2738 Comparision of the Toxicological Effects of Multi-Walled Carbon Nanotubes and Nitrogen-Doped Multi-Walled Carbon Nanotubes on Rat Lung Function

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The toxicological effects of multi-walled carbon nanotubes (MWCNT) have been widely investigated. Outcomes can range from initiating pulmonary inflammation and fibrosis to cardiovascular and central nervous system effects in animal models. Previous studies in our laboratory have shown that inhalation of MWCNT (Mitsui-7) results in airway hyperreactivity to methacholine (MCh) in rats. Nitrogen-doped multi-walled carbon nanotubes (N_2-MWCNT) are MWCNT that have been functionalized to incorporate nitrogen. They are utilized in applications such as lithium batteries and as matrix fillers in composite materials. N_2-MWCNT are shorter and more brittle than their MWCNT counterparts, which may reduce toxicity. The purpose of this study was to determine if exposure to N_2-MWCNT results in alterations in lung function that are comparable to those observed after MWCNT exposure. Sprague-Dawley rats were administered MWCNT or N_2-MWCNT (25, 50, or 250 µg) in dispersion medium by intratracheal instillation. Lung resistance (R_L), dynamic compliance (C_{dyn}), and reactivity to MCh aerosol were examined at 1 and 7 days post-exposure. Animals exhibited increased reactivity to MCh at 1 day after treatment with 25 and 250 µg MWCNT. Additionally, baseline R_L was decreased at 7 days after treatment with 50 µg MWCNT without a corresponding change in reactivity to MCh. The only alteration observed in N_2-MWCNT exposed animals was a decrease in baseline R_L at 7 days after 250 µg N_2-MWCNT exposure, with no corresponding change in reactivity to MCh. These results suggest that N_2-MWCNT are less toxic than MWCNT with respect to lung function.

2739 Pulmonary Toxicity and Gene Expression Changes in Response to Multi-Walled Carbon Nanotube Exposure in Rats

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Carbon-based nanomaterials have significant commercial and industrial applications and, therefore, human exposure to them potentially resulting in adverse health effects should be anticipated. Understanding the molecular mechanisms underlying the toxicity induced by carbon nanotubes has merit in the prevention of the adverse health effects potentially resulting from their exposures. Currently, a rat toxicity model was employed to investigate the molecular mechanisms underlying the pulmonary toxicity induced by exposure to multi-walled carbon nanotubes (MWCNT). Rats were exposed, by whole-body inhalation exposure, to air or an aerosol containing MWCNT particles (5 mg/m^3, 6 hours/day, 3 days). Toxicity and global gene expression profiles were determined in the lungs of the rats, 16-hours following the last exposure. MWCNT particles, often associated with alveolar macrophages (AMs), were detected in the lungs of the exposed rats. Lung histological changes resulting from MWCNT exposure were mild and consisted mainly of interstitial accumulation of macrophages. Bronchoalveolar lavage (BAL) toxicity parameters such as lactate dehydrogenase activity, number of AMs and PMNs, oxidant production by phagocytes, and levels of multiple cytokines (IL-1β, IL-10, MCP-1, MIP-2, and TNF-α) were significantly altered in response to inhalation exposure to MWCNT in the rats. Global gene expression profiling by next generation sequencing identified a large number of significantly differentially expressed genes (fold change >1.5 and FDR p value <0.05) in the lungs of the MWCNT exposed rats, compared with the controls. Bioinformatics analysis of the gene expression data identified significant enrichment of several diseases/biological functions (for example, cancer, organismal injury and abnormalities, cell cycle, respiratory disease, cellular movement, and inflammatory response) and canonical pathways (for example, LXR/RXR activation, granulocyte and agranulocyte adhesion and diapedesis, complement system, acute phase response, and atherosclerosis signaling). Taken together, the data provided insights into the molecular mechanisms underlying the pulmonary toxicity induced by inhalation exposure of rats to MWCNT.

