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Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0RQ, UK; School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK; Department of Cell and Molecular Biology, Northwestern University, Chicago, IL, USA; Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, UK

ABSTRACT

Cerium oxide nanoparticles (CeO2NPs), used in some diesel fuel additives to improve fuel combustion efficiency and exhaust filter operation, have been detected in ambient air and concerns have been raised about their potential human health impact. The majority of CeO2NP inhalation studies undertaken to date have used aerosol particles of larger sizes than the evidence suggests are emitted from vehicles using such fuel additives. Hence, the objective of this study was to investigate the effects of inhaled CeO2NP aerosols of a more environmentally relevant size, utilizing a combination of methods, including untargeted multi-omics to enable the broadest possible survey of molecular responses and synchrotron X-ray spectroscopy to investigate cerium speciation. Male Sprague–Dawley rats were exposed by nose-only inhalation to aerosolized CeO2NPs (mass concentration 1.8 mg/m3, aerosol count median diameter 40 nm) for 3 h/d for 4 d/week, for 1 or 2 weeks and sacrificed at 3 and 7 d post-exposure. Markers of inflammation changed significantly in a dose- and time-dependent manner, which, combined with results from lung histopathology and gene expression analyses suggest an inflammatory response greater than that seen in studies using micron-sized ceria aerosols. Lipidomics of lung tissue revealed changes to minor lipid species, implying specific rather than general cellular effects. Cerium speciation analysis indicated a change in Ce3+/Ce4+ ratio within lung tissue. Collectively, these results in conjunction with earlier studies emphasize the importance of aerosol particle size on toxicity determination. Furthermore, the limited effect resolution within 7 d suggested the possibility of longer-term effects.

INTRODUCTION

Nanomaterials offer unique mechanical, chemical, electrical and optical properties that are being exploited for an extensive range of applications; however, concerns remain about their potential impact on human health. Cerium oxide nanoparticles (CeO2NPs) are one of the manufactured nanomaterials currently receiving significant attention with respect to their potential health risks as they are used in a range of applications with the potential for direct human exposure, including polishing media (Reed et al. 2014) and fuel-borne catalysts, such as Eolys™, Platinum Plus™, and Envirox™ (Majestic et al. 2010). The benefits of CeO2NP-based fuel additives include reduced emissions of gaseous products of incomplete combustion and improved fuel combustion efficiency (Zhang et al. 2013). However, CeO2NPs have been detected in ambient air in locations where such additives are in use (Park et al. 2008a, Gantt et al. 2015) and there are concerns about their potential...
impact on health following inhalation (Geraets et al. 2012).

Reported effects of CeO$_2$NPs in vivo following intratracheal instillation include alveolar epithelial hyperplasia, lung inflammation, air-blood barrier damage, and fibrosis of the lungs (Ma et al. 2011; Ma et al. 2012; Peng et al. 2014; Rice et al. 2015). A number of in vivo inhalation studies have also reported similar effects, with indications that pulmonary inflammatory responses are slow to resolve post-exposure (Aalapati et al. 2014). Inhalation biodistribution studies have also found significant accumulation in pulmonary tissue and slow clearance (Geraets et al. 2012; Li et al. 2016).

Field studies have identified ceria particles emitted from vehicles using the EnviroxTM fuel additive, with a size range from 5 to 100 nm (Gantt et al. 2014). However, to date, the majority of reported CeO$_2$NP inhalation studies, as summarized in Table 1, including those mentioned above, have used aerosols in the micron-size range or at the upper end of the nano-size range and therefore, given the importance of particle size in driving biological effects (Oberdörster, Ferin, & Lehnert 1994; Braakhuis et al. 2016), may not be directly relevant to a hazard assessment of the effect of ceria particles emitted from engines using CeO$_2$NP fuel additives. The purpose of this study was, therefore, to undertake a short-term in vivo inhalation study using a ceria aerosol with a more environmentally relevant aerosol particle size, to investigate the effects on lung inflammation and injury. It was anticipated that comparing the results of this study with those in the literature, which have all used significantly larger aerosol particle sizes, would provide support for the hypothesis that aerosol particle size is an important toxicity driver.

We also utilized a multi-omics approach – applying microarray-based gene expression profiling and untargeted mass spectrometry-based lipidomics – to enable a broad measurement of thousands of molecular endpoints to provide insights into the toxicity mechanisms of CeO$_2$NPs (Taylor et al. 2016; Dekkers et al. 2018). Furthermore, we assessed the distribution of inhaled CeO$_2$NPs in various organs and their physicochemical properties using a range of approaches. Many studies have indicated that the physicochemical properties of nanomaterials have a significant influence on their biological activity. CeO$_2$NPs are an interesting case as it has been hypothesized that initial differences in the Ce$^{3+}$/Ce$^{4+}$ ratio on the NP surface, and changes in their anti-oxidant potential following exposure, may play a significant role in toxicity determination (Auffan et al. 2009). To explore this, synchrotron-based spectroscopy was applied to determine cerium oxidation states in exposed lung tissues.

**Materials and methods**

**Cerium oxide nanoparticles**

CeO$_2$NPs (primary particle size 8 nm) were provided by the University of Birmingham. Details of the synthesis are described in the Supplementary information (Chen et al. 2013). The powder was dispersed in MilliQ water (1 mg/mL) and sonicated briefly (~ 1 min) prior to use.

**Exposure system and aerosol characterization**

The nose-only inhalation exposure system and the aerosol characterization undertaken are described in the Supplementary information. Details of the dose estimation method are also described in the Supplementary information.

**Study design**

The experiments were performed within the legal framework of the United Kingdom under a Project License granted by the Home Office of Her Majesty’s Government. All procedures involving the animals were performed in accordance with the Animals (Scientific Procedures) Act 1986. Male Sprague–Dawley (SD) rats (10–12 weeks, 250–320 g) were purchased from Harlan, UK. Rats were randomly assigned into groups and exposed to aerosolized CeO$_2$NPs or water (controls) for 3 h per day, for 4 d per week, for 1 or 2 weeks. The 2-week exposure was chosen to explore the effect of a higher deposited dose within the lung, an alternative approach of using a higher aerosol concentration was discounted as this would have affected the aerosol particle size. Following exposure, the rats were returned to their cages for a period of either 3 d, to characterize the initial response, or 7 d to explore recovery and other effects in the short-
term. Assessment of adverse clinical signs is described in the Supplementary information.

**Analysis of bronchoalveolar lavage fluid (BALF)**

Rats were sacrificed by exsanguination by cardiac puncture under isoflurane anesthesia (induced at 5%, maintained at 1.5–2% in 100% oxygen). Bronchoalveolar lavage was performed in situ via tracheal cannula with 2 × 7 mL aliquots of phosphate buffered saline (PBS). Cells from both aliquots were pooled for cytological analysis as described in the Supplementary information. BALF supernatants from the first wash were collected for analysis of cytotoxicity and alveolar barrier damage as described in the Supplementary information. Cytokines and chemokines including IL-1β (interleukin-1β), TNF-α (tumor necrosis factor-α), TGF-β1 (transforming growth factor-β1), CXCL2 (CINC-3, cytokine-induced neutrophil chemoattractant 3), CXCL1 (CINC-1, cytokine-induced neutrophil chemoattractant 1), and CCL2 (MCP-1, monocyte chemoattractant protein-1) were measured in BALF supernatants using R&D Systems Quantikine® ELISA kits according to the manufacturer’s specifications.

**Lung tissue processing**

Following lavage, the apical and azygous lung lobes were tied off and snap frozen in liquid nitrogen for multi-omics analysis. The remainder of the lungs was removed and inflated and fixed with freshly made 4% paraformaldehyde via tracheal cannula, at a pressure of 30 cm of water and then processed into paraffin blocks. Cross/transverse sections of lung tissues chosen randomly were cut at a thickness of 5 μm before mounting onto microscope slices. For histopathology, staining with hematoxylin and eosin (H&E) was performed on lung tissue sections.

