples and CB had low to no surface reactivity as compared to a positive control (α-quartz). *In vitro*, ESR showed all Gr samples induced free radical production by mouse monocytes with significantly greater response in Gr20- and Gr5-treated cells compared to Gr1- and CB-treated cells. Indices of lung injury in lavage fluid were increased for the 40 μg doses of Gr20 and Gr5 on days 0, 1, and 7 when compared to control. Gr1 (40 μg) produced an increase only at day 7. Increased lung injury in the CB group was comparable to Gr20 and Gr5 on days 1 and 7. Injury decreased in all groups by day 28. Inflammation was elevated in the 40 μg Gr20, Gr5, Gr1 and CB groups on day 1, but only in the 40 μg Gr20 and Gr5 groups on days 0 and 7. In summary, the larger Gr particles appeared to produce more toxicity at the early time points post-exposure when compared to controls.

PS 459 A 28-Days Repeated Dose of Multiwalled Carbon Nanotubes (MWCNTs) in Sprague-Dawley Rats.

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There is a lack of available information on the human health and environmental hazards of MWCNTs. For this reason, the current study investigated the inhalation toxicity potential of MWCNTs. Eight-week-old rats were divided into 4 groups (10 rats in each group), including the fresh air control (0 mg/m^3), low-concentration group (0.2 mg/m^3), middle concentration group (0.5 mg/m^3), and high-concentration group (1.0 mg/m^3), and exposed to MWCNTs for 5 days (6 hrs/day) in nose-only inhalation exposure system. Then the rats were allowed to recover for 1 and 3 months by ceasing the exposure At the end of the study, the rats were subjected to a full necropsy. Cellular differential counts and inflammatory measurements, such as albumin, lactate dehydrogenase (LDH), total protein, and cytokines were also monitored in the a cellular bronchoalveolar lavage (BAL) fluid of the rats exposed to the MWCNTS for 28 days. Histopathological, hematological and clinical chemistry examinations indicated that there were no significant findings related to MWCNT exposure after 28 days of MWCNT inhalation exposure.

PS 460 Toxic Effects of MWCNT *In Vivo* and *In Vitro*.

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Multiwall carbon nanotubes (MWCNT) are discussed to exhibit a toxic potential depending on their length and fiber-like shape. For this reason, potential adverse biological effects in vivo (rat) and in vitro (human peritoneal mesothelial LP9/TERT-1 cells) of MWCNT are investigated in a project funded by the German BMBF (contract No. 03X0109A). In this project MWCNT data are compared with long amosite asbestos as a positive control and more particle-like MWCNT (Baytubes®, milled MWCNT, and Printex 90) as negative controls. For this study costum made MWCNT with different length and diameter were produced. To investigate the carcinogenic potential of these MWCNT, they were suspended in artificial lung-like medium using a sonotrode. The separated MWCNT were applied to the rats by intraperitoneal injection. In addition to the carcinogenicity study, the proliferation of cells in the diaphragm was investigated as a short time screening test after 3 month, using a BrdU method. To determine cytotoxicity in vitro LP9/TERT-1 cells were incubated for 24h with the same MWCNT, suspended in culture medium, and the toxic potential was estimated by cell counting and subsequent calculation of the relative increase in cell count (RICC). Suspension, size, and distribution of MWCNT were always monitored by SEM. CNT3 (length: 8.57 μm; diameter: 0,085 μm) and long amosite (length: 13.95 μm; diameter: 0.39 μm) led to significant thickening of the diaphragm, as compared to the negative control. With CNT2 (length: 10.24 μm; diameter: 0.04) a high amount of BrdU positive cells were noted. In the in vitro study part both CNT1 (length: 7.91 μm; diameter: 0.037 μm), CNT2, CNT3 and long amosite asbestos mediated strong reduction in cell number, compared to the particle controls, indicating a marked cytotoxic potential. In conclusion, some MWCNT mediate enhanced proliferation in rat diaphragm which may result in mesothelioma development and certain MWCNT exhibit a cytotoxic potential in mesothelial cells in vitro.

PS 461 Carbon Nanotubes Enhance Metastatic Growth of Lung Carcinoma via Up-Regulation of Myeloid-Derived Suppressor Cells.

