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Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Differences in Lung Deposition, Animal Species and Nanoforms.

Considerable differences in pulmonary responses have been observed in animals exposed to cerium dioxide nanoparticles via inhalation. These differences in pulmonary toxicity might be explained by differences in lung deposition, species susceptibility or physicochemical characteristics of the tested cerium dioxide nanoforms (i.e. same chemical substance, different size, shape, surface area or surface chemistry). In order to distinguish the relative importance of these different influencing factors, we performed a detailed analysis of the data from several inhalation studies with different exposure durations, species and nanoforms, namely published data on NM211 and NM212 (JRC repository), NanoAmor (commercially available) and our published and unpublished data on PROM (industry provided). Data were analyzed by comparing the observed pulmonary responses at similar external and internal dose levels. Our analyses confirm that rats are more sensitive to developing pulmonary inflammation compared to mice. The observed differences in responses do not result purely from differences in the delivered and retained doses (expressed in particle mass as well as surface area). In addition, the different nanoforms assessed showed differences in toxic potency likely due to differences in their physicochemical parameters. Primary particle and aggregate/agglomerate size distributions have a substantial impact on the deposited dose and consequently on the pulmonary response. However, in our evaluation size could not fully explain the difference observed in the analyzed studies indicating that the pulmonary response also depends on other physicochemical characteristics of the particles. It remains to be determined to what extent these findings can be generalized to other poorly soluble nanomaterials.

Keywords: cerium dioxide; nanomaterial; species differences; nanoform; inhalation; toxicity; lung deposition

Introduction

The number and variety of nanomaterials is expected to continue to increase because of the adaptability of their physicochemical properties to enhance their functionality for many different applications. However, manipulating the physicochemical properties of nanomaterials may also lead to undesirable behavior and, possibly, harmful effects in those exposed. A challenge for health risk assessment is the rapidly growing variety of nanomaterials and nanoforms (nanomaterials of the same chemical substance, but with different physicochemical characteristics such as size, shape, surface area and surface chemistry (EC 2017; ECHA 2017)). Thus, in recent years there is an increasing interest in strategies to compare assessments across different nanoforms, including (Quantitative) Structure Activity Relationships, grouping and read-across (Zhang et al. 2012; Stone et al. 2014; Arts et al. 2015; Oomen et al. 2015). A fuller understanding of the influence of basic (physicochemical) properties of nanomaterials on their potential toxicity is needed for further development, validation and specifically implementation of new or revised approaches in health risk assessment (Dekkers et al. 2016).

Recently, we have investigated the influence of zirconium doping of cerium dioxide nanoparticles (CeO_2 NPs) on their biodistribution, pulmonary and cardiovascular effects in mice following inhalation (Dekkers et al. 2017). Sub-acute (4 weeks) inhalation of the undoped CeO_2 NPs (4 mg/m^3, 3 hr/d, 5d/w) resulted in minimal pulmonary and cardiovascular effects in mice 4 weeks post-exposure. Other toxicity studies showed more pronounced effects after exposure to CeO_2 NPs under similar exposure conditions in rats (Geraets et al. 2012; Gosens et al. 2014; Keller et al. 2014; Arts et al. 2016) and mice (Aalapati et al. 2014). Possible explanations for the observed differences in toxicity of CeO_2 NPs after inhalation are: a) differences in kinetics (deposition and clearance) of the CeO_2 NPs, b) differences in the susceptibility of mice

compared to rats or c) differences in the toxicity of the different nanoforms of CeO_2, due to differences in certain physicochemical characteristics such as size, shape and surface chemistry. The objective of the analysis described in this paper is to investigate which of these possible explanations could explain the relatively mild toxicity of the undoped CeO_2 NPs observed in Dekkers et al. (2017) compared to the more pronounced toxicity observed in other inhalation studies.

The importance of toxicokinetics in interpreting pulmonary responses to inhaled NPs has been highlighted by several researchers (Kreyling et al. 2013; Kuempel et al. 2015; Pauluhn 2017). Previous studies, such as Braakhuis et al. (2016), indicate that the internal dose is more predictive of the toxicity of inhaled NPs than the external dose, suggesting that the deposition pattern (i.e. the regions of the respiratory tract in which NPs deposit) determines its interaction with the lung (e.g. lung lining fluid, cellular membranes and fluids), and the subsequent lung clearance and toxicity.

Differences in the susceptibility of different species have been observed in several studies, showing rats to be more susceptible than mice to inhaled TiO_2 NPs and carbon black (Bermudez et al. 2004; Elder et al. 2005; Warheit et al. 2016). This difference has been attributed to differences in the deposition as well as the pulmonary response, especially at concentrations where macrophage-mediated clearance is impaired (pulmonary overload conditions) (Bermudez et al. 2004; Warheit et al. 2016). Since the occurrence of lung tumors under pulmonary overload conditions in rats may not be representative for humans, the use of rats for particle toxicology has been criticized (Warheit et al. 2016). Although pulmonary overload generally occurs at higher exposure concentrations in mice and other experimental animals, there is no consensus as to the most appropriate species to predict particle induced lung toxicity in humans. One major

advantage of using mice is the availability of many disease models and relative ease of genetic modification to provide mechanistic insight.

Toxicokinetics and toxicodynamics of inhaled NPs are greatly influenced by their physical characteristics, such as the size and density of the primary particles, aggregates or agglomerates, as well as by their chemical composition, dissolution rate, surface reactivity, shape, charge and hydrophobicity (Nel et al. 2006; Braakhuis et al. 2014; Bakand and Hayes 2016).

To investigate the possible explanation(s) for the discrepancies between the findings of different CeO_2 NP inhalation studies, we have compared the results of exposure to undoped CeO_2 NPs (herein referred to as PROM) from our recent study (Dekkers et al., 2017) to the results of other 4 week inhalation studies with various nanoforms of CeO_2 (referred to as NM212, NM211 and NanoAmor in the subsequent tables and figures) in rats and mice (Geraets et al. 2012; Aalapati et al. 2014; Gosens et al. 2014; Keller et al. 2014). To investigate species differences in the susceptibility to CeO_2 NPs, two additional 5 day inhalation studies using the same undoped CeO_2 NPs as used in Dekkers et al. (2017) (PROM) were performed; one in rats and one in mice to bridge the results from the various studies. These additional studies are not presently published in peer-reviewed journals, but were performed within the NanoMILE project (http://nanomile.eu-vri.eu/) under GLP according a short term inhalation study (STIS) protocol, and were included in Deliverable D7.2 (NanoMILE 2017) which is available upon request. The STIS has been specifically designed for the testing of toxicity and kinetics of nanomaterials and has previously been applied for hazard assessment of 13 metal oxide nanomaterials, including the CeO_2 nanoforms NM211 and NM212 (Keller et al. 2014; Landsiedel et al. 2014).

To investigate the origin of the differences in toxicity of the different CeO_2 nanoforms, the results of these 5-day inhalation studies with the PROM CeO_2 NPs were compared to the results of previous 5 day inhalation studies with other nanoforms of CeO_2 (NM212 and NM211) in rats (Keller et al. 2014).

This paper describes the analysis of data from the aforementioned CeO_2 NP inhalation studies to investigate to what extent the observed differences in toxicity between various studies can be explained by differences in kinetics (section 3), species susceptibility (section 4) and/or physicochemical characteristics of the nanoforms (section 5).

We hypothesized that a lower internal dose and higher clearance of the CeO_2 NPs might explain the relatively mild effects observed 4 weeks post-exposure in our recent study (Dekkers et al. 2017). We also hypothesized that, similar to other poorly soluble particles (such as TiO_2 NPs and carbon black), rats would be more susceptible to exposure to CeO_2 NPs than mice. Lastly, based on the mild toxicity of PROM CeO_2 NPs observed in mice, we hypothesized that PROM CeO_2 NPs would also be less toxic compared to other nanoforms of CeO_2 NPs in rats.

Materials and methods

Selection of the Studies

The selected studies were limited to those where rats or mice were exposed via inhalation (either whole body or nose only) and in which the pulmonary toxicity as well as pulmonary dose (i.e. lung burden) were determined. Studies in which rats and mice were exposed for 4 weeks as well as for 5 days were selected because not all nanoforms of CeO_2 were tested in 4 week inhalation studies in both species. An overview of the inhalation studies that were analyzed can be found in Table 1. A summary of the pulmonary findings of these inhalation studies can be

found in Table S1 of the supplementary information, and further details on the 5-day inhalation studies with the PROM CeO_2 NPs can be found in Table 4 and Annex S2.

From External Concentration to Internal Lung or Pulmonary Burden

To investigate the role of kinetics in pulmonary toxicity of CeO_2 NPs, toxicity data of the studies was compared using a) the external exposure concentration, b) the internal retained dose in the lung (i.e. the measured lung burden) and c) the predicted internal retained dose in the pulmonary or alveolar regions of the lung (i.e. the expected pulmonary burden). In all studies, the rats or mice were exposed to CeO_2 NPs at concentrations between 0.5 and 25 mg/m^3 with similar particle size distributions (mass median aerodynamic diameter (MMAD) between 0.9 to 2.2 μm).

The measured lung burden (LB) was calculated from the measured Ce concentrations in the lung tissue (typically determined by ICP-MS) and total lung weight. For those studies in which no lung and body weights were reported (Aalapati et al. 2014; Keller et al. 2014), an estimation of the lung weight was made based on the lung weights of animals of the same strain and similar ages.

[Table 1 near here]

To verify if the different methods used to measure the LB resulted in comparable results, the anticipated LB was also estimated using the Multiple-Path Particle Dosimetry Model (MPPD V3.04, Applied Research Associates, Inc., https://www.ara.com/products/multiple-path-particle-dosimetry-model-mppd-v-304). The chosen MPPD airway morphometry model and input data used to estimate the retained dose in the lung of the studies can be found in Table S3 of the

supplementary information. For all studies the "deposition and clearance" option of the MPPD model was used to estimate the tracheobronchial retention (TBR) and the alveolar retention (AR). The density of the bulk material of CeO_2 (7.215 g/m^3) was used as input parameter as no information is available of the density of CeO_2 nanoforms occurring in the aerosols. Default settings where used unless stated otherwise.

The estimated LB was calculated using the following formula:

$$Estimated\ LB\ (\mu g/g) = \frac{TBR(\mu g) + AR(\mu g)}{Lung\ weight\ (g)}$$

The expected pulmonary burden (PB) was expressed as the surface area of the NPs per surface area of the pulmonary region of the lung (mm^2 NP/cm^2 lung) instead of µg NP/g lung, based on the findings of Braakhuis et al. (2016). PB was calculated using the measured LB, the fraction of deposition in the alveolar region of the lung (AD), the total deposition in the lung (alveolar deposition + tracheobronchial deposition; AD+TBD) (as estimated with the MPPD model), the surface area of the NPs (SA_{NP}; estimated using the reported BET measurement or calculated based on primary particle size) and the surface area of the pulmonary region of the lung (SA_{PR}; estimated with the MPPD model). The data used to calculate the PB of the selected studies can be found in Table S4 of the supplementary information. The PB was calculated using the following formula:

$$PB\ (mm^2/nm^2) = \frac{LB\ (\#)\ x\ \frac{AD\ (\%)}{(AD(\%) + TBD(\%))}\ x\ SA_{NP}\ (m^2)}{SA_{PR}\ (cm^2)}$$

To investigate if differences in LB or PB might explain the differences in toxicity, bronchoalveolar lavage fluid (BALF) and histopathological analyses of the studies were quantitatively compared using the measured exposure concentration (mg NP/m^3 air), LB (µg NP/g lung) and PB (mm^2 NP/cm^2 lung). A quantitative comparison of the effects on the BALF analyses among the studies was considered appropriate, since all studies used a similar method for lung lavage (2 lavages with a similar volume of saline solution). Alveolar histiocytosis (accumulation and infiltration of monocytes and macrophages in the pulmonary tissues) and increased neutrophil counts in BALF were also plotted against the exposure concentration, LB and PB to further examine dose response relationships, as these parameters were observed in most studies and are frequently identified as first evidence of adversity (Pauluhn 2017).

Differences in Susceptibility

To investigate species differences in susceptibility to the inflammatory effects induced by CeO_2 NPs, and differences in toxicity between PROM CeO_2 NPs and NM212, data from the two additional 5-day inhalation studies with the PROM CeO_2 NPs in rats and mice (see Table 4 and Annex S2) were compared.

