1326 Pulmonary Exposure to Respirable Cellulose Nanocrystals Caused Sustained Lung Damage and Male Reproductive Toxicity

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Cellulose nanocrystals (CNC) unveil an interesting combination of properties (e.g. mechanical, thermal, rheological and optical) and produced in large scale as nanofillers in polymer composites, building materials, cosmetics, food, and the drug industry. To date, there are only few studies investigating the potential adverse effects of nanocellulose materials. The present study was undertaken to investigate the pulmonary outcomes as well as alterations of the male reproductive system induced by repeated exposure to respirable CNC. C57BL6 male mice were treated by pharyngeal aspiration with CNC (40 µg/mouse) twice a week for 3 weeks. Three months after the last administration, exposure to respirable CNC resulted in pulmonary inflammation and damage, oxidative stress, elevated TGF-β and collagen in the lung. Additionally, CNC exposure significantly altered sperm concentration, motility, cell morphology, and sperm DNA integrity. These parameters correlated with elevated pro-inflammatory cytokines and myeloperoxidase activity in testes, as well as increase in oxidative stress in both testes and epididymis. Exposure to CNC also induced damage to testicular structure and imbalance in levels of testosterone and luteinizing hormone. Taken together, these results demonstrate that exposure to respirable CNC not only lead to pulmonary toxicity but also induces sustained adverse effects in spermatocytes/spermatozoa indicating male reproductive toxicity.

1327 Histological and Immunohistochemical Studies of a Nanocellulose Diester from Cotton Seeds in Rats

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Chemical modification of cellulose nanofibers is currently attracting attention as researchers attempt to take advantage of the abundance of hydroxyl groups on its surface to introduce extra biological functionality. However, the toxicity profile of this esterified nanocellulose has not been established in animal models. Thus, a 7-day repeated oral toxicity study of cotton seed nanocellulose diester (NCD) was conducted in male Wistar rats. A total of three groups (5 rats per group) were compared: (1) Control (normal saline), (2) 50 mg/kg NCD and (3) 100 mg/kg NCD. The effects of NCD in rats were investigated by assaying oxidative stress biomarkers, lipid peroxidation, plasma toxicity markers, nitric oxide and myeloperoxidase levels. The expressions of cyclo-oxygenase-2 and inducible nitric oxide synthase were also evaluated by immunohistochemical staining. Acute treatment of NCD had no adverse effect on enzymatic antioxidant status but significantly elevated the aminotransferases activities when compared with controls. Improvement in reduced glutathione levels was accompanied by a decrease in myeloperoxidase activity. Histological observations did not reveal any adverse effects on the liver at the lower concentration dose when compared with control. Treatment with NCD elicited a reduction in the expressions of cyclo-oxygenase-2 and inducible nitric oxide synthase when compared with controls. The present findings suggest that NCD appears to have minimal adverse oral toxicity effect on animals.

1328 Long-Term Inhalation Study with Nano Barium Sulfate: Unexpected Morphological Findings and Lung-Burden after 12 Months of Exposure

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Lung carcinogenicity and putative systemic effects of low-dose lifetime inhalation exposure to biopersistent nanoparticles were examined in a combined chronic/carcinogenicity inhalation study performed according to OECD test guideline no. 453 with several protocol extensions. Female rats were exposed to barium sulfate (NM-220; 50 mg/m^3) for 12 month (interim sacrifice, 10 animals), 24 month (final sacrifice, 50 animals) and 24 month plus 6 month exposure-free period (50 animals). A control group was exposed to clean air. Lung burdens and burdens in extrapulmonary tissues were measured at various time-point. In parallel, bronchioalveolar lavage fluid (BALF) was investigated and histopathology was performed. Range finding studies with 5d and 28d of inhalative exposure to Barium Sulfate (BaSO4; 50mg/m^3) revealed no histopathological findings in the lungs, low lung burden concentrations and no signs of inflammation in BALF examination. BaSO4 lung burdens were comparatively low (1 mg/g) within the first 13 weeks of exposure and steeply increased to > 10 mg/g lung tissue after one year, accompanied by severe inflammatory changes in the lung detected by BALF and histopathology. Whereas the excretion of BaSO4, after 13 weeks was comparable to control, a significant increase in feces after 12 and 24 months was measured. Histological examination of lungs revealed several adverse and non-adverse effects in the lung. The non-adverse effects comprised accumulation of particle-laden macrophages in alveolar/interstitial areas and in the BALT with an accentuation on interstitial accumulation and bronchiolo-alveolar hyperplasia (alveolar bronchiolization). The adverse effects included (mixed) alveolar/interstitial inflammatory cell infiltration without granulomatous inflammation, minimal interstitial fibrosis and alveolar lipoproteinosis. Neither pre-neoplastic nor neoplastic changes were observed after 12-months exposure. A no observed adverse effect concentration could not be established in this study. The comprehensive histopathological examinations of lungs and other tissues after 24 and 30 months of exposure will be finalized in 2017.

