

Unclassified

ENV/JM/MONO(2015)8

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

29-May-2015

English - Or. English

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

DOSSIER ON CERIUM OXIDE

**Series on the Safety of Manufactured Nanomaterials
No. 45**

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**ENV/JM/MONO(2015)8
Unclassified**

English - Or. English

OECD Environment, Health and Safety Publications

Series on the Safety of Manufactured Nanomaterials

No. 45

DOSSIER ON CERIUM OXIDE



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2015

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No. 44, *Dossier on Gold nanoparticles (2015)*

No. 46, *Dossier on Dendrimers (2015)*

No. 47, *Dossier on Nanoclays (2015)*

No. 48, *Dossier on Fullerenes (2015)*

No. 49, *Dossier on Multiwalled Carbon Nanotubes (MWCNTs) (2015)*

No. 50, *Dossier on Single-Walled Carbon Nanotubes (SWCNTs) (2015)*

No. 51, *Dossier on Silicon dioxide (2015)*

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

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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PREAMBLE

In November 2007, OECD's Working Party on Manufactured Nanomaterials (WPMN) launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The outcomes of the Testing Programme were intended to assess the applicability of the existing **test guidelines**¹ to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions. Understanding the properties of NMs is crucial to choose appropriate strategies for hazard identification, risk assessment or risk management measures. The Testing Programme involved delegations from OECD member countries, some non-member economies and other stakeholders. The broad international representation, from a range of delegations enabled the programme to pool expertise and resources without which this programme would not have been possible.

Before launching the Testing Programme, the WPMN first identified a broad list of possible nanomaterials, and the list was later adjusted to a final selection of eleven MNs for testing². This list comprised: i) fullerenes (C60); ii) single-walled carbon nanotubes (SWCNTs); iii) multi-walled carbon nanotubes (MWCNTs); iv) silver nanoparticles; v) titanium dioxide; vi) cerium dioxide; vii) zinc oxide; viii) silicon dioxide; ix) dendrimers; x) nanoclays; and xi) gold nanoparticles. One fundamental criterion for selecting these materials was that they should be either in commercial use at the time or expected to be in the near future. At the same time, other considerations were also given attention, such as the production volume of the materials, the likely availability of such materials for testing and the existing information that would readily be available on the materials.

It was also agreed that 59 endpoints would be addressed³ for each material corresponding to the following categories: i) nanomaterial information/ identification; ii) physical-chemical properties and material characterisation; iii) environmental fate; iv) toxicological and eco-toxicological effects; v) environmental toxicology; vi) mammalian toxicology; and vii) material safety. These endpoints were judged to be most important based largely on the general experience of testing chemicals, while taking into account the potentially different or new properties of nanomaterials. It is worth noticing that it was not expected that testing for all of the listed endpoints would be necessary for each of the selected MNs.

To assist with the Testing Programme, the WPMN developed two documents: i) a Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials [ENV/JM/MONO(2009)21]; and ii) Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme (Guidance Manual) in 2009, which was subsequently updated in 2010

¹ The OECD Test Guidelines are a collection of internationally agreed test methods used by government, industry and independent laboratories. They are used to determine the safety of chemicals.

<http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforhetestingofchemicals.htm>

² Originally Iron nanoparticles, Aluminium, Carbon black, and Polystyrene were suggested but later withdrawn and replaced by gold nanoparticles.

³ As specified in the Guidance Manual, "address" includes the term "completed" which provides that all dossiers will contain the identified endpoint information. Note that for some endpoints (for example, solubility) it is specified that the endpoint must be "completed". In such instances "completed" means that all Dossiers will be providing this endpoint information.

[ENV/JM/MONO(2009)20/REV]⁴. The objective of this Guidance Manual was to guide sponsors⁵ in the testing of the materials while ensuring that the information collected was reliable, accurate, consistent and therefore also comparable. The Guidance Manual addressed a whole range of issues including the organisation of the work.

