Effects of cerium oxide nanoparticle aerosol on human lung cells exposed at the Air-Liquid-Interface

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Most in vitro studies on aerosol health effects rely on submerged exposure of collected particulate matter, suspended in the medium. However this method does not represent the actual process in the human lung. It even changes the properties of the investigated aerosol. Particle exposure at the air-liquid interface can eliminate these disadvantages, but requires a well-engineered system to guarantee reproducible conditions (Paur et al., 2011).

Therefore, an advanced automated system for the exposure of cells to nanomaterial aerosols at the air-liquid interface was developed by KIT and VITROCELL Systems (Mülhopt et al., 2016). Airborne nanoparticles pass a size selective inlet and a conditioning reactor for flow-, temperature- and humidity-control before they are applied to the cells. The deposited particle mass is monitored by a quartz microbalance. The particle dose can be enhanced by an electrostatic field. An internal negative control using humidified synthetic air is implemented. A standard exposure protocol is executed automatically and all settings are controlled and the data are logged.

Aerosols of cerium oxide nanoparticles and two variants doped with zirconium oxide (provided by the NanoMILE consortium) were investigated. CeO_2 was chosen due to the fact that it can cycle between two redox states, Ce^{3+} and Ce^{4+}, which endows this nanomaterial with catalytic properties, and suggest a mechanism of activity based on oxidative stress. Doping with Zr was used to alter the redox activity. The use of CeO_2 nanoparticles in vehicle catalysts makes it relevant for exposure via inhalation.

For the ALI exposure a suspension of CeO_2 nanoparticles (primary particle size 20 nm) was aerosolized according the VDI guideline 3491 using a two phase nozzle with clean air. The dried aerosol was guided into the conditioning reactor and the particle size distribution was determined by Scanning Mobility Particle Sizer (Figure 1). A549 human cells were exposed to the aerosol for 4 hours and analyzed for viability and release of cytokines.

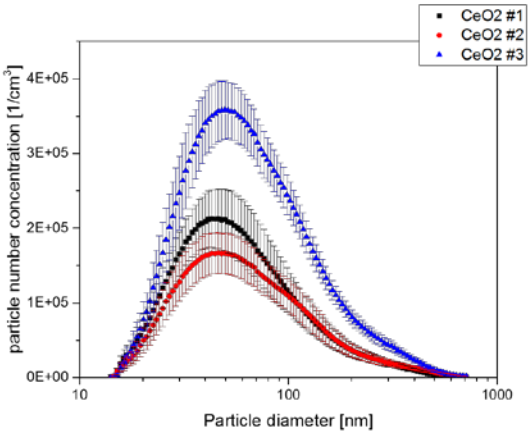

Figure 1. Number size distributions of different cerium oxide nanoparticles measured in the aerosol conditioning reactor of the VITROCELL Automated Exposure Station.

All CeO2 variants slightly enhanced LDH release after 4h exposure to a high dose but did not induce IL-8 release after 24 submerged post-incubation. We observed no differences between the CeO_2 variants with respect to their toxic properties.

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Paur, H.-R., Cassee, F.R., Teeguarden, J., Fissan, H., Diabate, S., Aufderheide, M., Kreyling, W.G., Hänninen, O., Kasper, G., Riediker, M., Rothen-Rutishauser, B., & Schmid, O. (2011). *Journal of Aerosol Science*, 42, 668-692.

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