DOSSIER ON CERIUM OXIDE - ANNEX 4

Series on the Safety of Manufactured Nanomaterials
No. 45

This document is only available in PDF format.

JT03377711

Complete document available on OLIS in its original format
This document and any map included herein are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.
OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 45

DOSSIER ON CERIUM OXIDE

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2015
Also published in the Series on the Safety of Manufactured Nanomaterials:

No. 44, Dossier on Gold nanoparticles (2015)
No. 46, Dossier on Dendrimers (2015)
No. 47, Dossier on Nanoclays (2015)
No. 48, Dossier on Fullerenes (2015)
No. 49, Dossier on Multiwalled Carbon Nanotubes (MWCNTs) (2015)
No. 50, Dossier on Single-Walled Carbon Nanotubes (SWCNTs) (2015)
No. 51, Dossier on Silicon dioxide (2015)
No. 52, Dossier on Zinc oxide (2015)
No. 53, Dossier on Silver nanoparticles (2015)
No. 54, Dossier on Titanium dioxide (2015)

© OECD 2015
Applications for permission to reproduce or translate all or part of this material should be made to: Head of Publications Service, RIGHTS@oecd.org,
OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France
ABOUT THE OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD’s work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD’s workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides; Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD’s World Wide Web site (www.oecd.org/chemicalsafety/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.
This publication is available electronically, at no charge.

For this and many other Environment, Health and Safety publications, consult the OECD’s World Wide Web site (www.oecd.org/chemicalsafety/)

or contact:

OECD Environment Directorate, Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80

E-mail: ehscont@oecd.org
Tissue Distribution of Inhaled Micro- and Nano-sized Cerium Oxide Particles in Rats: Results From a 28-Day Exposure Study

Liesbeth Geraets,*1 Agnes G. Oomen,* Jeffry D. Schroeter,† Victoria A. Coleman,‡ and Flemming R. Cassee*†


1To whom correspondence should be addressed at National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands. Fax: +31-30-274-4475. E-mail: liesbeth.geraets@rivm.nl.

Received January 12, 2012; accepted March 8, 2012

In order to obtain more insight into the tissue distribution, accumulation, and elimination of cerium oxide nanoparticles after inhalation exposure, blood and tissue kinetics were investigated during and after a 28-day inhalation study in rats with micro- and nanocerium oxide particles (nominal primary particle size: < 5000, 40, and 5–10 nm). Powder aerosolization resulted in comparable mass median aerodynamic diameter (1.40, 1.17, and 1.02 μm). After single exposure, approximately 10% of the inhaled dose was measured in lung tissue, as was also estimated by a multiple path particle dosimetry model (MPPD). Though small differences in pulmonary deposition efficiencies of cerium oxide were observed, no consistent differences in pulmonary deposition between the micro- and nanoparticles were observed. Each cerium oxide sample was also distributed to tissues other than lung after a single 6-h exposure, such as liver, kidney, and spleen and also brain, testis, and epididymis. No clear particle size–dependent effect on extrapulmonary tissue distribution was observed. Repeated exposure to cerium oxide resulted in significant accumulation of the particles in the (extra)pulmonary tissues. In addition, tissue clearance was shown to be slow, and, overall, insignificant amounts of cerium oxide were eliminated from the body at 48- to 72-h post-exposure. In conclusion, no clear effect of the primary particle size or surface area on pulmonary deposition and extrapulmonary tissue distribution could be demonstrated. This is most likely explained by similar aerodynamic diameter of the cerium oxide particles in air because of the formation of aggregates and irrespective possible differences in surface characteristics. The implications of the accumulation of cerium oxide particles for systemic toxicological effects after repeated chronic exposure via ambient air are significant and require further exploration.

Key Words: cerium oxide; nanoparticles; inhalation exposure; deposition; tissue distribution.

Cerium oxide micro- and nanoparticles are widely used in commercial settings. Both nano- and microcerium oxide variants are commonly used as polishing agents and in UV-absorbent coatings (Prospect, 2010). Currently, cerium oxide nanoparticles are also used as a fuel additive due to their catalyzing properties, resulting in a decreased emission (Park et al., 2007, 2008). Due to the growing prevalence of this material for a broad range of applications, human exposure to cerium oxide nanoparticles can be assumed to increase in upcoming years. At present, human health risk assessment of nanomaterials such as cerium oxide nanoparticles is a challenging task, due to a lack of relevant data (Cassee et al., 2011). Availability of data concerning the potential hazards of nanomaterials is limited, particularly in the areas of personal external and internal exposure.

The toxicological effects of nanoscale substances cannot—a priori—be considered comparable to the toxicological effects of the corresponding microscale substances. Smaller particle sizes accompanied by an increased specific surface area for either primary particles or aggregates, coupled with intentionally engineered structural properties of nanomaterials, result in different physico-chemical properties (Preining, 1998). In particular, an increased surface to volume ratio can result in stronger interaction with biological systems as compared with microscale materials, subsequently resulting in a different toxicological profile (Oberdorster et al., 2005).