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Expanding applications of nanomaterials, particularly carbonaceous nanoparticles (CNP), in new technologies, consumer products and biomedicine, imply their increasing levels of manufacturing. There are numerous attempts to utilize nanoparticles for better delivery of drugs and nucleic acid-based therapeutics to disease sites in the lung, particularly to the lung epithelium. The inhalation of drug nano-for-

mulations propelled the development of new strategies in therapy of several human lung diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease, lung cancer, tuberculosis, etc. Safety and lack of adverse health effects remain the major pre-requisites for broader applications of these novel technologies. Toxicological assessments of nano-particles typically are performed on normal animals. Thus possible effects of CNP on tumor growth have not yet been considered. The immune system safeguards the host from infections and malignancies. Recognition and undesirable interactions of CNP with cells of the immune system may lead to immunomodulation, hence increasing the host's susceptibility to infections and cancer. Here, we show that single wall carbon nanotubes (SWCNT) promote metastatic establishment and growth of Lewis lung carcinoma in C57BL6/J mice. The effect was mediated by increased local and systemic accumulation of myeloid-derived suppressor cells (MDSC), as their depletion abrogated pro-tumor activity in vivo. These data are important for the design of novel theranostics platforms with modules capable of depleting or functionally suppressing MDSC to ensure effective immunosurveillance in the tumor microenvironment.

PS 462 IL-33 Modulates Chronic Airway Resistance Changes Induced by Multiwalled Carbon Nanotubes.

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As the field of nanotechnology rapidly grows, the potential health hazards for human exposure rise. We have previously demonstrated that oropharyngeal instillation of multi-walled carbon nanotubes (MWCNTs) in C57BL/6 mice leads to increases in total respiratory system resistance (R) and Newtonian resistance (Rn), which is a measure of central airway resistance. In this study, we hypothesized that IL-33, a critical immune system alarmin, modulates mechanisms of pulmonary toxicity following exposure to MWCNTs. We assessed lung histology and pulmonary function in C57BL/6 and IL-33$^{-/-}$ mice 30 days following oropharyngeal aspiration of MWCNTs. The total number of bronchoalveolar lavage cells and the recruitment of neutrophils was increased in C57BL/6 mice following MWCNT exposure. In contrast, IL-33$^{-/-}$ mice exposed to MWCNTs did not demonstrate alterations in bronchoalveolar lavage cell content. Furthermore, C57BL/6 mice displayed increased inflammation around the airways demonstrated by histopathology which was unseen in IL-33$^{-/-}$ mice. To determine if these histopathological changes impact airway resistance, MWCNT exposed C57BL/6 were challenged with cumulative doses of methacholine (Mch) between 1.5 mg/ml and 24 mg/ml. Aerosolized Mch increased R and Rn in a dose-dependent manner in all groups with MWCNT instilled C57BL/6 mice responding with significantly higher R and Rn compared to control C57BL/6 mice. Importantly, increases in R and Rn induced by MWCNT were dependent on IL-33, as there was no significant difference between MWCNT treated and control IL-33$^{-/-}$ mice. In conclusion, these results indicate IL-33 plays an important role in pulmonary toxicity induced by MWCNT by influencing airway resistance via an inducible inflammatory response. This work supported by NIH RO1 ES019311.

PS 463 Pulmonary Toxicity Assessment of Multiwalled Carbon Nanotubes after Single Intratracheal Instillation in a One-Year Bioassay of Rats.

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Well-dispersed multi-wall carbon nanotubes (MWCNTs) were instilled intratracheally at dosage of 1.0 or 2.6 mg/kg body weight to male Wistar rats. A negative (vehicle) control, 0.5 mg/mL Triton X-100 was administered in a similar manner. After instillation, the bronchoalveolar lavage fluid (BALF) was assessed for the inflammatory biomarkers, and the lung, liver, kidney, spleen, and cerebrum were examined histopathologically at 1-day, 3-day, 1-week, 4-week, 3-month, 6-month, and 12-month post-exposure. Transient pulmonary inflammatory responses were observed up to 3-month post-exposure. In the histopathological examination, 1.0 and 2.6 mg/kg of MWCNTs deposited in the lungs were phagocytosed by the alveolar macrophages and these macrophages were accumulated in the alveoli up to 12-month post-exposure. There was no evidence of chronic inflammation, such as angiogenesis or fibrosis which induced by MWCNT instillation. These results suggest that MWCNTs were being processed and cleared by alveolar macrophages.

