

Workpage WP T-B: Toxicity in vivo

Lead: Harvard University

Objectives:

1. To evaluate the influence of surface modifications of nano-Ag and -CeO₂ in different aerosol formulations on their toxicological potential using a mouse PCLS system.
2. To evaluate the influence of surface modifications of nano-Ag and -CeO₂ in different aerosol formulations on their pulmonary toxic and inflammatory effects in intact mouse animal model.
3. To determine whether surface modifications of aerosolized nano-CeO₂ influence cerium lung clearance and extrapulmonary biokinetics in a mouse model.
4. To conduct a correlation study between in vitro and in vivo toxicity profiles
5. To assist WP M with recommendations for modeling.

Methodology :

1. Toxicological evaluations of surface-modified nano-Ag and -CeO₂ will be performed ex vivo using living mice PCLS-based assays. The PCLS will be prepared from 8 weeks old C57Bl/6 mice. The collapsed lung lobes will be infused with 1.5ml of 1.5% agarose in Hanks' balanced salt solution (HBSS) through the inserted catheter, followed by 0.5ml of air to clear the airways of agarose. Once the agarose solidified, the lungs will be removed from chest cavity and immersed and kept in cold HBSS. The left lobe will be sectioned into 250µm thick slices using a vibratome. The lung slices will be incubated in Dulbecco's Modification of Eagle's Medium/Ham's F-12 50/50 Mix (Corning) at 37°C overnight before each experiment or freezing for later testing. Lung slices will be cultured in 96-well plates. The first stage of this aim will make use of test materials collected on filters during aerosolization of various formulations (provided from WP E). The test materials will be added to each well, and at selected timepoints, several assays will be performed. The data obtained will guide the experimental design of exposure of PCLS to aerosols in real time.

This next stage will be performed at TU Dresden (WP E). Additionally, exposed PCLS will also be analyzed.

2. Assessment of in vivo pulmonary effects of surface-modified nano-Ag and -CeO₂ in C57Bl/6 mice using bronchoalveolar lavage and analyses. This experiment will determine whether and how surface modifications of nano-Ag and -CeO₂ in different aerosol formulations may modify nano-Ag and -CeO₂-induced lung toxicity and inflammation. Groups of mice will be instilled intratracheally with increasing doses of nano-CeO₂ or Ag suspension provided by WP E (Dresden). At selected timepoints later, mice will be euthanized and the lungs will be lavaged multiple times. The cells from all washes will be separated from the supernatant by centrifugation. Total cell count and hemoglobin measurements will be made from the cell pellets. Biochemical markers of lung injury (lactate dehydrogenase, myeloperoxidase, glucosaminidase and albumin) as well as inflammatory cytokines will be measured.
3. Biokinetic studies will be performed using nano-CeO₂ contained in collected aerosols provided by WP E (Partner 3). Collected nano-CeO₂ from the test rig developed in WP E will be used for evaluation of the cerium biokinetics. Samples from WP E group will be neutron activated at Massachusetts Institute of Technology (MIT) Nuclear Reactor Laboratory (Cambridge, MA, USA) with a thermal neutron flux of 5×10^{13} n/cm²/s for 24 hours. The activation process will generate the radioisotope ¹⁴¹Ce, useful for biokinetic studies. Mice will be used to determine if different MNM suspension preparations at BfR (described in WP PC) and aerosolized/characterized at Dresden (WP E) alter the pharmacokinetics of cerium after pulmonary exposure (intratracheal instillation).
4. The toxicity profiles obtained within this WP will be correlated with those gained in WP T-a of in vitro testing