2901 A Life Cycle Approach to Engineered Nanomaterial Toxicity to Provide Context to Potential Health Effects

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Pulmonary toxicity studies primarily focus on as-produced engineered nanomaterials and rarely are guided by a life cycle perspective or integration with exposure assessment. Understanding toxicity beyond the as-produced (AP) material is critical, due to modifications needed to overcome barriers to commercialization of applications. We evaluated the toxicity of AP multi-walled carbon nanotubes (MWCNT: 16 nm diameter, 1-2 µm length), their polymer-coated (PC) counterparts (polyurethane or a proprietary poly(arylene ethynylene)), and the respirable aerosols generated from sanding (fine, P320) the PC nanotube-embedded or neat composites from two separate companies. Polymer coating minimizes the percent MWCNT incorporation into the composite, permits ease in handling, and can decrease dustiness thereby reducing potential inhalation exposures downstream. Male C57BL/6J mice exposed by oropharyngeal aspiration to 4 or 40 µg were harvested 1, 7, 28, and 84 d post-exposure. Using workplace exposure assessment to guide *in vivo* study design (e.g. deposited dose and material preparations to emulate personal breathing zone collections), dose- and time-dependent measures of pulmonary cytotoxicity, inflammation, and histopathology were expectedly observed for the AP MWCNT. Polymer coating the MWCNT did not enhance the pulmonary toxicity of AP nanotubes and toxicity was significantly attenuated with one coating. The aerosols generated from sanding composites embedded with PC nanotubes (0.15-3% by weight) contained no evidence of free nanotubes, and were primarily micron-sized particles with some MWCNT protrusions. Following pulmonary exposure, if the percent weight incorporation of PC nanotubes and composite matrix utilized altered particle size distribution, acute *in vivo* toxicity was affected, and if unaltered, toxicity was not different. Our study provides perspective that while the number of workers and consumers increases along the life cycle, toxicity and/or potential for exposure to the as-produced material may greatly diminish.

2902 Deriving a Provisional Tolerable Intake for Intravenous Exposure to Silver Nanoparticles Released from Medical Devices

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Silver nanoparticles (AgNP) are incorporated into medical devices used intravenously (IV) for their anti-microbial characteristics and are produced with varying physico-chemical particle properties including size, shape, coating and agglomeration state. The potential exposure and toxicity of AgNPs to patients is unknown due to lack of toxicological data. A stringent battery of biological tests for each nanomaterial produced would be costly and time-consuming. The aim of this safety assessment is to derive a provisional tolerable intake (pTI) value for AgNPs released from blood-contacting medical devices. A comprehensive literature review of *in vivo* studies investigating critical adverse health effects induced from IV exposure to AgNPs was reviewed and evaluated by the Annapolis Accords principles. Key studies were further analyzed by the Toxicological Data Reliability Assessment Tool (ToxRTool) to determine the critical study for use in derivation of the pTI. The lowest statistically significant dose-dependent adverse health effect reported in the critical study served as the point of departure (POD). The POD was based on a 28-day IV repeated AgNP (20 nm) dose toxicity study that reported an increase in relative spleen weight in male and female rats with a lowest 5% lower confidence bound of the benchmark dose (BMDL) of 14 µg/kg bw/day. The POD was extrapolated to humans by applying a modifying factor (MF) of 1,000 (based on scientific review and analysis) to account for uncertainties in intraspecies variability (uncertainty factor (UF_1) = 10), interspecies differences (UF_2 = 10) and lack of long-term toxicity data (UF_3 = 10). The pTI for long-term IV exposure to 20 nm AgNPs released from blood-contacting medical devices was determined to be 0.14 µg/kg bw/day. This pTI may not be appropriate for nanoparticles of other physico-chemical properties or routes of AgNP administration. The methodology presented is deemed appropriate for deriving pTIs for nanoparticles in general.

