2684 Comparative Assessment of *In Vivo* Toxicity Induced by Multiwalled Carbon Nanotubes and Nanofibers from US Facilities

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Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F), known to induce inflammation, toxicity, or tumorigenesis, is a concern during production and dry powder handling. CNT/F represent a large class of materials and it is unclear if all confer similar toxicity. Our aim was to simultaneously test the pulmonary effects induced by CNT/F with variable physicochemical properties obtained from US facilities. Characterization was done for seven different multiwalled CNT and two CNF selected based on nominal diameter ranging from 10-150 nm. Cytotoxicity, inflammation, and histopathology was assessed in mice 1, 7, 28, and 84 d following oropharyngeal aspiration to 4 or 40 µg of each material. Utilized doses and material preparation for *in vivo* dosing were representative to ongoing occupational exposures. Lactate dehydrogenase (LDH) activity, a marker of cytotoxicity, was dose-dependently increased in bronchoalveolar lavage fluid (BALF) resolving toward baseline by 84 d in all groups. In materials with a diameter greater than or equal to 50 nm, LDH was persistently increased. Polymorphonuclear cell infiltration (%PMN), a marker of inflammation, was increased in all materials at 1 d post-exposure to 40 µg (<50 nm: 31.1%, ≥50 nm: 37.1%). With exposure to materials less than 50 nm, PMN influx mostly resolved by 7 d while materials greater than or equal to 50 nm induced persistent inflammation (7 d: <50 nm: 10.5%, ≥50 nm: 48.9%). For complement, inflammatory gene expression in lung tissue (e.g., *Il1b, Il6, Ccl22, Cxcl2*) and protein levels in BALF (e.g. Il1b, Il6, Il5, Ccl22, Cxcl1), were elevated to a greater extent in materials with a nominal tube diameter greater than or equal to 50 nm. In contrast, microscopic evaluation of lung sections at 84 d post-exposure indicated that histopathology does not appear distinguishable between CNT/F using diameter. In conclusion, general cytoxicity and inflammation exhibited a relationship with nominal diameter, with a threshold of sustained effects at approximately 50 nm and greater, that was dissimilar to histopathology. Ongoing research and modeling techniques will elucidate relationships between physicochemical characteristics and toxicities of various CNT/F.

2685 Valproic Acid and Sodium Butyrate Attenuates Manganese-Induced Repression of Tyrosine Hydroxylase Expression and Neurological Behavioral Deficits in Mice

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Chronic overexposure to manganese (Mn) induces neurological disorders with shared features of Parkinson's Disease (PD), referred to as manganism. Mn decreases astrocytic glutamate transporters GLT-1 and GLAST mRNA/protein levels in astrocytes and tyrosine hydroxylase, a dopaminergic neuronal marker in dopaminergic neurons. It has been shown that Mn enhanced interaction of transcription factor yin yang 1 (YY1) with epigenetic modifier histone deacetylases 1 (HDAC1) in repression of GLAST and GLT-1 promoter activities. Thus, the present study investigated whether HDAC inhibitors valproic acid (VPA) or sodium butyrate (NaB) attenuate Mn-induced decrease of tyrosine hydroxylase (TH) expression in the brain of C57BL/6 mice. One of the major target of Mn neurotoxicity is the nigrostriatal dopaminergic pathway including globus pallidus and substantia nigra. To determine this protective effects of HDAC inhibitors, C57BL/6 mice were administered with VPA (200 mg/kg, i.p.) or NaB (1200 mg/kg, i.p.) prior to intranasal instillation of Mn (30 mg/kg) daily for 21 days, followed by open-field and rota-rod behavioral tests and subsequent analyses of TH protein/mRNA levels in the several regions of the brain. The results showed that Mn significantly decreased locomotor activity as determined by total distance travelled and rota-rod activity reflecting altered motor coordination. Pretreatment with VPA and NaB reversed the effects of Mn on the locomotor activity and motor coordination and also attenuated the Mn-induced decrease in TH mRNA and protein levels in the striatum and substantia nigra. The immunohistochemistry experiments showed that VPA and NaB attenuated the Mn-induced decrease of TH in the substantia nigra pars compacta. These results suggest that VPA and NaB exert neuroprotective effects against Mn induced neurotoxicity in the nigrostriatal pathway.