**Multi-omics analysis of lung**

Lung tissues were homogenized in 8 mL/g (v/w wet mass) methanol and 2.5 mL/g (v/w) water using a bead-based homogenizer (Precellys 24; Strretton Scientific, Stretton, UK). Lung homogenate aliquots were then taken for both metabolite and RNA extractions. Total RNA was isolated using Qiagen’s mini RNeasy Kit and QIAshredder (Qiagen, Crawley, UK) according to the manufacturer’s protocol. RNA was quantified with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA), and the integrity of RNA was verified with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Lipids from the lung homogenate were extracted using a methanol:water:chloroform (2:2:1.8) solvent system (Wu et al. 2008), dried using a centrifugal concentrator and stored at −80°C until analysis.

Microarray-based gene expression profiling was performed according to the manufacturer’s protocol (Agilent Technologies, Santa Clara, CA). Further details are described in the Supplementary information. Mass spectrometry-based lipidomics was performed using a high-resolution spectral-stitching nanoelectrospray direct-infusion approach as previously reported (Southam et al. 2017). Further details are described in the Supplementary information.

**Cerium in tissues determined by ICP-MS and laser ablation ICP-MS**

The cerium content of liver and kidney samples was determined using inductively coupled plasma mass spectrometry (ICP-MS) as described in the Supplementary information. Elemental mapping of cerium in lung tissue samples was undertaken by laser ablation ICP-MS using a New Wave Research NWR213 laser ablation system (Electro Scientific Industries, Portland, OR) linked to an iCAP Q ICPMS (Thermo Fisher Scientific, Hemel Hempstead, UK). Further details are described in the Supplementary information.

**Transmission electron microscopy (TEM)**

Lung tissues from animals at 3 d after 2-week exposure (CeO2NP exposed and control) were prepared as described in the Supplementary information and TEM imaging was then performed using an FEI Tecnai Spirit G2.

**Synchrotron-based X-ray spectroscopy**

Synchrotron microfocus X-ray spectroscopy was carried out at the Diamond Light Source I18 Beamline (Oxfordshire, UK). Microfocus X-ray fluorescence (μ-XRF) analysis was undertaken to provide information on the spatial distribution of elements of interest for a number of lung sections. For identified areas of high cerium concentration, X-ray
absorption near-edge structure (XANES) spectrometry of the Ce L_{III}-edge was carried out to investigate chemical speciation (e.g., oxidation state). The spectra produced were compared with those for samples of some cerium compounds: Ce₂(CO₃)₃, Ce(OH)₄ (Sigma-Aldrich, St. Louis, MO), and CeO₂ (Acros Organics, Morris, NJ), and a sample of the CeO₂NPs. Further details are described in the Supplementary information.

**Results**

**Aerosol characterization**

Aerosol characteristics and lung dose estimates are presented in Table 2 and Figure 1, including representative images of agglomerated CeO₂NP aerosol particles, illustrating their spherical form and primary particle size consistent with the manufacturer's data (ca 8 nm) (Chen et al. 2013).
Acute pulmonary toxicity effects induced by inhaled CeO₂NPs

Inhalation exposure to CeO₂NPs did not affect the body weight development of the rats (data not shown). No adverse clinical signs were observed in any of the animals.

Cytology and cytotoxicity analysis of bronchoalveolar lavage fluid (BALF)

BALF analysis was undertaken at 3 d and 7 d post-exposure for both 1-week and 2-week exposure groups. The majority of BALF parameters showed significant increases for CeO₂NP exposed groups compared to the control groups (Figure 2, with details presented in Supplementary Figures S2 and S3). For the 1-week exposure group, there was a small but significant increase in the different cell populations between 3 and 7 d post-exposure, although the levels of total protein, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) showed a reduction over this period indicating limited recovery. For the 2-week exposure group, there was no significant change in the total protein, LDH or ALP levels between 3 and 7 d post-exposure, but significant increases in the different cell populations. The above indicates that the inhaled CeO₂NPs induced time- and dose-dependent inflammation and lung damage.

Cytokines/chemokines in BALF

Effects on several cytokines and chemokines relevant to inflammatory responses and allergic effects were considered. There was no significant change in IL-1β concentrations for any exposed group (Figure 3(A)), whereas for TNF-α and TGF-β1 there were significant increases in some groups (Figure 3(B,C)). For CXCL2, CXCL1, and CCL2, there were significant increases for all exposed groups (Figure 3(D–F)). The effects were the greatest for CXCL1 and CCL2, for which there was a clear dose-dependent effect at both post-exposure times. There was also some indication of
concentrations increasing with time post inhalation, although this was only significant for CCL2 for the 1-week exposure. For the others, patterns were less clear, with CXCL2 showing no dose- or time-dependence, TGF-β1 showing no time-dependence and dose-dependence for the 7-d groups only, and TNF-α...
Figure 6. Representative laser ablation inductively coupled plasma mass spectrometry elemental maps (isotopes $^{13}\text{C}$ and $^{140}\text{Ce}$) and light microscopy images ($\times12.5$) of lung tissues of negative controls (images A–C) and CeO$_2$NP aerosol exposed animals at 3 days (images D–F) and 7 d (images G–I) post-exposure. The light microscope images show the lung tissue section prior to undertaking laser ablation (which destroys the sample) and allow the overall shape and structures such as large airways and larger blood vessels to be seen for comparison purposes.

Figure 7. Transmission electron microscope images of lung tissue from rats exposed to H$_2$O aerosols (A) and CeO$_2$NP aerosols (B) for 2 weeks at 3 d post-exposure. The boxes with dotted lines denote the approximate locations of the corresponding magnified regions.
showing dose-dependence for the 3-d groups only and time-dependence for the 1-week exposure groups only.

**Histopathology – lung inflammatory changes**

Inhalation of CeO$_2$NPs resulted in mild inflammation for both exposure durations and at both 3 and 7 d post-exposure (Figure 4). The lung architecture was well preserved with inhaled CeO$_2$NPs but minor alveolar epithelial hyperplasia with accumulation of eosinophilic materials from macrophage breakdown was observed. The lung injury induced by CeO$_2$NPs appeared to increase with time for 1-week exposure based on the histopathological observations.

**Multi-omics analysis of lung**

Principal component analysis (PCA) score plots from the analysis of the gene expression and lipidomics measurements are shown in Figure 5. For the gene expression analysis, the score plot captured 53.6% of the variation in the dataset within the first two components; a significant separation between rats exposed to H$_2$O aerosols and CeO$_2$NP aerosols was discovered along PC1 (t-test of the scores values: $p = 4.0 \times 10^{-3}$, Figure 5(A)), confirming that the NP exposure induced perturbations to gene transcription. The PCA score plot derived from the lipidomics data captured 55.6% of the variation in the first two components, and also showed a significant separation between control and CeO$_2$NP aerosols, in this case along PC2 (t-test of the scores values: $p = 8.5 \times 10^{-4}$, Figure 5(B)). Limma was applied to the gene expression data to investigate the effects of CeO$_2$NP exposure (H$_2$O aerosol exposure as the controls) and it was found to be statistically significant with CeO$_2$NP exposure ($q < 0.05$ and log$_2$-fold change $>1.0$) for 67 genes (Supplementary Table S1). DAVID analysis on the 67 differentially expressed genes (of which 53 were matched to a gene present in DAVID) yielded 13 GO-categories that were significantly enriched ($q < 0.05$, Supplementary Table S2).

