cytotoxicity in the obese and lean groups exposed to CuO NPs. At the two-week post-exposure mark, macrophages were the predominant inflammatory cells, with continued cytotoxic effect in the obese mice exposed to CuO NPs. Additionally, we observed an increase in total protein concentration in the BALF and plasma. A suite of cytokines/chemokines, including eotaxin, MCP-1, IL-6, and MIP-1α, were persistently elevated in both lean and obese mice exposed to CuO NPs over a two-week period. Interestingly, IFN-γ, IL-1β, GM-CSF, and IL-4 also showed elevated levels in the plasma of the group exposed to CuO NPs. **Conclusions:** The findings indicate that CuO NP exposure elicits chemotactic activity in the pulmonary system and induces a sustained systemic inflammatory response, regardless of the presence of obesity. The obese group exposed to CuO NPs exhibits a slower resolution of inflammation compared to other groups. Future studies will focus on elucidating the underlying mechanisms of nanoparticle-induced inflammation, with a particular focus on exosomes as potential biomarkers. These exosomes may provide insight into the critical pathways of how inhalational exposure to CuO NPs causes systemic inflammation.

3716 Inhalation Exposure to Wood Sealant with Nano-Zinc Oxide Elicits Minimal Pulmonary Inflammation in Rats

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Background and Purpose: Increased use of engineered nanomaterials in construction and manufacturing adds new attributes to products and improves quality. New wood coating sealant products, used in spraying operations, that contain zinc oxide nanoparticles (nano-ZnO) help protect natural wood from UV-light-degradation and weathering. Therefore, a potential for inhalation exposure to nano-ZnO containing aerosols exists for workers and consumers. The objective of this study was to investigate the pulmonary responses to nano-ZnO-containing wood spray sealant *in vivo*. **Methods:** First, we sought to investigate the nanomaterials released during construction operations and performed real-time worker measurements of aerosol and particulates as previously measured by CPWR collaborators. After spraying operations, the respirable particles in the personal breathing zone were measured at 20.00 ± 3.52 mg/m^3 and contained 0.49 ± 0.09 mg/m^3 of zinc. Based off these measurements, Sprague Dawley rats (200 g) were exposed to respirable ZnO-containing wood spray sealant (total sealant aerosol 20 mg/m^3 14 ppm ammonia and propylene glycol) or filtered air (sham-control) for 4 hours per day for 1 and 14 days. Post-exposure time points assessed pulmonary toxicity at 1 day, 1 week, 1 month, and 3 months after the last exposure day. Bronchoalveolar lavage fluid (BALF) and lungs were collected at the time of sacrifice and assessed for lung inflammation (polymorphonuclear leukocytes [PMN] influx), lactate dehydrogenase activity levels (i.e. lung cytotoxicity), and histopathology. Lung associated lymph nodes and spleens were also collected to evaluate lymphocyte profiles, as zinc is a known immunotoxicant. **Results:** Rat weights between nano-ZnO exposed and sham-control were not different between either exposure at any of the different exposure durations or post-exposure time points. At all timepoints post-exposure, no significant differences in lung inflammation or cytotoxicity were found compared to sham-control, which indicates a lack of a pulmonary response. In addition, lung histopathological analyses indicated no differences between sham-control and nano-ZnO exposed rats at any post-exposure timepoint. Lymphocyte profiles in lymph nodes and spleen also did not differ. **Conclusions:** These results indicate that the wood spray sealant, which contained nano-ZnO, did not induce pulmonary inflammation after inhalation exposure at the concentrations tested in this study. Funding: 9390G1X (NIOSH-NORA)