In addition to exploring the hazards or intrinsic toxicological properties of nanomaterials, exposure assessment is critical for risk assessment. Internal exposure, in particular, will be crucial for the ultimate systemic adverse health effects, which can be quantified by absorption, distribution, metabolism, and excretion. Due to their smaller size and other physicochemical characteristics, nanoparticles can have a different kinetic profile, e.g., different absorption, tissue distribution and elimination. If absorption and subsequent distribution is insignificant, the potential risk upon exposure is expected to be limited to local effects at the exposure site and possibly to indirect effects. On the other hand, slow elimination or complete retention can result in accumulation upon repeated exposure and subsequent increasing internal exposure.

Cerium oxide has been detected in lung tissue and alveolar macrophages of subjects exposed via inhalation in occupational settings (as summarized in Cassee et al. (2011) and...
Environmental Protection Agency (2009). However, little is known about the fate of cerium oxide nanoparticles in the human body after a controlled inhalation exposure. The kinetics of cerium oxide have been studied both in vitro (Hirst et al., 2009; Limbach et al., 2005; Patil et al., 2007) and in vivo animal studies (Dan et al., 2012, 2011; Hardas et al., 2010; He et al., 2010; Hirst et al., 2009; Yokel et al., 2009, 2012). The distribution of nanocerium oxide in rats after a single intravenous administration has been demonstrated for tissues such as liver, spleen, and also brain (Dan et al., 2011; Hardas et al., 2010; Hirst et al., 2009; Yokel et al., 2009, 2012). Recently, extrapolmonary translocation of single intratracheally instilled cerium oxide in rats was demonstrated (He et al., 2010). Because human exposure is most likely via inhalation, and more importantly, may be chronic, more information on translocation after inhalation and potential accumulation in tissues is considered necessary.

To gain more insight into the deposition and kinetics of inhaled cerium oxide particles including retention and possible accumulation in the body, micro- and nanocerium oxide particles were used in a 28-day inhalation study in rats. We hypothesized that the biodistribution is dependent on the primary particle size and assessed the deposition, distribution, accumulation, and elimination in tissues at various time points during as well as after termination of the exposure. The results on the kinetics of cerium oxide are discussed in light of the in vivo kinetics of some other inhaled materials. Furthermore, recommendations are made with respect to future toxicological and kinetic studies on (cerium oxide) nanoparticles.

MATERIALS AND METHODS

Animals. Male Wistar rats (6 weeks old, 212 ± 14 g) were obtained from Charles River (Sulzfeld, Germany). Exposure started after at least 5 days of acclimatization. Animals were housed in standard laboratory cages (two or five animals per cage) under controlled conditions of temperature, humidity, and light. During exposure periods, the rats were individually housed in the exposure unit. Food and water were allowed ad libitum during the experiments, except during exposure. The experiments were conducted at TNO Triskelion (Zeist, The Netherlands) under a protocol approved by the Ethics Committee for Animal Experiments of TNO.

Characterization of test material. Three different sizes, i.e., nominally <5000, 40, 5–10 nm, of cerium oxide (CAS number 1306-38-3) were used in this study and were obtained from Sigma-Aldrich (United Kingdom), Unicore (Belgium), and Antaria (Australia), respectively. Solubility of the particles was extremely poor in water at neutral pH. In the present paper, no distinction is made between aggregated and agglomerated cerium oxide particles, and the term aggregates is used for both. This study was part of the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials Sponsorship Programme and specific codes were assigned to the different test materials (i.e., NM-213, NM-212, and NM-211 for Sigma-Aldrich, Unicore, and Antaria cerium oxide particles, respectively).

Scanning electron microscope (SEM) images from particles sampled from the test atmosphere on glass fiber filters during the inhalation study were obtained using a MIRA II LMH-LS field emission SEM from Tescan with a Quanta 800 EDX 25 mm detector from Bruker. Images were acquired at an accelerating voltage of 15 kV.

Primary particle size of the powders was studied by transmission electron microscope (TEM) and SEM. Bright field TEM images were generated on a Philips CM120 Bioflop (Eindhoven, NL) TEM at an accelerating voltage of 120 kV using a Gatan Imaging Filter camera (1024 × 1024 pixels). The SEM images were obtained using a Supra 40 field emission SEM from Carl Zeiss (Welwyn Garden City, Hertfordshire, U.K.). In-lens detector images were acquired at an accelerating voltage of 15 kV, a working distance of 3 mm, and a tilt angle of 0°. Image J software was used for analysis of both the TEM and SEM images. Outlines of at least 50–100 particles were traced manually, and the corresponding areas, and Feret diameters, were determined by the software. Equivalent circle diameters, i.e., diameters of circles with the same area as the projected particle images, were calculated from the measured areas (TEM images).

Particle number counts and size distributions were measured daily during the experimental period with a scanning mobility particle sizer (consisting of an electrostatic classifier model 3080, differential mobility analyzer model 3081, neutralizer model 3077A and condensation particle counter model 3022A; all TSI Inc, Shoreview MN) and an aerodynamic particle sizer (model 3321 with diluter 3302A, TSI Inc.).

