## 2216 Gender-Specific Biological Responses in Juvenile Rats Orally Exposed to Three Engineered Nanomaterials

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Engineered nanomaterials (ENMs) are widely used in medicine, food, and agriculture, as well as general household applications, and exposure to them is ubiquitous. Children represent a vulnerable population because perturbations in cell growth and signaling can disrupt temporally-sequenced developmental processes leading to long-term functional deficits. Little is known about the uptake, distribution, and biological responses of ENMs and their toxicity in developing animals. In this study three nanoparticles (NPs) provided by The NIEHS Consortium for Nanotechnology Health Implications Research were tested: $TiO_2$ P25, 30 nm $Al_2O_3$, and 50 nm ZnO. Three litters (five males and five females) of juvenile Sprague-Dawley rats were daily administered 10 mg/kg NP or vehicle control (water) by oral gavage between postnatal day (PND) 17-20. Basic neurobehavioral (acoustic startle response, locomotor activity, and rotarod) and cardiac (ECGenie) assessment were performed 4 hours post administration of the final dose. Animals were sacrificed on PND 21, and selected tissues were collected, weighted, and processed for histopathology or biochemical analysis. Neurotransmitter concentrations in brain tissues were quantified by HPLC with electrochemical detection. No change was observed for body weight (b.w.) or brain-to-b.w. ratio for pups. Liver-to-b.w. ratio were significantly increased for male pups receiving $TiO_2$ P25 (0.0417±0.0028) and $Al_2O_3$ NP and (0.0409±0.0021) and for female pups administered $TiO_2$ P25 (0.0420±0.0040) compared to control (male: 0.0389±0.0025; female: 0.0395±0.0021). No neurobehavioral effects were found. Heart rate was significantly decreased for female pups administered $TiO_2$ P25 (441±43.3 beats per minute [bpm]) compared to vehicle control (511±46.0 bpm). No significant changes were observed for neurotransmitter levels in brain tissue. Enhanced Darkfield and Hyperspectral imaging (CytoViva) are being used to evaluate the presence of NPs in tissue sections of the intestine, liver, spleen, kidney, and lymph nodes. The microscopy analysis is in progress, we have located $Al_2O_3$ in the liver. Gender-specific responses were observed in juvenile rats orally administered $TiO_2$ P25 and $Al_2O_3$ NP. These data suggest that the developing animal represents a valuable model for oral ENM exposure.

## 2217 Long-Term Effects of Inhaled Nanoparticles in Rats: Ceriumdioxide and Bariumsulphate

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Ceriumdioxide (NM212) and bariumsulphate (NM220) nanoparticles were tested according to the OECD test guideline no. 453; additions were made to the standard protocol to find evidence of inflammation and potential lung tumours with high sensitivity. Aerosol concentrations were concentrations 0.1, 0.3, 1 and 3 mg/m$^3$ and 50 mg/m$^3$ was tested, respectively. Levels of cerium measured in the organs increased with higher exposure concentrations and over time. However, the accumulation only reached a very low level. Lung burdens of barium were unexpectedly low during the first three months of exposure, due to fast clearance most probably by dissolution *in vivo*. Barium lung burdens increased thereafter. Animals in all exposure groups showed chronic inflammation of the lungs, with stronger inflammation at higher exposure concentrations. The level of particles present in the lungs was higher for bariumsulphate than for the highest level of ceriumdioxide. The nature of the inflammatory tissue changes varied between the two nanoparticles. Ceriumdioxide already triggered a chronic inflammation effect at the lowest dose, which was unexpected for insoluble nanoparticles without inherent toxicity. Despite chronic inflammation at all dose levels, no lung tumours were found which could be attributed to ceriumdioxide or bariumsulphate exposure. This may indicate that a particle-related, low-level chronic inflammation is not sufficient to cause tumour formation. Low-level accumulation in extrapulmonary organs did not lead to any pathological changes. No evidence of further health impacts was found for ceriumdioxide and bariumsulphate beyond those already known for granular particles. *Acknowledgement: The project was carried out and funded jointly by the German Federal Environment Ministry, the Federal Environment Agency, the Federal Institute for Occupational Safety and Health and BASF SE as well as the EU project NanoREG and Cefic LRI. Histopathological examinations were performed by Fraunhofer Institute for Toxicology and Experimental Medicine and the biodistribution was analysed by the Federal Institute for Risk Assessment.*