PS 464 Thirteen-Week Inhalation Toxicity Study with a Multiwall Carbon Nanotube Test Material in Wistar Rats.

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A subchronic inhalation toxicity study of an inhaled vapor-grown multiwall carbon nanotube (MWCNT) test substance was conducted in male and female Wistar rats. The test sample was composed of > 99.5% carbon, containing limited (Fe) catalyst metals; BET surface area measurements of ~25 m2/g; and average lengths/diameters of 9 μm and 100 nm, respectively. Four groups of rats per sex were exposed nose-only, 6 h/day, for 5 days/week to aerosol concs. of 0, 0.013 (low), 0.055 (mid) or 0.53 (high) mg/m3 MWCNT (MMAD ranging from 0.85 – 1.64 μm) over a 91-day period and evaluated 1 day later. Toxicity evaluations included clinical and histopathology methods, and bronchoalveolar lavage fluid (BALF) analyses. Additional control and high exposure groups were evaluated at 3 months PE. Results demonstrated that MWCNT exposures produced no significant adverse extrapulmonary effects. Absolute and relative lung weights were increased in high exposure conc. vs. controls and to a lesser extent after the recovery period. The results of BALF studies demonstrated increased GGT, LDH and ALK PHOS levels vs. controls in mid/high exposure groups. In addition, increased numbers of BALF cells were recovered at 0.53 mg/m3 MWCNT. Principal histopathological findings consisted of granulomatous lesions in centriacinar regions of male/female rats exposed to 0.53 mg/m3, and in some females at 0.055 mg/m3. The lesion was characterized by aggregation of pulmonary macrophages and focal pulmonary hypertrophy/hyperplasia of lung epithelial cells. In the nasal cavities, an increase of eosinophilic inclusions in the respiratory/olfactory epithelium was noted at 0.53 mg/m3 which was followed by the olfactory epithelial injury in the recovery animals. Based on the findings in respiratory tract tissues (lungs and nasal cavities), the overall LOAEL was considered to be 0.055 mg/m3, and the corresponding NOAEL was determined to be 0.013 mg/m3 under the conditions of this study.

PS 465 Carbon Nanotube Dosimetry: From Workplace Exposure Assessment to Inhalation Toxicology.

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Relevant dosimetry for toxicology studies involving multi-walled carbon nanotubes (MWCNT) has not been well described due to a lack of detailed occupational exposure assessments. In response, exposure assessment findings from U.S.-based MWCNT manufacturers and users were extrapolated to results of an inhalation study in mice. Inhalable and respirable personal breathing zone (PBZ) samples from 9 facilities were collected for the mass concentration of elemental carbon. Upon analysis, 95% of the PBZ samples found exposure concentrations to be <10 μg/m^3 with an average inhalable concentration of 8.5 μg/m^3. At facilities where respirable and inhalable PBZ samples were collected, respirable samples were approximately 25% of the inhalable size fraction. Using 10 μg/m^3, standard worker ventilatory parameters, and assuming 11% alveolar deposition, alveolar deposition was calculated to be 10.56 μg/d. Extrapolation to mouse equivalence by surface area equals 5.2 ng/d. In complement, a 19 d inhalation exposure to MWCNT with daily alveolar depositions of 1250 ng (=240 d of human exposure at 10 μg/m^3), 125 ng (=24 d), and 12.5 ng (=2.4 d) was conducted. Mice were sacrificed at day 0, 3, 28, and 84 post-exposure. Pulmonary cytotoxicity (LDH activity) and polymorphonuclear cell (PMN) influx were evident at the high dose through day 84. For the middle dose, no PMN influx was evident and cytotoxicity was significant only at day 0. Lung inflammatory gene expression was increased at the high and middle dose. Alveolar macrophages harvested after exposure and stimulated with LPS showed enhanced cytokine release at the high dose and day 0 for the middle dose. No exposure effects were observed at the lowest dose. These results show a no effect dose lies somewhere in between the middle (=456 d at 10 μg/m^3) and low dose (=45.6 d). The findings stress the importance of exposure assessment when extrapolating results of animal MWCNT exposures to potential human outcomes.

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