Statistical Analyses

GraphPad Prism v7.00 (GraphPad Software, San Diego, California, USA) was used to visualize and to analyze the data using linear regression analysis of the measured versus the estimated LB and non-linear regression analysis (curve-fitting) of the dose response curves using the various dose metrics.

Results and Discussion

Internal Lung and Pulmonary Burden

An overview of the measured and estimated LB and expected PB from the selected CeO_2 NP inhalation studies can be found in Table 2. Expressing the LB relative to the total lung weight and PB relative to the total surface area of the lung might lead to an underestimation of the LB and PB if exposure to the CeO_2 NPs also significantly increase the lung weight and/or the lung surface area. However, expressing the absolute LB and PB levels does not allow comparison of LB and PB levels of different studies, due to species, strain, age and bodyweight differences of the animals. Approximately 9 to18% of the inhaled (mass) dose was predicted to deposit in the lungs, of which between 50 and 70% was expected to deposit in the pulmonary region of the lung (see Table S4 in the supplementary information). The measured LB was lower than the estimated LB at nearly all exposure concentrations. This overestimation of the LB by the MPPD models might be due to an underestimation of the clearance rates or an overestimation of the deposition. The clearance rates of the MPPD model are based on LB measurements from studies in which various strains of rats (F344) and mice (BALB/C and B6C3F1) were exposed to various micro- and nanosized particles (TiO_2, C60-fullerenes and Fe_xO_y) at various concentrations (0.5, 2, 10 and 20 mg/m^3), exposure durations (4 h and 13 weeks) and recovery periods (0 and 6 h, 1 day, and 2, 4, 8, 13, 26 and 52 weeks). The animal strains (Wistar rats, C57Bl/6 J and CD-1 mice), NPs (CeO_2 NPs), concentrations (0.5, 2, 5, 10, 20 and 25 mg/m^3), exposure duration (5 days and 4 weeks) and recovery periods (0 days and 3-5 weeks) of the selected studies only partly overlap with those on which the clearance rates of the MPPD model are based. Therefore, the high LBs estimated by the MPPD model indicate that the clearance rates might have been underestimated by the MPPD model, especially for the mice. Additionally, the deposition may

have been overestimated. Next to differences in strains, concentrations and exposure durations between the selected studies and the studies on which the deposition in the MPPD model is based, also other ~~Although strain differences in respiratory tract geometry are taken into account in the MPPD model, the model makes~~ assumptions ~~which~~ may have led to an overestimation of deposition. For example, the model assumes spherical aerosols, while aerosols of NPs generally consist of non-spherical agglomerates of (spherical or nearly-spherical) primary particles, which tend to have a lower density than (solid) spherical particles of the same diameter. An overestimation of the density might lead to an overestimation of the amount deposited in the lungs.

Differences due to Deposition and Clearance

To investigate to what extent the differences in toxicity may be explained by differences in pulmonary deposition and clearance of the CeO_2 NPs, the LB and PB of several 4 week inhalation studies with various nanoforms of CeO_2 (NM211, NM212, PROM and NanoAmor) in rats and mice are compared. A comparison of the measured and estimated LB in rats and mice after 4 weeks of exposure to CeO_2 is presented in Figure 1.

[Table 2 near here]

[Figure 1 near here]

The measured LB correlated well (R^2=0.75) with the estimated LB (Figure 1), although this correlation was dominated by studies in rats. The measured LB was mostly lower or similar than

the estimated LB, except for mice exposed to 2 mg/mm^3 NanoAmor for which the measured LB was approximately 10 times higher than estimated (see black and white dot in Figure 1). There was no consistent relationship between the exposure concentration or particle diameter and the percentage of clearance (R^2<0.5; data not shown).

Subsequently, it was investigated whether the differences in toxicity observed in the different 4 week inhalation studies with various nanoforms of CeO_2 in rats and mice can be explained by differences in toxicokinetics (LB and PB). Therefore, a quantitative comparison was performed of the markers for toxicity (BALF and histopathological analyses) using external as well as internal concentrations expressed in two different dose metrics (Table 3 and Figure 2). All but one study found aggregates of particles and macrophages in the lungs, whereas only one study (in which rats were exposed to NM212) demonstrated an increase in the presence of eosinophilic material, interpreted to be indicative of cell damage. Granulomatous inflammation, indicative of macrophage mediated inflammation, was noted in only two studies (in which rats were exposed to NM212 and mice to NanoAmor). More importantly, neutrophilic inflammation was observed in all studies. The severity of the pulmonary toxicity (alveolar histiocytosis in 20% of the animals) observed in mice 4 weeks post-exposure to PROM CeO_2 NPs (Dekkers et al. 2017) was remarkably less than the severity of the pulmonary toxicity (increase in neutrophils and lymphocytes in BALF, alveolar histiocytosis in 100% of the animals and the occurrence of eosinophilic granular material, macrophage aggregates with particles and granulomatous inflammation) observed in the other 4 week inhalation studies at similar or even lower external and internal concentrations (i.e. in rats exposed to NM211 and NM212 and mice exposed to NanoAmor). These findings indicate that differences in toxicity cannot be explained by differences in toxicokinetics alone.

When the toxicological effects were expressed as percentages of neutrophils in the BALF at each exposure concentration, non-linear curve fitting did not provide a suitable fit (R^2=0.57 and 0.48; Figure 2a). Non-linear curve fitting was improved by plotting % neutrophils against internal dose, for the measured LB (R^2= 0.70 and 0.96; Figure 2c) but not for the PB (R^2=0.55 and 0.56; Figure 2e). However, since the LB of the Aalapati study was approximately 10 times higher than the estimated LB (Figure 1 and Table 2), the LB and PB of this study could be considered as outliers. Without the data of the Aalapati study, the non-linear curve fitting led to better goodness of fit values for both the LB (R^2=0.71 and 0.97) and PB (R^2=0.75 and 0.71) compared to the exposure concentration (R^2=0.66 and 0.52). Although these curve fitting analyses did not indicate whether LB (μg/g lung) or PB (mm^2/cm^2) is a better predictor for the observed toxicity, they are in line with previous studies that indicated that the internal dose was more predictive of the toxicity of inhaled NPs than the external dose (Kreyling et al. 2013; Kuempel et al. 2015; Braakhuis et al. 2016; Pauluhn 2017). Nevertheless, the differences in toxicity may to some extent also be explained by differences in susceptibility between mice and rats exposed to similar internal dose levels and/or differences in toxicity between the various nanoforms of CeO_2.

[Figure 2 near here]

[Table 3 near here]

Difference in Susceptibility between Mice and Rats

To further investigate differences in the susceptibility of mice compared to rats, two additional 5 day inhalation studies with the same nanoform of CeO_2 (PROM) were performed; one in mice

and one in rats. These additional studies are not presently published in peer-reviewed journals, but were performed within the NanoMILE project (http://nanomile.eu-vri.eu/) under GLP and were included in Deliverable D7.2 which is available upon request (NanoMILE 2017). The study design of these additional studies with PROM CeO_2 NPs follow the previously performed 5 day inhalation studies with NM212 (Keller et al. 2014), but used nose-only exposure instead of whole body exposure, to allow better comparison with the 4 week inhalation study with PROM CeO_2 NPs in mice (Dekkers et al. 2017). Animals were exposed to similar concentrations of CeO_2 NPs (0.5, 2 and 5 mg/m^3) with similar particle size distributions (MMAD between 1.0 and 1.3 μm for rats and between 0.9 and 1.2 μm for mice). A detailed description of these studies can be found in Annex S2 of the supplementary information.

In male Wistar rats, exposure to PROM CeO_2 NPs led to changes of several BALF parameters, including increased neutrophils, in animals exposed to 2 mg/m³ and 5 mg/m³ CeO_2 NPs (Table 4), the effects of which were still observed 3 weeks post-exposure. At these concentrations, the number of neutrophils was also increased in blood, as were blood monocyte counts. These findings indicate that inflammation in the lungs was persistent, and was accompanied by systemic inflammation. Histopathological findings after the recovery period were only observed at 5 mg/m³. The findings were consistent with a retarded lung clearance after 2 and 5 mg/m³ CeO_2 NP exposure.

In mice, exposure to PROM CeO_2 NPs up to 5 mg/m³ did not lead to any significant change in clinical pathology parameters in blood and in BALF, or treatment-related adverse effects based on histopathology in mice. These findings were consistent with the higher clearance in mice (approximately 30 to 50 % in 3 weeks) compared to rats (approximately 20 to 35 % in 3 weeks).

The measured LB of the two 5 day inhalation studies was much lower than the estimated LB, but showed a high correlation with the estimated LB (R^2=0.93 for rats and R^2=0.91 for mice; Figure 3). The high LBs estimated by the MPPD model indicate that some of the assumptions made by the MPPD model may not necessarily reflect the reality for the rats and mice in the selected studies and that the assumed clearance rate might have been underestimated by the MPPD model, especially in mice.

[Figure 3 near here]

The LB and PB was higher in rats compared to mice, especially 3 weeks post-exposure, an effect that is probably due to the higher clearance rates in mice compared to rats. To investigate if the modest toxicity of the PROM CeO_2 NPs can be explained by the species difference in toxicokinetics, a quantitative comparison of pulmonary effect parameters was performed using external as well as internal concentrations (Table 4 and Figure 4). Similar to the curve fitting analyses of the 4 week inhalation studies (Figure 2c and e), the curve fitting analyses of the 5 day inhalation studies with PROM CeO_2 NPs in rats and mice (Figure 4c and e) did not indicate whether LB (µg/g lung) or PB (mm^2/cm^2) is a better predictor for the observed toxicity. The differences in internal concentration (LB and PB) cannot fully explain the differences in the severity of the toxicological effects, since changes in BALF and adverse histopathological effects were observed at lower LB and PB levels in rats compared to mice. Furthermore, the dose response curves are steeper in rats compared to mice, indicating PROM CeO_2 NPs are more toxic to rats compared to mice (see Figure 4). This indicates that besides differences in kinetics (resulting in differences in LBs and PBs) between mice and rats, there are other differences in

susceptibility between these species. This is in line with outcomes from previous studies in which rats tended to be more susceptible to pulmonary inflammation induced by poorly soluble particles (Bermudez et al. 2004; Borm et al. 2015; Warheit et al. 2016). There are no *in vitro* studies available that compare the toxicity of CeO_2 NPs in mice and rat macrophages. However, the sensitivity of rat alveolar macrophages to various NPs has recently been tested in an *in vitro* assay to predict short-term inhalation of NPs. All four CeO_2 NPs tested in this study (including NM211 and NM212) showed a dose dependent cytotoxicity and were identified as being biologically active in rat alveolar macrophages (Wiemann et al. 2016).

[Table 4 near here]

[Figure 4 near here]

Differences due to Different Nanoforms

To investigate differences in toxicity between the different CeO_2 nanoforms , data from all 5 day inhalation studies in rats with NM211, NM212 and PROM were compared using exposure concentration, LB and PB and dose matrices. The measured LB was lower than the estimated LB but showed a strong correlation (R^2=0.99, Figure 5).

[Figure 5 near here]

Exposure to PROM CeO_2 NPs resulted in relatively high PB levels compared to NM211 and NM212 at similar exposure concentrations (Table 5 and Figure 6). This difference in PB in rats

is probably caused by differences in clearance, since the measured LB levels and the estimated fractions of the inhaled dose deposited in the lung and pulmonary region of the lung were largely similar across the different CeO_2 nanoforms (see Table S4). Clearance of particles deposited in the lungs is known to depend mainly on the dose, size, shape and dissolution rate of primary particles and their aggregates and agglomerates (Greim and Ziegler-Skylakakis 2007; Keller et al. 2014). Previous studies investigating the influence of particle size on the clearance have either found no influence of particle size on clearance (Semmler et al. 2004; Buckley et al. 2017) or a slower clearance of smaller particles (Geraets et al. 2012; Keller et al. 2014; Han et al. 2015). Indeed, the nanoforms with the smallest primary particle size (PROM: 4.7 nm) seem to have a slower clearance than the larger nanoforms (NM211: 12.5 nm and NM212: 40 nm; see Table S4 and S5 of supplementary information). However, the size of the aerosols has only a modest influence on the clearance rate, with the largest aerosols (NM211) showing a slightly higher clearance rate at all exposure concentrations compared to nanoforms with a smaller MMAD (NM212 and PROM) (see Table S4 and S5 of supplementary information).