The mass concentration was determined daily during the experimental period using gravimetric analysis. The surface area of the bulk material was analyzed by gas adsorption using the BET method with a TriStar 3000 from Micromeritics (Norcross, GA). Nitrogen was used as adsorbate. Prior to analysis, samples were purified under nitrogen for 6 h at 150°C. Assuming spherical particles, the equivalent diameter based on the surface area measurements was calculated, assuming a cerium oxide density of 7.65 g/cm³.

Experimental protocol. A 28-day inhalation study was performed in rats. Rats (3 per group) were exposed (6 h per day) by inhalation to cerium oxide in nose-only exposure units consisting of a cylindrical column, surrounded by a transparent hood. The test atmosphere was introduced at the top of the central column and was exhausted at the bottom of approximately 50-l exposure unit. Each column included four rodent tube sections of 20 ports each for animal exposure. The animals were secured in plastic animal holders (Battele) and positioned radially through the outer cylinder around the central column. Only the nose of the rats protruded into the interior of the column. The animals were placed in the exposure unit after stabilization of the test atmosphere. Particle number concentrations in the test atmosphere were targeted to be the same for each cerium oxide type.

Test atmosphere. To generate the test atmosphere, cerium oxide was aerosolized using a dust feeder, a Venturi, and a jetmill. We intentionally chose to aerosolize the particles from a powder to mimic realistic exposure scenarios rather than trying to get an aerosol consisting of only single primary particles. The generated test atmosphere was directed to the top inlet of the exposure unit, and at the bottom of the chambers, the test atmosphere was exhausted.

Test scheme. A schematic overview of the different experimental groups is shown in Table 1. At different time points during and after the study, tissue and blood samples were collected. For each cerium oxide, necropsy was performed within 1 h after finishing the final exposure for groups I, II, and III. Furthermore, for each cerium oxide, a recovery group was also included in the study (group IV). Rats in this group were repeatedly exposed (19–20×) followed by necropsy after 48 or 72 h instead of within 1 hour, in order to study elimination of cerium oxide.

Blood and tissue sampling. During the study, blood samples were taken at various time points after a single or repeated exposure. Blood sampling was performed in most cases well within the first hour after exposure but also at post-exposure periods of up to 67 h. Blood was sampled by orbital puncture under O2/CO2 anesthesia in EDTA-containing tubes and frozen (below −18°C) for analysis of cerium.

For tissue sampling, rats were sacrificed while under ether anesthesia, and tissues (lung, liver, spleen, kidney, testis, epididymis, and brain) were removed and frozen (below −18°C) for cerium analysis.
absolute amount of cerium oxide per tissue was calculated using organ weights. In order to study particle–dependent differences in tissue concentrations, the cerium oxide tissue concentrations were normalized for the total inhaled dose in similar because particle number concentrations were targeted to be the same, was extremely poor. This assumption is considered appropriate because results of the deposition model calculations. In order to estimate the dose deposited in the rat lung upon inhalation of cerium oxide, the multiple path particle dosimetry model (MPPD v 2.11; http://www.ara.com/products/mppd.htm) was used. The deposition fractions in the head, tracheobronchial, and pulmonary region were calculated. We used the default parameters of the model for rats, i.e., a forced respiratory capacity of 4 ml, head volume of 0.42 ml, nasal breathing, tidal volume of 2.1 ml, and a breathing frequency of 102/min. The inspiratory fraction was 0.5, and no pause was entered. Calculations were done using the measured mass median aerodynamic diameter (MMAD), count median diameter (CMD), geometric SD, the mass concentration, and a density of 7.65 g/cm³.

In addition, more detailed calculations were performed to estimate deposition in the olfactory epithelium because deposited particles may directly translocate to the brain, bypassing the blood brain barrier. A detailed anatomically accurate computational fluid dynamics (CFD) model of the rat nasal passages was used for these calculations. The CFD model was originally developed by Kimbell et al. (1997) and was subsequently smoothed using medical imaging software to provide a more realistic anatomic form according to Schroeter et al. (2012). Particle sizes from 1 nm to 5 μm were simulated, and nasal and olfactory deposition fractions were predicted for each particle size.

Data evaluation and statistical analysis. The measured cerium tissue levels were converted into cerium oxide tissue levels by multiplying the former with the ratio of the molecular masses of cerium oxide and cerium (i.e., 1.23), thus assuming that the deposited and distributed cerium oxide is still present as particles. This assumption is considered appropriate because results of solubility tests indicated that the solubility of the three cerium oxide particles was extremely poor.

As the mass concentrations of the different cerium oxide types were not similar because particle number concentrations were targeted to be the same, cerium oxide tissue concentrations were normalized for the total inhaled dose in order to study particle–dependent differences in tissue concentrations. First, the absolute amount of cerium oxide per tissue was calculated using organ weights. As no information on the total organ weights of the rats in the present study was available, data on organ weights from a different rat study were used (Lankveld et al., 2010). The rats used in the alternative study were similar to the animals in the present study with respect to strain, sex, age, and bodyweight. Second, the total inhaled dose of cerium oxide was calculated using the following formula: tidal volume × breathing frequency × exposure concentration × exposure duration (analogous to parameter settings of the MPPD model; the tidal volume was 2.10 ml and breathing frequency was 102/min). Finally, the absolute amounts of cerium oxide in tissue were then normalized by expressing these amounts as percentage of the total inhaled dose cerium oxide (i.e., 4.24, 1.54, and 0.83 mg cerium oxide for Sigma-Aldrich, Umicore, and Antaria particles, respectively). Results are presented as mean values ± SD unless otherwise indicated.