For the lipidomics data, two-way ANOVA (type II, unbalanced) was conducted on each peak to investigate the effects of inhaled CeO$_2$NPs and exposure dose (1-week versus 2-week exposure). CeO$_2$NP inhalation was statistically significant for 38 peaks ($q < 0.05$) of which 19 where putatively annotated with a lipid name from the Lipid Maps database (Supplementary Table S3 and Supplementary File S11). Exposure dose had no significant effect on any of the peak intensities. Enrichment analysis of categorical annotations of the significant and annotated lipids was conducted, using MBROLE (Lopez-Ibanez, Pazos, & Chagoyen 2016) (Supplementary Table S4) and LIPID Metabolites And Pathways Strategy (LIPID
MAPS), and revealed that several lipid classes (e.g. glycerophospholipids) and subclasses (e.g. diacylglycerophosphocholines, glycerophosphoethanolamines, and diacylglycerophosphoethanolamines) were significantly enriched.

Assessment of the inhaled CeO₂NPs

Detection of CeO₂NPs deposited in the lung

Elemental maps of lung tissues produced by laser ablation ICP-MS clearly indicate the presence of cerium, widely distributed within the lung lobes, without any significant association with the larger airways or larger blood vessels (Figure 6). TEM micrographs (Figure 7) indicate the presence of dense particle agglomerates of similar size to the CeO₂NP aerosol agglomerates in the alveolar macrophages, mainly in the lysosome-like intracellular vesicles.

Cerium in liver and kidney

The concentrations of cerium in the liver and kidney were measured by ICP-MS and were greater in exposed than control animals, higher for 2-week than 1-week exposure, and increased from 3 d to 7 d post-exposure, indicating the translocation of cerium from the lung to other organs after inhalation, however, these changes were only statistically significant for some groups (Supplementary Figure S5).

Cerium speciation by synchrotron X-ray spectroscopy

Cerium LIII XANES spectra of the cerium compounds Ce₂(CO₃)₃, CeO₂, and Ce(OH)₄ are shown in Figure 8(a). Both Ce⁴⁺ compounds (CeO₂, and Ce(OH)₄) show a doublet peak of roughly equal height, whereas the Ce³⁺ carbonate (Ce₂(CO₃)₃) shows a clearly different peak morphology. The XANES spectrum for the CeO₂NP sample appears qualitatively similar to the Ce⁴⁺ compounds (Supplementary Figure S6). A linear combination fitting (LCF) analysis was carried out for the CeO₂NP spectrum using the spectra from the cerium compounds as standards. The fit analysis indicated that the CeO₂NP sample was 100% CeO₂ with zero contributions from the other standards.

Discussion

We have investigated the pulmonary toxicity of nano-sized CeO₂NP aerosol agglomerate particles (CMD approximately 40 nm, GSD 1.7; primary particle size 5–10 nm). Histopathology indicated mild inflammation with alveolar epithelial hyperplasia (Figure 4), which increased with time after exposure, suggesting the potential for long-term effects. Consistent with this the BALF analysis indicates a significant inflammatory response increasing between 3 and 7 d (Figure 2 and Supplementary Figure S2 and S3). These changes were matched by significant increases in cytokines involved in inflammatory cell infiltration, such as CXCL1(CINC-1) and CCL2(MCP-1) (Figure 3), with levels increasing with dose for each post inhalation time (dose-dependence). Concentrations of total protein, LDH, and ALP in BALF increased significantly for all groups exposed to CeO₂NP aerosols (Figure 2 and Supplementary Figure S3) with some evidence of recovery only for the 1-week exposures, again suggesting the potential for longer-term effects. The gene expression results (Supplementary Table S2) are consistent with the above, with significant Gene Ontology terms including (GO:0030593) neutrophil chemotaxis, (GO:0048247) lymphocyte chemotaxis, (GO:0008009) chemokine activity, and (GO:0002548) monocyte chemotaxis.

Other CeO₂NP inhalation studies have also shown lung inflammation, cytotoxicity, and air-blood barrier damage; however, the majority of such studies have used aerosols with micron-sized aerosol
Summary of inhalation toxicity studies with CeO₂NPs.

Characterization of primary particles

| CeO₂NPs | Primary particle diameter (nm) | Surface area (m²/g) | CMD (μm)/GSD | Exposure dose (aerosol concentration × exposure duration) | Animal models | Brief biological findings                                                                 |
|---------|-------------------------------|---------------------|--------------|---------------------------------------------------------|--------------|-------------------------------------------------------------------------------------------|
| CeO₂NPs | 15-30 (manufacturer), 30-50 (manufacturer) | 30-50 (manufacturer) | MMAD (μm)/GSD 2.28/2.94 | 641 mg/m³ × 4 hrs | Wistar rats | 1, 2 and 14 d post Significant cytotoxicity, oxidative stress and inflammation in the lungs. |
| CeO₂NPs (NanoMorph) | 3-10 (manufacturer), 13.0 ± 3.2 (TEM), 44.9 ± 14.6 (SEM); | 63.95 ± 0.30 | MMAD (μm)/GSD 1.0 ± 0.04/1.82 | 10.79 ± 0.02 mg/m³ × 40 min | Wistar rats | 6/h exposure only - 1 d post plus 2/3 d post the 4-wk exposure groups |
| CeO₂NPs (NM-211) (Antaria); (NM-212) (Unisave) | 40 (manufacturer), 27.3 ± 13.6 (TEM), 28.4 ± 10.4 (SEM); | 27.15 ± 0.19 | MMAD (μm)/GSD 1.17 ± 0.34/2.07 | 19.95 ± 13.21 mg/m³ × 40 min | Wistar rats | 4-wk exposure only - 1 d post all exposure groups, and 28 d post the 6/h exposure groups |

Characterization of aerosols (MMAD – mass median aerodynamic diameter; CMD – count median aerodynamic diameter; GSD – geometric standard deviation)

| Exposure dose | Animal models | Brief biological findings |
|---------------|--------------|----------------------------|
| (aerosol concentration × exposure duration) | | |
| 641 mg/m³ × 4 hrs | Wistar rats | 1, 2 and 14 d post Significant cytotoxicity, oxidative stress and inflammation in the lungs. |
| 10.79 ± 0.02 mg/m³ × 40 min | Wistar rats | 6/h exposure only - 1 d post plus 2/3 d post the 4-wk exposure groups |
| 19.95 ± 13.21 mg/m³ × 40 min | Wistar rats | 4-wk exposure only - 1 d post all exposure groups, and 28 d post the 6/h exposure groups |

Animal models:
- Wistar rats
- Sprague-Dawley rats

Brief biological findings:
- Significant cytotoxicity, oxidative stress and inflammation in the lungs.
- No clear effect of the primary particle size or surface area on pulmonary deposition and extrapulmonary tissue distribution.
- Inhaled CeO₂NPs and Al-doped CeO₂NPs caused transient, concentration-dependent inflammation of the lung at all concentrations.
- Inhalation exposure of CeO₂NPs and Al-doped CeO₂NPs caused significant pulmonary and some extrapulmonary toxicity.
- Inhalation exposure of CeO₂NPs and Al-doped CeO₂NPs induced significant pulmonary and some extrapulmonary toxicity.
- Inhalation exposure of CeO₂NPs cleared from the lung with a half-time of 40 d at a concentration of 0.5 mg/m³, at higher concentrations clearance was impaired.
- Dose-dependent inflammatory response dominated by neutrophils and lymphocytes in BALF. Systemic effects were virtually absent.

Table 1. Summary of inhalation toxicity studies with CeO₂NPs.