[Figure 6 near here]

To investigate if differences in toxicokinetics across the various nanoforms of CeO_2 might explain the differences in toxicity, a quantitative comparison of parameters measured in BALF as well as histopathological analyses were performed using external as well as internal concentrations of NM211, NM212 and PROM (Table 5 and Figure 7). Similar to the curve fitting analyses of the 4 week inhalation studies (Figure 2c and e) and the 5 day inhalation

studies with PROM CeO_2 NPs in rats and mice (Figure 4c and e), curve fitting analyses of the 5 day inhalation studies in rats with the various CeO_2 NPs (Figure 7c and e) did not indicate whether LB (μg/g lung) or PB (mm^2/cm^2) is a better predictor for the observed toxicity. More data is needed to investigate the best dose metric.

Besides differences in toxicokinetics of CeO_2 NPs (resulting in differences in LB and PB), differences in the toxicodynamics (i.e. toxic potency) of the different nanoforms of CeO_2 may account for the observed differences in toxicity. Immediately after exposure (day 0) more severe changes in BALF parameters and lung histopathology were observed at lower exposure concentrations, LB and PB levels of NM211 and NM212 compared to PROM CeO_2 NPs (Table 5). This indicates that PROM CeO_2 NPs are less toxic than NM211 and NM212 when expressed as external concentration (mg NP/m^3 air), internal mass dose (μg NP/g lung), or internal surface area dose (mm^2 NP/cm^2 lung). Three weeks after exposure, more severe changes in BALF parameters and lung histopathology were observed after exposure to PROM CeO_2 NPs compared to NM212 at certain exposure concentrations (e.g. 5 mg/m^3) or LBs (60-70 μg NP/g lung). However, similar effects on BALF parameters and lung histopathology were observed at similar PB levels for all different nanoforms, indicating a similar toxic potency of all nanoforms when the dose is expressed as internal surface area (mm^2 NP/cm^2 lung).

[Table 5 near here]

[Figure 7 near here]

When expressed as exposure concentration (mg NP/m^3 air) or LB (μg NP/g lung) the dose

response curves of the percentage of neutrophils in the BALF are steeper for PROM CeO_2 NPs than for NM212 (see Figure 7a and c), indicating PROM CeO_2 NPs are more toxic than NM212 when the dose is expressed as external concentration or internal mass dose. Conversely, when expressed as PB (mm^2 NP/cm^2 lung) the dose response curves are steeper for NM212 compared to PROM CeO_2 NPs 1 day post-exposure (see Figure 7e), indicating NM212 is more acutely toxic than PROM CeO_2 NPs when the dose is expressed as internal surface area. Non-linear curve fitting did not result in a good fit for NM212 at 3 weeks post-exposure ($R^2 = 0.61$), which makes the comparison between the different nanoforms of CeO_2 based on dose response curves of the percentage of neutrophils in the BALF 3 weeks post-exposure difficult.

The observed differences between the different nanoforms of CeO_2 may reflect differences in physicochemical characteristics such as size, shape, surface chemistry (reactivity and charge), dissolution and hydrophobicity of the different nanoforms. Although there are no studies available in which the cytotoxicity of the NM212, NM211 and PROM are compared in the same *in vitro* assays, previous studies have shown that differences in physicochemical properties such as (primary) particles size, surface reactivity and shape may lead to differences in (cyto)toxicity.

Previous studies indicate that smaller particles, aggregates, agglomerates and aerosols result in higher deposition levels in the deep lung and slower clearance rates than larger particles (Braakhuis et al. 2014; Peng et al. 2014). In addition, smaller particles have a relatively large surface area (for an equivalent mass) available for interaction with the body, and potentially can more easily cross barriers or be taken up by macrophages (Nel et al. 2006; Geiser 2010; Arts et al. 2015; Bakand and Hayes 2016). These differences in toxicokinetics and toxicodynamics generally lead to smaller particles having a more potent pulmonary inflammation (acute and

chronic) after inhalation. However, differences in dose response for smaller TiO_2 and Ag NPs were found to disappear when the dose was expressed as surface area instead of mass (Oberdorster et al. 2005; Braakhuis et al. 2014). This is in contrast to the toxicity observed here immediately after exposure to CeO_2 NPs, which suggests that the nanoforms of CeO_2 with the smallest primary particle size (PROM) are less toxic compared to those with larger primary particle sizes and a similar or larger aggregate/agglomerate size (NM211 and NM212). However, the toxicity observed here 3 weeks post-exposure to CeO_2 NPs seems to confirm the findings of Oberdorster and Braakhuis, since the observed differences in toxicity between the nanoforms of CeO_2 NP with different primary particle and aggregate/agglomerate sizes seem to decrease when the dose is expressed as surface area (mm^2 NP/cm^2 lung) instead of mass (mg NP/m^3 air or μg NP/g lung).

Besides size, surface reactivity may also influence toxicity (Warheit et al. 2007; Braakhuis et al. 2014; Peng et al. 2014; Arts et al. 2015; Bakand and Hayes 2016). NPs with a higher surface reactivity are generally able to generate more reactive oxygen species (ROS) that may lead to oxidative stress and inflammatory responses. CeO_2 NPs have been shown to both induce ROS and oxidative stress (Lin et al. 2006; Park et al. 2008; Eom and Choi 2009) as well as protect against oxidant-induced effects (Xia et al. 2008; Celardo et al. 2011). Gandon et al. (2017) showed that NM212 and NM211 had similar reactivity in the Ferric Reduction Ability of Serum (FRAS) assay when the dose was expressed as NM surface area. This is in line with our findings showing a similar toxicity of NM212 and NM211 at similar external and internal concentrations. The reactivity PROM CeO_2 NPs is slightly higher than that of NM212 and NM211 (see Table S5). Although, this increasing reactivity with decreasing size is consistent with the increase of Ce^{3+} atoms on the surface of smaller particles, this higher reactivity is not in

line with our findings showing that PROM CeO_2 NPs are less toxic compared to NM212 and NM211 at similar external and internal concentrations. .

Previous studies show contradicting results with respect to the influence of particle shape on the toxicity of CeO_2 NPs. Wang et al. (2015) showed that cube-like and octahedron-like CeO_2 NPs induced higher cytotoxicity and lower anti-oxidative properties compared to rod-like CeO_2 NPs in HepG2 cells. Forest et al. (2017) on the other hand showed that rod-like CeO_2 NPs produced significantly and dose-dependently enhanced pro-inflammatory and cytotoxicity responses in RAW264.7 cells, that were not observed after exposure to cubic/octahedral NPs. Our findings cannot be compared to the results of these previous studies, as they didn't include cubic, octahedral or rod-like CeO_2 NPs. TEM images of the nanoforms used in the studies discussed here showed that the nanoform with the least toxic potency right after exposure (PROM) was spherical (Dekkers et al. 2017). Near-spherical NM211 and polyhedral shaped NM212 NPs showed similar toxicity. Because these three nanoforms differ in more than one physicochemical characteristic (Table S5), it is difficult to determine the influence of each individual characteristic on the observed toxicity.

Summary and Conclusion

In the present study we have used data from several inhalation studies in which mice or rats were exposed to various nanoforms of CeO_2 to investigate to what extent the observed differences in toxicity between various studies can be explained by differences in lung deposition, species susceptibility and/or physicochemical characteristics of the tested nanoforms. Considerable differences in pulmonary response were observed between mice and rats (Table 4) and between the various CeO_2 nanoforms tested (Table 5). Our evaluations demonstrate that the external exposure concentration, and the NP size and chemical composition (CeO_2) alone cannot fully explain the observed differences in health effects. The level at which an adverse effect starts to develop is also dependent on the internal dose, animal species and the specific nanoform. We have shown that rats are more sensitive than mice based on both external and internal concentrations. These differences do not result purely from differences in the delivered and retained doses (expressed in particle mass as well as surface area). In addition, the different nanoforms showed differences in toxic potency. Particle size is highly important to this response, but also other physicochemical properties of the CeO_2 NPs, such as surface reactivity and surface shape, may influence the toxicity of the nanoform. Based on these findings we conclude that the observed differences in toxicity of the CeO_2 NPs in various inhalation studies can be explained by differences in kinetics and susceptibility of rats compared to mice and differences in size as well as other physicochemical characteristics of the CeO_2 NPs. It remains to be determined to what extent these findings can be generalized to other poorly soluble nanomaterials.

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Declaration of Interest Statement

The contents of this paper do not necessarily reflect the views and polices of the RIVM or those of the Netherlands Ministry of Infrastructures and Water Management. The authors have no conflict of interest to disclose. Lan Ma-Hock, Jana Keller, Karin Küttler, Volker Strauss and Robert Landsiedel are employees of BASF SE, a chemical company producing and marketing nanomaterials (but not the test materials of the present study, which were selected based on purely scientific considerations).

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Supplementary Information

Table S1: Overview of the pulmonary findings (kinetics and effects)

Annex S2: Summary of the short term inhalation studies with PROM CeO_2 NPs

Table S3: Models and data used to calculate the lung burden (LB) or retained dose in the lung

Table S4: Data used to calculate the expected pulmonary burden (PB)

Table S5: Physicochemical characteristics of the CeO_2 NPs of the 5 day inhalation studies in rats

Table 1: Overview of the selected CeO_2 NP inhalation toxicity studies

Reference	Strain, species and sex of animals	Nanoform	Aerosol conc. (mg/m^3) [a]	MMAD ± GSD (µm)	Exposure method [B]	Exposure duration (hr/d)	Exposure duration (days)	Post-exposure period (weeks)
Keller et al., 2014	Wistar rat, female	NM211	0.45	1.9 ± 2.9	WB	6	5	3
			25.8	2.2 ± 2.4				
Keller et al., 2014	Wistar rat, female	NM212	0.5	1.4 ± 2.3	WB	6	5	3
			5.3	1.2 ± 2.1				
			25.9	1.0 ± 2.5				
NanoMILE, 2017	Wistar rat, male	PROM	0.57	1.3 ± 2.2	NO	6	5	3
			2.04	1.2 ± 2.4				
			4.85	1.1 ± 1.3				
Keller et al., 2014	Wistar rat, male	NM212	0.48	1.6 ± 2.1	WB	6	20	5
			5.2	1.3 ± 2.1				
			25.6	0.9 ± 2.5				
Geraerts et al., 2012	Wistar rat, male + female	NM211	10.8	1.0 ± 1.8	NO	6	20	4
Geraerts et al., 2012	Wistar rat, male + female	NM212	19.95	1.2 ± 2.1	NO	6	20	4
NanoMILE, 2017	C57BL/6 J mice, male	PROM	0.54	1.1 ± 2.5	NO	6	5	3
			2.04	1.1 ± 2.4				
			5.04	0.9 ± 3.0				
Dekkers et al., 2017	C57BL/6 J mice, female	PROM	3.98	0.3 ± 1.6[c]	NO	3	20	4
Aalipati et al., 2014	CD-1 mice, male	NanoAmor	2.0	1.4 ± 2.4	NO	6	20	2 and 4

[a] These aerosol concentrations are rounded to 0.5, 2, 5, 10, 20 and 25 mg/m^3 in the main text. [b] WB = whole body. [c] Mass median diameter (MMD) instead of MMAD, estimated based on the particle size distributions of the scanning mobility particle sizer (SMPS) measurements, assuming spherical aggregation around primary particles of 4.7 nm. NO = nose only. MMAD=mass median aerodynamic diameter. GSD=geometric standard deviation.

