An Evaluation of the Potential Phototoxicity of CeO_2 Nanoparticles in Retinal Pigment Epithelial Cells *in-vitro*

Pantlin, L., Orentzio, J., Holub, V., Degn, L., Zucker, R., Boyes, W.

Cerium dioxide (CeO_2) engineered nanoparticles (NP) are used as fuel-borne catalysts in off-road diesel engines, which can lead to exhaust emissions of respirable CeO_2 NP. Other metal oxides may act as photo-catalysts which induce the generation of free radicals upon exposure to UV radiation or visible light. The current study tested the hypothesis that CeO_2 NP would cause a dose-dependent phototoxic reaction in human-derived retinal pigment epithelial cells (ARPE-19) after UV exposure. Two samples of CeO_2 NP (Alfa Aesar, 36-99 nm; NanoAmor, 6-60 nm) were suspended in cell culture media with 10% fetal bovine serum (FBS) at concentrations: 0, 3, 10, 20, 30, 55, 100μg/ml or 20μg/ml TiO_2 NP (Degussa P25; positive control) and administered to ARPE-19 cells grown in 24-well plates. Plates were either exposed to UV radiation (t=90min) or kept in the dark. After 24hrs, cell viability was determined with a calcein-AM/propidium iodide stain. Exposure to higher concentrations of CeO_2 NP reduced cell viability in dark conditions. Exposure to UV light (290-400nm) reduced cell viability in CeO2 NP compared to dark plates. When a 2.5% CuSO4 solution filter restricted exposure to the UVA (320-400nm) range; there was no difference in viability between UVA exposed cells and dark controls. The results showed significant effects of irradiation ($F(2,165) = 3.87$, $p = 0.02$) and between the cerium samples ($F(2,165) = 1.91$, $p < .0001$) such that CeO_2 NP NanoAmor participated in phototoxic reactions with UVB, but not UVA wavelengths. Both samples of CeO_2 NP were less potent phototoxicants than TiO_2 NP. Dark-field microscopy confirmed CeO_2 NP uptake into ARPE-19 cells. At concentrations higher than 3μg/ml CeO_2 NP formed visible intracellular agglomerates, which were spatially arranged around the nucleus and associated with the endoplasmic reticulum and mitochondria. Thus, CeO_2 NP were taken up into ARPE-19 cells in culture, and showed slight phototoxicity in response to UV irradiation between 290-320 nm. *This does not reflect EPA policy.*