## 2218 Comparative *In Vivo* Assessment of Alveolar Fibrosis, Histopathology, and Systemic Translocation Induced by Carbon Nanotubes and Nanofibers from US Facilities

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Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F) is known to induce inflammation, toxicity, or tumorigenesis, and is a concern in the occupational setting. 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 U.S. facilities. Seven different multi-walled CNT and two CNF were characterized and evaluated for alveolar fibrosis, translocation, and histopathological changes in mice at 1, 7, 28, and 84 d following oropharyngeal aspiration to 4 or 40 µg of each material. Lung sections at 84 d post-exposure to 40 µg were microscopically evaluated to measure changes in histopathology. Moderate and multifocal granulomatous bronchopneumonia, bronchiolitis obliterans, bronchiolar epithelial hypertrophy, and peribronchial fibrosis were observed in most, but not all, high dose exposures. Variances in incidence and severity between the CNT/F correlate to physicochemical properties (e.g., particle agglomeration state, nominal tube diameter, etc.). Alveolar fibrosis was measured using morphometric point and intercept counting, and was generally increased in 7 of the 9 materials reaching significance in materials with nominal tube diameter greater than or equal to 50 nm. Tracheo-bronchial lymph node (TBLN) and liver sections at 84 d post-exposure were microscopically evaluated for particle translocation. A wide range of TBLN and liver accumulation patterns were observed, which reflect the ability of macrophages to phagocytose and clear particles dependent on size and agglomeration. Systemic translocation was limited to single tubes or fibers rather than agglomerates, meaning less systemic accumulation for smaller diameter, more agglomerated materials compared to larger diameter, and more fiber-like materials. In conclusion, histopathologic changes and translocation were dependent upon physicochemical properties such as particle agglomeration and size. Ongoing research and modeling techniques will elucidate relationships between physicochemical characteristics and toxicities of various CNT/F.

## 2219 Multiwalled Carbon Nanotubes Modulate Immune Responses and Pulmonary Injury without Increasing Influenza A Virus Titers in Infected Mice

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Extensive development and use of nanomaterials (NMs) have garnered concerns regarding their potential for exposure and adverse health effects. Many toxicological investigations have particularly focused on pulmonary injury that include fibrosis, asthma, and possibly lung cancer, but only a few reports have probed how exposure to NMs can affect host's susceptibility to pathogenic infections. Previously, we have found exposure of mice to pristine single-walled carbon nanotubes (SWCNTs) can significantly increase viral titers to influenza A virus (IAV) in mice in concert with repressed antiviral and pro inflammatory immune responses. In the present study, we investigated if pre-exposure to a different type of carbon nanotube, hydroxylated multiwalled carbon nanotubes (MWCNTs), would impact immune mechanisms and viral titers similarly. Male mice were randomly assigned to control, MWCNTs, IAV, and MWCNTs+IAV groups and exposed to either 20 µg of MWCNTs or control vehicle (pluronic) by pharyngeal aspiration. On day 3, animals were then given $3.14*10^4$ $TCID_{50}$ IAV or PBS by intranasal instillation. All animals were euthanized on day 7 and endpoints including immune cell profiles and cytokine and chemokine levels in bronchioalveolar fluid (BALF), lung histopathology and mRNA expression of innate immune and oxidative stress-related genes, were measured. Our results showed minimal lung tissue damage when mice were exposed to MWCNTs alone. Compared with mice that were exposed to IAV only, MWCNTs didn't significantly alter viral titers or immune cell profiles in BALF in the co-exposed group, but significantly increased cytokine and chemokine levels (IL-1β, TNFα, GM-CSF, KC, MIP-1α, MIP-1β, MIP-2, and RANTES), and inhibited expression of antiviral genes (*Tlr3, Rig-I, Mda5*, and *Ifit2*). Unlike pristine SWCNTs, hydroxyl functionalized MWCNTs do not increase viral titers to IAV infection, however they do cause molecular changes related to the immune response that are similar between CNT types.


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