2212 Effect of Particle Size on the Cytotoxicity of Zinc Oxide Nanoparticles in Rat and Human Intestinal Cell Models

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Zinc oxide nanoparticles (NPs) have several commercial applications ranging from catalysts to semiconductors. ZnO NPs are toxic to bacteria, aquatic organisms and human cells. There is a potential for human oral exposure to ZnO NPs following accidental or intentional ingestion, hand-to-mouth activity, or mucociliary transport following inhalation. This study assessed the cytotoxic effects of ZnO NPs (10 and 150 nm) in rat and human intestinal cells. The rat cells are a 2-dimensional model (IEC-6) while the human cells are a 3-dimensional highly differentiated model. Three-dimensional cell cultures offer greater predictability of *in vivo* toxicity than comparable 2-dimensional cell cultures because of their complexity and their overall functions are more similar to native tissues. The effect of dose (0.1 - 100 µg/mL rat; 1-100 µg/mL, human), time (4 and 24 h) and particle size were evaluated. $ZnSO_4$ (0.1-100 µg Zn/mL) was tested for 4 and 24 h to assess Zn ion toxicity. IEC-6 cells were plated at 60K/well in a 96 well plate 24 h before dosing. Media with 10% fetal bovine serum was the negative control. Triton X-100 (0.3%) was the positive control. ZnO NPs were suspended in media and probe sonicated before dosing. Following incubation, the cells were washed with media. Cytotoxicity was assessed using a colorimetric method that measures mitochondrial activity (MTS, rat; MTT, human). In IEC-6 cells, a significant dose-dependent ($p<0.0001$) decrease in viability was observed after 4- and 24-h incubation of the 10 and 150 nm ZnO NPs. For both particles in IEC-6 cells, at 4 h, viability decreased ~50% at ≥ 50 µg/mL; at 24 h, viability decreased ~75% at ≥ 25 µg/mL. In human cells, viability was significantly decreased, but no greater than 10% at 4 or 24 h, for both ZnO NPs. For $ZnSO_4$, similar results to the ZnO NPs were observed in both tissues at 4 and 24 h. Particle size does not appear to have a role in the cytotoxicity of ZnO NPs in these cells. In addition, the similar cytotoxicity profile of ZnO NPs and Zn^{2+} ions suggests dissolution of the NPs may have a greater impact than particle size. *This abstract does not necessarily represent US EPA policy.*

2213 Effects of Surface Modification of Graphene Quantum Dots on Differentiated THP-1 Human Macrophages

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Due to their unique chemical and physical properties, graphene quantum dots (GQDs; <10 nm in diam) are attractive for biomedical applications such as bio-imaging and drug delivery. Previous studies show that bare GQDs cause DNA damage and significantly increase the expression of proinflammatory cytokines (including TNF-α and IL-8) in macrophages at concentrations where no significant toxicity is observed (<50 µg/mL). However, these studies have been limited to bare GQDs without taking surface modification effects into consideration, which has the potential to passivate the GQDs surface and minimize toxicity. The objective of this study was to investigate the effects of bare, carboxyl-, and amino-coated GQDs on differentiated THP-1 human macrophages. Nanoparticle size distribution was assessed by transmission electron microscopy, atomic force microscopy, and dynamic light scattering in water and cell culture medium. GQDs exhibited an average diameter (TEM) of 5-10 nm and surface charges of -22 mV and +19 mV for carboxyl- and amino-coated GQDs, respectively. Aggregation was observed when GQDs were dispersed in the serum-rich cell culture medium, which is probably associated with protein corona formation. Cells were exposed to bare, carboxyl-, and amino-coated GQDs at concentrations of 15, 100, or 250 µg/mL for 24 and 48 hr. No significant cytotoxicity was observed for bare and carboxyl-coated GQDs at all concentrations. For amino-coated particles, a decrease of 40% in cell viability was observed at concentrations >100 µg/mL after 48 hr. The cytotoxicity pathway (e.g., apoptosis vs. necrosis) and release of cytokines were also assessed by flow cytometry and ELISA, respectively. Our findings indicate that the surface coating of GQDs significantly affects particle uptake and toxicity in human macrophages. Further studies are needed to build the toxicological profile of GQDs before use in biomedicine applications.

