A common European approach to the regulatory testing of nanomaterials

Organ burden, faeces analyses and particle detection pattern in other organs after chronic exposure Deliverable 4.6

Introduction

One of the key activities of the NANoREG project is a long term inhalation study according to OECD TG no. 453, aimed at filling knowledge gaps regarding the biokinetics, mode of action and possible carcinogenic effects of poorly soluble, biopersistant, nanomaterials.

The results of this study and sub studies are presented in a number of deliverables. Test design, exposure levels and operational parameters have been reported in Deliverable 4.1. Deliverables 4.2 to 4.7 report the results of sub studies. Deliverable 4.1 – 4.6 already have been submitted; deliverable 4.7 (histopathological evaluation) will be submitted in 2017.

The objective of the sub study reported in this deliverable was to investigate organ burden and particle distribution caused by chronic inhalation and also to compare the particle distribution in lung tissue by the use of imaging techniques. So far, no comprehensive data on the biodistribution of nanomaterials are available for chronic low dose exposure which is of particular relevance for consumer and occupational risk assessment.

Beside the estimation of the nanoparticle mass per organ, performed by inductively coupled plasma mass spectrometry (ICP-MS), this project included the assessment of the particle distribution by application of time of flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS allows for the visualization of particles on tissue level and enables for a size estimation of agglomerates. This is of particular relevance for risk assessment, because an important, currently still unanswered, question is if there is a de-agglomeration following to particle inhalation. The results will provide insight into nanospecific biokinetics and potential common characteristics of ultrafine dusts and nanoparticles.

Description of work

A two year inhalation study with cerium dioxide (NM-212 Ø 28 nm, source: JRC) has been performed according to OECD TG no. 453. Female Wistar rats were divided into five dose groups: 0 mg/m3 (control group), 0.1 mg/m3 (group 1), 0.3 mg/m3 (group 2), 1 mg/m3 (group 3) and 3 mg/m³ (group 4). After 3 and 12 months of exposure three animals per dose group were sacrificed. After 24 months of exposure another four animals per dose group (except control group: 2 animals) were sacrificed. The concentrations of CeO2 in liver, spleen, kidney, brain, heart, lymph nodes, small intestine, bone marrow, blood and faeces were determined after 3, 12 and 24 months of exposure for groups 1, 2, 3 and 4. In femur (named “bone” in the following) the concentrations were determined for all dose groups after 24 months of exposure only. Control groups were also examined to check the background of the tissue matrices.

Two in-house validations of analytical methods for sample preparation and quantification of cerium in bone and faeces were performed. All specified parameters and analytical conditions were met.

Main results and evaluation

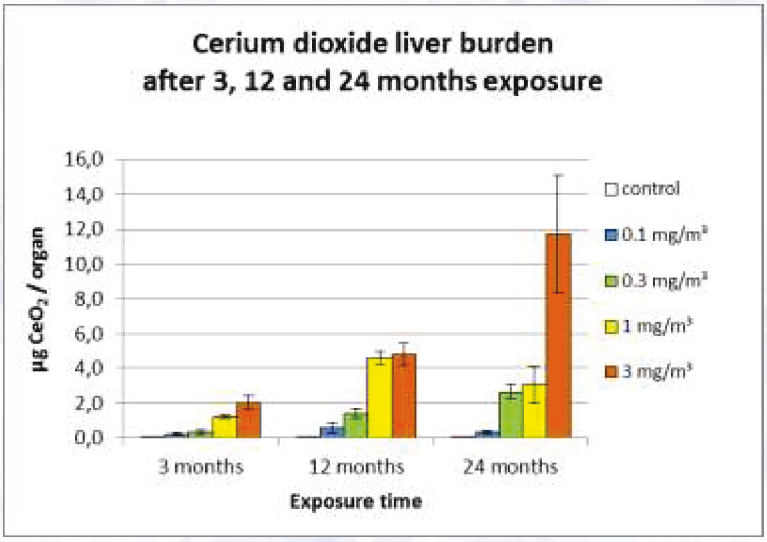
The validations of the sample preparation and quantification of cerium in bone and faeces were successful.

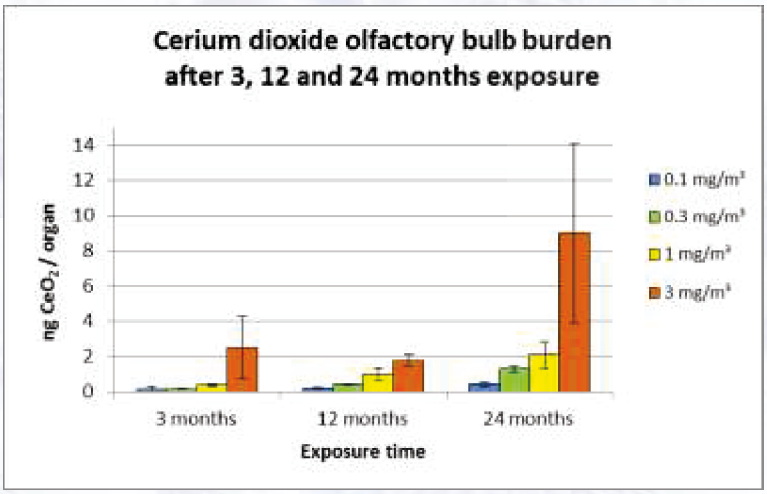
The quantification of cerium dioxide nanoparticles in tissues of lung, liver, spleen, kidney, brain, olfactory bulb, heart, mesenteric and lung associated lymph nodes, small intestine, bone marrow, bone, blood and faeces were performed in groups of 1, 2, 3, 4 and the control groups after inhalation for 3, 12 and 24 months.

The examination with inductively coupled plasma mass spectrometry (ICP-MS) revealed the following average CeO_2 distribution [CeO_2/organ] in the analyzed matrices within each dose group, except the control group:

- after 3 months: lung** > lung associated lymph nodes (LALN) > liver > kidney > spleen > bone marrow* > small intestine* > mesenteric lymph nodes > heart > brain > olfactory bulb** > blood*
- after 12 months: lung associated lymph nodes > lung** > liver > kidney > spleen > bone marrow* > small intestine* > mesenteric lymph nodes > heart > brain > olfactory bulb** > blood*
- after 24 months: lung associated lymph nodes > lung** > liver > bone > kidney > spleen > bone marrow* > small intestine* > mesenteric lymph nodes > heart > brain > olfactory bulb** > blood*

* referred to [CeO_2/g]
** reported in deliverable 4.5





The results show that there is a significant correlation between inhaled aerosol concentration, organ burden and faecal concentration, respectively. In all tissues despite blood, significant amounts of CeO_2 could be detected. Among all extrapulmonary matrices examined, concentrations of CeO_2 were highest in LALN as expected. Faeces, bone, bone marrow, liver, kidney and spleen contained lower concentrations. Only traces of the substance were detected in olfactory bulbs, blood, brain, heart and mesenteric lymph nodes for all dose groups 1-4.

Generally, CeO_2 concentrations increased with exposure time and with increasing dose. However, compared to the progression of lung burden between months 3 and 24 (deliverable 4.5), no linear increase of CeO_2 burden could be observed for any other organs or faeces.

Questions that remain to clarify are represented by the storage of CeO_2 in bone and the dissolution of CeO_2 under circumstances of low pH-values like in the liver.

For more details about NANoREG please visit the official website [www.nanoreg.eu.](http://www.nanoreg.eu)

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