2686 Size and Shape of Multiwalled Carbon Nanotubes Influence Epigenetic Changes and Lung Disease in a Mouse Model

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The diversity in physicochemical properties of engineered multiwalled carbon nanotubes (MWCNT) are a major human exposure concern. Studies indicate that mechanistically, epigenetic changes could be at least partially involved in MWCNT-induced pro-inflammatory and fibrotic lung pathology. However, the precise mechanisms have not yet been defined. Furthermore, toxicological studies have not yet addressed all variables of exposure. Therefore, this project aimed to identify possible epigenetic biomarkers of MWCNT exposure and disease progression and to characterize distinct epigenetic changes in response to MWCNT of varied size and shape relevant to lung disease. Adult 2-month C57BL/6 mice were exposed via oropharyngeal instillation to one dose (50 microgram) of either dispersion media only (DM), or to a different diameter and length MWCNT: "Wide Short" (WS), "Narrow Short" (NS), and "Narrow Long" (NL). Lung lavage fluid (LLF) and lung tissue (LT) were collected after 24 hours and 7 days exposure in order to examine pulmonary inflammatory responses. At 7 days post-exposure, LT airway thickening and MWCNT distribution within LT were measured using laser scanning cytometry and darkfield hyper-spectral imaging, respectively. From LT, global DNA and promoter methylation of inflammation and fibrosis-related genes were measured using the luminometric methylation assay (LUMA) and the pyrosequencing assay, respectively. HMGB1 was non-significantly elevated in all MWCNT groups, with significant increases in NS and NL after 24 hours in LLF. After 7 days, a hierarchy in airway thickening was observed: NS<WS<NL, with statistical significance in NL and borderline in NS & WS. Overall, different patterns of LT methylation were observed among the different MWCNT, however, the gene-specific methylation trends after 24 hours did not consistently appear to persist after 7 days. The observed methylation trends suggest that dynamic epigenetic changes and multi-gene mechanistic processes are occurring within the acute to sub-acute immune response to MWCNT exposure. These epigenetic changes are sensitive to the variables of MWCNT size and shape. The results suggest that methylation alterations might not correspond directly to MWCNT bioactivity.

2687 Investigations on Classification and Mechanisms of MWCNT-Induced Carcinogenicity

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Carbon nanotubes (CNT) are of great interest for toxicological research due to similarities to asbestos as shape, size, biopersistency, and toxic modes of action. It is widely accepted that fiber-specific characteristics like diameter or rigidity can affect their toxic potential. Larger diameters (> 35 nm), for instance, have been shown to contribute to tumor development. Although there are still uncertainties about the exact role of CNT thickness to carcinogenicity, a classification based on morphological characteristics could help to improve risk assessment and safe use of CNTs. This study therefore aims at providing data on the toxicity of multiwalled CNTs with narrow diameter ranges, in order to test whether a threshold for tumor development could be set for a specific fiber thickness. For this purpose, rats will be administered a single intraperitoneal injection of CNTs. Two different doses will be tested, 0.1 and 1.0 x 10^9 WHO-fibers, and five different types of CNT within and outside of the tumor-sensitive diameter or length range will be compared. Those include one single-walled CNT, one multiwalled CNT (MWCNT) with an average diameter of 10 nm and a length of > 5 µm, two customized MWCNT with an average diameter of 20 and 30 nm and > 5 µm in length, as well as one short MWCNT (max. 4-5 µm) with a diameter of > 40 nm. Amosite will be used as positive control. Approximately two years post-application animals will be histopathologically examined for tumors. Additionally, an interim group will be included for investigation of inflammatory reactions in the peritoneum three months after substance administration. Detailed characterization of the test materials will be presented, including determination of aspect ratios. Rigidities will be used to test whether differentiation between the five fiber types and observed responses can be achieved. The approach also aims at supporting the determination of guidelines for a standardized characterization procedure for CNTs. *The project is funded by the German Federal Institute for Occupational Safety and Health (F2376).*

