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-

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