Table 2: Measured and estimated lung burden and expected pulmonary burden of the selected inhalation studies

Reference	Species	CeO_2 nanoform	Exposure concentration (mg/m^3) [a]	Exposure duration (weeks or days)	Estimated fraction of inhaled dose deposited in the lung (%)	Lung burden 0 days after exposure (μg/g lung)			Expected pulmonary burden 0 days after exposure (mm^2/cm^2 lung) [d]	Lung burden 3 to 5 weeks after exposure (μg/g lung)		Expected pulmonary burden 3-5 weeks s after exposure (mm^2/cm^2 lung) [d]
						Estimated [b]	Estimated [c]	Measured		Estimated [c]	Measured	
Keller et al., 2014	rat	NM211	0.45	5 days	9.8	17	11	6	0.07	5	3	0.04
			25.8	5 days	9.4	942	522	269	2.8	327	206	2.6
Keller et al., 2014	rat	NM212	0.5	5 days	11.6	23	13	11	0.06	7	5	0.03
			5.3	5 days	14.3	295	170	96	0.54	96	70	0.47
			25.9	5 days	16.2	1640	997	510	3.0	647	317	2.3
NanoMILE, 2017	rat	PROM	0.57	5 days	10.3	32	17	11	0.38	8	7	0.3
			2.04	5 days	10.8	120	71	36	1.4	44	30	1.3
			4.85	5 days	12.0	310	179	79	2.9	94	63	3.5
Keller et al., 2014	rat	NM212	0.48	4 weeks	10.7	76	33	37	0.22	15	18	0.12
			5.2	4 weeks	13.1	1005	508	473	2.8	319	444	3.0
			25.6	4 weeks	17.8	6735	4053	2382	15	3033	1960	14
Geraerts et al., 2012	rat	NM211	10.8	4 weeks	14.8	3281	1494	639	6.8	1177	x	x
Geraerts et al., 2012	rat	NM212	19.9	4 weeks	13.0	5592	2776	1260	4.9	2334	x	x
NanoMILE, 2017	mice	PROM	0.54	5 days	10.7	46	29	6	0.23	12	4	0.14
			2.04	5 days	10.6	183	116	18	0.61	44	9	0.32
			5.04	5 days	12.8	522	338	60 [e]	2.1 [e]	144	29	0.94
Dekkers et al., 2017	mice	PROM	3.98	4 weeks	11.5	749	341	x	x	103	82	3.4
Aalipati et al., 2014	mice	Nano Amor	2.0	4 weeks	9.3	405	171	1353	17	52	615	7.9

[a] These aerosol concentrations are rounded to 0.5, 2, 5, 10, 20 and 25 mg/m^3 in the main text. [b] Estimated with the MPPD model (deposition only). [c] Estimated with the MPPD model (deposition and clearance). [d] Calculated using the measured lung burden, estimated pulmonary deposition fractions and estimated surface area of the pulmonary region of the lung, as described in the materials and methods section. [e] Estimated using the measured lung burden 21 days after exposure and the expected clearance (≈50%). The measured lung burden 0 days after exposure (of 495 μg/g lung) was considered unreliable, and was probably a preparation artefact (e.g. big bronchus included in the sample). x=no data available.

Table 3: Comparison of pulmonary effects in rats and mice after 4 weeks of exposure to various CeO_2 NPs (NM211, NM212, NanoAmor and PROM)

Reference	Nanoform of CeO_2 NP	Species	Measured exposure concentration (mg/m^3) [a]	LB (µg/g lung)	PB (mm^2/cm^2 lung) [b]	BALF analysis (statistically significant difference compared to the controls)	Neutrophils in BALF (% total cells)	Alveolar histiocytosis (% of animals)	Eosinophilic granular material	Macrophage aggregates with particles	Granulomatous inflammation
0-1 days after exposure											
Keller et al. 2014	NM-212	rats	0.48	37	0.22	-	2.3%	100%	-	+	-
	NM-212	rats	5.2	473	2.8	increased TC, NEU, LYM and MON	49%	100%	+	+	-
	NM-212	rats	25.6	2382	15	increased TC, NEU, LYM and MON	75%	100%	+	+	-
Gerearts et al., 2012; Gosens et al., 2013	NM-211	rats	10.8	639	6.8	increased TC, NEU, LYM and MAC	36%	100%	-	+	-
	NM-212	rats	19.9	1260	4.9	increased TC, NEU, LYM and MAC	31%	100%	-	+	-
Aalapati et al., 2013	NanoAmor	mice	2.0	1353	17	increased TC, NEU, LYM and MAC	74%	100%	-	+	+
3-5 weeks post-exposure											
Keller et al. 2014	NM-212	rats	0.48	18	0.12	-	4%	0%	-	+	-
	NM-212	rats	5.2	444	3.0	increased NEU, LYM and MON	43%	100%	+	+	-
	NM-212	rats	25.6	1960	14	increased TC, NEU, LYM and MON	73%	100%	+	+	+
Dekkers et al., 2017	PROM	mice	4.0	82	3.4	-	1.1%	20%	-	-	-
Aalapati et al., 2013	NanoAmor	mice	2.0	615	7.9	increased NEU, LYM and MAC	48%	100%	-	+	+

[a] These aerosol concentrations are rounded to 0.5, 2, 5, 10, 20 and 25 mg/m^3 in the main text. [b] Calculated using the measured lung burden, estimated pulmonary deposition fractions and an estimated surface area of the pulmonary region of the lung, as described in the materials and methods section. TC=total cell count, NEU=neutrophils, LYM=lymphocytes, MON=monocytes, MAC=macrophages, +=increased incidence compared to the controls, -=no increased incidence compared to the controls.

Table 4: Comparison of effects in rats and mice after 5 day exposure to the same CeO_2 NPs (PROM)

Reference	Nanoform of CeO_2 NP	Species	MMAD (µm)	GSD	Measured exposure concentration (mg/m^3) [a]	Lung burden (µg/g lung)	Pulmonary burden (mm^2/cm^2 lung) [b]	BALF analysis (statistically significant difference compared to the controls)	Neutrophils in BAL (% total cells)	Alveolar histiocytosis (% of animals)	Eosinophilic granular material	Macrophage aggregates with particles
0 days after exposure												
NanoMILE, 2017	PROM	mice	1.1	2.5	0.54	6.2	0.23	-	0.1%	0%	-	+
NanoMILE, 2017	PROM	rats	1.3	2.2	0.57	11	0.38	-	5%	0%	-	+
NanoMILE, 2017	PROM	mice	1.1	2.4	2.04	18	0.61	-	0%	0%	-	+
NanoMILE, 2017	PROM	rats	1.2	2.4	2.04	36	1.4	increased TC and NEU	7%	0%	-	+
NanoMILE, 2017	PROM	mice	0.9	3.0	5.04	60[c]	2.1[c]	-	0.1%	0%	-	+
NanoMILE, 2017	PROM	rats	1.1	2.3	4.85	79	2.9	increased TC, NEU, MON and EPI	29%	0%	-	+
3 weeks after exposure												
NanoMILE, 2017	PROM	mice	1.1	2.5	0.54	4.2	0.14	-	0.2%	0%	-	+
NanoMILE, 2017	PROM	rats	1.3	2.2	0.57	7.1	0.30	-	2%	0%	-	+
NanoMILE, 2017	PROM	mice	1.1	2.4	2.04	9.2	0.32	-	0.2%	0%	-	+
NanoMILE, 2017	PROM	rats	1.2	2.4	2.04	30	1.3	increased TC and MON	9%	20%	-	+
NanoMILE, 2017	PROM	mice	0.9	3.0	5.04	29	0.94	-	0.7%	0%	-	+
NanoMILE, 2017	PROM	rats	1.1	2.3	4.85	63	3.5	increased TC, NEU, MON, LYM and MAC	50%	100%	+	+

[a] These aerosol concentrations are rounded to 0.5, 2 and 5 mg/m^3 in the main text. [b] Calculated using the measured lung burden, estimated pulmonary deposition fractions and an estimated surface area of the pulmonary region of the lung, as described in the materials and methods section. [c] Estimated using the measured lung burden 21 days after exposure and the expected clearance based on other dose levels (≈50%). The measured lung burden 0 days after exposure (of 495 µg/g lung) was considered unreliable, and was probably a preparation artefact (e.g. big bronchus included in the sample). TC=total cell count, NEU=neutrophils, LYM=lymphocytes, MON=monocytes, MAC=macrophages, +=increased incidence compared to the controls, -=no increased incidence compared to the controls.

Table 5: Comparison of effects in rats after 5 day exposure to various CeO_2 NPs (NM211, NM212 and PROM)

Reference	Nanoform of CeO_2 NP	Measured exposure concentration (mg/m^3) [a]	Lung burden (µg/g lung)	Pulmonary burden (mm^2/cm^2 lung) [b]	BALF analysis (statistically significant difference compared to the controls)	Neutrophils in BAL (% total cells)	Alveolar histiocytosis (% of animals)	Eosinophilic granular material	Macrophage aggregates with particles
0 days after exposure									
Keller et al. 2014	NM211	0.45	5.8	0.07	increased NEU	6.2%	0%	-	+
Keller et al. 2014	NM211	25.8	270	2.8	increased TC, NEU, LYM and MON	79%	100%	+	+
Keller et al. 2014	NM212	0.5	11	0.06	increased NEU	5%	0%	-	+
Keller et al. 2014	NM212	5.3	96	0.54	increased TC, NEU, LYM and MON	50%	0%	-	+
Keller et al. 2014	NM212	25.9	510	3.0	increased TC, NEU and LYM and MON	81%	100%	+	+
NanoMILE, 2017	PROM	0.57	11	0.38	-	5.4%	0%	-	+
NanoMILE, 2017	PROM	2.04	36	1.4	increased TC and NEU	6.5%	0%	-	+
NanoMILE, 2017	PROM	4.85	79	2.9	increased TC, NEU, MON and EPI	29%	0%	-	+
3 weeks after exposure									
Keller et al. 2014	NM211	0.45	3.0	0.04	increased NEU	4.8%	0%	-	+
Keller et al. 2014	NM211	25.8	206	2.6	increased NEU and LYM	31%	80%	-	+
Keller et al. 2014	NM212	0.5	4.8	0.03	-	3%	0%	-	+
Keller et al. 2014	NM212	5.3	70	0.47	-	12%	60%	-	+
Keller et al. 2014	NM212	25.9	318	2.3	increased NEU	10%	80%	-	+
NanoMILE, 2017	PROM	0.57	7.1	0.30	-	2%	0%	-	+
NanoMILE, 2017	PROM	2.04	30	1.3	increased TC and MON	9%	20%	-	+
NanoMILE, 2017	PROM	4.85	63	3.5	increased TC, NEU, MON, LYM and MAC	50%	100%	+	+

[a] These aerosol concentrations are rounded to 0.5, 2 and 5 mg/m^3 in the main text. [b] Calculated using the measured lung burden, estimated pulmonary deposition fractions and an estimated surface area of the pulmonary region of the lung, as described in the materials and methods section. TC=total cell count, NEU=neutrophils, LYM=lymphocytes, MON=monocytes, MAC=macrophages, +=increased incidence compared to the controls, -=no increased incidence compared to the controls.

Figure captions

Figure 1: Measured lung burden plotted against the estimated lung burden in rats and mice after 4 weeks of exposure to various CeO_2 NPs (NM211, NM212, PROM and NanoAmor). The estimated lung burden was assessed using the MPPD V3.04 model.

Figure 2: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 4 weeks of exposure to various CeO_2 NPs plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f). Data from all 4 week inhalation studies in rats and mice with various nanoforms of CeO_2 (NM211, NM212, PROM and NanoAmor, as described in Table 3) were used.

Figure 3: Measured lung burden plotted against the estimated lung burden in rats and mice after 5 days of exposure to PROM CeO_2 NPs. The estimated lung burden was determined using the MPPD model.

Figure 4: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 5 days of exposure of rats and mice to PROM CeO_2 NPs plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f).

Figure 5: Measured lung burden plotted against the estimated lung burden in rats after 5 days of exposure to various CeO_2 NPs (NM211, NM212 and PROM). The estimated lung burden was determined using the MPPD model.

Figure 6: Expected pulmonary burden plotted against the measured exposure concentration in rats after 5 days of exposure to various CeO_2 NPs. Each bar represents the expected pulmonary burden either 1 day (white bars) or 3 weeks (black bars) after exposure to a specific exposure concentration of NM211, NM212 or PROM CeO_2 NPs.

Figure 7: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 5 days of exposure of rats to various CeO_2 NPs (NM211, NM212 and PROM) plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f).