The cerium oxide tissue concentration data were not normally distributed. The nonparametric Mann-Whitney U test was used to evaluate the effect of particle size on cerium oxide tissue content, to study potential tissue–dependent differences in cerium oxide levels and to assess the potential effect of number of exposures and recovery period on the cerium oxide tissue levels. Two-tailed p values of 0.05 or less were considered statistically significant.

## RESULTS

### Characteristics of Test Atmospheres and Dose

The physical characteristics of the aerosols are summarized in Table 2. The mean mass concentration for the different exposure protocols were 55.00, 19.95, and 10.79 mg/m³ for Sigma-Aldrich, Umicore, and Antaria cerium oxide particles, respectively. The actual concentrations of the test material in the atmosphere were measured at least six times daily by gravimetric analysis.

The total airflow through the unit was monitored at least three times a day and was at least 1 l/min for each rat. The air entering the unit was controlled at 22 ± 2°C, and the relative humidity was targeted between 30 and 70%.

TEM analysis and SEM analysis were used to verify the manufacturer’s nominal sizes of < 5000, 40, and 5–10 nm. Figure 1A–C presents TEM images from the bulk material, acquired at a magnification of 41,000× (scale bar is 200 nm). The Sigma-Aldrich sample contained very large particles (> 500 nm), and overall was unsuitable for size characterization by TEM. It is worth noting, however, that together with the larger particles, there was a non-negligible proportion of sub-100 nm–sized particles present in this sample, with some particles as small as ~10 nm visible. These small particles appeared to “decorate” the large particles present in the sample. Overall, the sample appeared to have an extremely broad size distribution. TEM analysis of the Umicore sample revealed crystals of polydisperse size and shape, with a mean Feret diameter of 27.3 ± 13.6 nm (100 particles). The Antaria sample revealed small aggregate clusters of primary particles with much greater size and shape homogeneity. A mean Feret diameter of 13.0 ± 3.2 nm (100 particles) was determined for this sample.

SEM analysis showed actual primary particle sizes of 615.3 ± 430.5, 28.4 ± 10.4, and 44.9 ± 14.6 nm for Sigma-Aldrich, Umicore, and Antaria particles, respectively.

Both TEM and SEM analyses revealed that the difference between the two nanoparticles was smaller than anticipated. The mean particle size determined from TEM measurements

<table>
<thead>
<tr>
<th>Cerium oxide particle type</th>
<th>Experimental group</th>
<th>Number of exposures (6 h/exposure)</th>
<th>Time period between termination of exposure and sacrifice (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigma-Aldrich and Umicore</td>
<td>I</td>
<td>1 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>11 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>19 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>19 ×</td>
<td>72</td>
</tr>
<tr>
<td>Antaria</td>
<td>I</td>
<td>1 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>12 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>20 ×</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>20 ×</td>
<td>48</td>
</tr>
</tbody>
</table>
shows good equivalence with the average particle size calculated from the BET surface area measurements.

Although the nano- and microcerium oxides differ in primary particle size (i.e., size of a single particle), the aerodynamic diameter for all three particles is similar, indicating that in particular the Umicore and Antaria samples were present in the aerosol as aggregates. Figure 1D–F presents SEM images of the three different cerium oxide particles sampled from the test atmosphere during the inhalation study on a glass fiber filter.

**Tissue Content**

Lung, liver, spleen, kidney, testis, epididymis, brain, and blood were analyzed for the presence of cerium. After single and repeated exposure, cerium could be detected in all investigated organs, although substantial higher levels were measured in the lungs. Cerium tissue levels in control rats were very low and were very close to and partly below the tissue-specific detection limits (data not shown). Cerium concentration in blood after single or repeated exposure was below the detection limit (≤ 5 μg/kg) for most samples (data not shown).

Figure 2 shows the cerium oxide tissue amounts after single inhalation exposure expressed as percentage of the total inhaled dose. Though small differences in pulmonary deposition efficiencies of cerium oxide were observed, no obvious size-dependent differences in the pulmonary deposition of cerium oxide particles occurred. The percentage of inhaled cerium oxide measured in lung tissue for Umicore particles (5.6%) was significantly lower ($p = 0.05$) than the lung content for Sigma-Aldrich particles (12.2%) and Antaria particles (9.4%), indicating that the Umicore particles were less efficiently deposited in the lung as compared with the Sigma-Aldrich and Antaria particles.