(continued)
| Characterization of primary particles | Characterization of aerosols (MMAD – mass median aerodynamic diameter; GSD – geometric standard deviation; CMD – count median diameter; GMD – geometric mean diameter) | Exposure dose (aerosol concentration × exposure duration) | Animal models | Animal sacrifice time points post-exposure | Brief biological findings | Reference (* nose-only inhalation; ** whole-body exposure) |
|--------------------------------------|-------------------------------------------------|-------------------------------------------------|---------------|------------------------------------------|--------------------------|-------------------------|
| **CeO$_2$NPs** (Wako Chemical Ltd) | GMD (nm) 110 ± 12.5 (high dose), 87.6 ± 7.9 nm (low dose) | 10.2 ± 1.38 mg/m$^3$ (high dose) and 2.09 ± 0.29 mg/m$^3$ (low dose) × 6 h/d × 5 d/wk × 4 wks | Sprague-Dawley rats | 15 min, 1 d and 7 d post | CeO$_2$NPs (either through inhalation or intratracheal instillation) induced not only acute but also chronic inflammation in the lung. | (Li et al., 2016) * |
| **CeO$_2$NPs** (produced in-house) | 2-3 (TEM); 6.67 ± 0.06 (XRD) | N/A | GMD (nm) 146 (fresh 1), 195 (fresh 2), 174 (aged 1), 151 (aged 2) | Sprague-Dawley rats | 24 hrs and 13 wks post | The biodistribution of fresh and aged CeO$_2$NPs followed the same patterns, with the highest amounts recovered in the faeces and lungs. | (Larsen et al., 2016)** |
| **CeO$_2$NPs** (Evonik-Degussa) | 13.0 ± 12.1 | 56.7 | GMD (nm) 372 (high dose), 295 (low dose) | BALB/cJ mice | 24 hrs and 13 wks post | BALF cell analyses revealed both neutrophilic and lymphocytic inflammation 24 h post exposure. CeO$_2$ gave rise to a more persistent inflammation; both neutrophilic and lymphocytic inflammation was seen at 13 weeks post. | (Larsen et al., 2016)** |
| **CeO$_2$NPs** (produced in-house) | 4.7 ± 1.4 (TEM) | N/A | CMD (nm)/GSD 182 ± 10/1.88 | CS7BL/6J mice (ApoE$^{-/-}$) | 4 wks post | CeO$_2$NP exposure had no major toxicological affects apart from modest inflammatory histopathology in the lung. | (Dekkers et al., 2017)* |
| **CeO$_2$NPs** (NM-212) (provided by Fh-IME) | 28.4 | 27.2 (BET) | MMAD (µm)/GSD 0.71/3.59, 0.63/3.83, 0.68/4.32, 0.79/3.50 | Wistar rats | 1 d post all exposure groups, Lung burden values increased with increasing nanoparticle dose levels and ongoing exposure. CeO$_2$NPs penetrate the alveolar space and affect the respiratory tract mainly in terms of dose-dependent inflammation, with post-exposure persistence. | (Schwotzer et al., 2017) * |
| **CeO$_2$NPs** (produced in-house) | 8 nm (TEM) | N/A | CMD (nm)/GSD 43.8/1.72 (1-wk exposure); 43.8/1.72 (2-wk exposure) | Sprague-Dawley rats | 3 and 7 d post | Changes in gene expression in AEII cells (30 of 391 genes investigated) indicate that the cells contribute to CeO$_2$NP caused inflammatory and oxidative stress reactions in the respiratory. | (Schwotzer et al., 2018) * |

**Table 1. Continued.**
agglomerates (Table 1), significantly larger than used in this study, and the resulting effects are typically less marked than indicated here. To achieve similar or relatively comparable toxicological effects, inhalation studies with micron-sized CeO$_2$NP aerosols usually used higher aerosol concentrations or longer exposure durations (Table 1). Landsiedel et al. (2014), for example, used a similar short-term repeated exposure study design (6 h/d for 5 d with sacrifice at 3 d) with a micron-sized aerosol, which at the low concentration (0.8 mg/m$^3$) delivered a very similar ‘exposure dose’ (i.e. concentration × duration) to our one-week exposure, and yet their results at this concentration indicate a significantly lower response, with small but significant changes for only three endpoints, including neutrophil and lymphocyte number but not LDH. Only at the medium and high concentrations, delivering exposure doses >4 times higher, were significant changes seen, similar to those here. Keller et al. (2014) also used a micron-sized aerosol in a short-term repeated dose study using three concentrations, the lowest of which delivered a similar exposure dose to our 1-week study. However, it was only at the medium and high concentrations, delivering exposure doses >5 times higher than our study, that a broad pattern of changes was seen, similar to the results presented here. It is clearly more difficult to make direct comparisons with the results from studies with longer exposure durations; however, it is instructive to note that levels of CCL2(MCP-1), which were significantly enhanced in this study, were only significantly enhanced in a 28-d study (6 h/d, 5 d/week, 4 weeks) (Schwotzer et al. 2017, 2018) at the two highest concentration levels, which would have resulted in exposure doses >3 times higher than this study, and for neutrophil numbers and LDH levels only the highest concentration (3 mg/m$^3$), delivering an exposure dose ~9 times greater, produced similar effects to those reported here. Schwotzer et al. (2018) also assessed the effect of exposure on the expression of 391 genes in alveolar epithelial type II cells and identified significant regulatory changes to a total of 30 genes, many of which (e.g. inflammatory cytokines: CCL2, CCL22, CCL7, and CCL17; markers of lung cancer: Mmp12, indicators of oxidative stress: Fabp4 and Noxo1) were also identified in this study, but again significant changes to gene regulation were only seen at exposure doses markedly higher than used here.

The above comparison supports a conclusion that nano-sized aerosols of CeO$_2$NPs are more effective than micron-sized at generating effects. A few other in vivo inhalation studies have used nano-sized CeO$_2$NPs aerosols, albeit with larger aerosols than used here (40 nm). For example, Demokritou et al. (2013) used a 90 nm aerosol and a similar study design (2.7 mg/m$^3$ × 2 h/d × 4 d), resulting in a very similar exposure dose which generated significant neutrophilia and cell damage (LDH), similar to this study. Comparing the two studies, it is possible to argue that the levels of PMN infiltration and LDH are higher in the current study suggesting greater effects for the smaller aerosol; however, this may be a result of other factors including differences in the sacrifice time post-exposure (1 d versus 3 d). Morimoto et al. (2015) used an approximately nano-sized (90–110 nm) aerosol in a 28-d study (2 and 10 mg/m$^3$) and found a pattern and degree of inflammatory effects at 3 and 7 d post-exposure similar to the current study despite significantly greater levels of exposure, again suggesting a greater effect for the smaller aerosol particles. It is also interesting to note that Morimoto et al. (2015) saw a reduction in effects between 3 and 7 d for some endpoints, which was not seen for our 2-week exposure. The results of our study are, therefore, broadly consistent with the small number of studies using nano-sized aerosols, but suggestive of a greater biological potency for our smaller aerosol.

It is generally understood that aerosol particle size influences the resulting biological effects following inhalation for a number of related reasons: level and pattern of deposition within the lung depends in detail on the aerosol particle size distribution in a non-linear manner, but with aerosol particles below 100 nm (peak around 20 nm) generally depositing more efficiently in the alveolar region than larger particles (Asgharian et al. 2009); smaller

| Exposure group | CMD (nm) | GSD | Number conc. (particles/cm$^3$) | Mass conc. (mg/m$^3$) | Dose (lung) (µg) | Dose (alveolar) (µg) |
|----------------|---------|-----|-------------------------------|----------------------|-----------------|---------------------|
| 1-week exposure (3 h/d, 4 d/week) | 43.4 | 1.70 | $2.16 \times 10^6$ | 1.80 | 79 | 49 |
| 2-week exposure (3 h/d, 4 d/week) | 43.8 | 1.72 | $1.96 \times 10^6$ | 1.82 | 161 | 100 |
particles may also be less efficiently phagocytosed and, therefore, cleared from the lung than larger particles, thus leading potentially to prolonged exposure and build-up; and smaller particle size also implies a greater surface area per unit deposited mass, which a number of *in vitro* and *in vivo* studies have linked to greater toxicity (e.g. Oberdörster et al. 2005; Stoeger et al. 2006). All these factors may have contributed to the greater apparent short-term toxicity of the aerosols used in this study in comparison with others in the literature.