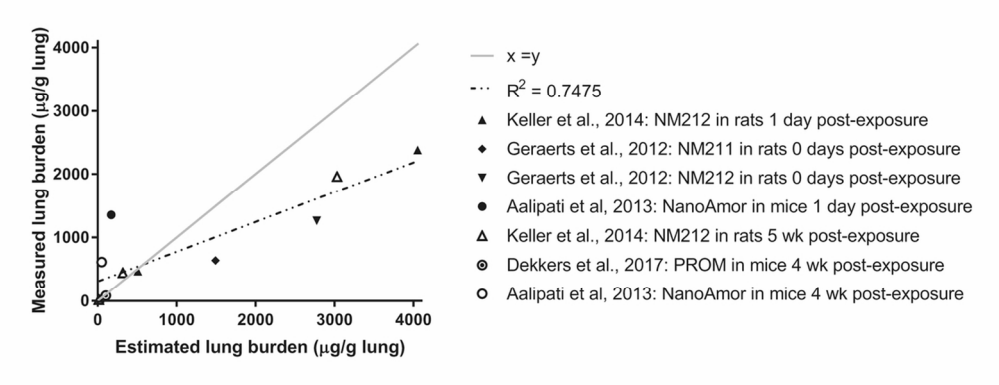

Figure 1: Measured lung burden plotted against the estimated lung burden in rats and mice after 4 weeks of exposure to various CeO_2 NPs (NM211, NM212, PROM and NanoAmor). The estimated lung burden was assessed using the MPPD V3.04 model.

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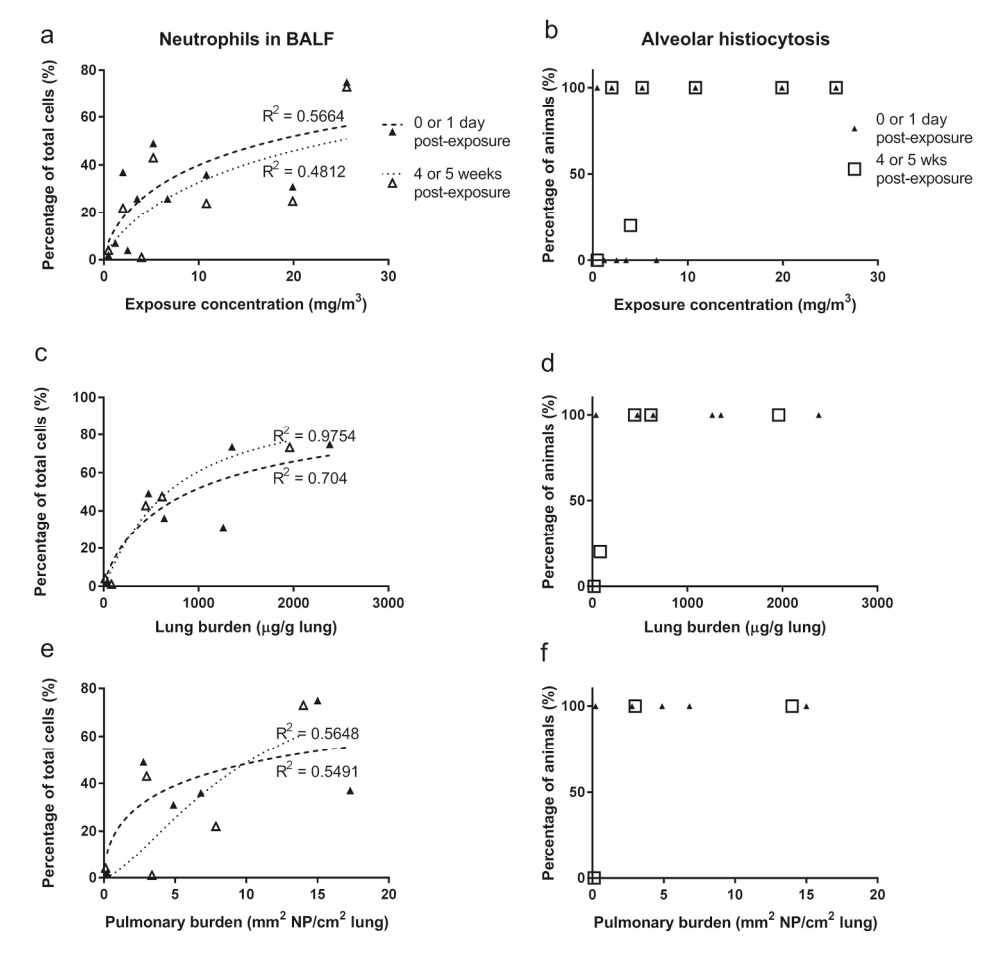

Figure 2: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 4 weeks of exposure to various CeO_2 NPs plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f). Data from all 4 week inhalation studies in rats and mice with various nanoforms of CeO_2 (NM211, NM212, PROM and NanoAmor as described in Table 3) were used.

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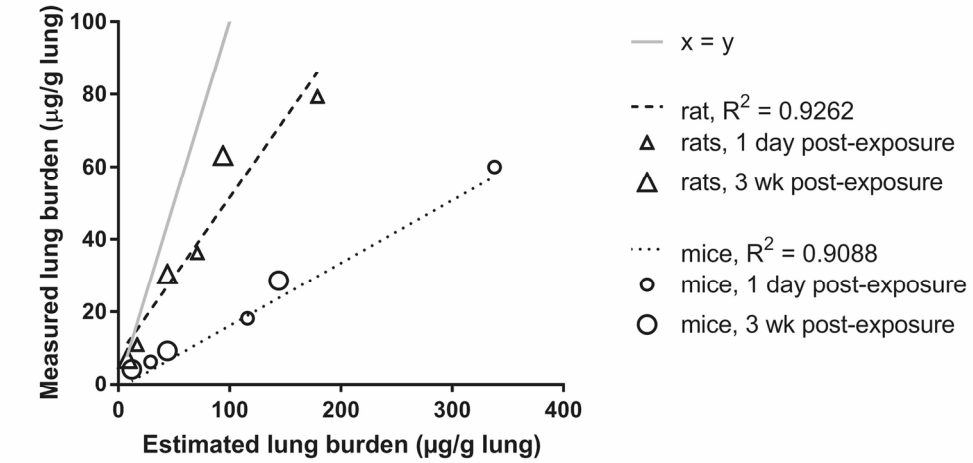

Figure 3: Measured lung burden plotted against the estimated lung burden in rats and mice after 5 days of exposure to PROM CeO_2 NPs. The estimated lung burden was determined using the MPPD model.

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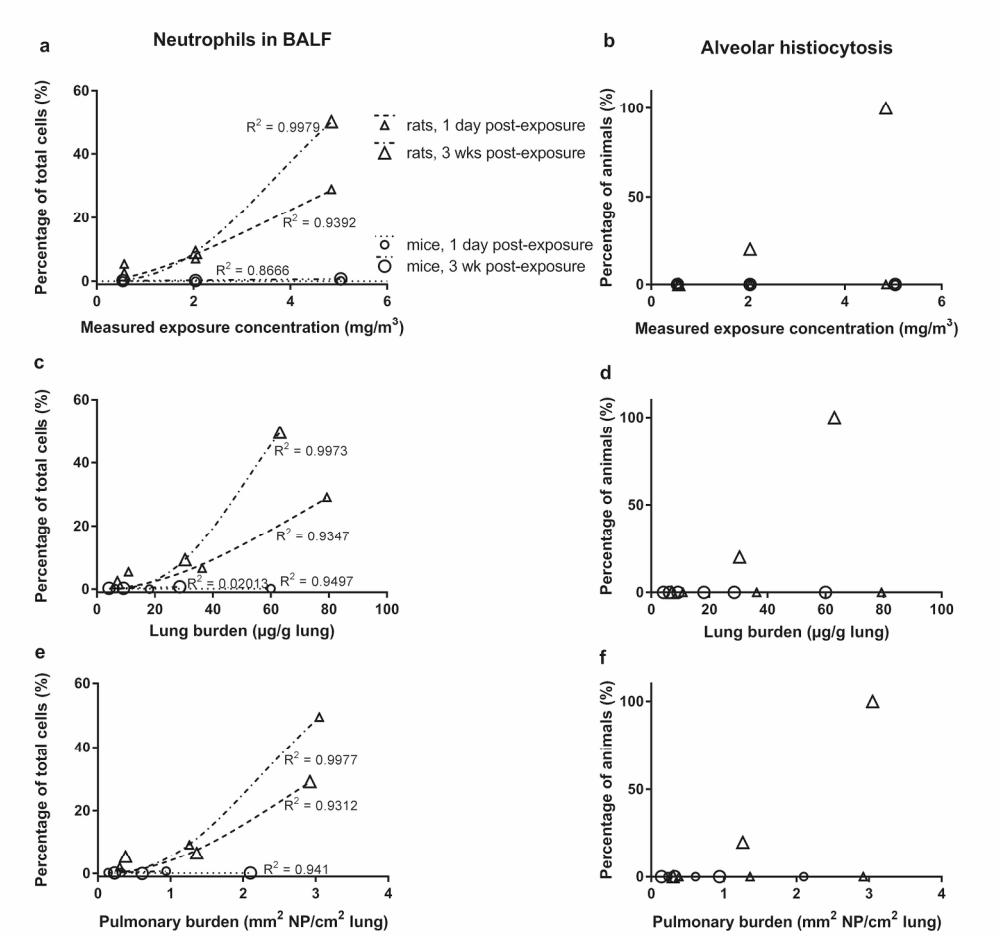

Figure 4: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 5 days of exposure of rats and mice to PROM CeO_2 NPs plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f).

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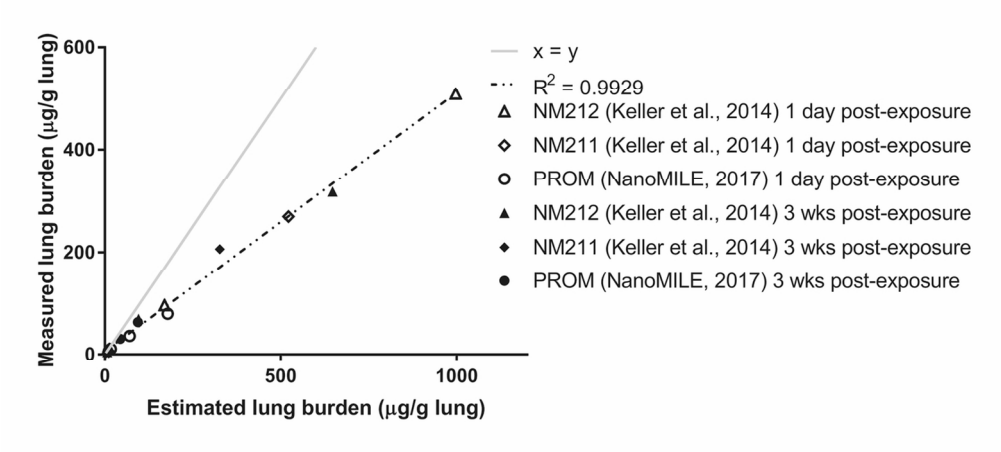

Figure 5: Measured lung burden plotted against the estimated lung burden in rats after 5 days of exposure to various CeO_2 NPs (NM211, NM212 and PROM). The estimated lung burden was determined using the MPPD model.