Cerium oxide detected in the extrapulmonary tissues was less than 0.2% of the inhaled dose for all cerium oxide particles. Unlike the deposition in the lung, the extrapulmonary cerium oxide tissue levels for Umicore particles were slightly (though not statistically significant) increased as compared with the extrapulmonary tissue levels of rats exposed to Sigma-Aldrich and Antaria particles, indicating a moderately better distribution to extrapulmonary organs.

**Tissue Distribution**

In order to study tissue-dependent differences in cerium oxide concentrations, tissue cerium oxide concentrations were expressed per kg tissue after single (Fig. 3A) and repeated exposures (Figs. 3B and C) and after the recovery period (Fig. 3D).

For Sigma-Aldrich particles, cerium oxide levels in lung tissue were significantly higher than the other investigated tissues and were at least 1000× higher than the extrapulmonary tissue levels. After single exposure, cerium oxide particles were distributed to the extrapulmonary tissues with an order of concentration of epididymis > brain > liver > kidney > testis. After repeated exposures, the differences in distribution were less clear.

For Umicore particles, the highest cerium levels were also found in lung tissue. After a single exposure, cerium oxide particles were distributed to the extrapulmonary tissues with an order of concentration of epididymis > brain > liver = spleen.

### Table 2

**Characteristics of Cerium Oxide Particles**

<table>
<thead>
<tr>
<th>Type of cerium oxide</th>
<th>Sigma-Aldrich</th>
<th>Umicore</th>
<th>Antaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM-number in OECD Sponsorship</td>
<td>NM-213</td>
<td>NM-212</td>
<td>NM-211</td>
</tr>
<tr>
<td>Nominal primary particle size (nm)</td>
<td>&lt; 5000</td>
<td>40</td>
<td>5–10</td>
</tr>
<tr>
<td>Mean primary particle size (100 particles), measured with TEM (Feret diameter, nm)</td>
<td>Sample too large for TEM measurement (&gt; 500 nm)</td>
<td>27.3 ± 13.6(22.4 ± 10.9)</td>
<td>13.0 ± 3.2(10.7 ± 2.6)</td>
</tr>
<tr>
<td>Equivalent circle diameter given in square brackets (nm)</td>
<td>Smallest size observed: 9 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary particle size, measured with SEM (Feret diameter, nm)</td>
<td>615.3 ± 430.5</td>
<td>28.4 ± 10.4</td>
<td>44.9 ± 14.6</td>
</tr>
<tr>
<td>MMAD on aerodynamic particle size (μm)</td>
<td>1.40 ± 0.11(1.64)</td>
<td>1.17 ± 0.34(2.07)</td>
<td>1.02 ± 0.04(1.82)</td>
</tr>
<tr>
<td>CMD based on scanning mobility particle sizer (μm)</td>
<td>0.34 ± 0.06(1.52)</td>
<td>0.25 ± 0.07(1.78)</td>
<td>0.21 ± 0.03(1.76)</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>3.73 ± 0.01</td>
<td>27.15 ± 0.19</td>
<td>63.95 ± 0.30</td>
</tr>
<tr>
<td>Diameter from BET surface area (nm)</td>
<td>213</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>Number concentration (10⁶ particles/cm³)</td>
<td>0.68 ± 0.46</td>
<td>1.1 ± 0.70</td>
<td>1.79 ± 0.42</td>
</tr>
<tr>
<td>Mass concentration (mg/m³)</td>
<td>55.00 ± 6.20</td>
<td>19.95 ± 13.21</td>
<td>10.79 ± 0.82</td>
</tr>
<tr>
<td>Total estimated inhaled dose (mg)</td>
<td>4.24</td>
<td>1.54</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Note. Results are mean ± SD of multiple measurements.

aGeometric SD.

bCalculated using the formula $d = 6/(\text{surface area} \times \text{density})$.

C) Calculated using the formula: tidal volume $\times$ breathing frequency $\times$ exposure concentration $\times$ exposure duration (analogous to parameter settings of the MPPD model; the tidal volume was 2.10 ml and breathing frequency was 102/min).
Kidney was the second highest organ in distribution after single exposure, except for slightly reduced distribution to testis. In repeated exposure, the levels of cerium oxide in kidney were not different from single exposure, though testis levels remained the lowest.

Lung cerium oxide levels were also the highest of all investigated tissues for the Antaria particles. Except for a slightly reduced distribution to testis, epididymis, and brain, no clear differences in distribution pattern to the various extrapulmonary organs were observed after single and repeated exposures.

Tissue Accumulation and Elimination

In Figure 4, the cerium oxide tissue levels are normalized for the tissue levels after single exposure in order to evaluate potential accumulation after repeated exposure. Repeated exposure to all cerium oxide particle-types was found to result in significant increasing cerium oxide concentrations, indicating that accumulation occurred. This was most evident and significant in lung tissue. Increasing the number of repeated exposures was found to result in higher fold of tissue accumulation for rats exposed to Sigma-Aldrich and Umicore, indicating that steady state was not yet reached after 11 exposures. Particularly, significant increases in cerium oxide lung (Sigma-Aldrich and Umicore) and liver (Umicore) levels could be observed.