2740 Characterization of Pulmonary Toxicity following Graphene Inhalation

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Graphene is a 2D nanomaterial with unique physicochemical properties making it highly marketable for applications in numerous industries. Our previous studies have shown that toxicity varies depending on size and oxidative form of graphene following a single bolus-dose aspiration in mice, with exposure to reduced graphene oxide (rGO) resulting in the greatest degree of lung injury and inflammation. The goal of this study was to characterize pulmonary toxicity following subacute inhalation of rGO. C57BL/6J mice were exposed to rGO (2.41 µm MMAD, 2.69 GSD) by whole body inhalation at 5.0 mg/m^3 for 5 h/d for 19 d, 0.5 mg/m^3 for 5 h/d for 19 d, or 0.5 mg/m^3 for 0.5 h/d for 19 d to achieve depositions with a two order of magnitude range. Controls were exposed to filtered air. Lung burden of rGO, parameters of lung toxicity and inflammation, and histopathology was analyzed 0 d, 3 d, 1 m, and 3 m post-exposure. Lung burden at 0 d was 0.78, 6.47, and 34.4 µg per lung for the low, middle and high dose. Following high dose exposure, lactate dehydrogenase (LDH) in lung lavage fluid (LLF, lung injury) and total lavage cells (inflammation) were increased up to 1 m compared to control. The cellular increase was due primarily to macrophage influx. Neutrophils were also significantly increased; however, this cell population accounted for less than 1% of the total cells. Inflammation and injury resolved by 3 m. Inflammatory and tissue remodeling proteins in LLF followed a similar pattern. Pathologic analysis indicated increased macrophages in the lungs at 0 and 3 d scored as minimal, resolving over time. Particle burden in macrophages was also observed to decrease over time. Following middle dose exposure, total lavage cells increased at 3 d, due primarily to macrophage influx, and resolved by 1 mo. Significantly fewer inflammatory proteins were elevated in LLF following exposure to the middle dose compared to the high dose, with resolution by 1 m. There was no difference in lung cellularity following low dose exposure. No lung pathology or increased LDH in LLF were observed following middle or low dose exposures. Exposure to rGO led to an acute dose-dependent increase in lung injury and inflammation, which resolved over time, with 0.5 mg/m^3 causing a minimal degree of inflammation representing the low observable effect level.

2741 Developmental Toxicity Assessment of Four Different Preparations of Multi-Wall Carbon Nanotubes in Mice after Repeated Intratracheal Instillation

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Some studies have reported that maternal exposure to manufactured nanomaterials, including carbon nanotubes, may induce reproductive and developmental toxicity, such as teratogenicity. In order to initiate grouping and read across for filling data gaps in the developmental toxicity of carbon nanotubes via airway exposure, we conducted repeated intratracheal instillation studies of various preparations of multi-walled carbon nanotubes (MWCNTs) in pregnant mice. Four types of MWCNT dispersions (bulk, heat-treatment, single dispersion by Taquann method, and heat-treatment after single dispersion) were repeatedly administered to pregnant Crlj:CD1(ICR) mice on gestational days 6, 9, 12, and 15 at dosages of 4.0 mg/kg/day. Ten pregnant mice per group were dissected on gestational day 17, and then developmental toxicity was evaluated. The body weights of the heat-treatment MWCNTs exposed mice significantly decreased. In the other 3 groups, the body weights of MWCNTs exposed mice also decreased, although the changes were not statistically significant. Body weight of fetuses was significantly decreased in the bulk, the heat-treatment, and the heat-treatment after single dispersion MWCNT exposed group. External malformations of fetuses were observed in the bulk and the heat-treatment MWCNT exposed groups. No statistically significant difference was observed between the control group and all MWCNT exposed groups in the numbers of corpora lutea, number of implantations, and placental weights. These results suggested the teratogenic effects caused by the intratracheal exposure of MWCNTs are dependent on their dispersion methods. Further examinations are needed to clarify the mechanism of the developmental toxicity by MWCNTs.