2903 Proteomic Analysis of Hippocampal Proteins of Rats Exposed to Acrylamide

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Objectives: Acrylamide is used industrially and also found in certain foods cooked at high temperatures. There are human cases intoxicated with acrylamide through well water, showing ataxia, memory impairment or illusion. The present study investigated change in expression of proteins in rats exposed to acrylamide orally. Comparison with our previous proteomic study on 1-bromopropane is expected to suggest the common mechanism of neurotoxicity of soft electrophiles, which both acrylamide and 1-bromopropane belong to. Methods: Male Wistar rats were exposed by gavage to acrylamide at 0, 2.0, 20.0mg/kg for 1 week or at 0, 0.2, 2.0, 20.0mg/kg for 5 weeks. At the end of the experiments, rats were decapitated, and hippocampus was dissected out and flash-frozen. Proteins were extracted from the hippocampus and applied to two-dimensional difference in gel electrophoresis (2D-DIGE) combined with matrix-assisted laser-desorption ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF MS). Results: The 2D-DIGE detected 2 spots in the 2.0, 20.0mg/kg group of 1-week exposure and 33 spots in the 0.2, 2.0, 20.0mg/kg of 5-week exposure. Among them, MALDI-TOF/TOF MS identified 2 proteins of 1-week exposure and 21 proteins of 5-week exposure. The identified proteins included 8 up-regulated proteins and 13 down-regulated proteins in 20.0mk/kg group. The identified proteins could be categorized into biological processes of metabolic pathway, cellular process, cellular component organization or biogenesis, localization, developmental process, response to stimulus, immune system process, biological regulation and multicellular organismal process. Conclusion: When compared to the previous proteomic study on hippocampal proteins in rats exposed to 1-bromopropane, the result shows commonality of acrylamide and 1-bromopropane on their effects on expression of proteins associated with glycolysis and mitochondria. The study suggests the common mechanism in neurotoxicity of soft electrophiles.

2904 Comparison of Dynamic Light Scattering Instruments in Size Analysis of Nanoparticles

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Dynamic light scattering (DLS) is a technique for nanoparticle (NP) size characterization in a liquid environment (e.g. cell culture medium). It provides crucial information for toxicological assessment as it gives insight into dynamic changes, aggregation and agglomeration vs. dissolution, of NPs and their evolution along with time. Besides electron microscopy DLS is a powerful tool for understanding NP behavior in physiologically relevant media within toxicity testing as well as efficiency of a dispersion procedure. Two state-of-art DLS instruments were compared in this study - a NanoBrook Omni (Brookhaven Instruments Corporation, USA) and a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The integrated EU project NANoREG - "A common European approach to the regulatory testing of Manufactured Nanomaterials" - provides diverse well characterized NPs in order to compare toxicity results among EU laboratories. Comparative tests were performed on standard NPs (NANOSPHERE Size Standards, Thermo Scientific) and batch dispersions (2.56 mg/ml) of the NANoREG project NPs under identical experimental conditions. The standard was measured undiluted and gradually diluted. NANoREG NP dispersions were prepared using probe sonication according to the generic NANOGENOTOX dispersion protocol. The standard and NANoREG NPs were measured by back-scattering to prevent issues arising from sample opacity. Standard size was correctly measured in undiluted form, approx. 10mg/ml, by the Zetasizer Nano ZS; the NanoBrook Omni was able to measure the size correctly at a concentration of 0.4mg/ml or lower. Not only that it is uncomfortable for the operator, the dilution itself also affects the extent of aggregation and agglomeration of the suspended NPs. Differences among sizes of the NANoREG NPs measured by the two instruments increased with rising sample opacity. Intensity distributions of the NANoREG samples were more stable with the Zetasizer Nano ZS. We concluded that under identical experimental conditions, the Zetasizer Nano ZS is suitable for quick and reliable DLS measurements of NP suspensions, while the NanoBrook Omni requires dilution of more concentrated suspensions, and thus it is not applicable for higher concentrations often used in *in vitro* toxicity assays.


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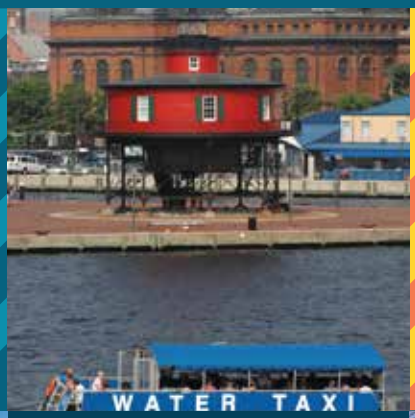

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