In an additional experimental group of rats, cerium oxide tissue levels were measured 48 h (Antaria) or 72 h (Sigma-Aldrich and Umicore) after finishing the last exposure in order to study elimination of cerium oxide. As observed in Figure 4, the elimination of Sigma-Aldrich and Umicore cerium oxide particles from the investigated tissues was slow, with, in almost all cases, insignificant amounts of cerium oxide eliminated in 72 h. Rats exposed to Sigma-Aldrich cerium oxide particles showed significant decreases in brain cerium levels. Umicore-exposed rats showed significant decreases in lung and liver cerium levels after recovery. Elimination of Antaria cerium oxide particles appeared to be slower.

Modeling of Lung Deposition

The deposition fractions estimated with MPPD are presented in Table 3 for MMAD, CMD, and primary particle size (as measured with SEM analysis).

The cerium oxide particles are expected to be mainly deposited in the head region, and solely a small part (≤10%) is expected to be deposited in the pulmonary region. Based on MMAD, the smallest aggregates resulted in the highest deposition fraction in the pulmonary region, though the differences were very small: 7, 8, and 9% of the inhaled Sigma-Aldrich, Umicore, and Antaria cerium oxide particles, respectively, were expected to be deposited in the lung.

Model calculations indicate that should the diameter in the aerosol be equal to the primary particle size, significant differences would have been expected between the nano-sized particles (28 and 45 nm, based on SEM measurements) and the micro-sized particles (615 nm based on SEM measurements). MPPD calculates a pulmonary deposition of around 30% for these nano-sized particles and only 7% for the micro-sized particles. Similar results are obtained for tracheobronchial deposition, which is in the order of 7–10% and 3.5% for nano- and micro-sized particles, respectively.

Additional olfactory deposition calculations showed that the most efficient olfactory deposition is expected for particles with much smaller diameter (i.e., approximately 7 nm) as compared with the aggregates of the cerium oxide particles as studied in the present study (data not shown). Though the deposition in the head region was estimated to be approximately 33–52% (i.e., 73–89% of the total deposition), the olfactory deposition of the three cerium oxide particles is expected to be very low.

DISCUSSION

The present study describes the tissue distribution and elimination of cerium oxide nano- and micro-particles of different primary particle size in rats. Animals were exposed by inhalation to cerium oxide. Inhalation of cerium oxide aerosol provided a relevant and natural route of exposure, as exposure in occupational settings is mainly via inhalation.
Differences Between Cerium Oxides Tested

Despite the fact that the micro- and nanocerium oxide particles clearly differed in primary particle size, the size distributions of the aerosol generated for the inhalation exposure were very similar due to aggregation of the primary particles. This was confirmed by electron microscopy. An aerosolization regime that mimics what can occur due to (re)suspension of cerium oxide in air at a workplace, for example, was intentionally applied.

Lung deposition of airborne particles is highly dependent on the aerodynamic diameter (Carvalho et al., 2011). Because, in the present study, the three types of cerium oxide did not differ substantially in the MMAD or CMD, it seems safe to assume that the deposition efficiency and rate and the distribution in the...

---

**FIG. 2.** Cerium oxide levels in (A) lung, (B) liver, (C) spleen, (D) kidney, (E) testis, (F) epididymis, and (G) brain of Wistar rats exposed by single inhalation exposure (6 h per exposure) to cerium oxide Sigma-Aldrich (white), Umicore (gray), and Antaria (black) particles. Cerium oxide levels are expressed as percentage of the total dose inhaled cerium oxide during corresponding single exposure in order to compare the pulmonary deposition and extrapulmonary tissue distribution of the three cerium oxide particles. Results are mean ± SD of three rats. *p ≤ 0.05 versus Umicore.
upper respiratory tract, trachea, conducting airways, and alveoli are more or less similar, as was also estimated by the MPPD deposition model. Measurement of the cerium oxide content of lung tissue confirmed this assumption. Though small differences in pulmonary deposition efficiencies of cerium oxide were observed, no consistent relationship between the primary particle size or MMAD and the pulmonary dose was noted. These findings do not support the hypothesis that the BET surface area is a good predictor of either the pulmonary or extrapulmonary dose or for adverse effects (unpublished findings).

As aggregates may break up upon contact with body fluids, it is remarkable that the extrapulmonary distribution was more or less similar for the three samples examined in this study. This may indicate that (1) the aggregates are not broken up by body fluids and remain intact during distribution, (2) the nanoparticles and microparticles distribute similarly, (3) the particles dissolve and behave as molecules, or (4) the fraction distributed to other organs aside from the lung is too small to show the differences clearly. Solubility tests conducted with the three cerium oxide particles showed very low solubility, and thus the third interpretation of the distribution data can be ruled out. Currently, no information is available on the condition of the cerium oxide in the tissues (i.e., if it is present as aggregates or single particles).

