Code Number and Title

LRI-N5: Biokinetics and long-term effects of inhaled nanoparticles (Bariumsulfate)

Background

A lot of data are known regarding acute and subacute toxicity of nanomaterials, whereas the long term outcome of inhalative exposure to nanomaterials is still unclear. Long-term inhalation exposure data are only available for nano-TiO_2 and Carbon black particles at high aerosol concentrations. These studies indicated a chronic inflammation and subsequent tumor formation in the lungs. It is under discussion which mode of action is causative for the tumor formation.

Within the European NANoREG project and under the umbrella of the BMU (The Federal Environment Ministry, Germany) a cooperation project between BASF SE, Germany and the BAuA (Federal Institute for Occupational Safety and Health), BfR (Federal Institute for Risk Assessment) and UBA (Federal Environment Agency) for conducting and evaluation of a chronic inhalation study with nanomaterials was set up. Goal of this study is to derive profound conclusions of the outcome of inhalative long term exposure with selected nanomaterials. The test compounds are Ceroxid (CeO_2) at several dose levels, and Bariumsulfate ($BaSO_4$) at one high dose. The two particles are expected to cover a wider range of different biokinetic behaviours and toxicological responses with Bariumsulfate being more rapidly cleared and less toxic.

Objectives

CeO_2 and $BaSO_4$ are tested in the long-term inhalation study. While CeO_2 exposed animals are evaluated within the EU project NanoREG, this Cefic-LRI call will only concern $BaSO_4$ exposed animals. The objective of this project is the investigation of the (i) effects and (ii) biokinetics of nanoparticles inhaled for a life-time.

<u>Investigations of effects:</u>

This includes histopathological processing (from wet formalin-fixed tissue to stained HE slides) as well as light microscopic evaluation and assessment of the groups treated with $BaSO_4$ after 24 and 30 months of treatment (50 animals/time-point). The spectrum of histopathology has to be performed according to the following guidelines OECD 453 (Combined Chronic Toxicity\ Carcinogenicity Studies; 2009) and OECD 413 (Subchronic Inhalation Toxicity: 90-Day Study; 2009). The organ trimming has to be performed according to the published RITA guidance documents (“Revised guides for organ sampling and trimming in rats and mice”; Ruehl-Fehlert C et al. 2003, Kittel B et al. 2004, Morawietz G et al. 2004). The diagnostic criteria have to be used according to INHAND (International Harmonization of Nomenclature and Diagnostic Criteria). The respiratory tract, esp. lungs, has to be evaluated in an extended way (serial sections of lung tissue).

The study pathologist should be a certified veterinary toxicopathologist with extensive experience in lung toxicopathology. A histopathological peer-review by a second pathologist is highly recommended. It should be possible to present all results within a PWG (Pathology Working Group), if necessary.
This works have to be done strictly under GLP. A GLP-conform pathology phase report with all data (summary and single data) has to be written.
Because the original study is a GLP study, the provided finalized pathology phase report will be part of the overall study report.

Investigations of biokinetics:
The lung deposition, lung clearance and potential extrapulmonary translocation of particles (or material from particles) in the lung is one key factor in understanding the long-term effects. Hence investigations on lung burdens, burdens of lung associated lymph nodes and extrapulmonary organs shall be performed and provide a mass balance of the body's total burden (using e.g. ICP-MS methods). Moreover, the localisation of the material in the tissue shall be determined and the identity of the material (original particle, alter particle or ions released from particles) should be elucidated (using e.g. MALDI-TOF analysis or neutron-activation analysis).

Work Plan
104 female Wistar rats are exposed to Bariumsulfate aerosols at 50 mg/m^3 by whole body exposure for two years. 50 Animals will be examined after two years of exposure and another 50 animals after additional 6 months without further exposure.

For histopathological examinations: The wet formalin-fixed tissue will be provided by BASF SE, Germany, within 3 months after final sacrifice of the animals (final sacrifice June and December 2015). The histotechnique has to be performed in an adequate time frame (ca. 6 month). All histopathological evaluation and assessment has to be performed under GLP and shall be finished 18 months after receipt of the wet tissue. The finalized pathology phase report shall be available 24 months after receipt of the wet tissue.

For biokinetic investigations: The wet formalin fixed tissue is available (as far as it is not used for histopathology) as well as additional 4 animals which can be processed as needed. Information on Bariumsulfate mass burdens of the lung, burdens of lung associated lymph nodes and burden of RES tissues as well as other relevant organs are to be measured by appropriate analytical methods. Information on the localisation of the material in the respective tissues as well as discrimination of Bariumsulfate particles and Barium-ions in the respective tissues should be obtained by appropriate methods. These investigations have to be finished 18 months after receipt of the tissues. The finalized report shall be available 24 months after receipt of the tissue.

Deliverables

Researchers are encouraged to present the results of their work at scientific symposia and publish in peer reviewed scientific journals and submit their data to appropriate on-line repositories. An outline of delivery of the science should be included in the proposal. Progress of the research will be reviewed periodically at agreed times with CEFIC LRI.

Cost and Timing

Budget in the order of € 280,000
Start in 2015, duration 2 years

Partnering/Co-funding

Applicants should provide an indication of additional partners and funding opportunities that can be appropriately leveraged as part of their proposal. Partners can include, but are not limited to industry, government/regulatory organizations, research institutes, etc. Statements from potential partners should be included in the proposal package.

Fit with LRI objectives/Possible regulatory and policy impact involvements/ Dissemination

Applicants should provide information on the fit of their proposal with LRI objectives and an indication on how and where they could play a role in the regulatory and policy areas. Dissemination plans should also be laid down.

References:

Ruehl-Fehlert C, Kittel B, Morawietz G, Deslex P, Keenan C, Mahrt CR, Nolte T, Robinson M, Stuart BP, Deschl U (2003) Revised guides for organ sampling and trimming in rats and mice - Part 1. *Exp Toxicol Pathol* 55: 91–106

Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, O'Sullivan MG, Roth DR, Wadsworth PF (2004) Revised guides for organ sampling and trimming in rats and mice - Part 2. Exp *Toxicol Pathol* 55: 413–431

Morawietz G, Ruehl-Fehlert C, Kittel B, Bube A, Keane K, Halm S, Heuser A, Hellmann J (2004) Revised guides for organ sampling and trimming in rats and mice - Part 3. *Exp Toxicol Pathol* 55: 433–449

DEADLINE FOR SUBMISSIONS: 31 January 2015

Please visit www.cefic-lri.org for general information about the LRI funding programme, guidelines for grant applications and links to application documents.

For further assistance do not hesitate to contact the LRI Secretariat by e-mail at lri@cefic.be or by phone on 0032 (0)2 676 7368.