2688 Dose Response of Multiwalled Carbon Nanotube (MWCNT)-Induced Lung Tumors

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Mitsui-7 MWCNTs (MWCNTs) are strong lung tumor promoters in B6C3F1 mice. B6C3F1 mouse lung tumors have many molecular and morphological similarities to human pulmonary tumors. In previous work, we demonstrated that exposure to inhaled MWCNTs following exposure to a DNA damaging agent caused potent promotion of lung tumors. To investigate a possible threshold for MWCNT-induced carcinogenesis, we exposed B6C3F1 mice to 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 2, 5 or 10 days. At 17 months post-exposure, mice were euthanized and examined for lung tumor formation. Thirty six percent of the filtered air controls, 33% of the MWCNT- exposed, and 47% of the MC-exposed, had a mean of 0.33, 0.33 and 0.4 tumors per mouse, respectively. By contrast, 94% of mice receiving MC followed by 10 days MWCNT had an average of 2.9 tumors per mouse while 81% of mice exposed to MWCNTs for 5 days had an average of 1.9 tumors per mouse, and 73% of mice exposed to MWCNTs for 2 days had an average of 1.2 tumors per mouse. Additionally, mice exposed to MWCNTs or MC followed by MWCNTs had larger tumor volumes than their corresponding control groups. Preliminary data indicate a dose response in the percent of animals with tumors as well as the number of tumors per animal following exposure to MC and MWCNTs. 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.

2689 Label-Free Quantitation of Proteomic Responses Triggered by Rutile Titanium Dioxide Nanoparticles

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Proteomics can serve as a promising tool in establishing gene, protein or metabolite changes which indicate perturbations that are caused by a foreign body (compound or chemical) on the organisms. However, considerable information on protein profiling using proteomic approaches in earthworms in response to TiO_2 nanoparticles is scanty when compared to vertebrate sentinels. In light of this scenario, invertebrate based proteomic approach is followed in the present study to identify proteins that portray toxicological effects of rutile titanium dioxide nanoparticles (r-TiO_2-ENP). Earthworms were exposed to r-TiO_2-ENP via skin contact as per OECD-207 guidelines. Label free quantitation technique was employed to demonstrate specific signatures of the proteins in *Eisenia fetida* exposed to LC_{50} (0.15 mg/cm^2) and LC_{10} (0.05 mg/cm^2) of r-TiO_2-ENP via skin contact. Relative quantitation of the non-conflicting peptides was carried out to identify the proteins. Sequence coverage of the peptides and proteins was assessed through MASCOT analysis that matched 100% with databases against *Eisenia fetida*. Fold change in the proteins is presented in terms of average normalized abundancies. From the results, it was observed that 22 proteins that were identified through label free quantitation, met the requirements of 1 fold change (either decrease or increase) greater than 1 ($p<0.05$). It was observed that 16 proteins were upregulated, whereas 6 proteins were down regulated when compared to their respective controls. This study underlines the role of various proteins that affect stress-responsive system and behavior upon exposure to r-TiO_2-ENP in earthworms. This is true with regard to upregulation of (i) heat shock protein 70 (ii) beta-adrenergic receptor kinase 1-4 (iii) valosin containing protein-2 (iv) pyruvate carboxylase (v) HSP60 (vi) lombricine kinase (vii) cytochrome c oxidase subunit II and (viii) protein kinase C1 which indicate the significance of mitochondria when metabolism gets disturbed. Besides these proteins, this study also identified down regulation of metallothionein, valosin-containing protein, precursor protein EEP-2 and cyclophilin-A that assist in paraphrasing toxicity assessment of r-TiO_2-ENP. Outcomes of the study also assist in better understanding of the r-TiO_2-ENP toxicity and concomitant survival mechanisms in earthworms.

2690 Biochemical and Histopathological Evaluation of Graphene Oxide in Sprague-Dawley Rats

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Graphene Oxide (GO) due to its unique physico-chemical properties is a promising candidate for biomedical applications. However, the available reports on potential toxicity of GO are limited. The aim of the study was to determine the hepatotoxic and oxidative stress potential of GO in male Sprague-Dawleys rats. Five male rats per group were orally administered GO, once a day for 5 days with doses of 10, 20 and 40 mg/Kg GO respectively. Deionized water was used as a control. Twenty four hours after the last treatment blood and liver were collected following standard procedures. Exposure to GO was shown to enhance the induction of reactive oxygen species (ROS), activities of certain liver enzymes (alanine (ALT), aspartate (AST) aminotransferases, alkaline phosphatases (ALP), lipid hydro peroxide (LHP) concentration and damage to liver tissue compared to control. Statistically significant ($p<0.05$) increases in the above mentioned results were evident in the highest two doses 20 mg/Kg and 40 mg/Kg GO respectively. Aspartate aminotransferases (AST) activity showed no effect on GO exposure. Our results indicate that hepatotoxicity induced by GO might be mediated through the mechanism of oxidative stress. Although it is most likely that this impairment in hepatotoxicity biomarkers is associated with GO toxicity, further experiments are needed to elucidate the biochemical mechanisms involved.