2214 Molecular Toxicity Analysis of Citrate Gold Nanoparticle-Treated Human Intestinal CaCo-2 Cells by Array Gene Expression Profiling

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Some *in vitro* studies have shown citrate gold nanoparticles (cAuNPs) to be safe for nanomedicine applications (not toxic) via viability assay, while some have shown that toxicity is size-dependent. Meanwhile, in a previous study (Sibuyi et al., 2017), the application of peptide functionalised cAuNPs was established for selective induction of apoptosis in CaCo-2 cells. Unfunctionalized cAuNPs were shown to accumulate inside the cells with no discernible toxicity. However, very little is known about how the uptake of cAuNPs may affect gene expression patterns within cells. Any adverse effects of cAuNPs on gene expression may hamper the use of AuNP in nanomedicine. Consequently, this study aimed to investigate the effects of cAuNPs on gene expression in CaCo-2 cells. Monodisperse, 14 nm spherical cAuNPs were synthesised using the Turkevich method. CaCo-2 cells were treated for 24 hrs with 12.5 nM cAuNPs. The viability and uptake of cAuNPs in CaCo-2 cells were monitored using WST-1 assay and ICP-OES, respectively, and the expression levels of a panel of 84 genes that are related to molecular toxicity were evaluated using quantitative RT-PCR and the RT2 Profiler PCR array. Although the WST-1 assay shows no toxicity of cAuNPs to CaCo-2 cells, gene expression profiling revealed that internalisation of cAuNPs affects the expression of several genes associated with endoplasmic reticulum stress, DNA damage and repair, immunotoxicity, oxidative stress and antioxidant and mitochondrial energy metabolism pathways.

2215 Evaluation of the Skin Sensitizing Potential of Gold Nanomaterials and the Impact of Established Dermal Sensitivity to Gold on the Pulmonary Immune Response with Respect to Dose Mass and Surface Area

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The easily-manipulated physico-chemical properties of gold nanomaterials (AuNM) have proven useful in many biomedical applications. However, the historical use of gold for the treatment of rheumatoid arthritis and the known sensitizing capacity of gold salts suggest that AuNM may exhibit immunomodulatory properties. To address this, three studies were performed using female C57BL/6 mice. First, the skin sensitizing capacity of different forms of gold was assessed in the Local Lymph Node Assay (LLNA) using soluble gold salt (AuCl, 10%) and increasing concentrations (2.5, 5, 10%) of gold particles (Au, 12.1 um) and spherical AuNM (30 nm). Next, the pulmonary immune effects of AuNM (10, 30, or 90 ug) were assessed 1 d, 4 d, and 8 d post-aspiration. Finally, the impact of existing dermal sensitivity to gold on the pulmonary immune response to different forms of gold was assessed. Mice were dermally sensitized to gold by three dermal exposures (1 d, 2 d, 3 d) to 10% AuCl or vehicle control (VC). Mice were then aspirated once (10 d), twice (10 and 14 d), or three times (10, 14, and 18 d) with VC, 30 ug Au, or a dose of AuNM normalized to the mass or surface area of the 30 ug Au (30 ug or 0.2 ug, respectively) and euthanized 1 d later. In the LLNA study, AuCl had a stimulation index (SI) of 10.9, in accordance with its known potent sensitizing capacity. Although the SI of AuNM (2.3) was higher than that of Au (1.1), a three-fold increase in lymphocyte proliferation was not observed for either particle, indicating minimal risk for dermal sensitization. In the dose-response study, AuNM induced only minimal lung injury and inflammation. However, exposure to the 90 ug dose did induce a significant increase in total number and percent activated CD4+ T-cells and B-cells in the mediastinal lymph nodes at 4 and 8 d. In the allergy study, after two and three aspirations, mice sensitized to gold exhibited elevated lung lymphocyte numbers which correlated to dose surface area. Moreover, serum IgE levels were significantly increased only in dermally-sensitized mice aspirated with AuNM, irrespective of dose, indicating a potential for increased susceptibility to the development of an IgE-mediated adaptive immune response following respiratory exposure. Collectively, the results from these studies suggest existing dermal sensitivity to gold may exacerbate the pulmonary immune effects resulting from AuNM inhalation.

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