A generally accepted paradigm is that the toxicity of nanomaterials arises primarily as a result of their ability to generate reactive oxygen species (ROS) and thus oxidative stress damage (Fu et al. 2014). Studies have demonstrated that CeO$_2$NPs can induce oxidative stress and resulting toxicity *in vivo* (Nemmar et al. 2017) and *in vitro* (Park et al. 2008b; Eom and Choi 2009). The hierarchical model of oxidative stress (Damoiseaux et al. 2011) proposes that cells and tissues respond to increasing levels of NP-induced oxidative stress via various enzyme systems - mild oxidative stress results in transcriptional activation of phase II antioxidant enzymes via nuclear factor (erythroid-derived 2)-like (Nrf2) induction; and at an intermediate level, redox-sensitive mitogen-activated protein kinase (MAPK) (family includes p38 MAPK and growth factor-regulated extracellular signal-related kinases (ERK)) and nuclear factor kappa-light-chain enhancer of activated B cells (NF-kappaB) cascades mount a pro-inflammatory response. Compatible with this hypothesis, *in vitro* studies have shown that CeO$_2$NPs induce toxicity in human bronchial epithelial cells via oxidative stress and the p38-Nrf2 signaling pathway (Eom & Choi 2009) and in human hepatoma cells via oxidative stress and the activation of MAPK signaling pathways (Cheng et al. 2013). One of the significantly enriched terms in the Gene Ontology analysis of the 67 differentially expressed genes in this study (*Supplementary Table S2*) was GO:0070374 – positive regulation of ERK1 and ERK2 cascade, which is also consistent with the above model. MAPK pathways have also been shown to mediate toxicity for other nanomaterials, e.g. titanium dioxide (Park et al. 2008c) and other inhaled pollutants, e.g. cadmium (Cormet-Boyaka et al. 2012).

**Cerium speciation**

Cerium can exist in both 3$^+$ and 4$^+$ oxidation states. Typically the cerium in CeO$_2$NPs is predominantly Ce$^{4+}$, however, surface defects mean that Ce$^{3+}$ is also found at the surface, and indeed for CeO$_2$NPs (3–30 nm) the majority of the surface ceria is Ce$^{3+}$ (Deshpande et al. 2005). It has been hypothesized that initial differences in the Ce$^{3+}$/Ce$^{4+}$ ratio on the NP surface and changes to this following exposure may play a significant role in toxicity determination (Auffan et al. 2009). In our study, to our knowledge the first to investigate cerium speciation in rat lung after inhalation exposure to CeO$_2$NPs, analysis of the XANES cerium spectra (Figure 7) indicates that the cerium in the nanoparticles was largely Ce$^{4+}$; however, the cerium in the tissue samples was 15–21% Ce$^{3+}$, indicating a change in speciation following delivery to the lung. This speciation change is consistent with *in vitro* studies, which have indicated higher Ce$^{3+}$ speciation inside rather than outside cells (Szymanski et al. 2015). Some studies have found that the presence of phosphate ions in aqueous systems significantly affects the reactivity of ceria and it has been hypothesized that the formation of cerium phosphate in biological systems would ‘trap’ cerium in the 3$^+$ state and thus block potential cycling between Ce$^{3+}$ and Ce$^{4+}$ (Singh et al. 2011). Further studies are required to investigate the potential links between speciation and toxicity mechanisms, especially for long-term pulmonary effects, which, given the ability of CeO$_2$NPs to generate ROS in a number of *in vitro* and *in vivo* systems (Lin et al. 2006; Park et al. 2008b; Nemmar et al. 2017) may relate to ROS generation capacity.

**Lipidomic analysis**

The most abundant lipid classes in the lung lipidome are glycerophosphocholines (PC), due to their significant role in lung surfactant, glycerophosphothanolamines (PE), glycerophosphoserines (PS), glycerolphosphoglycerols, and sphingolipids (Titz et al. 2016). Few studies have investigated the effect of inhaled nanomaterials on the lung lipidome and to date, all have been targeted. Gold nanoparticles (3-week exposure) had no effect on total lung surfactant, sphingolipids or glycerophospholipids, except
a decrease in one PS species (Yu et al. 2007). Single-walled carbon nanotubes (4-d exposure) produced no changes in total phospholipids, no oxidized PC or PE species, but some oxidized minor phospholipid species, suggesting selective rather than nonspecific effects (Tyurina et al. 2011). Recently, alterations in numerous phosphorylcholine-containing lipid species were identified in rat lung following inhalation of ZnO particles (35 nm and 250 nm), with the analysis suggesting that these included lipids involved in membrane conformation and cellular signaling (Lee et al. 2016, 2018). In this study, untargeted mass spectrometry-based lipidomics revealed that the most marked effect of CeO$_2$NP exposure is the significant increase of some lipids within the category of glycerophospholipids, dominated by PC and PE (Supplementary Tables S3 & S4 and Supplementary File SI1). Comparison of the putatively annotated lipids that were perturbed, with information on individual lipid species usually identified within the lung (Griese et al. 2015; Titz et al. 2016; Zemski Berry et al. 2017), revealed that the majority of the perturbed species were not those usually identified at significant concentrations within the lung. Comparing the annotated lipids against a comprehensive profile of lung glycerophospholipids suggests that of the 80 lipids listed by Zemski Berry et al. (2017) only three (PC(14:0/16:0), PC(16:0/16:1) and PE(16:0/18:1)) were significantly modified by CeO$_2$NP exposure. The results of the global lipidomics analysis, the first to be undertaken for an in vivo nanotoxicology inhalation study, suggest minor changes to a small number of commonly found lipids within the lung, but potentially more marked changes to less abundant lipids, which may indicate effects for specific cells/pathways. This could usefully be investigated further using a targeted analysis of these putatively annotated lipids.

Relevance of aerosol concentration and characteristics

Cerium concentrations in ambient air in Newcastle (UK) post-introduction of Envirox™ to local bus fleets were approximately 0.5 ng/m$^3$ (Park et al. 2008a; Cassee et al. 2011; Gantt et al. 2015). A number of modeling studies have estimated potential future concentrations based on the more extensive use of the additives, including 1.25 × 10$^3$ ng/m$^3$ (HEI 2001), 5–80 ng/m$^3$ (Park et al. 2008a), and 22 ng/m$^3$ (Erdakos et al. 2014). These concentrations are lower than used in this study (1.8 × 10$^6$ ng/m$^3$), however, short-term exposures were used rather than the chronic exposures that would arise in the general population from the use of these fuel additives. Ma et al. (2011) estimated a lifetime lung burden of 936 µg/kg and Park et al. (2008a) estimated a 20-year lung burden of 30 µg. The doses delivered in our study were of this order, i.e. comparable to estimated lifetime exposure levels.

Laboratory studies have found ceria particles emitted from various engines using CeO$_2$NP fuel additives with a range of sizes, including: majority <80 nm (Skillas et al. 2000); ca. 75 nm (Gantt et al. 2015); and typically 60–300 nm (Dale et al. 2017), all broadly consistent with measurements made during field studies (5–100 nm) (Gantt et al. 2014) and with the size of the aerosol agglomerates used in this study. It is important to note, however, that emitted ceria particles are typically single crystals (or small groups thereof) formed within the engine from the smaller particles (8–10 nm) within the additive, rather than agglomerates as used here. This may impact on the resulting effects and could usefully be investigated further, but in this context it is important to note that studies using a range of sizes of primary particles producing aerosol agglomerates of a similar size have found that the resulting biological effects were very similar at concentrations below overload (Gosens et al. 2014; Keller et al. 2014), suggesting that aerosol particle size, rather than primary particle size, is a key factor driving biological effects. It is also necessary to acknowledge that in the environment some ceria particles would be attached to larger micron-sized soot particles, which may also affect their biological effects, although the importance of this is unclear with one study indicating 40% of ceria particles associated with larger soot particles (Gantt et al. 2015) but another suggesting significantly lower levels (Skillas et al. 2000).

