31 nm particle having the highest potency. Only one of the three cerium dioxide nanoparticles, 8 nm, showed a significant dose-response relationship ($p<0.001$) with respect to cytotoxicity. The cytotoxicity for this nanoparticle at 500 μg/ml was 40.7%. For the titanium (31 nm) and cerium dioxide (8 nm) nanoparticles with the highest cytotoxicity at a concentration of 500 μg/ml, the relative intensity of oxidant formation was increased 151- and 47-fold, respectively, relative to control. Reactive oxygen species appear to be formed in response to exposure to titanium and cerium dioxide nanoparticles and contribute to cytotoxicity in HaCaT cells. (This abstract does not necessarily represent U.S. EPA policy.)

## PS 1939 *In Vitro* Cardiotoxicity Screening of Silver and Metal Oxide Nanoparticles Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Exposure risk to silver and metal oxide nanoparticles (NPs) continues to increase due to their widespread use in products and applications. *In vivo* studies have shown Ag, $TiO_2$ and $CeO_2$ NPs translocate to the heart following various routes of exposure. Thus, it is critical to assess NP systemic toxicity including their effects on the heart and identify properties regulating NP cardiotoxicity. This study examined the cardiotoxicity of 4 Ag (10 or 110 nm,) citrate (cit) or polyvinylpyrrolidone (PVP) coated, 3 $CeO_2$ (<7 - 105 nm), 3 $TiO_2$ (10 - 40 nm) NPs using human induced pluripotent stem cell-derived cardiomyocytes (CM). Metal oxide NPs were sonicated in medium containing 20% fetal bovine serum while Ag NPs were resuspended without sonication. Cytotoxicity was determined using Cell Titer Blue, MitoTracker Deep Red, and nuclear staining assays at 48 h post-exposure to 3 – 50 μg/ml of NP or $AgNO_3$. CM function was monitored using microelectrode array technology measuring field potential duration, beat period, sodium spike amplitude, beat rate prior to exposure and at 1, 24, and 48 h post-exposure to each NP at 3 or 25 μg/ml, or $AgNO_3$ at 3 μg/ml. CM isoproterenol (ISO) (25 and 50 nM) responses were assessed at 48 h post-exposure. Ten nm Ag cit or PVP NPs were cytotoxic to CM at 50 μg/ml and $AgNO_3$ was cytotoxic at 6.3 μg/ml. At 3 μg/ml, only the <7nm $CeO_2$, 10 nm Ag cit or PVP coated NPs decreased all CM functional endpoints and ISO responses at 24 and 48 h post-exposure, while $AgNO_3$ effects were not identical to Ag NPs. Our results demonstrate: i) altered electrophysiology is a sensitive endpoint to assess human NP CM toxicity; ii) size, composition and coating regulate human NP cardiotoxicity; and iii) establishment of an alternative model for human CM NP toxicity testing. (This abstract does not reflect Agency Policy)

## PS 1940 Tracking Translocation of Industrially Relevant Engineered Nanomaterials across Alveolar Epithelial Monolayers *In Vitro*

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Relatively little is known about the fate of industrially relevant engineered nanomaterials (ENMs) in the lungs regarding translocation across the epithelial lining layer. Such processes may lead to subsequent effects on particle clearance, toxic effects or both. To allow precise quantitation of translocation across lung epithelial cells, we developed a method for tracking metal oxide ENMs *in vitro* using neutron activation. The versatility and sensitivity of the proposed *in vitro* epithelial translocation (INVET) system was demonstrated using a variety of industry relevant ENMs including CeO2 of various primary particle diameter, ZnO, and SiO2-coated CeO2 and ZnO particles. ENMs were neutron activated, forming gamma emitting isotopes 141Ce and 65Zn, respectively. Calu-3 lung epithelial cells cultured to confluency on transwell inserts were exposed to neutron-activated ENM dispersions at sub-lethal doses to investigate the link between ENM properties and translocation potential. The effects of ENM exposure on monolayer integrity was monitored by various methods. ENM translocation across the cellular monolayer was assessed by gamma spectrometry following 2, 4 and 24 h of exposure. Our results demonstrate that ENMs translocated in small amounts (e.g.<0.01% of the delivered dose at 24 h), predominantly via transcellular pathways without compromising monolayer integrity or disrupting tight junctions. It was also demonstrated that the delivery of particles in suspension to cells in culture is proportional to translocation, emphasizing the importance of accurate dosimetry when comparing ENM–cellular interactions for large panels of materials. The reported INVET system for tracking industrially relevant ENMs while accounting for dosimetry can be a valuable tool for investigating ENM–cell interactions in the future.

