Session I-5: Nanotoxicology and the Three Rs

Session I-5: Oral presentations

I-5-648

Alternative *in vitro* assays in nanomaterial toxicology

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Nanomaterials are acclaimed for their novel properties, for which broad new uses are being discovered with increasing frequency. It is obvious that, as the properties change, unwanted properties (toxicity) are to be expected as well. Today's toxicology, however, is already overwhelmed with the challenge of addressing new chemicals, not to mention the enormous number of old chemicals never properly assessed. Limitations of traditional approaches range from animal welfare issues, which were a strong driving force for alternative approaches (the 3Rs concept) over the last two decades, to aspects of throughput and accuracy of the predicted toxicities. The latter has prompted discussion about a revolutionary change in chemical safety assessment, now known as Toxicology for the 21st Century (Tox-21c). The multitude of possible formulations of nanomaterials to be assessed for novel toxic properties makes these alternative approaches especially attractive, given the well-recognized limitations of traditional animal-based approaches – limitations that might be even more pronounced for nanomaterials, which have notably altered biokinetics.

I-5-053

Three Rs and the safety assessment of nanotech drugs

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Many drug products are nanoparticles. Some were originally designed as nanoparticles, including monoclonal antibodies, and others were originally larger size particles that were later milled to nanosize to improve bioavailability. Drug nanosize products may include base particles, carriers or encapsulators, therapeutics, contrast agents, targeting molecules, chelators, diagnostics, or a combination of all of these. Judicious use of *in vitro* data can reduce the use of animals to assess the safety of nanodrugs. Before any studies are conducted, the material needs to be made reproducibly and be carefully characterized for critical attributes. *In vitro* assays can assess comparability across particle size ranges and stability in various media, and dermal penetration. Nanoparticles may distribute to tissues or organs that larger particles do not, such as crossing the placenta or the blood-brain barrier. Mouse stem cell assays might be used to bridge embryofetal effects of larger particles to nanosize particles, and various *in vitro* systems can assess penetration of the blood-brain barrier. Small bridging studies may be used to assess nanoparticles milled from larger particles.

I-5-351

Development of an integrated aerosol measurement system in the i-Lung

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Respiratory research can be divided into medical device testing and research of particle deposition in lungs, like aerosol inhalation. The necessity of testing the effects of aerosols on human health gained even more importance when the topics of fine dust, nano-particles and the safety of chemicals emerged. Amongst others, these issues have to be tested by using realistic models of the respiratory tract. Different kinds of mechanical lung simulators and numerical simulation models have been derived in the course of time.

The presented aerosol application and measurement system, using the novel lung simulator (i-Lung) as the core element, can be employed for the reduction of necessary laboratory animals according to the EU Cosmetics Directive. Additionally it allows the realisation of extensive test series, as demanded by the EU REACH regulation, with an appropriate number of animals. The simulation of a physiologically or pathologically breathing human lung can be performed by using different lung equivalents, like latex bags of different sizes or primed porcine lungs. In the presented test setting, the simulation device has been synchronised and combined with an aerosol spectrometer with a white light aerosol sensor in order to detect in- and exhaled particles in a size range of 0.2-10 μm. The used lung equivalents respired a DEHS aerosol produced beforehand. Particles entering and exiting the lung were counted. The measurement results show a remarkable degree of separation of the aerosols during in- and exhalation, if a primed porcine lung is used as human lung equivalent.

I-5-425

A simple method for testing the toxicity of nanomaterials on 3D air-liquid interface human airway epithelia (MucilAir™)

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We developed a simple method to deliver nanoparticles to air-liquid interface (ALI) culture systems. This patented method (PCT/IB2010/053956) uses Dextran as carrier, which allows testing a wide range of doses of nanoparticles. Briefly, the nanoparticles were diluted and mixed with the Dextran powder; small pellets were made and then applied onto the apical surface of the ALI culture. We tested the toxicity of several nanoparticles, such as ZnO and $Fe(IO_3)_3$, on an *in vitro* cell model of the human airway epithelium (MucilAir™). MucilAir™ closely mimics the morphology and functions of the normal human airway epithelium. Moreover, it has a unique shelf-life of one year, allowing chronic/long term toxicity testing. Using multiple endpoints, like trans-epithelial electrical resistance (TEER), cell viability assay (LDH), cilia beating frequency, morphology, cytokine release, etc., we determined the dose response curve of ZnO and $Fe(IO_3)_3$ nanoparticles on MucilAir™. Toxicity of ZnO (9 nm) was observed at doses higher than 0.1% (9 $\mu g/cm^2$). Interestingly, at 0.1% of ZnO, the epithelia had the potential to recover/repair after the exposure, while at 0.5% (45 $\mu g/cm^2$) of ZnO, this was not the case. Effects of two forms of $Fe(IO_3)_3$ from 10 to 20 nm were also compared, namely spheroids and nanoneedles.

Our results showed that the Dextran-carrier method is an easy and efficient way to deliver the nanoparticles *in vitro*. MucilAir™ is a relevant *in vitro* system for assessing the toxicity of nanoparticles.

I-5-432

Use of normal human 3-dimensional (NHu-3D) tissue models (EpiDerm, EpiAirway) for nanotoxicology applications

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Nanomaterials are increasingly utilized in numerous commercial applications where dermal contact, inhalation or oral ingestion is likely. However, their toxicological properties are largely unknown. Potential adverse effects of nanoparticle exposure include allergenicity, cytotoxicity and genotoxicity. Nanomaterials may enter the body by interacting with, and eventually crossing, epithelial barriers including skin, airway and intestinal epithelium. Once inside the body, additional interactions with internal organs, such as heart, liver, brain, kidney and others, are possible. Therefore, there is an urgent need for animal alternative tissue models that can be utilized for toxicological evaluation of nanoparticle materials. This poster summarizes use of *in vitro* NHu-3D skin (EpiDerm, EpiDerm-FT) and airway (EpiAirway) models for nanotoxicology applications. Notable applications to date include the use of: 1) the EpiDerm model to study potential health implications of cerium oxide nanoparticulates (SafePharm Laboratories, UK), 2) EpiDerm to investigate skin irritation/toxicity potential of nanosilica particles (Korea University College of Medicine), 3) the EpiDerm-FT skin model to evaluate skin penetration of quantum dot nanoparticles (Korea University College of Medicine), 4) EpiDerm-FT for investigations of single-walled carbon nanotubes (NIOSH, USA), 5) EpiDerm-FT to investigate the effect of nanoparticle formulations on wound healing (Free University Berlin) and 6) the EpiAirway model for *in vitro* determination of nanoparticle translocation through airway epithelium (Procter and Gamble, USA). These studies demonstrate that *in vitro* NHu-3D models are useful tools for the study of nanoparticle interactions and potential toxicologic effects on epithelial tissues.