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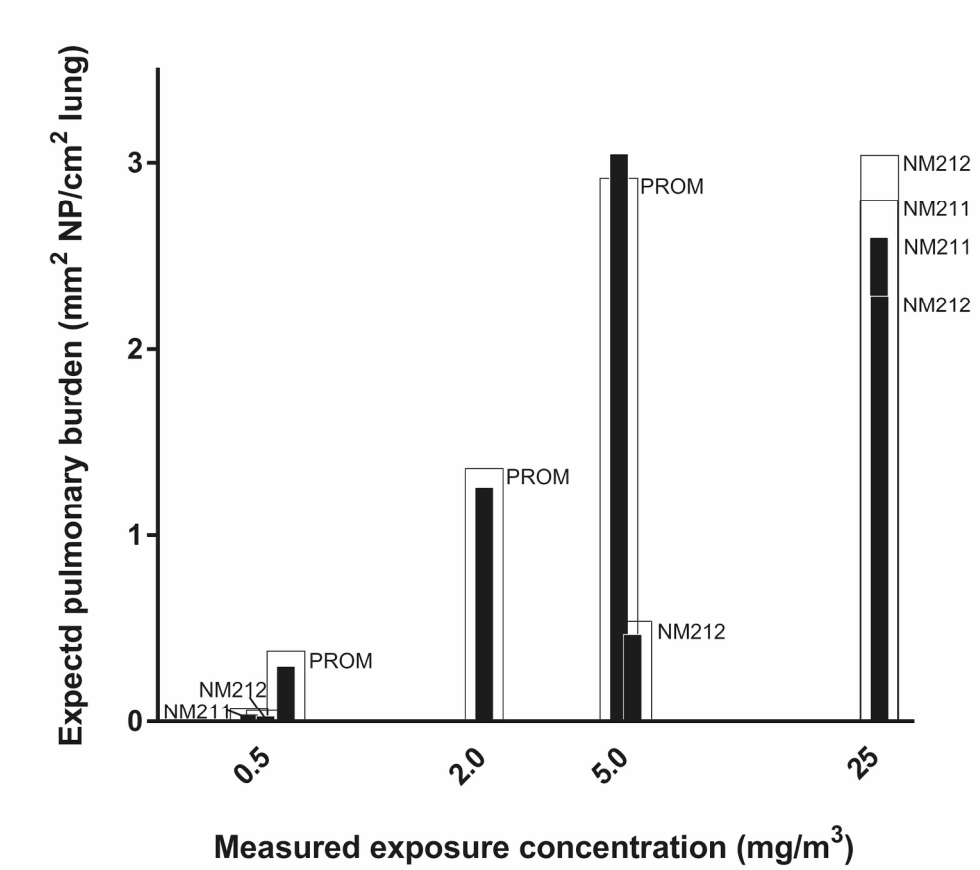

Figure 6: Expected pulmonary burden plotted against the measured exposure concentration in rats after 5 days of exposure to various CeO_2 NPs. Each bar represents the expected pulmonary burden either 1 day (white bars) or 3 weeks (black bars) after exposure to a specific exposure concentration of NM211, NM212 or PROM CeO_2 NPs.

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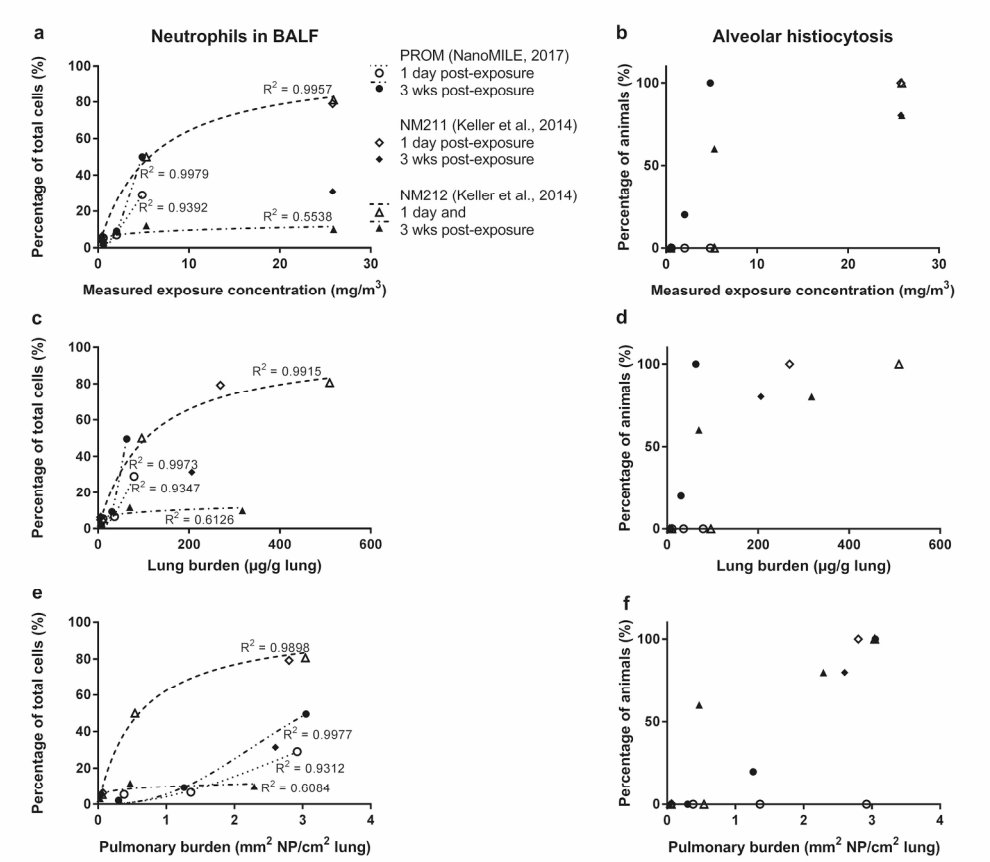

Figure 7: The percentage of neutrophils in the BALF (a, c, and e) and the percentage of animals with alveolar histiocytosis (b, d, and f) after 5 days of exposure of rats to various CeO_2 NPs (NM211, NM212 and PROM) plotted against the measured exposure concentration (mg NP/m^3 air) (a and b), lung burden (µg NP/g lung) (c and d) and expected pulmonary burden (mm^2 NP/cm^2 lung) (e and f).

193x171mm (300 x 300 DPI)

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Table S1: Overview of the pulmonary findings (kinetics and effects) of the selected inhalation studies

Reference	Nanoform of CeO_2 NP	Species	Measured exposure concentration (mg/m^3)	Exposure duration (days or weeks)	LB (µg/g lung)	PB (mm^2/nm^2 lung)	BALF analysis (statistically significant difference compared to the controls)	NEU in BALF (% total cells)	Alveolar histiocytosis (% of animals)	Eosinophilic granular material	Macrophage aggregates with particles	Granulomatous inflammation
0-1 days after exposure												
Keller et al. 2014	NM211	rat	0.45	5 days	5.8	0.07	increased NEU	6.2	0	-	+	-
			25.8	5 days	270	2.8	increased TC, NEU, LYM and MON	79	100	+	+	-
Keller et al. 2014	NM212	rat	0.5	5 days	11	0.06	increased NEU	5	0	-	+	-
			5.3	5 days	96	0.54	increased TC, NEU, LYM and MON	50	0	-	+	-
			25.9	5 days	510	3.0	increased TC, NEU and LYM and MON	81	100	+	+	-
NanoMILE, 2017	PROM	rat	0.57	5 days	11	0.38	-	5.4	0	-	+	-
			2.04	5 days	36	1.4	increased TC and NEU	6.5	0	-	+	-
			4.85	5 days	79	2.9	increased TC, NEU, MON and EPI	29	0	-	+	-
NanoMILE, 2017	PROM	mice	0.54	5 days	6.2	0.23	-	0.1	0	-	+	-
			2.04	5 days	18	0.61	-	0	0	-	+	-
			5.04	5 days	60[a]	2.1[a]	-	0.1	0	-	+	-
Keller et al. 2014	NM212	rats	0.48	4 weeks	37	0.22	-	2.3	100	-	+	-
			5.2	4 weeks	473	2.8	increased TC, NEU, LYM and MON	49	100	+	+	-
			25.6	4 weeks	2382	15	increased TC, NEU, LYM and MON	75	100	+	+	-
Gosens et al., 2013	NM211	rats	10.8	4 weeks	639	6.8	increased TC, NEU, LYM and MAC	36	100	-	+	-
	NM212	rats	19.9	4 weeks	1260	4.9	increased TC, NEU, LYM and MAC	31	100	-	+	-
Aalapati et al., 2013	Nano Amor	mice	2.0	4 weeks	1353	17	increased TC, NEU, LYM and MAC	74	100	-	+	+

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Reference	Nanoform of CeO_2 NP	Species	Measured exposure concentration (mg/m^3)	Exposure duration (days or weeks)	LB (µg/g lung)	PB (mm^2/nm^2 lung)	BALF analysis (statistically significant difference compared to the controls)	NEU in BALF (% total cells)	Alveolar histiocytosis (% of animals)	Eosinophilic granular material	Macrophage aggregates with particles	Granulomatous inflammation
3-5 weeks post-exposure												
Keller et al. 2014	NM211	rat	0.45	5 days	3.0	0.04	increased NEU	4.8	0	-	+	-
			25.8	5 days	206	2.6	increased NEU and LYM	31	80	-	+	-
Keller et al. 2014	NM212	rat	0.5	5 days	4.8	0.03	-	3	0	-	+	-
			5.3	5 days	70	0.47	-	12	60	-	+	-
			25.9	5 days	318	2.3	increased NEU	10	80	-	+	-
NanoMILE, 2017	PROM	rat	0.57	5 days	7.1	0.30	-	2	0	-	+	-
			2.04	5 days	30	1.3	increased TC and MON	9	20	-	+	-
			4.85	5 days	63	3.5	increased TC, NEU, MON, LYM and MAC	50	100	+	+	-
NanoMILE, 2017	PROM	mice	0.54	5 days	4.2	0.14	-	0.2	0	-	+	-
			2.04	5 days	9.2	0.32	-	0.2	0	-	+	-
			5.04	5 days	29	0.94	-	0.7	0	-	+	-
Keller et al. 2014	NM-212	rats	0.48	4 weeks	18	0.12	-	4	0	-	+	-
			5.2	4 weeks	444	3.0	increased NEU, LYM and MON	43	100	+	+	-
			25.6	4 weeks	1960	14	increased TC, NEU, LYM and MON	73	100	+	+	+
Dekkers et al., 2017	PROM	mice	4.0	4 weeks	82	3.4	-	1.1	20	-	-	-
Aalapati et al., 2013	NanoAmor	mice	2.0	4 weeks	615	7.9	increased NEU, LYM and MAC	48	100	-	+	+

TC=total cell count, NEU=neutrophils, LYM=lymphocytes, MON=monocytes, MAC=macrophages, +=increased incidence compared to the controls, -=no increased incidence compared to the controls. [a] Estimated using the measured lung burden 21 days after exposure and the expected clearance (≈50%). The measured lung burden 0 days after exposure (of 495 µg/g lung) was considered unreliable, and was probably a preparation artefact (e.g. big bronchus included in the sample)

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Annex S2: Summary of the short term inhalation studies with PROM CeO_2 NPs

S2.1 Study design and exposure

The study design for these additional studies with PROM CeO_2 NPs followed that of previously performed 5 day inhalation studies with NM212, but used nose-only exposure instead of whole body exposure, to allow better comparison with the 4 week inhalation study with PROM CeO_2 NPs in mice as reported previously (Dekker et al., 2017). Briefly, male Wistar rats and male C57BL/6 mice were nose-only exposed to target concentrations of 0.5, 2 and 5 mg/m³ CeO_2 NPs in respirable aerosols for 6 hours per day, on 5 consecutive days. The actual exposure concentrations were similar for rats (0.57, 2.04 and 4.85 mg/m^3) and mice (0.54, 2.04 and 5.04 mg/m^3) with similar particle size distributions (MMAD between 1.0 and 1.3 μm for rats and between 0.9 and 1.2 μm for mice; see Table S3). The concurrent control group was exposed to clean air only. During the study, the animals were monitored for mortality and clinical signs of toxicity. Body weights were determined twice weekly. Autopsy was performed and toxicity parameters were evaluated either 24 hours after the last exposure or after a post-exposure recovery period of 3 weeks (see Table S2). Blood was sampled, selected organs were weighed and a broad set of organs and tissues were preserved. The respiratory tract was examined histologically in 5 animals per group. Clinical chemistry parameters, hematology parameters and acute phase proteins were examined in blood of the same 5 animals per group for rats and 3 or 5 additional animals per group for mice. After blood sampling these animals underwent bronchoalveolar lavage of the left lung. Bronchoalveolar lavage fluid (BALF) was examined for cytological and biochemical parameters including selected antigens. Lung burdens were determined in 3 additional animals per group for both rats and mice.

Table S2: Overview of number of animals per autopsy group

Species	Post-exposure	Histopathology lung	Blood sampling	BALF	Lung burden
Rat	24 hours	5 animals/group			3 animals/group
	3 weeks	5 animals/group			3 animals/group
Mice	24 hour	5 animals/group	3 animals/group		3 animals/group
	3 weeks	5 animals/group	5 animals/group		3 animals/group

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

S2.2 Lung burden

The results of lung burden measured 24 hours after the exposure and after a 3 week recovery period are shown in Figures S2.1 and S2.2, in rats and mice, respectively.