## PS 1941 Effects of Metal Oxide Nanomaterials on Cytotoxicity and Immune Response in THP-1 Cells

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[Purpose] Nanomaterials are now widely used in various fields of science, technology and medicine. However, there are many unclear safety issues because they are new materials. An *in vitro* cellular toxicological study using well-characterized nanomaterials is conducted for the evaluation of the biological effects of nanomaterials. In this study, we examined cytotoxicity and immune response of metal oxide nanomaterials in human hematopoietic cell line THP-1. [Methods] $Al_2O_3$, $CeO_2$, ITO, $SiO_2$, $TiO_2$, CuO, NiO and ZnO were examined in this study. The physicochemical properties, such as the size distribution and the zeta potential were measured by dynamic light scattering. After the nanomaterials were exposed to THP-1 cells, the cytotoxicity was assayed by using intracellular ATP method and the immune response was evaluated by measuring cytokines in the culture medium. [Results and Discussion] The hydrodynamic diameter of nanomaterials observed in suspension and culture medium were 41 to 224 nm and 153 to 407 nm, respectively. Although the zeta potentials of nanomaterials measured in suspension were positive (+45 to +62 mV), except $SiO_2$ and ZnO(Alfa Aesar) (-54 and -7.5 mV), all of the zeta potentials measured in culture medium were negative (-22 to -8.2 mV). Cytotoxicity in THP-1 cells was observed following treatment with CuO, ZnO and NiO. As the results of cytokine measurement in the cell culture supernatant, IL-8 content was increased following treatment with CuO, ZnO and NiO in a dose-dependent manner. TNF-α content was slightly increased by ZnO and NiO treatment. On the other hand, CuO, ZnO and NiO did not affect IL-6 and IL-1β productions. In conclusion, THP-1 cell showed both cytotoxicity and cytokine release following treatment with CuO, ZnO and NiO, but the effect on these cellular responses was different in each nanomaterial. A further molecular-level analysis would help the better understanding of the relation between biological effects and the physicochemical properties of nanomaterials.

## PS 1942 Nanoparticles Exacerbate Drug-Induced Phospholipidosis

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Nanoparticles (NP) have been applied to load small molecules and to target specific sites in both *in vitro* and *in vivo* studies. However, there is a possibility that drugs may be retained together with NP due to the intracellular accumulation of NP in which drugs becomes trapped. Amiodarone (A) and ketoconazole (K) are small molecules that can induce phospholipidosis and wereloaded onto silica dioxide, graphene and graphene quantum dot NP. The aim was to investigate the effects of NP on drug-induced phospholipidosis. These two drugs were loaded onto three NP followed by incubation with Raw264.7 and HepG2 cells for 48h. N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-snglycero-3-phosphoethanolamine (NBD-PE) was used as a marker for quantifying phospholipidosis. These results showed that all three types of NP induced phospholipidosis in Raw264.7 and HepG2 cells. NP/drugs A and K co-incubation enhanced drug-induced phospholipidosis, especially at the low concentrations of A and K like 2μg/ml. In addition, the culture medium containing A and K and NP were removed and replaced with fresh medium, to determine if phospholipidosis could be attenuated. Phospholipidosis in cells incubated with A and K loaded NP were attenuated slower than cells incubated with drugs only. Lastly, real-time PCR analysis showed that NP loaded with A and K further altered the expression of several phospholipidosis markers such as LSS, FABP1, HPN, INHBE compared to cells treated with drug only. Therefore, these findings suggest the potential risk of NP for drug delivery especially when drugs might have side effects on human cells.

## PS 1943 Proteome Alteration and Toxicity Induced by Ag Nanoparticles in an Intestinal Coculture Model

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Introductions and objectives: This study aims at establishing a more physiological intestinal co-culture model with the use of Caco-2 and HT29 cells in order to evaluate the effects of Ag particles (20nm and 200nm) on the intestinal compartment *in vitro.* Methods: Metabolic activity was evaluated by alamar blue assay,

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