## PS 1270 INHALATION EXPOSURE STUDY OF TITANIUM DIOXIDE NANOPARTICLES ON BLEOMYCIN-INDUCED PULMONARY INFLAMMATION IN MICE.

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Titanium dioxide nanoparticle (nTiO2) is widely used in many industrial fields. It is known that may cause to pulmonary inflammation by inhalation. In this study, we examined the effects of inhaled nTiO2 on pulmonary inflammation in animal model. Mice were induced lung inflammation by intratracheal instillation (ITI) of bleomycin (1 mg/kg). 7 days after instillation, mice were exposed one time for 4 hours by inhalation of nTiO2 (0.12, 1.2, 12 mg/m3). The change of inflammation was evaluated by cytokine analysis, histopathology and immunochemistry in lung tissue. mRNA expression of IL-1β, MCP-1 and fibronectin was decrease in nTiO2 exposure groups. Histopathological changes of inflammation were inflammatory cell infiltration, bronchiole-alveolar formation and bronchiole-alveolar hyperplasia by bleomycin-treated. In nTiO2 exposure groups, these findings were dose-dependently decreased. Also, the decrease of IL-6 was observed in nTiO2 12 mg/m3 exposure group comparing to bleomycin-treated control group. These results suggest that nTiO2 may inhibit progress of inflammation by bleomycin.

## PS 1271 THE ROLE OF CCR5 IN INFLAMMATORY RESPONSES TRIGGERED BY SINGLE-WALLED CARBON NANOTUBES.

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Due to the development of new materials and technology, the pollutants in the environment are becoming more varied and complex over time. Recently, holding toxicologists' attention is the outbreak of unforeseen adverse health effects, as a result of rapid expansion in the application of nanoparticles. In our previous study using ICR mice, we suggested that a single intratracheal instillation of single-walled carbon nanotubes (SWCNTs) induced early lung fibrosis and subchronic tissue damage. In this study, to investigate the role of CCR5 in inflammatory responses by the inflow of SWCNTs, we compared BAL cell composition, cell cycles, cytokines, cell phenotypes, inflammatory response-related proteins, cell surface receptors and histopathology in CCR5 knockout (KO) mice and CCR5 wild-type (WT) mice. Results showed that the distribution of neutrophils in BAL fluid was significantly decreased in KO mice. The expression of apoptosis-related proteins including caspase-3, p53, phospho-p53, p21 and cleaved PARP, TGF beta l and mesothelin were markedly increased in KO mice compared with WT mice. Histopathological lesions were also more frequently noted in KO mice. Moreover, the secretion of IL-13 and IL-17 with IL-6 was significantly increased in KO mice compared to WT mice, whereas that of IL-12 significantly decreased in comparison to WT mice. The distribution of B cells and CD8+ T cells was predominant in the inflammatory responses in KO mice, whereas that of T cells and CD4+ T cells was predominant in the inflammatory responses in WT mice. Furthermore, the expression of CCR4 and CCR7 was significantly increased in KO mice. Based on these results, we suggest that the absence CCR5 delays the resolution of inflammatory responses triggered by SWCNTs inflowing into the lungs and shifts Th1-type inflammatory response in the normal state to Th2-type inflammatory response.