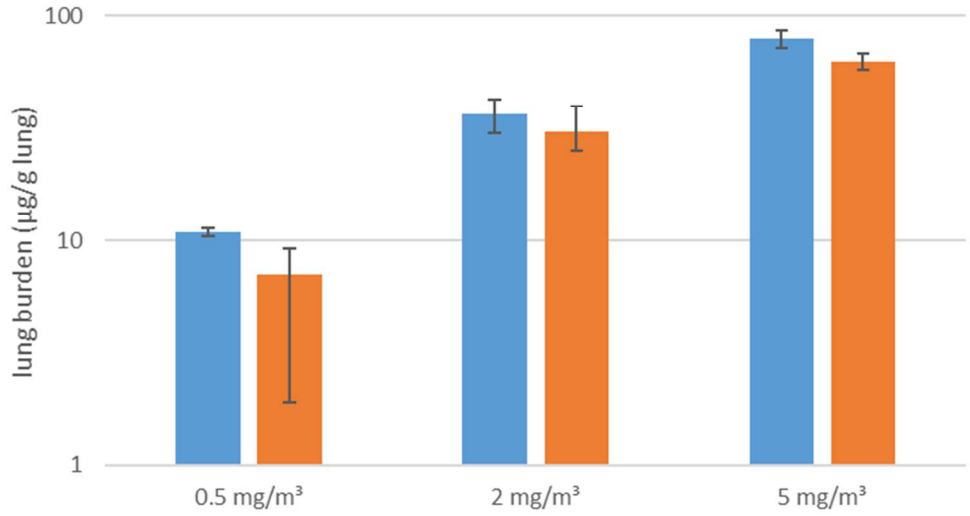

Figure S2.1: Lung burden after 5 days of exposure to CeO_2 NPs 24 hours (blue bars) and 3 weeks (orange bars) post-exposure in rats.

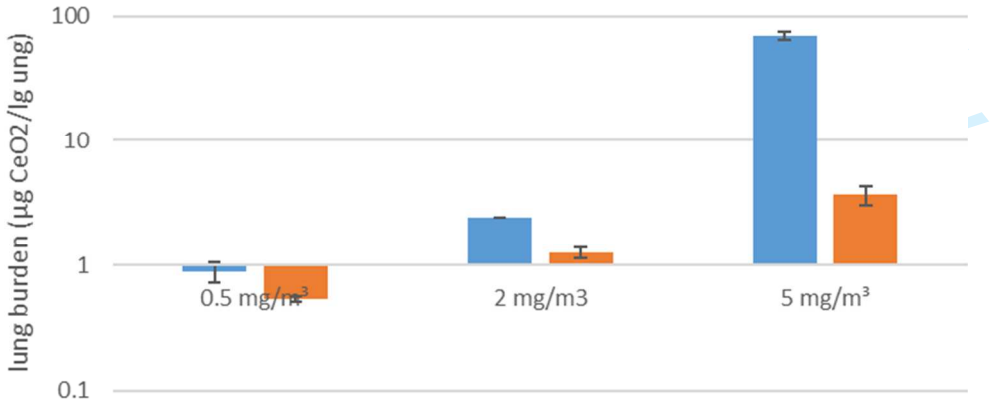

Figure S2.2: Lung burden after 5 days of exposure to CeO_2 NPs 24 hours (blue bars) and 3 weeks (orange bar) post-exposure in mice.

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Ce could not be detected in the lung of the control animals (detection limit: 0.3 µg per sample). In rats a modest clearance was observed in animals exposed to 0.5 mg/m³ CeO_2 NPs the after 3 weeks recovery, whereas the clearance of 2 and 5 mg/m³ seemed minimal (Figure S2.1). In mice the lung burden was low (0.9 & 2.4 µg Ce/lung) in animals exposed to 0.5 mg/m³ and 2 mg/m CeO_2 NPs after exposure, whereas the mean lung burden in mice exposed to 5 mg/m³ CeO_2 NPs was extraordinary high (~69 µg Ce/lung; Figure S2.2). The high value (of 495 µg/g lung) of mice exposed to 5 mg/m^3 CeO_2 NPs on study day 5 (last blue bar) was out of range and probably a preparation artefact (e.g. big bronchus included in the sample). In the mice exposed to 0.5 and 2 mg/m^3 CeO_2 NPs a clearance of approximately 50% was observed within the 3 weeks recovery period. A similar clearance rate was assumed for mice exposed to 5 mg/m^3 CeO_2 NPs.

S2.3 Hematology and clinical chemistry

No treatment-related effects were observed for hematology or clinical chemistry parameters, except for a slight, but statistically significant increase in the absolute neutrophil counts in rats exposed to 2 and 5 mg/m^3 CeO_2 NPs after the three weeks recovery period (see NanoMILE Deliverable D7.2), indicating a mild systemic inflammation.

S2.4 Bronchoalveolar lavage fluid (BALF)

A moderate increase in neutrophil counts was found in the BALF of rats exposed to 2 or 5 mg/m^3 CeO_2 NPs 24 h after exposure (Figure S2.3). This was associated with higher alkaline phosphatase (ALP) activity and cytokine-induced chemoattractant-1 (CINC-1/IL8) levels. ALP is released by neutrophils and CINC-1 is a cytokine which attracts neutrophils into the lung tissue. Epithelial cell counts in BALF were marginally increased in rats exposed to 5 mg/m^3 CeO_2 NPs, indicating an effect on the bronchioles. There was also in increased monocyte number and in the cytokines osteopontin and macrophage colony stimulating factor (M-CSF) in rats exposed to 5 mg/m^3 CeO_2 NPs. Increased monocyte chemoattractant protein-1 (MCP-1) in rats exposed to 2 and 5 mg/m^3 CeO_2 NPs also indicates that more monocytes and macrophages were attracted into the lung. A higher permeability of the capillaries in the alveoli can be assumed because of slightly higher total protein levels in BALF of rats exposed to 5 mg/m^3 CeO_2 NPs. The enzyme activities of lactate dehydrogenase (LDH) and γ-glutamyl transferase (GGT) in BALF of rats exposed to 2 and 5 mg/m^3 CeO_2 NPs were only marginally, but statistically significantly, increased.

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

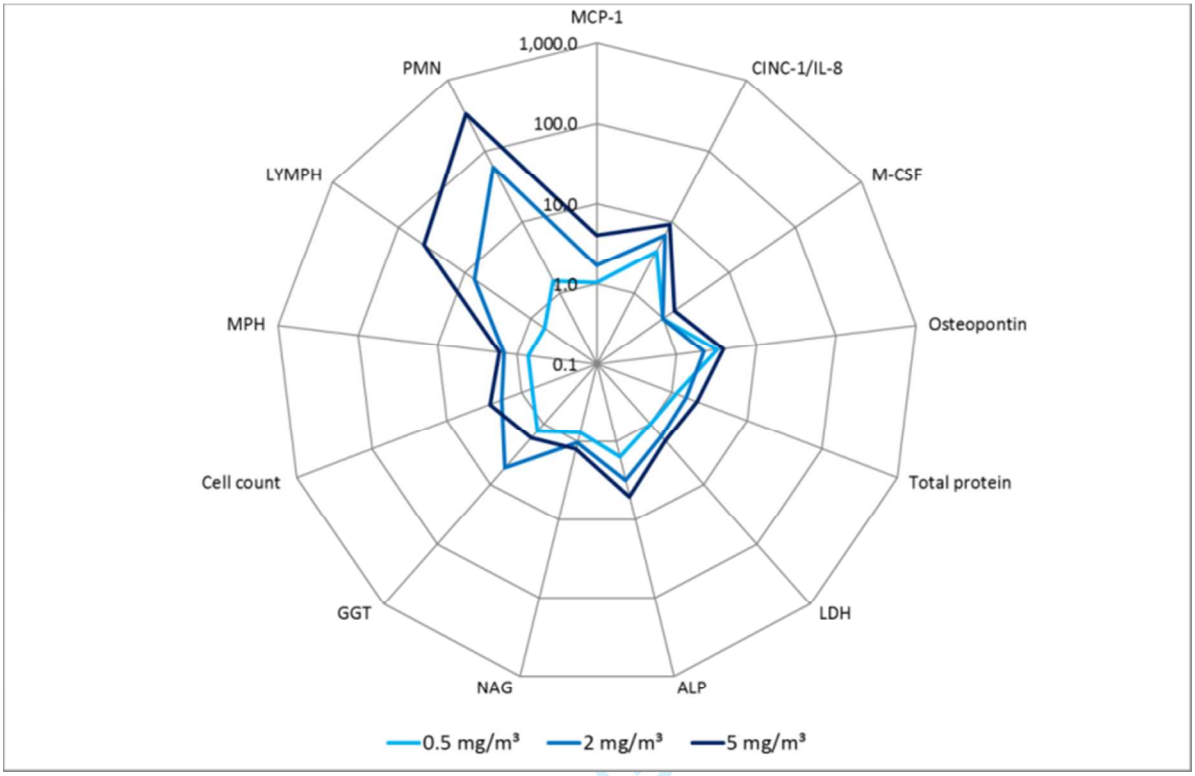

Figure S2.3: Changes in various BALF parameters in rats on study day 5 (24 hours after last exposure). Results are presented as x-fold changes compared to concurrent controls. Cell count = total cell count, MPH = alveolar macrophage, LYMPH = lymphocyte, PMN = polymorphnuclear neutrophilic granulocyte, GGT = γ-Glutamyl-transferase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; NAG = β-N-Acetyl glucosaminidase, GGT = γ-Glutamyl-transferase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; NAG = β-N-Acetyl glucosaminidase, CINC-1/IL-8 = cytokine-induced neutrophil chemoattractant-1; MCP-1 = monocyte chemoattractant protein-1; M-CSF = macrophage colony stimulating factor

In the BALF, monocyte cell counts were increased in rats exposed to 2 and 5 mg/m^3 CeO_2 NPs (Figure S2.4). Absolute neutrophil counts were higher in the BALF of rats exposed to 5 mg/m^3 CeO_2 NPs 3 weeks post-exposure compared to 24 hours post-exposure. Lymphocyte counts were also increased in rats exposed to 5 mg/m^3 CeO_2 NPs, indicating an ongoing inflammatory process in the lungs. This was also reflected by high levels of MCP-1 in rats exposed to 5 mg/m^3 CeO_2 NPs. CINC-1/IL-8 levels in rats exposed to 5 mg/m^3 CeO_2 NPs were lower compared to 24 hours post-exposure, but were still raised above control levels. The total protein, LDH and GGT levels in BALF of rats exposed to 5 mg/m^3 CeO_2 NPs 3 weeks post-exposure were marginally increased compared to the controls, with levels similar to those 24 hours post-exposure. ALP activity was lower compared to the 24 hours post exposure, but still marginally above control levels.