The *Guidance Manual* contains detailed information on the selected endpoints for testing and recommendations on sample preparation and dosimetry.

The *Guidance Manual* also described the development of *Dossier Development Plans* (DDPs). These plans were prepared by Lead sponsors, Co-sponsors together with contributors to describe the specific plan for the testing of each nanomaterial including when and where the testing will be undertaken and by whom. The DDPs also included information on the materials to be tested as well as information on issues such as sample preparation and dosimetry. Each of the DDPs was prepared and reviewed by the WPMN before testing work began.

Based on the lessons learned during the Testing Programme, the WPMN also developed *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials* [ENV/JM/MONO(2012)40]. This latter document is an update of an earlier text first published in 2010.

The work on OECD's Testing Programme was completed by the end of 2013. In June 2014 the WPMN agreed that for each nanomaterial the dataset would be published in IUCLID printed format^{6 7}. The document will include the protocols and methods to allow their wider use (regulators and researchers).

The dataset in this document has been declassified and made publicly available and it is expected regulators and researchers will wish to use it. Due to a broad dissemination of the data and the exploratory setting in which they were developed there are a number of limitations in using the data of which potential users should be aware. The programme focused on answering scientific questions in the field of the OECD test guidelines but not to provide conclusions on the hazard or risk of the materials selected. The absence of data for some endpoints may be a gap for some endpoints but for other end points there may not if the data was not considered necessary. Although the programme ensured a broad participation of many stakeholders it was not intended to arrive at any pre-defined regulatory datasets requirements or risk assessment decisions. It was recognised from the beginning that the exploratory nature of the work would require subsequent follow-up work for example to review the specific needs that may arise when performing risk assessment of nanomaterials. In this context, the programme's ultimate goal, to add to the knowledge of the properties of nanomaterials, would form a cornerstone.

⁴ It is worth noting that while the *Guidance Manual for Sponsors* was primarily intended as a guide to WPMN's Testing Programme, it is also expected that it will be of value to anyone involved in testing NMs.

⁵ The Guidance Manual noted, for example, that there could be three levels of participation to the programme. Lead sponsors, who would assume responsibility for conducting or coordinating all of the testing, determined to be appropriate for each of the endpoints for a specific nanomaterial. In some cases, "joint lead" arrangements were developed. Co-sponsors conducted some of the testing determined to be appropriate and feasible to address the endpoints for a specific listed nanomaterial. Contributors provided test data, reference or testing materials or other relevant information to the lead and co-sponsors.

⁶ IUCLID is a software programme for the administration of data on chemical substances. Although it was originally developed to fulfill requirements in the EU for the evaluation and control of the risks of existing chemical substances, it is used by many others.

⁷ SIAR = SIDS Initial Assessment Report (SIDS = Screening Information Data Set)

FOREWORD

As part of its Programme on the Safety of Manufactured Nanomaterials, OECD launched the Sponsorship Programme for the Testing of Manufactured Nanomaterials (hereafter the Testing Programme). The objective was to conduct specific tests, relevant to human health and environmental safety endpoints, on a variety of manufactured nanomaterials (MN). The Testing Programme mainly aimed to assess the applicability of the existing test guidelines to nanomaterials, as well as to provide useful information on any intrinsic properties of MNs, which are different from the same bulk material with greater external dimensions.

This document presents the Dossier of Cerium dioxide. This nanomaterial has been tested for a number of endpoints for: i) Nanomaterials Information / Identification; ii) Physical-Chemical Properties; iii) Environmental Fate; iv) Environmental Toxicology; v) Mammalian Toxicology; and vi) Material Safety. They have been analysed using OECD Guidelines for the Testing of Chemicals (TG)⁸. The data is presented in an IUCLID⁹ style format and includes the protocols and methods used (see Preamble).

