Pathological effects and biokinetics of life-time inhaled Barium sulfate nanoparticles

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Introduction

A lot of data are known regarding acute and subacute toxicity of nanomaterials, whereas the long term outcome of inhalation exposure to nanomaterials is still unclear. Long-term inhalation exposure data are only available for nano-TiO2 and Carbon black particles at high aerosol concentrations. These studies indicated a chronic inflammation and subsequent tumor formation in the lungs (Heinrich et al., 1995).

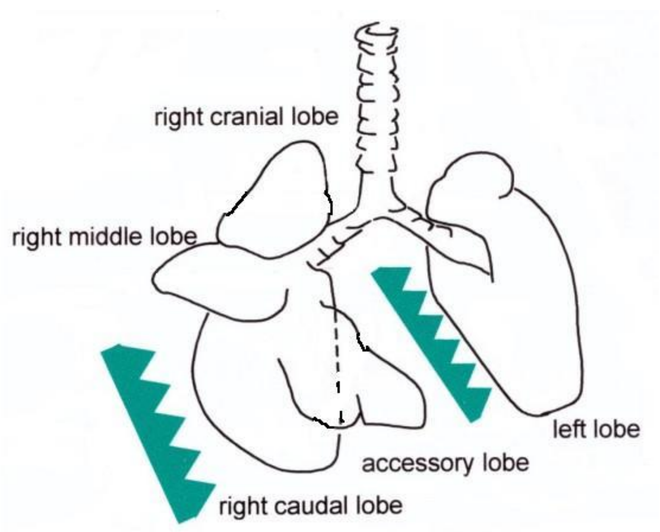
A co-operation project was set up between the BMU (The Federal Environment Ministry, Germany), BASF SE, Germany, and competent authorities as BAuA (Federal Institute for Occupational Safety and Health), BfR (Federal Institute for Risk Assessment), and UBA (Federal Environment Agency) for conducting and evaluation of a chronic inhalation study with nanomaterials which was amended by the European project NANoREG (7th framework programme) and also by the Cefic LRI project. Goal of this study is to derive profound conclusions based on the outcome of long term inhalation exposure with selected nanomaterials. The ongoing inhalation study with nanomaterials is the first and only study providing data on the type and potency of long-term exposure to nanoparticles. The outcome of the study will be a basis of future regulations of 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. The project has an immediate impact on the regulation of nanomaterials: the results of the project will be the basis of future regulations of nanomaterials both on occupational exposure levels and cancer classifications. The chronic $BaSO_4$ exposed animals (12 month interim sacrifice) within this combined Chronic Toxicity\-Carcinogenicity Study (OECD 453) are already sacrificed and first investigations (bronchoalveolar lavage, organ burden, histopathology) are performed and published by BASF (Keller et al., 2014). Some unexpected results after 52 weeks of $BaSO_4$ exposure might be solved in the context of this proposal after life-time exposure. Previous work with very high technical standards and harmonized histopathologic nomenclature over several studies (1 week, 4 week, 13 week, 52 week of exposure) is already done.

Workpackages

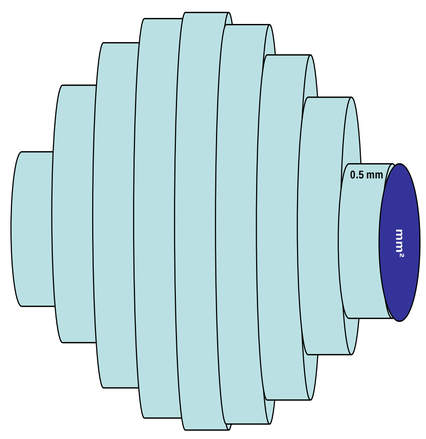
Histopathology:

For the investigation of biological (i) effects, all organs of all animals of the exposed $BaSO_4$ groups (24 month and 30 month time-point of scheduled sacrifice as well as all animals, unscheduled died or sacrificed animals, in total 100 animals) will be processed histotechnically and assessed by light microscopy. The scope of investigation will cover all organs recommended by OECD Guideline 453 (Combined Chronic Toxicity\-Carcinogenicity Studies; 2009) and OECD 413 (Subchronic Inhalation Toxicity: 90-Day Study; 2009). The lungs will be evaluated in an extended way (at intervals of 500 µm to produce multiple up to 60 per lung/rat step sections) to ensure all relevant lesions are included and to calculate the volume of possible pre-neoplastic and neoplastic lesions.

Trimming of the lung:



Calculation of approximate tumor volume:



To determine the tumor volume, all sections from a particular tumor are added up:

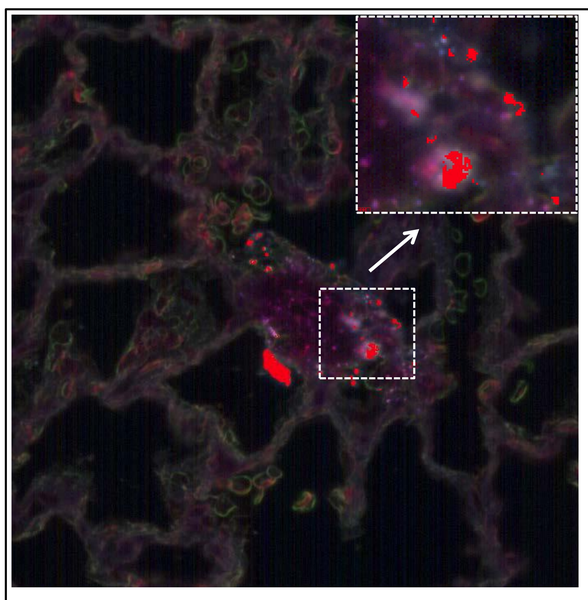
$$V = D * \sum_{i}^{n} Ai$$

n = number of section planes; A = surface area measured; D = section interval; i = respective section plane