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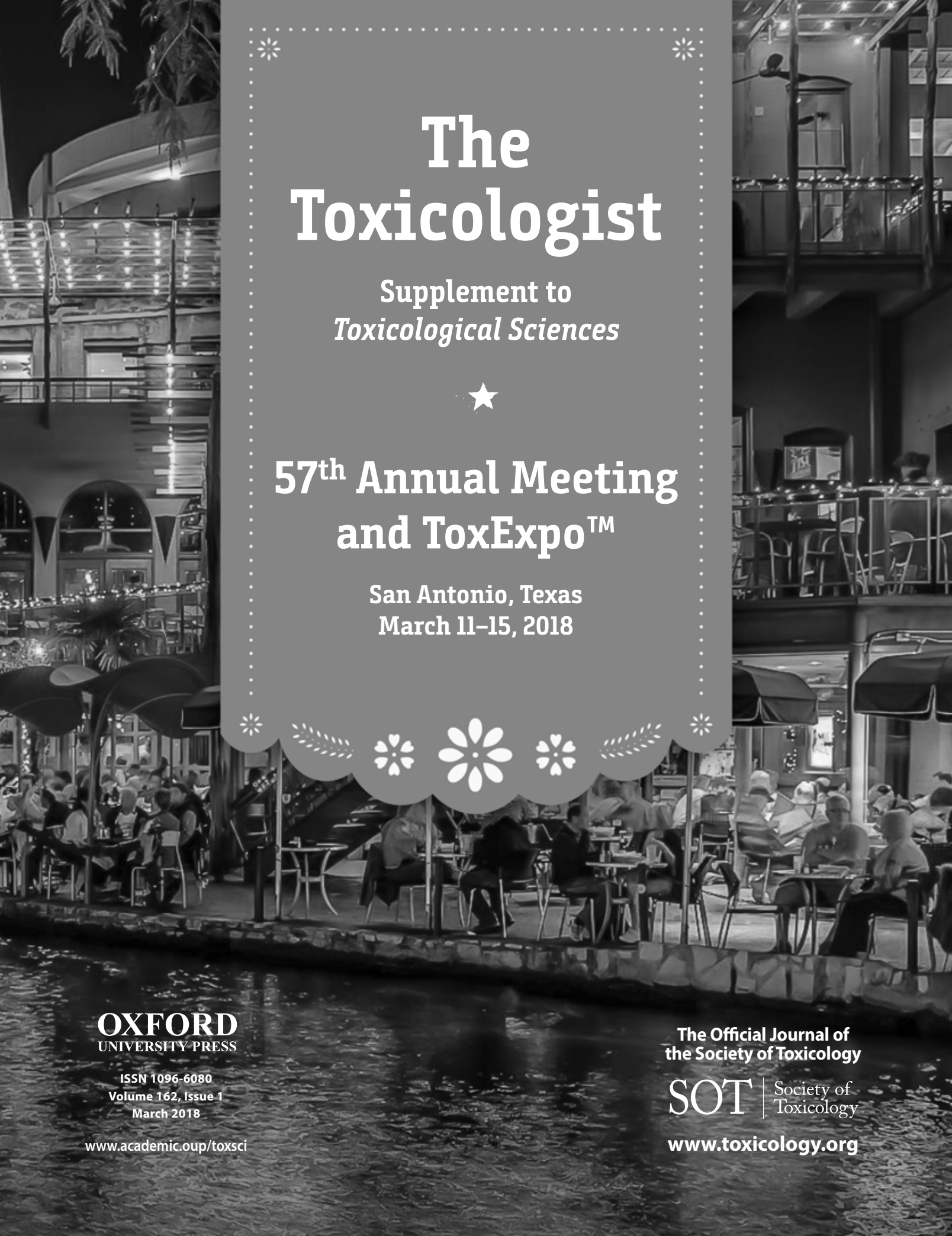

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