2667 Evaluating the Cytotoxicity of Tin Dioxide Nanofibers

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Tin dioxide nanofibers (SnDNFs) are small fibers with many applications in areas such as cosmetics, solar cells, toxic gas release sensors, and air pollution control. There have been few studies on the cytotoxicity of SnDNFs. The goal of this research was to evaluate the toxicity of electrospun SnDNFs in a lung cancer cell line (A549). Occupational exposure to SnDNFs, primarily through inhalation, has been linked to pulmonary disease, thus we used the A549 cell line, which has been used in both nanoparticle and pulmonary toxicity studies. Synthesized SnDNFs were characterized using scanning electron microscopy (SEM), Raman spectroscopy, and powder X-ray diffraction (PXRD). SEM images confirmed that the fibers were 200-300 nm in diameter, which is consistent with the fiber size used in industry. Raman spectroscopy and PXRD verified that the fibers were also in the rutile phase, a smaller and more stable crystalline structure. Following confirmation of the physical properties, A549 cells were treated with fiber concentrations ranging from 0.02-500 µg/mL. Cell proliferation was determined by comparing data from the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures Complex II activity in mitochondria, with general cell death, as measured by lactate dehydrogenase (LDH) release. Results from the MTT assay indicated an IC_{50} of 0.02 mg/mL, while the LDH assay showed that significant cell death did not occur until the fifth day of exposure ($^{*}p = 0.05$). Gene transcription analysis using qRT-PCR of genes linked to oxidative stress, inflammation, and apoptosis was conducted, and showed inflammatory genes to be differentially expressed compared to controls. Taken together, these results suggest that toxicity due to rutile SnDNF exposure in A549 cells line likely occurs via inflammation, but requires least a five-day exposure to these nanofibers *in vitro*.

2668 Iron Oxide Nanoparticle-Induced Changes in Epithelial-to-Mesenchymal Transition and Cellular Transformation May Be Ablated with Amorphous Silica Coating

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Iron oxide nanoparticles (IONP) are emerging as unique components of drug delivery systems, imaging techniques, environmental catalysts, components of thermoplastics, and more. Workers in IONP manufacturing facilities are known to be exposed to low doses of these particles over long periods of time. However, very few studies have assessed potential adverse outcomes following this type of occupationally relevant exposure. Our previous research suggests that IONPs may induce a neoplastic-like cellular transformation, likely due to particle dissolution, release of free iron ions, and disruption of iron homeostasis. Other studies suggest that an amorphous silica coating may reduce particle dissolution, thereby reducing subsequent adverse outcomes. We hypothesized that an amorphous silica coating (SiO_2-nFe_2O_3) would ablate iron homeostasis disruption induced by an uncoated but otherwise identical particle (nFe_2O_3), and would therefore reduce nFe_2O_3-induced subsequent neoplastic-like cellular transformation and oxidative stress. To test this hypothesis, we used an occupationally relevant low dose/long term exposure *in vitro* model system which utilized a normal human bronchial epithelial cell line (Beas2B). The cells were continuously treated at 0.6 µg/cm^2 to SiO_2-nFe_2O_3 or nFe_2O_3 for six months, and were evaluated for oxidative stress, epithelial to mesenchymal transition (EMT), and neoplastic-like cellular transformation throughout. Our results show an nFe_2O_3 induced time dependent cellular transformation, evaluated by colony formation assay, beginning at about 3 months post exposure. nFe_2O_3 transformed cells also possess a pro-inflammatory phenotype, including increased oxidative stress, EMT marker proteins, and changes in select inflammatory cytokines. These outcomes are not seen with SiO_2-nFe_2O_3 or non-treated control cells. Overall, our results show that nFe_2O_3-induced iron homeostasis disruption, oxidative stress, and cellular transformation may be significantly reduced with an amorphous silica coating. This may suggest this coating as being a useful component of safe by design hazard reduction strategy.

