A common European approach to the regulatory testing of nanomaterials

# Organ burden and particle detection pattern in other organs after subacute exposure
# Deliverable 4.4

## Introduction

The NANoREG project includes a long term (two year) inhalation study on Granular Biodurable Particals (GBP), aimed at elucidating the carcinogenicity of this group of nanomaterials under the conditions of chronic low dose exposure (task 4.1). Part of this study will be the analysis of different tissues as e.g. spleen, liver and kidney aimed at determining the accumulation and distribution of particles (task 4.3).

This Deliverable (together with Deliverable 4.3) is part of a 28 day inhalation study on $CeO_2$. It will serve as a basis for analysis of organ tissues out of the 2-year inhalation study. The study was also used to develop and validate all necessary methods.

## Description of Work

A 28-d inhalation study with cerium dioxide (NM212, Ø 28 nm) according to OECD TG 412, has been performed using four doses: 0 mg/m$^3$ (CG; control group), 0.5 mg/m$^3$ (LD; low dose), 5 mg/m$^3$ (MD; mid dose) and 25 mg/m$^3$ (HD; high dose). The selected $CeO_2$ is considered to be exemplary for the group of Granular Biodurable Particles without known significant specific toxicity (GBP).
The concentration of $CeO_2$ in the different organs was determined at the end of the exposure period (day 28) as well as on days 30, 36, 62, 92 and 156.

Slices of organs, which showed significant $CeO_2$ burden have been analyzed regarding the particle distribution (agglomerates, aggregates and their size).

## Main Results

With exception of liver, where $CeO_2$ occurred in the µg range, burden of peripheral organs was found to be in the ng range. For all peripheral organs analyzed, no linear correlation between dosage and $CeO_2$ burden was found.

The $CeO_2$ concentrations measured in peripheral organs confirm that a translocation of the substance into organs behind the respiratory tract occurs following to inhalation. However, measured concentrations are orders of magnitude below the amount of $CeO_2$ analyzed in lung. Contrastingly to the situation in the lung, no clear decrease of secondary organ burden over the post exposure period was observed.

## Evaluation of the results

The process of clearance appears to be similar for all dose groups. For all three dose groups there was an increase of $CeO_2$ from the last day of inhalation till day 8 post exposure (MD and HD) and day 34 post exposure (LD), respectively. After that, the $CeO_2$ levels decreased till day 64 post exposure, before the $CeO_2$ levels increased again (day 128 post exposure). This suggests a continuous translocation of $CeO_2$ from lung to liver, spleen and kidney. Phagocytosis is assumed as main mechanism, responsible for clearance of particles.

Out of the investigation of tissue of the 28 day study we conclude that it is possible to determine the localization of $CeO_2$ particles in lung and liver tissue sections using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). Based on this finding, the ToF-SIMS technique will be used as a tool to

study particle uptake and fate in organ tissues out of the 2-year inhalation study. The results achieved within ToF-SIMS investigations further demonstrate, that the technique is a suitable tool for identification of specific areas within the organ, where particle accumulation occurs. The results achieved with Inductively Coupled Plasma Mass Spectrometry (ICP-MS, reported in D 4.3 and D 4.4) are in line with the ToF-SIMS results, a higher $CeO_2$ concentration was detected in lung compared to liver.

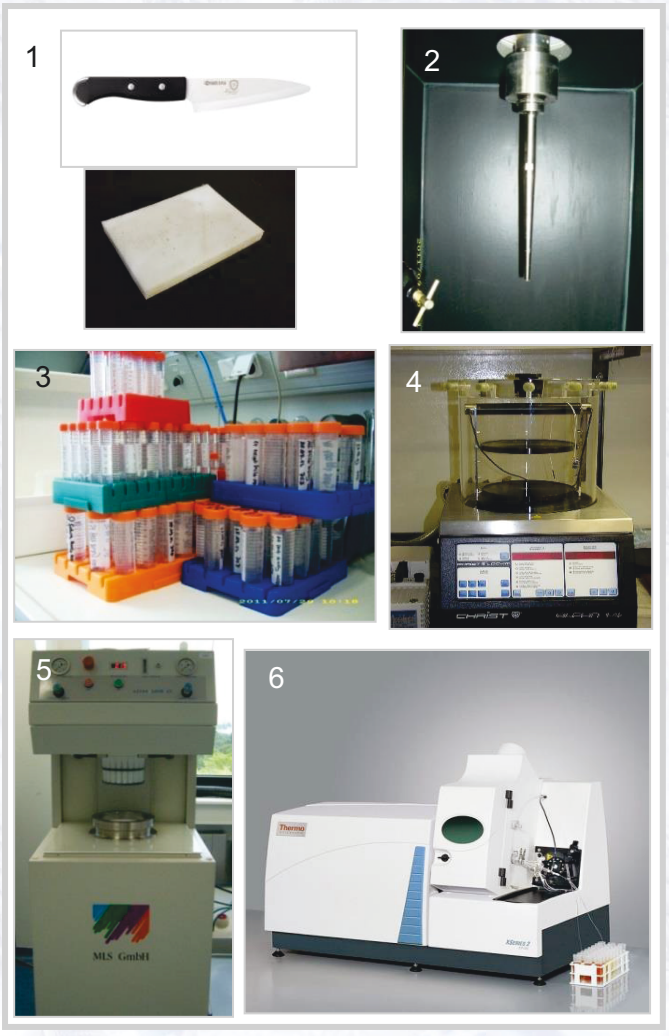
The findings obtained on organ burden so far confirm that a translocation of $CeO_2$ to liver, spleen and kidney occurs following to inhalation.

The comparison of organ burden (ICP-MS) and particle distribution pattern (ToF-SIMS and IBM), revealed that the $CeO_2$ found in secondary organs is mostly present in the form of particles. The pictures of particle distribution generated with ToF-SIMS and IBM suggest particle deposition in the lung followed by phagocytosis and translocation of agglomerates to peripheral organs.

**Working steps for the estimation of $CeO_2$ organ burden by ICP-MS:**

Tissue samples cut to size

1. Homogenization
2. Sample dilution
3. Lyophilisation
4. Microwave digestion
5. Analysis by Inductively Coupled
6. Plasma Mass Spectrometry (ICP-MS)




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

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