## PS 1272 EFFECTS OF CARBON-BASED NANOMATERIALS ON PRIMARY HUMAN IMMUNE-COMPETENT CELLS.

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Due to their novel electrical, optical, mechanical and chemical properties, carbon-based nanomaterials are currently of great interest for a variety of technological as well as biomedical applications. In this study, we investigated the interaction of three carbon-based nanomaterials with immune-competent cells namely graphene oxide (GO), a 2-D nanomaterial composed of layers of carbon atoms forming six-member rings; single-walled carbon nanotubes (SWCNT), a 1-D nanomaterial formed by the rolling of graphene sheets into hollow tubes, and 3-D hollow carbon spheres (HCS). The materials were first confirmed to be free from endotoxin contamination. We then compared the effect of these nanomaterials on cell viability by Trypan Blue exclusion using primary human monocyte-derived macrophages (HMDM). No significant loss of cell viability was seen in cells treated with SWCNTs or GO up to 100 μg/ml up to 48 h, while a dose-dependent cytotoxic effect was seen for HCS. Cellular uptake of the three nanomaterials was monitored by transmission electron microscopy. We also studied the production of the pro-inflammatory cytokines interleukin (IL)-1β and IL-1α, using LPS-primed HMDM. Dose- and time-dependent activation of IL-1β was noted for cells incubated with SWCNT and HCS, while GO only induce the secretion of IL-1β, but to a lesser degree, at the highest dose (100 μg/ml). This work reveals immunotoxicity and/or immunostimulatory effects of three carbon-based nanomaterials.
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## PS 1273 POLYAMIDOAMINE (PAMAM) DENDRIMERS INDUCE EPIGENETIC CHANGES IN A549 CELLS.

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Dendrimers are promising nanomaterials that have inspired a vast range of applications in biomedicine. Polyamidoamine (PAMAM) dendrimers are the most common class of dendrimers and toxicological studies of PAMAMs are mostly based on the analyses of acute cytotoxicity/loss of cell viability. However, more subtle effects occurring at lower doses that are potentially more relevant to human exposure also need to be addressed. Importantly, whether PAMAMs could induce epigenetic changes still remains to be investigated. In the present study we exposed human alveolar epithelial cells (A549) to 4th generation PAMAM dendrimers with hydroxyl (-OH) and amino (-NH2) terminating groups and measured cell viability, DNA damage, histone modifications, and global DNA methylation. Global changes in DNA methylation were observed using the Illumina Infinium methylation array. Interestingly, PAMAM dendrimers were found to regulate DNA methylation even at low doses (0.01 μM) that do not induce a loss of cell viability, as measured by the LDH release assay. Cells exposed to a higher concentration (1 μM) of PAMAM-OH displayed more hypomethylation of genes compared to cells exposed to the lower dose (0.01 μM). However, exposing cells to 1 μM of PAMAM-NH2 resulted in more hypermethylation of genes compared to the 0.01 μM dose. Hence, specific changes occur depending on the surface charge (cationic versus neutral) of PAMAMs. Furthermore, immunohistochemistry and western blotting indicated that PAMAMs may induce histone modifications. Finally, DNA damage was observed with the comet assay after exposure to 1 μM doses of both PAMAMs. This study suggests that PAMAMs cause DNA damage and may alter gene expression in cells through epigenetic mechanisms which could, in turn, lead to alterations in cell function.

## PS 1274 DOSE-METRICS FOR NANOPARTICLES IN *IN VITRO* TOXICITY TESTS.

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Traditionally, safe exposure limits to chemical substances are based on mass concentrations, for example, as a maximum tolerable daily intake of chemical substance Y in mg per kg body weight. For nanomaterials, characteristics other than chemical composition (e.g. size, shape) may also determine their toxic potential, implying that information on the administered mass of the nanomaterial consisting of chemical substance Y may not be a sufficient description of the dose. As a result, risk assessors are faced with the question of what dose description to use when setting exposure limits for nanomaterials. A simple dose-metric summarizing the material properties to a single number (i.e. administered total number of particles, total mass or total surface area) would be most pragmatic for risk assessment and regulatory purposes, since only one exposure limit would have to be derived for different nanomaterials consisting of chemical substance Y. However, it needs to be demonstrated that the use of such a simple dose-metric is appropriate.
We tested whether the dose of spherical silver (20, 80 and 113 nm) and silica (11, 34 and 248 nm) nanoparticles could be appropriately described by one of the simple dose-metrics administered total number of particles, mass or surface area, using a novel graphical method. Data from *in vitro* assays on markers of cytotoxicity, inflammation and inhibition of stem cell differentiation was used to obtain equi-response curves: continuous curves connecting concentrations of particles leading to the same response level in a test system. These curves were compared to those of

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