2669 Cytotoxicity Screening and Cytokine Profiling Using the Human Macrophage-Like THP-1 Cell Line Enables Hazard Ranking and Grouping of Engineered Nanomaterials and Nanocelluloses

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The increasing number of applications of engineered nanomaterials (ENMs) greatly increases the potential of direct human exposure to ENMs. However, the number of new ENMs presents a challenge in risk assessment. Grouping and ranking according to their potential hazard seems a promising approach. Here we tested a panel of nineteen ENMs procured from the Joint Research Centre (JRC) and commercial sources focusing on cytotoxicity and cytokine responses in the human macrophage-differentiated cell line THP-1. Macrophages are key players of the innate immune system. Physicochemical characterization of ENMs was performed using dynamic light scattering; moreover, all ENM samples were shown to be endotoxin-free prior to testing. Following cytotoxicity screening in THP-1 cells using the Alamar Blue cell viability assay and ranking on the basis of IC_{50} values, the multi-walled carbon nanotubes (MWCNTs), ZnO, Ag, and SiO_2 NMs were found to be the most cytotoxic while single-walled carbon nanotubes (SWCNTs), TiO_2, $BaSO_4$, and CeO_2 NMs, as well as the nanocellulose materials, were non-cytotoxic (at doses up to 100 µg/mL). Profiling of cytokine and chemokine secretion using a multi-plex assay indicated that the TiO_2, SiO_2, $BaSO_4$, CeO_2, and nanocellulose materials induced potent inflammatory responses at sub-cytotoxic doses. Hierarchical clustering of cytokine responses coupled with pathway analysis demonstrated that the panel of ENMs could be segregated into two distinct groups characterized by activation and deactivation, respectively, of PPAR (peroxisome proliferator-activated receptor)/LXR (liver X receptor/retinoid X receptor) nuclear receptor pathways. Both nuclear receptors are well-known for modulating inflammatory responses. Furthermore, using rosiglitazone, a selective PPAR-γ agonist, we could show that PPAR-γ played an important role in the activation of inflammatory responses in THP-1 cells exposed to TiO_2 and SiO_2 NMs. These studies have shown that ENMs of different chemical composition can be grouped according to their inflammatory potential when tested under *in vitro* conditions.

2670 Evaluation of Toxicity of Erbium Oxide Nanoparticles in Rat Pleural Mesothelial Cells

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Erbium oxide nanoparticles (Er_2O_3 NPs) are used in various products from simple clay bowls to amplifiers for lasers and fiber optics. Such particles are also used in fuel cells and other electrical components. The increased use of this compound has elevated the risk of occupational exposure. In the present study the toxicity of Er_2O_3 NPs was assessed using rat pleural mesothelial cells (RPMCs), CCL-216 (ATCC), cultured in HAM's F12 medium supplemented with FBS, L-glutamine and penicillin-streptomycin. Cultures were exposed to 50, 100, 200, 400, or 800 µg/ml concentrations of Er_2O_3 NPs for 24 hours. MTT and LDH assays were carried out to determine cell viability. TUNEL assay along with measuring caspase 3 levels were carried out to examine the possible mechanism of cell injury. Caspase 8 and 9 levels were determined to indicate a possible pathway for initiation of apoptosis. MTT assay indicated a significant ($p<0.05$) reduction of cells in cultures treated with 400 or 800 µg/ml of Er_2O_3 NPs, to 71% ± 4% or 57% ± 6% respectively of the control values. The LDH levels increased significantly ($p<0.0001$) by 410% ± 60% or 570% ± 70% for cultures exposed to 400 or 800 µg/ml Er_2O_3 NPs respectively as compared to the controls. All additional testing was carried out on cell cultures treated with 400 µg/ml of Er_2O_3 NPs. TUNEL assay of cultures treated with 400 µg/ml of Er_2O_3 NPs had a significant ($p<0.0001$) increase in the number of apoptotic cells, to 61.8 ± 2.6 cells/field when compared to controls at 14.6 ± 0.6 cells/field. Caspase 3 levels were increased significantly ($p<0.05$) by 250% ± 20% for the cultures treated with 400 µg/ml Er_2O_3 NPs when compared to the control cultures. The data also show a significant ($p<0.005$) increase of caspase 8 levels of 200% ± 2% for the treatment group 400 µg/ml as compared to the controls. Caspase 9 levels were also significantly ($p<0.05$) increased in cultures by 120% ± 8% for cells treated with 400 µg/ml of Er_2O_3 NPs when compared to the control. Scanning electron micrographs of cells treated with Er_2O_3 NPs had surface blebbing and adherent particles. Er_2O_3 NPs significantly reduced RPMCs' cell viability as: measured by MTT and LDH, activated the apoptotic pathway as demonstrated by elevations of caspase 3 levels and increased TUNEL positive cells, and may act through both intrinsic and extrinsic apoptotic pathway as indicated by increases in caspase 8 and 9 proteins.