**Distribution of Cerium Oxide**

The highest levels of cerium oxide were found in lung tissue, which was not surprising given exposure was via inhalation. Furthermore, the deposition model estimations are consistent with the actual cerium oxide levels in lung tissue. Cerium could not be detected in blood in the present study, indicating fast distribution from blood to tissues. Cerium oxide was detected in all investigated extrapulmonary tissues, i.e., liver, spleen, kidney, testis, epididymis, and brain. This indicates that after inhalation exposure, toxicological effects can also be expected beyond local pulmonary effects. Although the fraction of cerium oxide that becomes systemically available is small, systemic effects should be considered when assessing the health risk of inhaled cerium oxide nanoparticles. No differences among the three cerium oxides were seen in

---

**FIG. 3.** Cerium oxide concentrations in lung, liver, spleen, kidney, testis, epididymis, and brain of Wistar rats exposed by single or repeated inhalation (6 h per exposure) to cerium oxide Sigma-Aldrich (white), Umicore (gray), and Antaria (black) particles. A = experimental group I, B = experimental group II, C = experimental group III, D = experimental group IV. See Table 2 for specification of the experimental groups. Cerium oxide levels are expressed as µg of cerium per kg of tissue. Results are mean ± SD of three rats. *p ≤ 0.05 versus liver, spleen, kidney, testis, epididymis, and brain cerium oxide levels; +p ≤ 0.05 versus epididymis cerium oxide levels; #p ≤ 0.05 versus testis cerium oxide levels; x p ≤ 0.05 versus brain cerium oxide levels; o p ≤ 0.05 versus kidney cerium oxide levels.
term of pathology or other markers of toxicity (unpublished findings).

However, the total amount of cerium measured in the tissues was far below the inhaled dose. This might be explained by (1) the selection of investigated organs for cerium analysis (i.e., not all tissues were investigated), (2) possible elimination of inhaled cerium via exhalation during, or shortly after the exposure period, and (3) rapid elimination of deposited cerium oxide via the mucociliary escalator of the bronchia and conducting airways. Remarkably high levels were detected in brain, epididymis, and testis, indicating that further attention should be paid to potential central nervous system effects and reprotoxic effects. Potential important routes responsible for extrapulmonary tissue distribution are: (1) translocation to the lymphatic and circulatory system or (2) activity of the mucociliary escalator and subsequent oral exposure. The high cerium oxide levels in the brain could have furthermore been a consequence of deposition of cerium oxide in the olfactory epithelium in the nose and subsequent translocation via the olfactory nerve. This effect has previously been described for other materials, both soluble and insoluble (Dorman et al., 2002; Elder et al., 2006; Henriksson et al., 1999; Oberdorster et al., 2004). Additional modeling of the deposition showed that, though the deposition in the head region is relatively high (deposition fractions of 0.33–0.52, corresponding to 73–89% of the total deposition), the olfactory deposition of the three cerium oxide particles is expected to be minimal. Though translocation via the olfactory nerve is possible, this route should not contribute extensively to the relatively high levels of cerium oxides observed in the brain as compared with particles with a smaller diameter. Distribution across protecting membranes such as the blood cerebrospinal fluid barrier, blood brain barrier, and blood testis barrier might be an additional explanation for the relatively high brain and testis levels.

FIG. 4. Accumulation of cerium oxide in lung, liver, spleen, kidney, testis, epididymis, and brain tissue of Wistar rats exposed by single and repeated inhalation (6 h per exposure) to cerium oxide Sigma-Aldrich (A), Umicore (B), and Antaria (C) particles. See Table 2 for specification of the experimental groups I, II, III, and IV. Accumulation was expressed as fold increase over cerium oxide levels after single exposure (i.e., cerium oxide levels after single exposure = 1). Results are mean ± SD of three rats. * p ≤ 0.05 versus experimental group I; + p ≤ 0.05 versus experimental group II; # p ≤ 0.05 versus experimental group III.

Kinetic Profile of Nanoparticles Versus Cerium Oxide
Extrapulmonary and extensive systemic distribution to liver, spleen, kidney, testis, and brain was previously observed after intratracheal instillation and intravenous administration of nanocerium oxide (Dan et al., 2011; Hardas et al., 2010; He et al., 2010; Yokel et al., 2009, 2012). These studies and the present study are generally thus in agreement with the broad distribution of cerium oxide particles. The present study is the first to employ the most relevant exposure route (inhalation) and to show that cerium oxide after inhalation may become systemically bioavailable.

Extrapulmonary distribution has also been demonstrated after inhalation of other nanoparticles, including: elemental carbon (Oberdorster et al., 2002), iridium (Kreyling et al., 2002), ferric oxide (Zhu et al., 2009), fullerences (Shinohara et al., 2010) and gold (Yu et al., 2007). Although the nanoparticle concentrations were always highest in lung tissue, nanoparticles were also recovered from other extrapulmonary tissues such as liver, spleen, kidney, heart and brain after inhalation exposure (Kreyling et al., 2002; Oberdorster et al., 2002; Shinohara et al., 2010; Yu et al., 2007; Zhu et al., 2009).

Systemic Exposure Via the Oral Route
Potential exposure via the oral route following inhalation exposure of particles (e.g., via activity of the mucociliary escalator mediated by macrophages) might also contribute to extrapulmonary tissue deposition. The deposition model used in this work estimated that the deposition of cerium oxide in
the head region would be high (i.e., 73–89% of the total deposition). This suggests that oral exposure might be an important contributor to systemic tissue deposition.