Diesel emissions are complex mixtures of gases and particles, and ultimately a complete assessment of the overall impact of using CeO$_2$NP fuel additives on human health needs to consider all aspects of the changes in the emission characteristics (Zhang et al. 2013). Some studies have used diesel exhaust
from machines using fuel additives. Cassee et al. (2012) exposed atherosclerosis-prone mice to exhaust from a diesel engine using standard fuel (DE) or fuel containing CeO$_2$NP (DECe) and found a trend of increasing atherosclerotic plaques following DE exposure that was not evident in the DCEe group. Overall there were no clear pathological changes in either group. These results contrast with those of Snow et al. (2014), who exposed rats to diesel exhaust (DE) from a generator run using diesel fuel, with (DECe) and without (DE) the addition of CeO$_2$NP and found that DECe induced greater adverse pulmonary effects. Clearly such studies are extremely useful in evaluating the overall effects of the introduction of CeO$_2$NP fuel additives, however, the influence of engine type, engine abatement, running conditions, and exhaust ageing may be significant in determining the characteristics of the output. Thus, studies investigating the biological effects of relevant CeO$_2$NPs remain of use in understanding their contribution to overall effects.

Conclusions

Short-term inhalation exposure to nano-sized cerium oxide agglomerates induced a significant pulmonary inflammatory effect with a limited resolution by 7d post-exposure, indicating the potential for longer-term effects, which is worthy of further investigation. Comparison with the results of previous studies using larger aerosol particle sizes supports a hypothesis that aerosol particle size is a key toxicity driver and thus emphasizes the need for future in vivo inhalation studies to use nanomaterial aerosol sizes of environmental relevance.

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Disclosure statement

The authors report no conflict of interests with the work in this article. The authors alone are responsible for the content and writing of the manuscript.

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References

Aalapati, S., S. Ganapathy, S. Manapuram, G. Anumolu, and B. M. Prakya. 2014. “Toxicity and Bio-accumulation of Inhaled Cerium Oxide Nanoparticles in CD1 Mice.” Nanotoxicology 8 (7): 786–798.

Asgharian, B., O. Price, F. Miller, R. Subramaniam, F. R. Cassee, J. Freijer, L. Van Bree, and R. De Winter-Sorkina. 2009. MPPD, Multiple-Path Dosimetry Model v2.11. Applied Research Associates (ARA), The Hamner Institutes for Health Sciences, the National Institute of Public Health and the Environment (RIVM), the Netherlands, and the Ministry of Housing, Spatial Planning and the Environment, the Netherlands.

Auffan, M., J. Rose, T. Orsiere, M. De Meo, A. Thiill, O. Zeyons, O. Proux, et al. 2009. “CeO$_2$ Nanoparticles Induce DNA Damage towards Human Dermal Fibroblasts in Vitro.” Nanotoxicology 3 (2): 161–171. doi: 10.1080/17435390902788086.

Braakhuis, H. M., F. R. Cassee, P. H. Fokkens, L. J. De La Fonteyne, A. G. Oomen, P. Krystek, W. H. De Jong, H. Van Loveren, and M. V. Park. 2016. “Identification of the Appropriate Dose Metric for Pulmonary Inflammation of Silver Nanoparticles in an Inhalation Toxicity Study.” Nanotoxicology 10:63–73. doi:10.3109/17435390.2015.1012184.

Cassee, F. R., A. Campbell, A. J. Boere, S. G. Mclean, R. Duffin, P. Krystek, I. Gosens, and M. R. Miller. 2012. “The Biological Effects of Subacute Inhalation of Diesel Exhaust following Addition of Cerium Oxide Nanoparticles in Atherosclerosis-prone Mice.” Environmental Research 115: 1–10. doi:10.1016/j.envres.2012.03.004.

Cassee, F. R., E. C. Van Balen, C. Singh, D. Green, H. Muijser, J. Weinstein, and K. Dreher. 2011. “Exposure, health and Ecological Effects Review of Engineered Nanoscale Cerium and Cerium Oxide Associated with Its Use as a Fuel Additive.” Critical Reviews in Toxicology 41 (3): 213–229. doi:10.3109/10408444.2010.529105.

Chen, H.-S., J. Mazzolini, J. Ayers, J. Rappoport, J. Lead, and J. Preece. 2013. Synthesis and characterization of nano ceria for biological applications. Nano-Bio Sensing, Imaging and Spectroscopy SPIE, 7.
Cheng, G., W. Guo, L. Han, E. Chen, L. Kong, L. Wang, W. Ai, N. Song, H. Li, and H. Chen. 2013. “Cerium Oxide Nanoparticles Induce Cytotoxicity in Human Hepatoma SMMC-7721 Cells via Oxidative Stress and the Activation of MAPK Signaling Pathways.” *Toxicology in Vitro* 27 (3): 1082–1088. doi:10.1016/j.tiv.2013.02.005

Correia-Boyaka, E., K. Jolivet, A. Bonnegarde-Bernard, J. Rennolds, F. Hassan, P. Mehta, S. Tridandapani, J. Webster-Marketon, and P. N. Boyaka. 2012. “An NF-kappaB-independent and Erk1/2-dependent Mechanism Controls CXCL8/IL-8 Responses of Airway Epithelial Cells to Cadmium.” *Toxicological Sciences* 125 (2): 418–429. doi:10.1093/toxsci/kfr310.

Dale, J., S. Cox, M. Vance, L. Marr, and M. Hochella. 2017. “Transformation of Cerium Oxide Nanoparticles from a Diesel Fuel Additive during Combustion in a Diesel Engine.” *Environmental Science & Technology* 51: 1973–1980.

Damoiseaux, R., S. George, M. Li, S. Pokhrel, Z. Ji, B. France, T. Xia, et al. 2011. “No Time to Lose – High Throughput Screening to Assess Nanomaterial Safety.” *Nanoscale* 3 (4): 1345–1360. doi:10.1039/c0nr00618a.

Dekkers, S., M. R. Miller, R. P. F. Schins, I. Romer, M. Russ, R. J. Vandenbriel, I. Lynch, et al. 2017. “The Effect of Zirconium Doping of Cerium Dioxide Nanoparticles on Pulmonary and Cardiovascular Toxicity and Biodistribution in Mice after Inhalation.” *Toxicology* 11, 794–808. doi:10.1080/17435390.2017.1357214.

Dekkers, S., T. D. Williams, J. Zhang, J. Zhou, R. J. Vandenbriel, L. J. De La Fonteyne, E. R. Gremmer, et al. 2018. “Multimicroscopy Approaches Confirm Metal Ions Mediate the Main Toxicological Pathways of Metal-bearing Nanoparticles in Lung Epithelial A549 Cells.” *Environmental Science: Nano* 5: 1506–1517. doi:10.1039/C8EN00071A

Demokritou, P., S. Gass, G. Pyrgiotakis, J. M. Cohen, W. Goldsmith, W. Mckinney, D. Frazer, et al. 2013. “In vivo and in Vitro Toxicological Characterisation of Realistic Nanoscale CeO(2) Inhalation Exposures.” *Nanotoxicology* 7 (8): 1338–1350. doi:10.3109/17435390.2012.739665.

Deshpande, S., S. Patil, S. V. N. T. Kuchibhatla, and S. Seal. 2005. “Size Dependency Variation in Lattice Parameter and Valency States in Nanocrystalline Cerium Oxide.” *Applied Physics Letters* 87 (13): 133113. doi:10.1063/1.2061873.

Eom, H. J., and J. Choi. 2009. “Oxidative Stress of CeO2 Nanoparticles via p38-Nrf-2 Signaling Pathway in Human Bronchial Epithelial Cell, Beas-2B.” *Toxicology Letters* 187 (2): 77–83.

