3487 Lack of Genotoxicity in the Comet and Micronucleus Assays of Graphistrength© C100 Multiwalled Carbon Nanotubes (MWCNT) After A 90-Day Nose-Only Inhalation Exposure of Rats

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Graphistrength© C100 provides superior electrical and mechanical properties for various applications. Graphistrength© C100 is formed of MWCNT (ca. 12 walls, outer mean diameter ca. 12 nm, length ca. 1 µm) agglomerated in particles with a granulometry centered on 400 µm. A general feature of MWCNT after inhalation or intratracheal exposures is the induction of an inflammatory reaction in the lungs sometimes associated with local genotoxic effects. Most of the *in vitro* and *in vivo* genotoxicity data available on Graphistrength© C100 are negative. However, a weak DNA damage activity in the *in vitro* and *in vivo* FPG-modified Comet assays and a weak clastogenic effect in the *in vitro* micronucleus test were reported. To evaluate the genotoxic potential of Graphistrength© C100 in the cells at the site of contact and those distant from it, Wistar rats were exposed by nose-only inhalation (6h/d, 5d/week) for 13 weeks to target concentrations of 0.05, 0.25 and 5.0 mg/m3 air of a respirable aerosol (MMAD < 3 µm). Twenty-four hours post-exposure, chromosomal aberrations in the bone marrow cells of males and females were evaluated by the micronucleus test (OECD TG 474) and DNA damage in the lung, kidney and liver cells of males were assessed by both the standard and the human 8-oxoguanine DNA N-glycosylase 1 (hOGG1)-modified comet assay (OECD TG 489). Broncho-alveolar lavage fluid (BALF) was also collected and analyzed for inflammatory parameters. Neither increase in the number of micronucleated polychromatic erythrocytes nor increase in percent DNA damage were observed at any concentration whereas an inflammatory lung reaction and the release of inflammatory factors in the BALF were observed in all rats exposed to 5.0 mg/m3 (see companion poster of the same author for details). Therefore, Graphistrength© C100 appears of low concern in term of local and systemic genotoxicity. Published in: Pothmann D et al. Lung inflammation and lack of genotoxicity in the comet and micronucleus assays of industrial multiwalled carbon nanotubes Graphistrength © C100 after a 90-day nose-only inhalation exposure of rats. Particle and Fibre Toxicology 2015, 12:21.

3488 The Occupational Life Cycle of MWCNT: Toxicity Evaluation from As-Produced to Post-Production Modifications and Composites

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Companies synthesize or purchase as-produced multi-walled carbon nanotubes (AP-MW), apply a polymer coating (PC-MW), then embed the PC-MW into an epoxy or fiberglass matrix. Workers are potentially exposed at each production stage from dry powder handling of the MW or aerosols from sanded composites. To date, toxicity studies have focused primarily on AP-MW. The aim was to characterize the toxicity of these additional materials. Materials were prepared to emulate workers personal breathing zone collections and male C57BL/6J mice were then dosed by oropharyngeal aspiration with vehicle, AP-MW, or PC-MW from two separate companies at 4 µg or 40 µg. Mice were sacrificed at 4 h, 1, 7, 28 and 84 d post-exposure with collection of lavage fluid, lung, tracheobronchial lymph node (TBL), kidney, and liver. Both AP-MW induced dose- and time-dependent measures of pulmonary cytotoxicity, inflammatory cell influx, and inflammatory proteins. Histopathology identified small granulomas at terminal bronchioles at 84 d but no significant alveolar fibrosis at the 40 µg dose. In neither case did PC-MW enhance the effects of AP-MW. With mass as a dose metric, inflammation was less from exposure to PC-MW from company #2 and, at 84 d, remaining material was localized to large extracellular aggregates with minimal or no inflammation. All MW were mostly cleared by 84 d. Translocation to the TBL was more prevalent for materials from company #2 but unlike larger diameter MW, no translocation to systemic organs was detected for any material. Lastly, the PC-MW containing composites with 0.15% and 3% PC-MW by weight were subjected to an industrial sanding process. Assessment of generated aerosols show primarily micron size particles with some MW protrusions (evidence of free MW under evaluation). These findings provide toxicity context at different stages of the MW production chain.