The Business & Industry Advisory Committee to the OECD (BIAC) via the Nanotechnology Industries Association (NIAC) and the US co-led the Testing Programme on the Cerium dioxide. This included the determination of the tests that were appropriate, performing a number of tests, as well as coordinating tests and results obtained by other participating stakeholders from Australia, Denmark, European Commission, Germany, Japan, Netherlands, Spain and Switzerland.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

⁸ <http://www.oecd.org/env/testguidelines>

⁹ IUCLID is a software programme for the administration of data on chemical substances. It was originally developed to fulfil requirements in the EU for the evaluation and control of the risks of existing chemical substances. It is specifically relevant in the context of an international programme for the initial assessment of chemical substances.

ACKNOWLEDGMENTS

The OECD Secretariat and the Working Party on Manufactured Nanomaterials wish to thank the Business & Industry Advisory Committee to the OECD (BIAC) and the US for co-leading the Testing Programme for Cerium dioxide. They are specifically grateful to David Carlander from the Nanotechnology Industries Association. In addition, we appreciate the efforts made by other countries that participated in the Testing Programme, in particular to Australia, Denmark, European Commission, Germany, Japan, Netherlands, Spain and Switzerland.

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SUBSTANCE: CERIUM DIOXIDE NANO

1. GENERAL INFORMATION

1.1 Identification

Substance identification

Chemical name Cerium dioxide nano

Legal entity Nanotechnology Industries Association / Brussels / Belgium

Reference substance

Cerium dioxide nano

Type of substance

Composition mono constituent substance

1.2 Composition

Substance composition

Name	NM211 Cerium dioxide nano
Brief description	Ceria dry CeO ₂ material Antaria, Australia
Degree of purity	> 99.5 % (w/w)
Name	NM212 Cerium dioxide nano
Brief description	Nanograin CeO ₂ material, Umicore, Belgium
Degree of purity	> 99.5 % (w/w)
Name	NM213 Cerium dioxide
Brief description	Micron CeO ₂ material, Sigma Aldrich, United Kingdom
Degree of purity	> 99.9 % (w/w)

1.3 Identifiers

1.4 Analytical information

1.5 Joint submission

1.6 Sponsors

1.7 Suppliers

1.8 Recipients

1.9 Product and process orientated research and development

2. CLASSIFICATION & LABELLING AND PBT ASSESSMENT

2.1 GHS

Classification and Labelling according to GHS

General information	
Name	Cerium dioxide nano
State / form of the substance	nanomaterial
Remarks	Nanomaterials NM211, NM212, NM213 used in the OECD sponsorship programme
Classification	
Physical hazards	

	Hazard category	Hazard statement	Reason for no classification
Explosives			data lacking
Flammable gases and chemically unstable gases			data lacking
Aerosols			data lacking
Oxidising gases			data lacking
Gases under pressure			data lacking
Flammable liquids			data lacking
Flammable solids			data lacking
Self-reactive substances and mixtures			data lacking
Pyrophoric liquids			data lacking
Pyrophoric solids			data lacking
Self-heating substances and mixtures			data lacking
Substances and mixtures which in contact with water emit flammable gases			data lacking
Oxidising liquids			data lacking
Oxidising solids			data lacking
Organic peroxides			data lacking
Corrosive to metals			data lacking

Health hazards

	Hazard category	Hazard statement	Reason for no classification
Acute toxicity - oral			data lacking
Acute toxicity - dermal			data lacking
Acute toxicity - inhalation			data lacking
Skin corrosion/irritation			data lacking
Serious eye damage/eye irritation			data lacking
Respiratory sensitisation			data lacking
Skin sensitisation			data lacking
Aspiration hazard			data lacking
Reproductive toxicity			

Reproductive toxicity	data lacking
Specific effect	
Route of exposure	
Effects on or via lactation	data lacking

Germ cell mutagenicity

	Hazard category	Hazard statement	Reason for classification	no
Germ cell mutagenicity			data lacking	
Route of exposure				

Carcinogenicity

	Hazard category	Hazard statement	Reason for classification	no
Carcinogenicity			data lacking	
Route of exposure				