1329 Food Grade Titanium Dioxide (E171) Consumption in Diet Induces Higher DNA Damage in Colon than in Spleen and Liver of Mice

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Food grade titanium dioxide, known as E171, is used as a food additive and in personal care products. The Food and Drug Administration allows the use of E171 in up to 1% of the final product weight. However, the IARC has classified TiO_2 as a posible carcinogen to humans by inhalation but the oral route has been poorly investigated. It is known that after oral administration, TiO_2 can be absorbed into blood stream and can be taken up by different organs such as colon, liver and spleen. However, the toxic efects in these organs are still unknown. Here we investigated the potential genotoxic effect of E171 in colon, liver and spleen after oral administration to mice in the diet. For this purpose, Balb/C mice were fed with 0.5% E171 through diet during 4 and 10 weeks and DNA damage in these organs was measured using the γ-H2AX immuno-staining assay. We found that 0.5% E171 in the diet induced a 1.5-fold of increase of fluorescence in liver, 2-fold in spleen and 3-fold in colon after 4 weeks and the DNA damage was sustained at least until 10 weeks. Also, two of the mice treated for 10 weeks developed adenomas in the distal colon. These results suggest that oral consumption of 0.5% E171 in the diet caused DNA double strand breaks in these organs, but also, that probably colon tissue could have higher susceptibility followed by spleen and liver.

1330 Differences in Nickel Oxide Nanoparticle-Induced Pulmonary Toxicity and Exacerbated Allergic Response following Acute Respiratory Exposure

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Particle size and morphology play critical roles in nanomaterial-induced lung inflammation, but the relationship of these factors to augmentation of allergic response in the respiratory tract is largely unknown. To address this concept, two different sizes of nickel oxide (NiO) particles were characterized and investigated *in vivo*. Dynamic light scattering showed the average particle sizes (APS) were 27 nm for NiO-1 and 190 nm for NiO-2. NiO-1 particles were spherical while NiO-2 particles were more irregular and plate-like. The goal of the study was to assess effects of the different NiO particles on augmentation of allergic response using an ovalbumin (OVA) model. Effects of NiO on the lung were also assessed at critical time points correlating to the OVA model in the absence of OVA. Female BALB/c mice were given a single dose of 40 micrograms of NiO-1, NiO-2, or dispersion medium (DM; vehicle control) by oropharyngeal aspiration (OPA) and euthanized at 1, 10, 19, and 29 d post-exposure in the absence of OVA. In the OVA allergy model, mice were similarly exposed to particles or DM on day 0, sensitized to OVA via i.p. injection at 1 and 10 d, challenged with OVA at 19 and 28 d via OPA, and euthanized at 29 d. In the absence of OVA, only NiO-2 induced significant and persistent increases in lung injury and inflammation in the lung, but both NiO-1 and NiO-2 increased mediastinal lymph node

(LN) size as compared to controls. In the OVA allergy model, the smaller nanoparticles (NiO-1), resulted in an exacerbated airway response following OVA challenge, and increased serum OVA-specific IgE levels as compared to vehicle and allergy controls. Differentially, exposure to NiO-2 significantly increased LN size, yet reduced OVA-specific IgE levels. Overall, results demonstrate that size, and possibly particle morphology, contributed to nickel-based particle-induced pulmonary inflammation and modulation of immune responses in the lung. The larger plate-like particle was capable of inducing a greater degree of pulmonary inflammation; however, the smaller NiO particle exacerbated the allergic response to OVA to a greater degree. Further studies are needed to elucidate the mechanisms related to these findings.