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

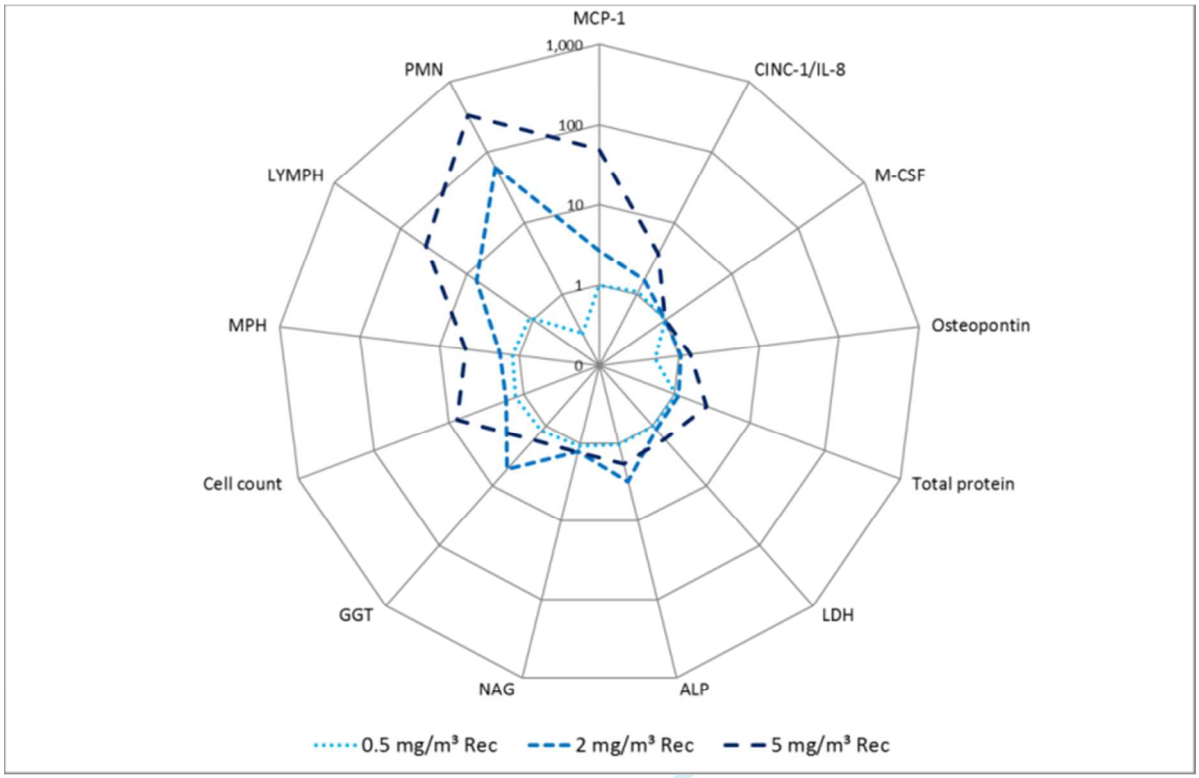

Figure S2.4: Changes in various BALF parameters in rats after a three weeks recovery period on study day 26. Results are presented as x-fold changes compared to concurrent controls. Cell count = total cell count, MPH = alveolar macrophage, LYMPH = lymphocyte, PMN = polymorph nuclear neutrophilic granulocyte, GGT = γ-Glutamyl-transferase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; NAG = β-N-Acetyl glucosaminidase, GGT = γ-Glutamyl-transferase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; NAG = β-N-Acetyl glucosaminidase, CINC-1/IL-8 = cytokine-induced neutrophil chemoattractant-1; MCP-1 = monocyte chemoattractant protein-1; M-CSF = macrophage colony stimulating factor

No treatment-related changes in cytology parameters, enzyme activities or total protein levels in BALF were observed in mice.

S2.5 Pathology

Histopathological finding in the lungs

In all exposed groups of both rats and mice, characteristic particles, either single or very few, were observed in the cytoplasm of alveolar macrophages (histiocytes) or free in the alveoli. These particles were considered to represent test substance. They occurred in all animals of all treatment groups, and

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

were still present after the recovery period in all rats exposed to 0.5 mg/m^3 CeO_2 NPs, in 4 rats exposed to 2 mg/m^3 CeO_2 NPs and all mice of all exposed groups. In rats, the particles were also seen in the bronchus-associated lymphoid tissue (BALT) or in macrophages in the BALT in two rats exposed to 0.5 mg/m^3 CeO_2 NPs and in all rats exposed to 2 and 5 mg/m^3 CeO_2 NPs. Particles were also present in the BALT after the recovery period in 3 rats exposed to 0.5 mg/m^3 CeO_2 NPs and in all rats exposed to 2 and 5 mg/m^3 CeO_2 NPs.

After the recovery period, a minimal alveolar histiocytosis with particles occurred in one rat exposed to 2 mg/m^3 CeO_2 NPs and in all rats exposed to 5 mg/m^3 CeO_2 NPs. In addition, all rats exposed to 5 mg/m^3 CeO_2 NPs showed very occasional eosinophilic granular material, most probably debris of degraded macrophages, free in the alveoli.

The occurrence of alveolar histiocytosis with particles in the recovery group in combination with the occurrence of eosinophilic granular material in rats exposed to 5 mg/m^3 CeO_2 NPs was regarded to be an indicator of an adverse reaction, whereas the occurrence of particles in single histiocytes or in the BALT in the lungs was considered non-adverse, as there were no signs of any cytotoxicity.

Single or very few particles could be noted within single histiocytes in all exposed mice after the recovery period. The occurrence of these particles in the lungs was considered to be treatment-related, but because there were no signs of an inflammatory response, it was regarded to be non-adverse.

Histopathological findings in the mediastinal and tracheobronchial lymph nodes

In rats exposed to the 5 mg/m^3 CeO_2 NPs, single or very few particles were observed in the mediastinal lymph node in one animal and in the tracheobronchial lymph nodes in 3 rats. After the recovery period single or very few particles were seen in the mediastinal lymph node of one rat exposed to 2 mg/m^3 CeO_2 NPs and in 4 rats exposed to 5 mg/m^3 CeO_2 NPs, as well as in the tracheobronchial lymph nodes of 2 rats exposed to 2 mg/m^3 CeO_2 NPs and in all rats exposed to 5 mg/m^3 CeO_2 NPs. In mice, particles were seen free in the lymphoid tissue of the tracheobronchial and mediastinal lymph nodes after the recovery period, and in one mouse exposed to 2 mg/m^3 CeO_2 NPs and in one mouse exposed to 5 mg/m^3 CeO_2 NPs. Because there was no activation or aggregation of macrophages, the occurrence of these particles was regarded to be non-adverse.

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Table S3: Models and data used to calculate the lung burden (LB) or retained dose in the lung in the selected inhalation studies

Reference	MPPD model [a]	body weight (g)	Nanoform of CeO_2 NP	MMAD (µm)	GSD	aerosol conc. (mg/m^3)	Breathing frequency (#/min)	Tidal volume (mL)	Breathing scenario [b]	exposure duration (hr/d)	exposure duration (weeks)	post-exposure period (days)	lung weight (g)	lung weight after recovery (g)
Keller et al., 2014	ASD	270	NM211	1.9	2.9	0.45	119	1.89	WB	6	1	0, 21	1.04	1.26
				2.2	2.4	25.8								
Keller et al., 2014	ASD	270	NM212	1.4	2.3	0.5	119	1.89	WB	6	1	0, 21	1.04	1.26
				1.2	2.1	5.3								
				1.0	2.5	25.9								
NanoMILE, 2017	ASD	270	PROM	1.3	2.2	0.57	166	1.88	NO	6	1	0, 22	1.03	1.22
				1.2	2.4	2.04							1.03	1.15
				1.1	2.3	4.85							1.05	1.40
Keller et al., 2014	ASD	270	NM212	1.6	2.1	0.48	119	1.89	WB	6	4	0, 35	1.10	1.26
				1.3	2.1	5.2								
				0.9	2.5	25.6								
Geraerts et al., 2012	ASD	224	NM211	1.0	1.8	10.8	166	1.49	NO	6	4	0	0.87	-
Geraerts et al., 2012	ASD	212	NM212	1.2	2.1	19.95	166	1.39	NO	6	4	0	0.73	-
NanoMILE, 2017	MB6	24	PROM	1.1	2.5	0.54	326	0.19	NO	6	1	0, 21	0.142	0.131
				1.1	2.4	2.04							0.134	0.139
				0.9	3.0	5.04							0.139	0.128
Dekkers et al., 2017	MB6	20	PROM	0.29 [c]	1.6	3.98	353	0.20	NO	3	4	28		0.158
Aalipati et al., 2014	MB6	35	Nano Amor	1.4	2.4	2.00	277	0.17	NO	6	4	0, 28	0.160	0.160

[a] ASD: Asymmetric Sprague Dawley rat, MB6: Mouse B6C3F1; [b] WB: whole body, NO: nose only; [c] Mass median diameter (MMD) instead of mass median aerodynamic diameter (MMAD), estimated based on the particle size distributions of the scanning mobility particle sizer (SMPS) measurements, assuming spherical aggregation around primary particles of 4.7 nm. -: no data available

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Table S4: Data used to calculate the expected pulmonary burden (PB) in the selected inhalation studies

Reference	Species	Nanoform of CeO_2 NP	Primary particle size (nm)	Aerosol conc. (mg/m^3)	Exposure duration (days or weeks)	Estimated fraction of inhaled dose deposited in lung (%)	Estimated fraction of inhaled dose deposited in pulmonary region (%)	Estimated fraction of lung deposition deposited in pulmonary region (%)	Lung burden 0 days after exposure (µg/lung)	Lung burden 3-5 weeks after exposure (µg/lung)	Surface area per particle (nm^2)	Surface area per g NP (m^2/g)	Surface area of pulmonary region of the lung (nm^2)	Pulmonary burden 0 days after exposure (mm^2/nm^2)	Pulmonary burden 3-5 weeks after exposure (mm^2/nm^2)
Keller et al., 2014	rat	NM211	12.5	0.45	5 days	9.8	6.1	63	6.0	3.8		53	2864	0.07	0.04
				25.8	5 days	9.4	5.1	54	280	260				2.80	2.60
Keller et al., 2014	rat	NM212	40	0.5	5 days	11.6	6.9	59	11	6.0		27	2864	0.06	0.03
				5.3	5 days	14.3	8.1	57	100	88				0.54	0.47
				25.9	5 days	16.2	9.9	61	530	400				3.04	2.29
NanoMILE, 2017	rat	PROM	4.7	0.57	5 days	10.3	5.7	55	11	8.7				0.38	0.30
				2.04	5 days	10.8	6.3	58	38	35	69		2860	1.36	1.26
				4.85	5 days	12.0	6.8	57	83	87				2.92	3.05
Keller et al., 2014	rat	NM212	40	0.48	4 weeks	10.7	6.0	56	41	23				0.22	0.12
				5.2	4 weeks	13.1	7.4	57	520	560		27	2864	2.77	2.99
				25.6	4 weeks	17.8	10.9	61	2650	2470				15.2	14.3
Geraerts et al., 2012	rat	NM211	12.5	10.8	4 weeks	14.8	7.4	50	557	-		64	2612	6.80	-
Geraerts et al., 2012	rat	NM212	40	19.95	4 weeks	13.0	6.4	50	923	-		27	2543	4.88	-
NanoMILE, 2017	mice	PROM	4.7	0.54	5 days	10.7	7.2	67	0.9	0.5				0.23	0.14
				2.04	5 days	10.6	7.2	67	2.4	1.3	69		470	0.61	0.32
				5.04	5 days	12.8	8.7	68	8.3	3.7				2.15	0.94
Dekkers et al., 2017	mice	PROM	4.7	3.98	4 weeks	11.5	8.0	70	-	13	69		476	-	3.37
Aalipati et al., 2014	mice	Nano Amor	45	2.0	4 weeks	9.3	6.1	65	216	98		56	459	17.3	7.86

Supplementary Information to Differences in the Toxicity of Cerium Dioxide Nanomaterials after Inhalation can be explained by Lung Deposition, Animal Species and Nanoforms. Dekkers et al., 2018.

Table S5: Physicochemical characteristics of the CeO_2 nanoparticles used in the 5-day inhalation studies in rats.

Characteristic ↓ Nanoform of CeO_2 NP→	**NM211**	**NM212**	**PROM**
Shape	near spherical	polyhedral	spherical
Primary particle size (nm)	12.5	40	4.7
Surface area (BET: m^2/g)	64	27	-
Surface reactivity (nmol TEU/m^2 NP)[a]	14 ± 0.7	13 ± 0.6	17 ± 03
Charge (mV)	16	42	50
MMAD (µm) ± GSD at an aerosol conc. of 0.5 mg/m^3	1.9 ± 2.9	1.4 ± 2.3	1.3 ± 2.2
MMAD (µm) ± GSD at an aerosol conc. of 2 mg/m^3	-	-	1.2 ± 2.4
MMAD (µm) ± GSD at an aerosol conc. of 5 mg/m^3	-	1.2 ± 2.1	1.1 ± 2.3
MMAD (µm) ± GSD at an aerosol conc. of 25 mg/m^3	2.2 ± 2.4	1.0 ± 2.5	-

[a] The surface reactivity was measured with the ferric reduction ability of serum (FRAS) assay (Gandon et al., 2017). The read-out of the FRAS assay is calibrated by a concentration series of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a Vitamin E analogon) and the result expressed as nmol Trolox Equivalent Unites (TEU)/m^2NP.
BET= Brunauer, Emmett and Teller method to measure surface area, MMAD=mass median aerodynamic diameter, GSD=geometric standard deviation, -: no data available