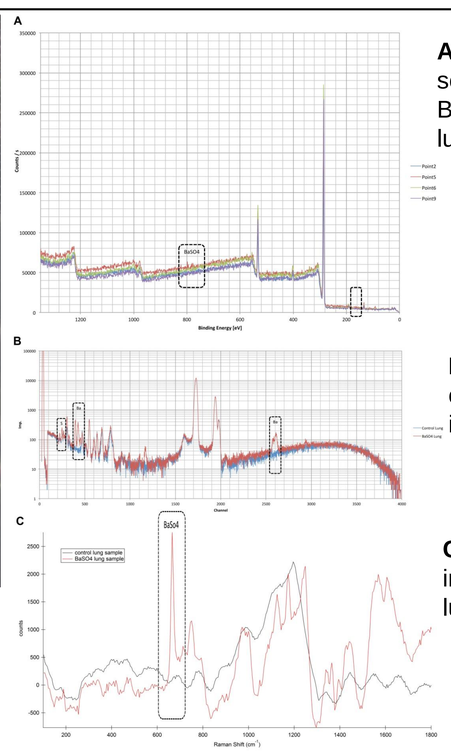
Biokinetics and Bioprocesses:

The tissues will be examined at different levels of resolution (gross – microscopic - ultrastructure) using the following technics:

- ICP-MS - to measure the total barium content of selected organs
- Light microscopic analysis utilizing hyperspectral imaging in the visible near-infrared and in the short wave infrared wavelength ranges (CytoViva® technology) (Husain M. et al., 2013) – to demonstrate the presence of particulate form of barium sulfate in tissue sections
- X-ray photoelectron spectroscopy (XPS) - to demonstrate the presence of barium sulfate in tissues
- X-ray fluorescence spectroscopy (XRF) - to demonstrate the presence of the element barium in tissues
- Raman Spectroscopy - to demonstrate the presence of barium sulfate particles in tissues
- Advanced electron microscopy including quantitative methods, ultra-structural analysis, atomically-resolved spectroscopy and imaging: HR-TEM/STEM with electron energy loss spectroscopy (EELS) elemental quantification (Graham et al., 2014).



CytoViva® image showing $BaSO_4$ particles (red) in rat lung 60 days post-intratracheal instillation of 5 mg/kg $BaSO_4$ nanoparticles.



A. XPS spectra of 4 selected points in $BaSO_4$-instilled rat lungs.

B. XRF spectra of control and $BaSO_4$-instilled rat lungs.

C. Raman spectra in $BaSO_4$-instilled lung.

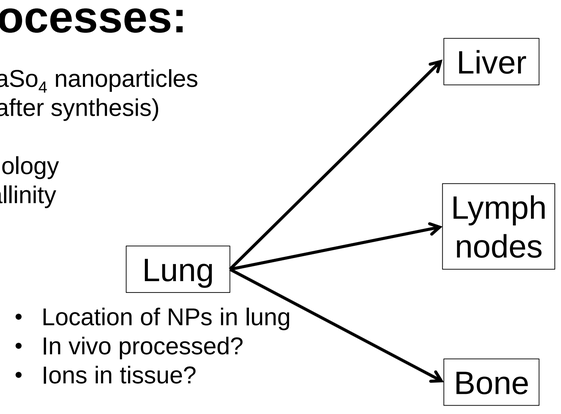
Since each of the techniques has varying sensitivity (limit of detection), additional rats instilled with 1, 10 or 50 mg/kg $BaSO_4$ NPs will be analysed over time (1 day-4 weeks) using the proposed techniques. We will examine the lungs, liver, bone and lymph nodes, the major sites of barium retention shown in previous study (Konduru, et al., 2014). The experimental plan is summarized in the table below:

Treatment	# of rats	Tissue for analyses	Sample Preparation	Analyses
Aerosol-exposed to $BaSO_4$	4	▪ Lungs ▪ Liver ▪ Bone ▪ Lymph nodes	▪ Formalin-fixed ▪ Glutaraldehyde-fixed ▪ Frozen	▪ ICP-MS ▪ CytoViva ▪ XPS ▪ XRF ▪ Raman spectroscopy ▪ HR-TEM/ STEM with EELS
Filtered air-exposed	2			
IT-instilled* with I, 10, 50 mg/kg $BaSO_4$	12			
IT-instilled* with dH_2O	4			

***intratracheally-instilled**

Bioprocesses:

Image $BaSo_4$ nanoparticles ex vivo (after synthesis)
- Size
- Morphology
- Crystallinity



Lung
- Location of NPs in lung
- In vivo processed?
- Ions in tissue?

Liver
- Location of NPs in liver
- In vivo processed?
- Ions in tissue?
- Imaging of NPs
- Analysis (EDS/EELS)

Lymph nodes
- Can we detect Ba?
- Form/Shape/Size
- Location of NPs in lymph nodes

Bone
- Location of NPs in bone
- In vivo processed?
- Ions in tissue?
- Imaging of NPs
- Analysis (EDS/EELS)
- Chemical mapping (EELS) of bone