1331 Nanoparticle-Induced Pulmonary Toxicity–Effects from Subchronic Exposure to Cerium Dioxide and Barium Sulfate

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Manufactured nanomaterials are widely used in industrial and commercial products. However, detailed information especially on long-term effects are limited. This subchronic study aimed on the investigation of effects caused by cerium dioxide and barium sulfate nanoparticle inhalation. To create a link to potential chronic effects, experiments were examined under similar conditions as a combined chronic inhalation toxicity and carcinogenicity study (BASF, Ludwigshafen, Germany). Obligatory endpoints (OECD 413) combined with gene expression analysis should provide information on effects at early stages of nanoparticle exposure (within 90 days). Respective observations and the identification of biomarkers should help to predict potential long-term effects. Up to 90-day exposure of rats to low concentrations of nano-CeO_2 (0.1 - 3.0 mg/m^3) caused inflammatory reactions in respiratory organs, detected by histopathological examinations and analysis of bronchoalveolar lavage fluid. Effects remained persistent after end of exposure. One high concentration of $BaSO_4$ (50.0 mg/m^3) nanoparticles, a typical inert dust, was also examined. Induced inflammatory effects were less severe compared to CeO_2. Elevated levels declined during post-exposure. Expression analysis of more than 300 genes in pneumocytes type II suggested an influence of nanoparticles on inflammatory mediators including chemokines and interleukines. Also, mRNA expression of biochemical markers related to oxidative stress, apoptosis and DNA repair was modulated. - Inhaled nanoparticles affect the rat's respiratory system mainly in terms of inflammation. Currently available data might predict potential chronic effects like cell proliferation or even tumor development following low-concentration exposure to CeO_2. *This project is funded by the German Federal Ministry of Education and Research (BMBF) - 03X0149A.*

1332 Quantum Dot-Induced Histopathological Changes and the Roles of the Release of Free Cadmium Ions and Hydroxyl Radicals Remain to Be Clearly Elucidated

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Background: A complete understanding of the toxicological behaviors of quantum dots (QDs) *in vivo* is of great importance and is a prerequisite for their applications in humans. In contrast to the numerous cytotoxicity studies investigating QDs, a paucity of *in vivo* studies have been reported, and the issue remains controversial. The overall aim of our study was to understand QD-mediated toxicity across different time points and to explore the roles of free cadmium (Cd) and ROS in tissue damage. Methods: Male ICR mice were administered a single intravenous dose (1.5 μmol/kg) of aqueous synthesized CdTeQDs, and the liver and kidney functions and morphology were subsequently examined at 1, 7, 14 and 28 d. Furthermore, hydroxyl radical (•OH) production in the tissues was quantified by trapping •OH with salicylic acid (SA) as 2,3-dihydroxybenzoic acid (2,3-DHBA)detected using an HPLC-fluorescence method. We used the induction of tissue metallothionein (MT) levels and 2,3-DHBA/SA ratiosas markers for elevated free cadmium (Cd) from the degradation of QDs and ROS generation in the tissues, respectively. Results: The degree of tissue damage gradually increased with time, reached a maximum at 14 d and gradually recovered by 28 d.The MT levels and 2,3-DHBA/SA ratios significantly increased and peaked at 1 and 7 d, respectively, but were not elevated at 14 d. The histological changes of the liver and kidneys, released free Cd and ·OH levels exhibited different trends over the 28-d period, with the free Cd and ·OH exhibiting delayed effects in terms of histopathological abnormalities. Conclusions: The QD-induced histopathological changes were time-dependent. The tissues can recover from the histopathological abnormalities. The histological assessments performed at multiple time points and the temporal analyses of the potential toxic effects of QDs might contribute to the evaluation of the biological safety of QDs. Moreover, our study further determined that the underlying mechanism *in vivo* after low-dose QD exposure might include cellular defense mechanisms and tissue adaptive mechanisms. The QD-induced histopathological changes and the roles of free cadmium ions and ROS will need to be more completely elucidated in the future.