Erdakos, G. B., P. V. Bhave, G. A. Pouliot, H. Simon, and R. Mathur. 2014. “Predicting the Effects of Nanoscale Cerium Additives in Diesel Fuel on Regional-scale Air Quality.” *Environmental Science & Technology* 48 (21): 12775–12782. doi:10.1021/es504050g.

Fu, P. P., Q. Xia, H.-M. Hwang, P. C. Ray, and H. Yu. 2014. “Mechanisms of Nanotoxicity: Generation of Reactive Oxygen Species.” *Journal of Food and Drug Analysis* 22 (1): 64–75. doi:10.1016/j.jfda.2014.01.005.

Gantt, B., S. Hoque, K. M. Fahey, R. D. Willis, J. M. Delgado-Saborit, R. M. Harrison, K. M. Zhang, et al. 2015. “Factors Affecting the Ambient Physicochemical Properties of Cerium-Containing Particles Generated by Nanoparticle Diesel Fuel Additive Use.” *Aerosol Science and Technology* 49 (6): 371–380. doi:10.1080/02786862.2015.1027809.

Gantt, B., S. Hoque, R. D. Willis, K. M. Fahey, J. M. Delgado-Saborit, R. M. Harrison, G. B. Erdakos, et al. 2014. “Near-road Modeling and Measurement of Cerium-containing Particles Generated by Nanoparticle Diesel Fuel Additive Use.” *Environmental Science & Technology* 48 (18): 10607–10613. doi:10.1021/es502169p.

Geraets, L., A. G. Oomen, J. D. Schroeter, V. A. Coleman, and F. R. Cassee. 2012. “Tissue Distribution of Inhaled Micro- and Nano-sized Cerium Oxide Particles in Rats: Results from a 28-day Exposure Study.” *Toxicological Sciences* 127 (2): 463–473. doi:10.1093/toxsci/kfs113.

Gosens, I., L. E. Mathijssen, B. G. Bokkers, H. Muijser, and F. R. Cassee. 2014. “Comparative Hazard Identification of Nano- and Micro-sized Cerium Oxide Particles Based on 28-day Inhalation Studies in Rats.” *Nanotechnology* 8 (6): 643–653. doi:10.3109/17435390.2013.815814.

Griere, M., H. G. Kirmeier, G. Liebsch, D. Rauch, F. Stuckler, G. Schmitz, R. Zarbock, and I.-B. W. G. O. T. Kids-Lung-Register. 2015. “Surfactant Lipidomics in Healthy Children and Childhood Interstitial Lung Disease.” *PLoS One* 10 (2): e0117985. doi:10.1371/journal.pone.0117985.

HEI 2001. *Evaluation of Human Health Risk from Cerium Added to Diesel Fuel*. Communication 9. Boston, MA: Health Effects Institute (HEI).

Keller, J., W. Wohlleben, L. Ma-Hock, V. Strauss, S. Groters, K. Kuttler, K. Wiensch, et al. 2014. “Time Course of Lung Retention and Toxicity of Inhaled Particles: Short-term Exposure to nano-Ceria.” *Archives of Toxicology* 88 (11): 2033–2059. doi:10.1007/s00204-014-1349-9.

Landsiedel, R., L. Ma-Hock, T. Hofmann, M. Wiemann, V. Strauss, S. Treumann, W. Wohlleben, S. Groters, K. Wiensch, and B. van Ravenzwaay. 2014. “Application of Short-term Inhalation Studies to Assess the Inhalation Toxicity of Nanomaterials.” *Particle and Fibre Toxicology* 11: 16.

Larsen, S. T., P. Jackson, S. S. Poulsen, M. Levin, K. A. Jensen, H. Wallin, G. D. Nielsen, and I. K. Koponen. 2016. “Airway Irritation, inflammation, and Toxicity in Mice following Inhalation of Metal Oxide Nanoparticles.” *Nanotoxicology* 10 (9): 1254–1262. doi:10.1080/17435390.2016.1202350.

Lee, S.-H., T.-Y. Wang, J.-H. Hong, T.-J. Cheng, and C.-Y. Lin. 2016. “NMR-based Metabolomics to Determine Acute Inhalation Effects of Nano- and Fine-sized ZnO Particles in the Rat Lung.” *Nanotoxicology* 10 (7): 924–934. doi:10.3109/17435390.2016.1144825.

Lee, S. H., C. H. Tang, W. Y. Lin, K. H. Chen, H. J. Liang, T. J. Cheng, and C. Y. Lin. 2018. “LC-MS-based Lipidomics to Examine Acute Rat Pulmonary Responses after Nano- and Fine-sized ZnO Particle Inhalation Exposure.” *Nanotoxicology* 114: 439–452.

Li, D., M. Morishita, J. G. Wagner, M. Fatouraie, M. Wooldridge, W. E. Eagle, J. Barres, U. Carlander, C. Emond,
and O. Jolliet. 2016. “In Vivo Biodistribution and Physiologically Based Pharmacokinetic Modeling of Inhaled Fresh and Aged Cerium Oxide Nanoparticles in Rats.” Particle and Fibre Toxicology 13 (1): 45.

Lin, W., Y. W. Huang, X. D. Zhou, and Y. Ma. 2006. “Toxicity of Cerium Oxide Nanoparticles in Human Lung Cancer cells.” International Journal of Toxicology 25 (6): 451–457.

Lopez-Ibanez, J., F. Pazos, and M. Chagoyen. 2016. “MBROLE 2.0-functional Enrichment of Chemical Compounds.” Nucleic Acids Research 44: W201–W204. doi:10.1093/nar/gkw253.

Ma, J. Y., R. R. Mercer, M. Barger, D. Schwegler-Berry, J. Scabillon, J. K. Ma, and V. Castranova. 2012. “Induction of Pulmonary Fibrosis by Cerium Oxide Nanoparticles.” Toxicology and Applied Pharmacology 262 (3): 255–264. doi:10.1016/j.taap.2012.05.005.

Ma, J. Y., H. Zhao, R. R. Mercer, M. Barger, M. Rao, T. Meighan, D. Schwegler-Berry, V. Castranova, and J. K. Ma. 2011. “Cerium Oxide Nanoparticle-induced Pulmonary Inflammation and Alveolar Macrophage Functional Change in Rats.” Nanotoxicology 5 (3): 312–325. doi:10.3109/17435390.2010.519835.

Majestic, B. J., G. B. Erdakos, M. Lewandowski, K. D. Oliver, R. D. Willis, T. E. Kleindienst, and P. V. Bhave. 2010. “A Review of Selected Engineered Nanoparticles in the Atmosphere: Sources, transformations, and Techniques for Sampling and Analysis.” International Journal of Occupational and Environmental Health 16 (4): 488–507. doi:10.1179/eoh.2010.16.4.488.

Morimoto, Y., H. Izumi, Y. Yoshiura, T. Tomonaga, T. Oyabu, T. Myojo, K. Kawai, et al. 2015. “Pulmonary Toxicity of Well-dispersed Cerium Oxide Nanoparticles following Intratracheal Instillation and Inhalation.” Journal of Nanoparticle Research 17: 442.

Nemmar, A., P. Yuvaraju, S. Beegam, M. A. Fahim, and B. H. Ali. 2017. “Cerium Oxide Nanoparticles in Lung Acutely Induce Oxidative Stress, Inflammation, and DNA Damage in Various Organs of Mice.” Oxidative Medicine and Cellular Longevity 2017:12. doi:10.1155/2017/9639035.

Oberdörster, G., J. Ferin, and B. E. Lehnhert. 1994. “Correlation between Particle Size, in Vivo Particle Persistence, and Lung Injury.” Environmental Health Perspectives 102 (Suppl 5): 173–179. doi:10.1289/ehp.94102s5173.

Oberdörster, G., A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, et al. 2005. “Principles for Characterizing the Potential Human Health Effects from Exposure to Nanomaterials: Elements of a Screening Strategy.” Particle and Fibre Toxicology 2 (1): 8. doi:10.1186/1743-8977-2-8.