A recent rat in vivo study by He et al. (2010) showed that after intratracheal instillation of cerium oxide nanoparticles (primary particle size: 6.6 ± 0.9 nm, BET surface area: 86.85 m²/g), approximately 25% of the given intratracheal dose was cleared via the feces, pointing toward particle transport from the lung to the gastrointestinal (GI) tract (He et al., 2010). Furthermore, approximately 90% of the oral dose was excreted in feces in the first day post-exposure, and nearly 100% of the given oral dose was excreted in feces after 3 days post-exposure (He et al., 2010). This suggests a limited GI absorption of cerium oxide nanoparticles. However, absorption and subsequent excretion via bile and feces could also result in fecal excretion. Hence, in the present study exposure via the oral route following the mucociliary escalator may also be possible. Analysis of cerium in the feces after inhalation of cerium oxide could thus aid in clarification of this exposure route. The contribution from the oral route can be quantified if the oral bioavailability of cerium oxide and the fecal cerium content is known.

**Elimination**

In the present study, the elimination half-life could not be reliably determined. At 48 and 72 h exposure, there was no significant reduction of cerium oxide in either lung tissue or other investigated tissues, indicating a long elimination half-life, which was also recently demonstrated by He et al. (2010). Additionally, significant accumulation of cerium oxide in tissues was observed after repeated exposure in the present study, which was also recently reported following repeated intravenous and intraperitoneal administration of cerium oxide particles (Hirst et al., forthcoming). In addition, elimination from the body of intravenously administered 30 nm cerium oxide in a different rat study was found to be <1% of the total dose during the first 2 weeks post-exposure (Yokel et al., 2012).

The negligible elimination and subsequent accumulation of cerium oxide particles observed after repeated exposure therefore highlight the importance of assessing the potential toxicity after chronic exposure to this material.

**Limitations**

In this study, we included a limited set of time points to get rough perspective on the biodistribution of three cerium oxide particles that differ in the primary particle sizes. For this reason, the data are insufficient to predict the biodistribution in rodents and humans. The main aim, however, was to identify if primary sizes had a major impact on the biodistribution. Another limitation is that very small amounts of cerium were detected in organs other than the lung. We cannot rule out that contamination has occurred during autopsy of these organs, for instance, due to the perfusion of the organs. On the other hand, the blood did not contain detectable amounts of cerium nor did we detect a pattern that suggests contamination of organs via scalpels or tweezers.

**CONCLUSIONS AND RECOMMENDATIONS**

In the present study, rats were exposed via inhalation to cerium oxide particles with similar aerodynamic sizes but varying primary particle size. It can be concluded that after exposure, the cerium oxide particles were mostly retained in lung tissue. Distribution of the inhaled cerium oxide particles to various extrapulmonary tissues was also observed. Though there were small differences in the pulmonary deposition efficiencies of the three cerium oxide materials, no clear effect of the primary particle size or surface area on pulmonary deposition and extrapulmonary tissue distribution was observed. Repeated exposure resulted in significant accumulation of the particles in both the pulmonary as well as the extrapulmonary tissues. Furthermore, tissue clearance of the particles was slow, and insignificant amounts of cerium oxide were eliminated in 48–72 h.

A lack of strong correlation between primary particle size and pulmonary deposition or extrapulmonary distribution is
likely to be explained by the presence of aggregates rather than single nano-sized particles in the test atmosphere resulting in similar aerodynamic diameter of the micro- and nanocerium oxide but with different surface characteristics, though this latter is also known to affect particle distribution. At present, no work has been undertaken to establish whether the cerium oxide measured in tissues is present as aggregates or single particles. Such information would provide insight into the differences and similarities between micro- and nanocerium oxide.

The present results provide a solid foundation for further studies on the fate of inhaled cerium oxide (nano)particles in the body. Additional studies, such as information on the extent of oral exposure after inhalation exposure, are necessary to clarify the exposure pathways. Finally, future toxicological studies should include potential central nervous system effects and reprotoxic effects and, given the poor tissue clearance, should focus on chronic exposure.

FUNDING

This work was supported by the Dutch Ministry of Infrastructure and Environment providing the funds to perform the studies within the scope of the OECD Working Party on Manufactured Nanomaterials Sponsorship Programme. TEM measurements were supported by the Australian Center for Microscopy and Microanalysis at the University of Sydney (Australia).

ACKNOWLEDGMENTS

We would like to thank Hans Muijser and coworkers from TNO Triskelion (Zeist, The Netherlands) for conducting the animal studies. We would like to thank Ratna Tantra (National Physical Laboratory, United Kingdom) for providing SEM-data on particle size measurements. We also acknowledge Jan van Eijkeren (RIVM, The Netherlands), Jos Bessem (RIVM, The Netherlands), and Wolfgang Kreyling (Helmholtz Zentrum München, Germany) for contributing to the study and for critical evaluation of the results and manuscript, respectively.

REFERENCES