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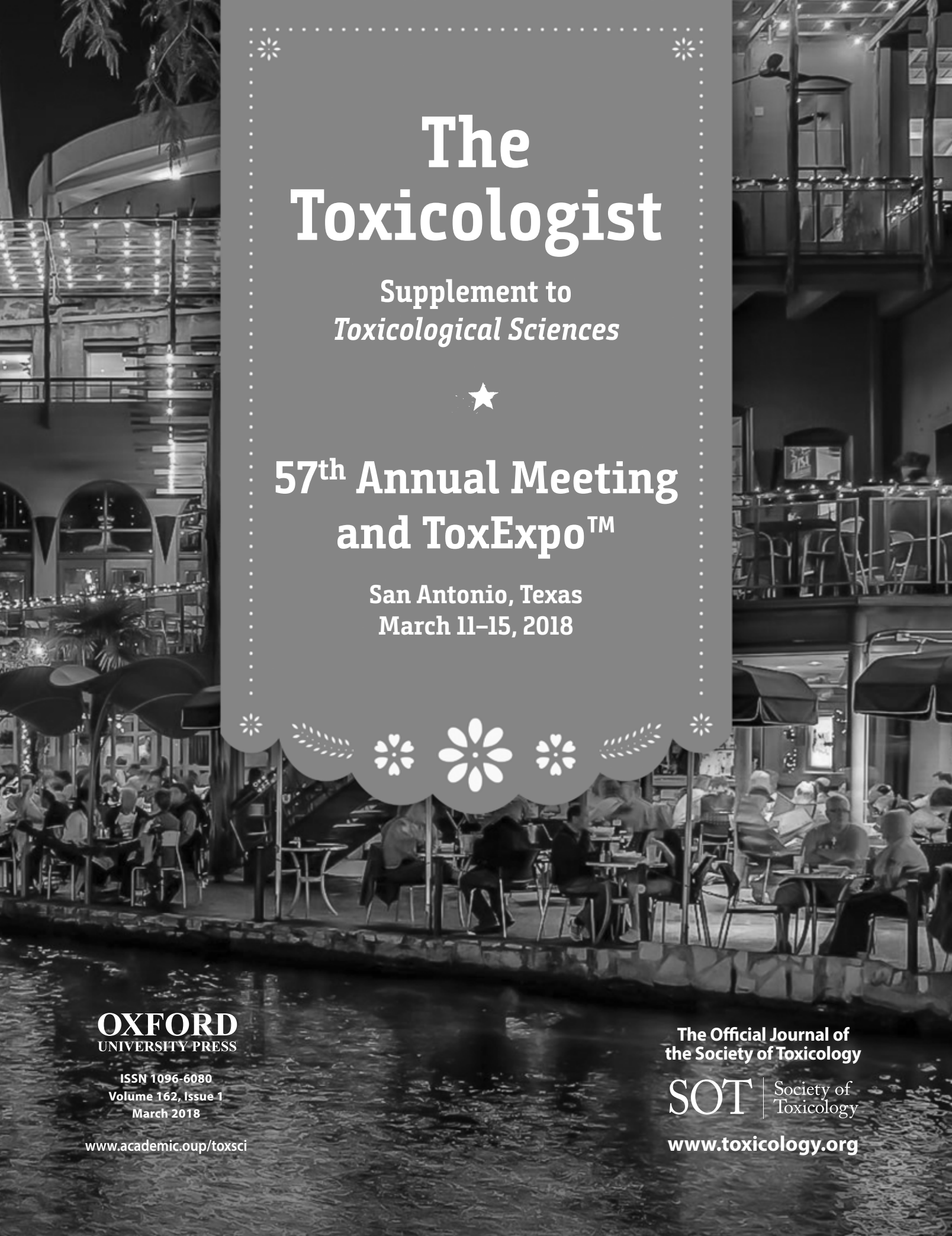

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