2691 Adjuvant Effects of Redox-Modified Cerium Oxide Nanomaterials to Promote Airway Sensitization in BALB/c Mice

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Inhalation of ambient ultrafine particles and engineered nanomaterials are associated with adverse airway responses, including allergic asthma. Generation of reactive oxygen species by inhaled nanoparticles (NPs) may activate immunological adjuvant pathways and enhance local sensitization to environmental and occupational allergens. To test the hypothesis that NP redox status is associated with adjuvant activity, we modified the redox activity of cerium dioxide (CeO2) NPS by incorporating increasing quantities of zirconium (Zr) into the crystalline structure of the CeO_2 NPs. Female BALB/c mice were intranasally sensitized with ovalbumin (OVA) or OVA + 200 µg CeO_2 (doped with 0%, 27% or 78% Zr) on days 1, 3, 6, and 8, and then challenged with OVA alone on days 22 and 23. Twenty-four hours later serum was collected to assess OVA-specific IgE and IgG1, bronchoalveolar lavage fluid (BALF) collected for quantitation of inflammatory cells, and lungs processed for detection and morphometric evaluation of eosinophils and intraepithelial mucosubstances (IM). OVA-sensitized and -challenged mice had minimal increases in serum IgE or IgG1 compared to non-sensitized mice (PBS control). However, marked increases in IgE and IgG1 were observed after co-sensitization with CeO_2 (doped with 0%, 27% and 78% Zr) compared to OVA alone. OVA-sensitized and -challenged mice had increased BALF macrophages and eosinophils compared to controls. Sensitization with OVA + CeO_2 (0% Zr) enhanced BALF total cells (2.3-fold increase), macrophages (2.8-fold), eosinophils (15-fold), and lymphocytes (3.8-fold) compared to OVA alone. Doping of CeO_2 with 78%, but not 27% Zr further enhanced BALF total cells, macrophages, and eosinophils by 50-100% compared to CeO_2 without Zr doping. Allergic inflammatory, epithelial, and mucous cell responses were minimal in lung tissues of OVA-sensitized and challenged mice, but marked eosinophilic alveolitis and bronchiolitis, and a modest increase in IM were induced by co-sensitization with OVA and all zirconium-doped CeO_2 NPs. Mice sensitized with CeO_2 (78%Zr) had more parenchymal eosinophils than CeO_2 (0%Zr), but mucous and inflammatory cell responses were similar for all CeO_2 NPs. Our results suggest that CeO_2 can act as potent airway adjuvant for allergic sensitization, and that the redox activity of engineered NPs can affect the character and severity of allergic airway responses.

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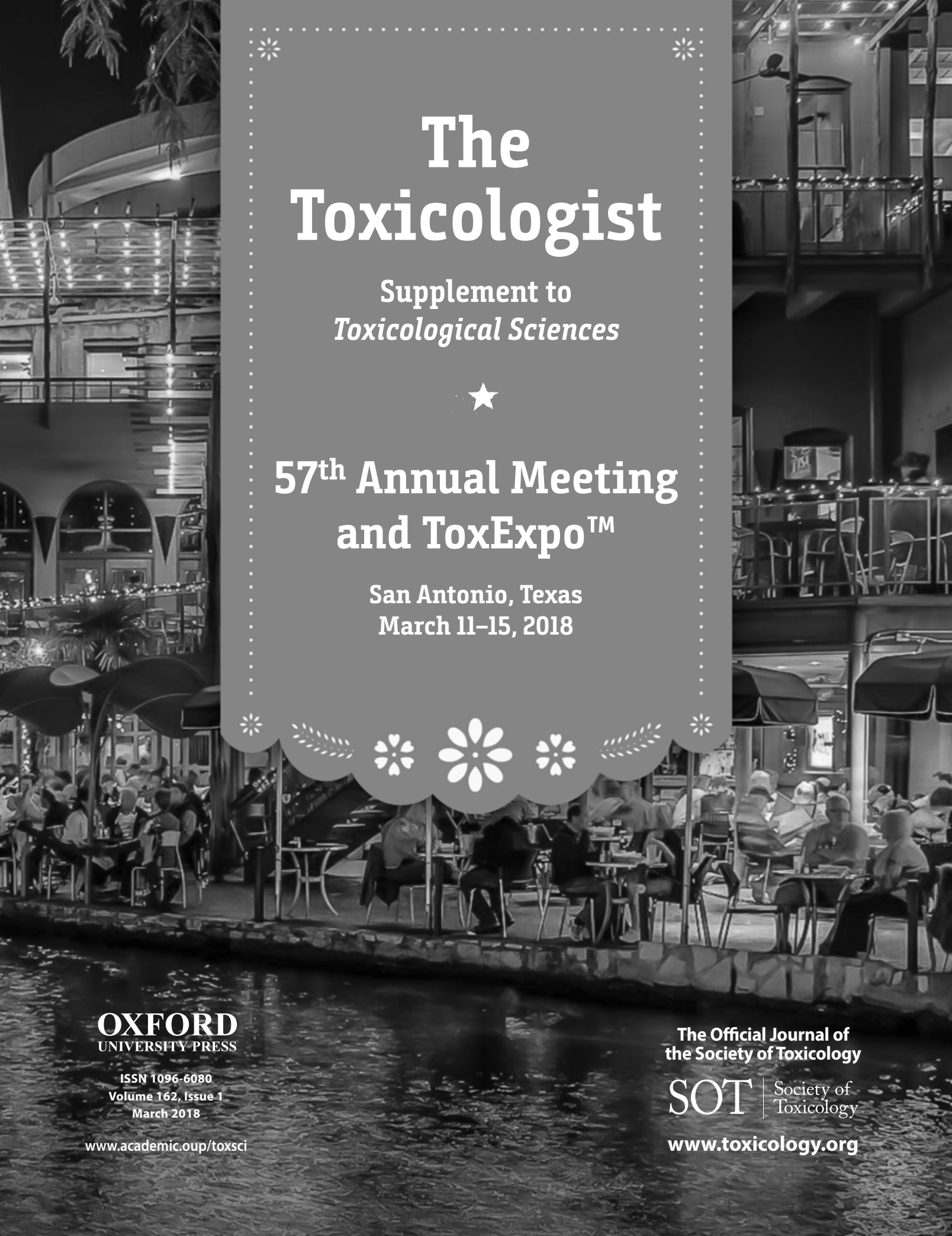

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