Park, B., K. Donaldson, R. Duffin, L. Tran, F. Kelly, I. Mudway, J. P. Morin, et al. 2008a. “Hazard and Risk Assessment of a Nanoparticulate Cerium Oxide-based Diesel Fuel Additive – a Case Study.” Inhalation Toxicology 20 (6): 547–566. doi:10.1080/08958370801915309.

Park, E. J., J. Choi, Y. K. Park, and K. Park. 2008b. “Oxidative Stress Induced by Cerium Oxide Nanoparticles in Cultured BEAS-2B Cells.” Toxicology 245 (1–2): 90–100. doi:10.1016/j.tox.2007.12.022.

Park, E. J., J. Yi, K. H. Chung, D. Y. Ryu, J. Choi, and K. Park. 2008c. “Oxidative Stress and Apoptosis Induced by Titanium Dioxide Nanoparticles in Cultured BEAS-2B Cells.” Toxicology Letters 180 (3): 222–229. doi:10.1016/j.toxlet.2008.06.869.

Peng, L., X. He, P. Zhang, J. Zhang, Y. Li, J. Zhang, Y. Ma, et al. 2014. “Comparative Pulmonary Toxicity of Two Ceria Nanoparticles with the Same Primary Size.” International Journal of Molecular Sciences 15 (4): 6072–6085. doi:10.3390/ijms15046072.

Reed, K., A. Cormack, A. Kulkarni, M. Mayton, D. Sayle, F. Klaessig, and B. Stadler. 2014. “Exploring the Properties and Applications of Nanoceria: Is There Still Plenty of Room at the Bottom?” Environmental Science: Nano 1: 390–405. doi:10.1039/C4EN00079J.

Rice, K. M., S. K. Nalabetu, N. D. Manne, M. B. Kolli, G. Nandyala, R. Arvapalli, J. Y. Ma, and E. R. Blough. 2015. “Exposure to Cerium Oxide Nanoparticles Is Associated with Activation of Mitogen-activated Protein Kinases Signaling and Apoptosis in Rat Lungs.” Journal of Preventive Medicine and Public Health 48 (3): 132–141. doi:10.3961/jpmph.15.006.

Schwotzer, D., H. Ernst, D. Schaudien, H. Kock, G. Pohlmann, C. Danenbrock, and O. Creutzenberg. 2017. “Effects from a 90-day Inhalation Toxicity Study with Cerium Oxide and Barium Sulfate Nanoparticles in Rats.” Particle and Fibre Toxicology 14: 23.

Schwotzer, D., M. Niehof, D. Schaudien, H. Kock, T. Hansen, C. Danenbrock, and O. Creutzenberg. 2018. “Cerium Oxide and Barium Sulfate Nanoparticle Inhalation Affects Gene Expression in Alveolar Epithelial Cells Type II.” Journal of Nanobiotechnology 16: 16.

Singh, S., T. Dosani, A. S. Karakoti, A. Kumar, S. Seal, and W. T. Self. 2011. “A Phosphate-dependent Shift in Redox State of Cerium Oxide Nanoparticles and Its Effects on Catalytic Properties.” Biomaterials 32 (28): 6745–6753. doi:10.1016/j.biomaterials.2011.05.073.

Skillas, G., Qian, Z., Baltensperger, U., Matter, U. & Burtscher, H., 2000. The Influence of Additives on the Size Distribution and Composition of Particles Produced by Diesel Engines. Combustion Science and Technology, 154, 259–273.

Snow, S. J., J. Mcgee, D. B. Miller, V. Bass, M. C. Schladweiler, R. F. Thomas, T. Krantz, et al. 2014. “Inhaled Diesel Emissions Generated with Cerium Oxide Nanoparticle Fuel Additive Induce Adverse Pulmonary and Systemic Effects.” Toxicological Sciences 142 (2): 403–417. doi:10.1093/toxsci/kfu187.

Southam, A. D., R. J. Weber, J. Engel, M. R. Jones, and M. R. Viant. 2017. “A Complete Workflow for High-resolution Spectral-stitching Nanoelectrospray Direct-infusion Mass-spectrometry-based Metabolomics and Lipidomics.” Nature Protocols 12: 310–328.
Srinivas, A., P. J. Rao, G. Selvam, P. B. Murthy, and P. N. Reddy. 2011. "Acute Inhalation Toxicity of Cerium Oxide Nanoparticles in Rats." *Toxicology Letters* 205 (2): 105–115.

Stoeger, T., C. Reinhard, S. Takenaka, A. Schroeppe, E. Karg, B. Ritter, J. Heyder, and H. Schulz. 2006. "Instillation of Six Different Ultrafine Carbon Particles Indicates a Surface Area Threshold Dose for Acute Lung Inflammation in Mice." *Environmental Health Perspectives* 114 (3): 328–333. doi:10.1289/ehp.8266.

Szymanski, C. J., P. Munusamy, C. Mihai, Y. Xie, D. Hu, M. K. Gilles, T. Tyliszczak, S. Thevuthasan, D. R. Baer, and G. Orr. 2015. "Shifts in Oxidation States of Cerium Oxide Nanoparticles Detected inside Intact Hydrated Cells and Organelles." *Biomaterials* 62: 147–154. doi:10.1016/j.biomaterials.2015.05.042.

Taylor, N. S., R. Merrifield, T. D. Williams, J. K. Chipman, J. R. Lead, and M. R. Viant. 2016. "Molecular Toxicity of Cerium Oxide Nanoparticles to the Freshwater Alga Chlamydomonas reinhardtii Is Associated with Supra-environmental Exposure Concentrations." *Nanotoxicology* 10: 32–41. doi:10.3109/17435390.2014.1002868

Titz, B., S. Boue, B. Phillips, M. Talikka, T. Vihervaara, T. Schneider, C. Nury, et al. 2016. "Effects of Cigarette Smoke, Cessation, and Switching to Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in C57BL/6 and Apo−/− Mice-An Integrative Systems Toxicology Analysis." *Toxicological Sciences* 149 (2): 441–457. doi:10.1093/toxsci/kfv244.

Tyurina, Y. Y., E. R. Kisin, A. Murray, V. A. Tyurin, V. I. Kapralova, L. J. Sparvero, A. A. Amoscato, et al. 2011. "Global Phospholipidomics Analysis Reveals Selective Pulmonary Peroxidation Profiles upon Inhalation of Single-Walled Carbon Nanotubes." *ACS Nano* 5 (9): 7342–7353. doi:10.1021/nn202201j.

Wu, H., A. D. Southam, A. Hines, and M. R. Viant. 2008. "High-throughput Tissue Extraction Protocol for NMR- and MS-based Metabolomics." *Analytical Biochemistry* 372 (2): 204–212.

Yu, L. E., L.-Y. Lanry Yung, C.-N. Ong, Y.-L. Tan, K. Suresh Balasubramaniam, D. Hartono, G. Shui, M. R. Wenk, and W.-Y. Ong. 2007. "Translocation and Effects of Gold Nanoparticles after Inhalation Exposure in Rats." *Nanotoxicology* 1 (3): 235–242. doi:10.1080/17435390701763108.

Zemski Berry, K. A., R. C. Murphy, B. Kosmider, and R. J. Mason. 2017. "Lipidomic Characterization and Localization of Phospholipids in the Human Lung." *Journal of Lipid Research* 58 (5): 926–933. doi:10.1194/jlr.M074955.

Zhang, J., Y. Nazarenko, L. Zhang, L. Calderon, K. B. Lee, E. Garfunkel, S. Schwander, et al. 2013. "Impacts of a Nanosized Ceria Additive on Diesel Engine Emissions of Particulate and Gaseous Pollutants." *Environmental Science & Technology* 47 (22): 13077–13085. doi:10.1021/es402140u.