3717 Toxicity of inhaled amorphous silicon dioxide nanoparticles *Golden Syrian Hamsters*

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Background and Purpose: Amorphous Silicon Dioxide Nanoparticles (SiO_2 NPs) are widely used in the industrial processes, chemical, and cosmetics industries. Amorphous SiO_2 NPs are abundant in the earth's crust and can be released into the air through industrial and manufacturing activities. Such particles are overlooked and not as commonly studied when compared to their crystalline counterpart. Amorphous SiO_2 NPs are pulmonary toxicants; however, the mechanism of toxicity is uncertain. **Methods:** In the current study, toxicity was assessed using an *in vivo* system. Concentrations of particles for the treated groups were selected based on NIOSH's PEL for silica, Time Weighted Average (TWA) of 6mg/m^3. SiO_2 particles were characterized by dynamic light scattering, zeta potential and particle size measured by TEM. Golden Syrian Hamsters were exposed by inhalation to SiO_2 NPs in a whole-body exposure chamber. Experimental animals were divided into 4 groups: Group 1: room air control, Group 2: aerosolized water vehicle control, Group 3: 6mg/m^3 SiO_2 NPs and Group 4: 12mg/m^3 SiO_2 NP treated groups. BALF was removed, and lungs inflated and fixed with formalin or harvested and stored in -80 degrees C for biochemical analyses. Tissue sections were cut for histological examination and stained with hematoxylin and eosin (H&E) and the TUNEL Assay. Lung Mean Linear Intercept (MLI) was measured on H&E sections to determine the extent of airspace enlargement. TUNEL was done to quantify apoptotic bodies. Prior *in vivo* experiments have shown increased inflammatory cells and significantly increased cell injury markers. To explore cellular mechanism of toxicity the pathways of Autophagy, Pyroptosis, Apoptosis, and Necroptosis were evaluated. Autophagy was evaluated using Western Blot of Beclin-1. To measure Pyroptosis, NLRP3, Cathepsin B, and Caspase 1 were evaluated by Western Blots. Caspases 3, 8, and 9 were measured by Western Blots to determine if Apoptosis was a possible mechanism of cell death. P-RIP3 levels were measured to evaluate Necroptosis. HSP70 was evaluated to measure cellular stress. **Results:** Beclin-1, NLRP3, Cathepsin B, and Caspase 1, showed no change when compared to controls. Caspases 3 and 8 were significantly increased in Group 4 when compared to controls (1.39 and 1.41-fold, respectively). Caspase 9 levels remained unchanged. P-RIP3 levels measured to evaluate the progression of Necroptosis were unchanged. HSP70 measured to evaluate cellular stress was significantly increased in Group 4 compared to controls (6.7-fold). MLI was significantly increased in both Group 3 and 4 compared to controls (1.9 & 1.8-fold, respectively). The TUNEL assay showed a significant increase in apoptotic bodies in Group 4 (5.9-fold) compared to controls. **Conclusions:** Results from this study indicate that toxicity from inhalation exposure to amorphous SiO_2 NPs was not mediated by Autophagy, Pyroptosis, nor Necroptosis as indicated by unchanged levels of biomarkers associated with these processes. However, markers for the extrinsic apoptotic pathway were increased in a statistically significant fashion when compared to controls. Such increases strongly indicate the apoptotic pathway as the principle mechanism of lung injury in animals exposed to inhaled Amorphous SiO_2 NPs. The airspace enlargement and increase in apoptotic bodies reported here following exposure to Amorphous SiO_2 NPs suggests an apoptotic mechanism for such tissue destruction.

3718 Long-term exposure to food-grade silicon dioxide from *in utero* life until adulthood led to sex-specific alterations of the microbiota-gut-immune system axis with development of metabolic disorders

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Background and Purpose: The food-grade (*fg*) SiO_2 is used as anticaking and antifoaming agent in powdered food (e.g., sugar, salt, milk, instant soup, infant formulae), with chronic dietary exposure in Humans (0.8-74 mg/kg bw/day). This food additive is composed of aggregated nanoparticles (NPs) that cross biological barriers, like the intestine and placenta, and accumulate in systemic organs raising public health issues. Following ingestion, SiO_2-NPs could also alter the intricate dialogue linking the intestinal microbiota to various functions under its control, including the intestinal barrier, immune functions, and host metabolism, with health consequences. Through a longitudinal study from *in utero* life to adulthood in mice, the aim of this study is to explore the consequences of chronic oral exposure to *fg*-SiO_2 on the microbiota-intestine-immune system axis, and on metabolic functions of the descendants. **Methods:** Female mice were exposed to a control or *fg*-SiO_2-enriched diet at a human relevant level (10 mg/kg bw/day) during pregnancy and lactation until weaning of pups, then the descendants (F1) were fed the same diet as their mother until postnatal day (PND) 150. Intestinal permeability to macromolecules (oral FITC-Dextran 4kD) was determined *in vivo* at PND136 while oral glucose tolerance was assessed at PND143. At PND150, intestinal and systemic production of pro- and anti-inflammatory cytokines was measured by ELISA, and gut microbiota composition was assessed by 16S gene sequencing. All animal experiments were approved by the Local Animal Care and Use Committee. **Results:** Chronic *fg*-SiO_2 exposure starting *in utero* increased the intestinal permeability and the production of the pro-inflammatory cytokines TNF-α and IL-6 and of the anti-inflammatory cytokine IL-10 in the colon of adult F1 males. In F1 females exposed to *fg*-SiO_2 a decrease in TGF-β and an increase in IFN-γ secretion were observed in gut mucosa without intestinal permeability alteration. The systemic immune response following exposure to the food additive also differed between the sexes, with a decreased production of TNF-α, IL-6, IFN-γ, IL-17, IL-10 and TGF-β in males, while an increase in the secretion of IL-17 and TGF-β was found in females only. Changes in gut microbiota composition occurred in *fg*-SiO_2-exposed males only, exhibiting increased β-diversity and decreased proportion of the Actinobacteria and of the species *Akkermansia muciniphila* and *Bifidobacterium pseudolongum*, both known to alleviate metabolic disorders. Accordingly, an increased glucose intolerance was observed in F1 males exposed to *fg*-SiO_2, while no alteration of glucose metabolism was reported in *fg*-SiO_2-exposed F1 females. **Conclusions:** These results showed that long-term exposure to *fg*-SiO_2 from *in utero* life until adulthood initiate the development of metabolic disorders in a sex-dependent manner *via* an alteration of the gut microbiota and of systemic immune response associated with gut inflammation and permeability in males only. This

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