1333 Physicochemical Properties of Zinc Oxide Nanoparticles Differentially Influence Microvascular Function and Proteomic Responses

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Engineered nanomaterials' (ENM) physicochemical properties have been shown to impact the toxicity associated with *in vitro* nanomaterial exposure; however, it is unknown if ENM physicochemical properties alter *in vivo* responses such as microvascular function, inflammatory responses, and endothelium activation. Therefore, we hypothesize that ENMs with unique physicochemical properties will lead to differential levels of microvascular dysfunction following exposure. Zinc oxide nanoparticles (ZnO NP) were treated with argon, hydrogen, and oxygen to modify their reductive and oxidative states. Male C57BL/6 mice were intravenously injected with these modified ZnO NP (0.58 mg/kg) or saline. Twenty-four hours post exposure, plasma and mesenteric arterioles were harvested. Endothelium-dependent and -independent reactivity was assessed with acetylcholine (ACh, 10^{-9}-10^{-4} M), and sodium nitroprusside (SNP, 10^{-9}-10^{-4} M) via pressure myography. Vascular smooth muscle reactivity was assessed with phenylephrine (10^{-9}-10^{-4} M). After initial assessments, the arterioles were intraluminally exposed to control plasma to determine if microvascular function could be restored. The plasma was processed for proteomic analysis to analyze changes in circulating proteins that are associated with inflammation and endothelial activation. Following ZnO NP exposure, there were no significant differences in microvascular reactivity compared to control mice. Argon treated ZnO NP caused a significant reduction in endothelium-dependent (10 ± 1% vs. control 64 ± 3%) and -independent dilation (32 ± 6% vs. control 80 ± 5%), that was partially restored after plasma exposure for endothelium-dependent dilation only (40 ± 12%). Finally, there were significant microvascular impairments following oxidized ZnO NP exposure, which were endothelium-dependent (26 ± 7% vs. control 64 ± 3%) and -independent (5 ± 11% vs. control 80 ± 5%). These responses were significantly improved following plasma exposure (ACh 54 ± 8% and SPR 56 ± 14%). Taken together, these results indicate that modification of ZnO NP' physicochemical properties differentially influence microvascular function and these impairments may be due to changes in circulating proteins associated with inflammatory and/or endothelial activation. *R01-ES019311 (JMB)*

1334 Lung Toxicity following a Dose-Response *In Vivo* Study of Dispersed Boron Nitride Nanotubes

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Boron nitride nanotubes (BNNT) are multi-walled nanotubes composed of hexagonal B-N bonds and are an emerging nanomaterial for which markets are rapidly expanding. Although not rigid in nature, the high aspect ratio (HAR) raises concern for potential toxicity that may be associated with lung exposure. The goal of the study was to assess lung toxicity using an *in vivo* dose-response time course model. The BNNT in this study were originally manufactured to be 5 nm wide and up to 200 μm long. Dispersion by sonication in ethanol, drying, and suspension in physiological dispersion medium (DM) yielded byproducts with surface area ~180 m^2/g and size distribution on average of 13-23 nm in diameter x 0.6-1.6 μm in length lowering the original HAR by up to 99.9%. Male C57BL/6J mice received 4 or 40 μg of dispersed BNNT or DM by oropharyngeal aspiration. Bronchoalveolar lavage (BAL) was performed and blood collected at 4 hour, 1 day, 7 day, 1 month, and 2 month post-exposure. Lung lymph nodes (LN) and spleens were also harvested. Lung injury (BAL lactate dehydrogenase) was significantly elevated in the dispersed high-dose BNNT group up to 1 month post-exposure with resolution by 2 month. BAL neutrophils and eosinophils, a measure of inflammation and irritant response, were elevated in the high-dose group up to 7 day post-exposure, peaking at 1 and 7 day, respectively. BAL lymphocytes were significantly elevated at 1 and 7 day post-expo-


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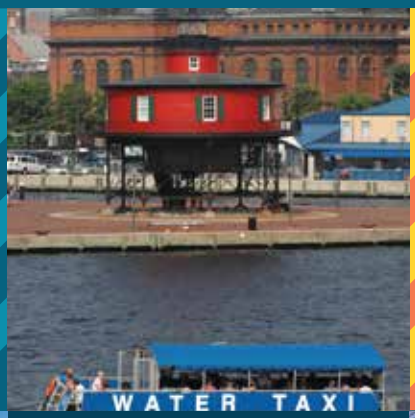

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