3489 Pulmonary Effects of Different Sized and Oxidized Forms of Graphene Nanoparticles

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Risk of graphene (Gr) nanoparticle inhalation by workers during the manufacture of composite materials is a growing concern as new graphene nanomaterials are generated and utilized. Gr-induced cytotoxicity *in vitro* (PC12 cells, fibroblasts) and lung inflammation *in vivo* have been demonstrated, and Roberts et al (2013) showed that lung inflammation was increased up to 7 d after aspiration of non-oxidized Gr particles with lateral dimensions >5 µM and greater number of layers (~20) compared with that of smaller particles (<1 µM, ~4 layers). We reported previously that a single exposure of graphene [5 µm lateral x 7-10 nm thick (Gr5)] transiently increased lung resistance (RL), dynamic lung compliance (CDyn) and reactivity to inhaled methacholine (MCh) in mice. In this study we examined the effects two additional sizes of non-oxidized Gr particles [20 µm lateral x 7-10 nm thick (Gr20), and <2 µm lateral x 1-2 nm thick (Gr1)] as well as a reduced form of oxidized Gr (rGO), on basal RL and CDyn and reactivity to inhaled methacholine (MCh). Mice were given 40 µg Gr (Gr1, Gr20 or rGO) suspended in dispersion medium (DM; control) via aspiration. Basal levels and RL and CDyn responses to increasing concentrations of inhaled MCh were measured 4 h - 2 mo after Gr exposure. Gr1 increased basal RL on day 7 and basal RL decreased at 2 mo. Gr1 increased Cdyn responses to MCh 1 mo post-exposure, which returned to control levels at 2 mo. However, basal Cdyn was increased by 2 mo post-exposure. Gr20 increased Cdyn responses to MCh 1 mo post-exposure, but Cdyn returned to control by 2 mo. rGO had no effect on basal RL or Cdyn, nor MCh reactivity. Our results indicate that exposure to non-oxidized Gr nanoparticles resulted in transient changes in RL or Cdyn responses to MCh, while the reduced form rGO had no significant effect on airway reactivity or basal RL or Cdyn. Basal Cdyn was increased 2 mo after exposure to Gr1. Our findings suggest that the pulmonary effects of graphenes are influenced by particle size, number of layers and oxidative/reduced state.

3490 Identification of Biomarkers for Nano-Safety Assessment through Proteomic Analysis of Multi-Walled Carbon Nanotubes Functionalized by Atomic Layer Deposition Coating

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Introduction: Multi-walled carbon nanotubes (MWCNT) are commonly surface-functionalized post-synthesis to enhance their novel properties for use in electronics and engineering. Since MWCNT are known to cause lung fibrosis in rodents, it is important to determine how functionalization affects pro-fibrotic potential in order to predict and prevent fibrosis. Preventative measures can be acquired through the identification and monitoring of groups of protein biomarkers. The goal of this work was to use proteomics to identify biomarkers of fibrosis unique to MWCNT atomic layer deposition (ALD) coating type. Methods: Mouse epithelial cell culture was established using E10 cells derived from normal type-II epithelial cells. E10 cells were exposed to control media, uncoated (U)-MWCNT and two ALD-coated MWCNTs: aluminum oxide (A), and zinc oxide (Z). Following a 24 hour exposure, cells were harvested, lysed, trypsin digested, and the peptides were isolated. Nanoflow LC-MS/MS was conducted using a ThermoScientific Q-Exactive Plus, and further validation of significant proteins was achieved using a triple quadrupole mass spectrometer, Quantiva. Spectra were searched using Sequest implemented through Proteome Discoverer. Skyline-daily was used to evaluate significant proteins of interest by selected reaction monitoring. Results: The E10 exposure yielded several differentially expressed proteins across coating types that perturbed pathways by different mechanisms; including: disruption in oxidative phosphorylation, and complement activation. A two-sample t-test found the following number of significant proteins for each exposure group compared to control (α=0.05): 167 Z-MWCNT (2.5 µg/mL), 199 A-MWCNT (100 µg/mL), and 142 U-MWCNT (100 µg/mL). Conclusion: *In vitro* proteomic experimentation was able to elucidate differential pathway disruption as a function of MWCNT coating type. Funding: Supported by NIEHS grants T32 ES007046 and R01ES020897.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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