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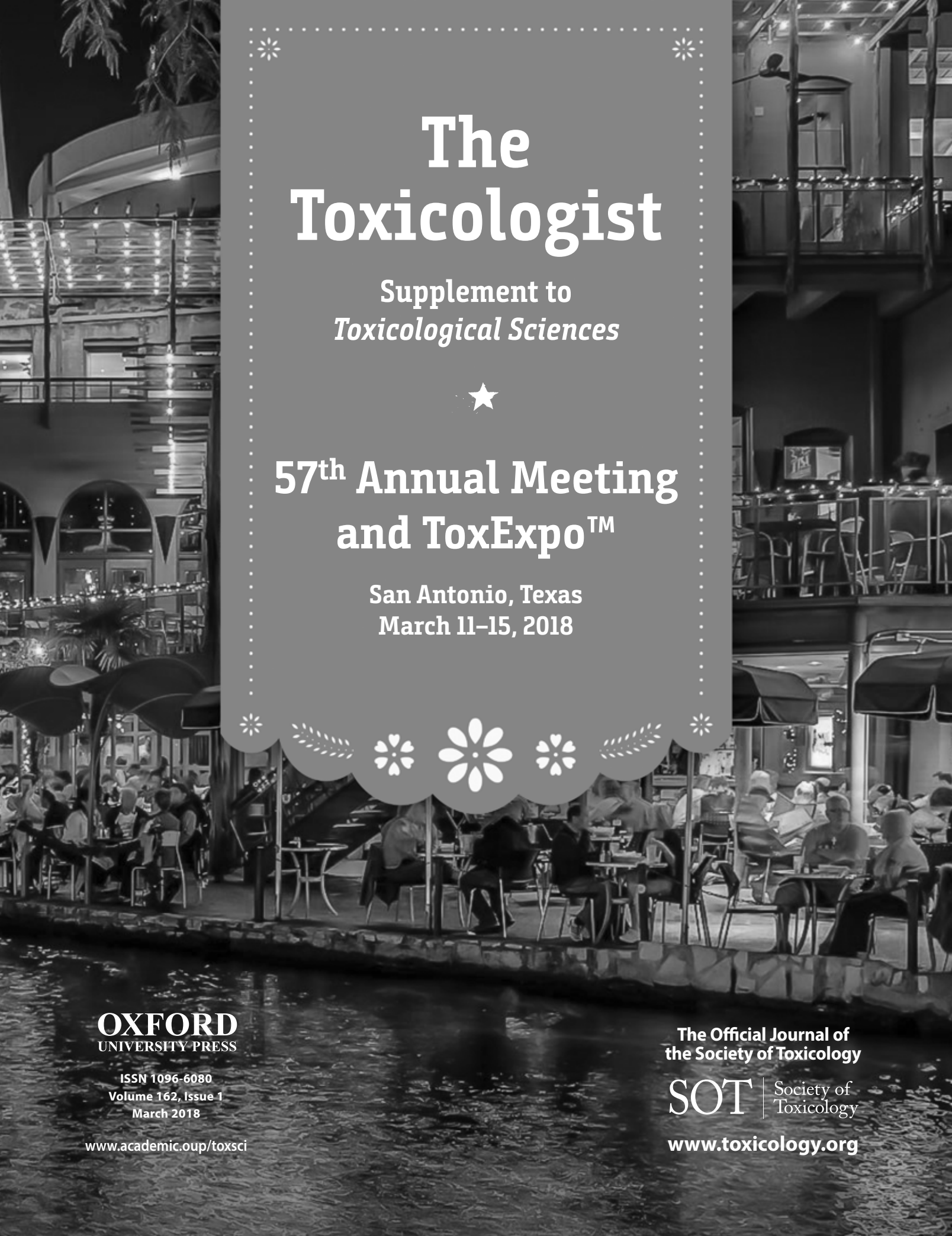

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