Specific target organ toxicity - single

	Hazard category	Hazard statement	Reason for classification	no
Specific target organ toxicity - single			data lacking	
Affected organs				
Route of exposure				

Specific target organ toxicity - repeated

	Hazard category	Hazard statement	Reason for classification	no
Specific target organ toxicity - repeated			data lacking	
Affected organs				
Route of exposure				

Environmental hazards

	Hazard category	Hazard statement	Reason for classification	no
--	------------------------	-------------------------	----------------------------------	-----------

Hazardous to the aquatic environment (acute/short-term)		data lacking	
Hazardous to the aquatic environment (long-term)		data lacking	
	Hazard category	Hazard statement	Reason for no classification
Hazardous to the ozone layer			data lacking

2.2 DSD - DPD

3. MANUFACTURE, USE AND EXPOSURE

3.1 Technological process

3.2 Estimated quantities

3.3 Sites

3.4 Information on mixtures

3.5 Life Cycle description

3.6 Uses advised against

3.7 Exposure Scenarios, exposure and risk assessment

3.7.1 Exposure scenarios and local assessment

3.7.2 Environmental assessment for aggregated sources

3.7.3 Generic exposure potential

3.8 Biocidal information

3.9 Emissions Information

3.10 Application for authorisation of uses

4. PHYSICAL AND CHEMICAL PROPERTIES

4.1 Appearance/physical state/colour

Endpoint study record: Appearance/physical state/colour.001

Administrative Data

Materials and methods

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)
yes

Test material form

nanomaterial

Endpoint study record: 2012-02-29_Australia_CSIRO_Apperance_TEM

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Rationale for reliability incl. Appearance was assessed from a limited number of TEM images

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Principles of method if other than guideline

Used transmission electron microscopy to image the appearance of particles at high resolution

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)
yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

The three OECD CeO₂ samples were sourced from three different commercial suppliers (Antaria, Umicore, Sigma-Aldrich) and labeled as NM211, NM212 and NM213 respectively. Australia received NM211 and NM213 from the NPL (UK), and NM212 (same batch as supplied to the OECD Program) directly from the supplier (Umicore). The samples represented a range of particle sizes, based on information from the suppliers, which varied from ultrafine to bulk. Supplier details, product names and other pertinent information regarding these are summarized in Table 1. Sub samples of these master samples were distributed across different laboratories in Australia for various analyses. The physico-chemical results presented here were mainly obtained in various CSIRO laboratories in Australia.

Table 1 Summary of product details provided by supplier

Identity Supplier Product name Purity Particle Size (nm)

NM211 Antaria Cerium (IV) Oxide 99.5wt% 10 nm

NM212 Umicore Nanograin Ceria Total REO >99.95wt% Na, Fe, Cr, Ni, Cu, Al, K < 10 ppm 20 to 25 nm

NM213 Sigma-Aldrich 211575 99.9% trace metals basis <5µm

Any other information on materials and methods incl. tables

For all samples, carbon-coated grids (copper, 300 mesh) were glow discharged in nitrogen for 30 seconds to render them hydrophilic. Samples were dispersed by briefly sonicating a few mg of the material in approximately 20µl ethanol to form a milky dispersion. 5µl of dispersion was applied to the freshly glow-discharged grids. After 2 mins adsorption time, excess dispersion was wicked off using filter paper (Whatman 541) and the grids were air-dried for 15 minutes. Grids were examined using a Tecnai 12 TEM (FEI, Eindhoven, Netherlands) operating at 120 kV, and micrographs were recorded using an Olympus Megaview III CCD camera (Tokyo, Japan) running AnalySiS imaging software (Olympus) at a variety of magnifications chosen to show both the aggregation/agglomeration state of the samples (lower magnifications eg 6000x) as well as particle morphology (higher magnifications eg 100 000x - 360 000x).

Overall remarks, attachments

Remarks on results including tables and figures

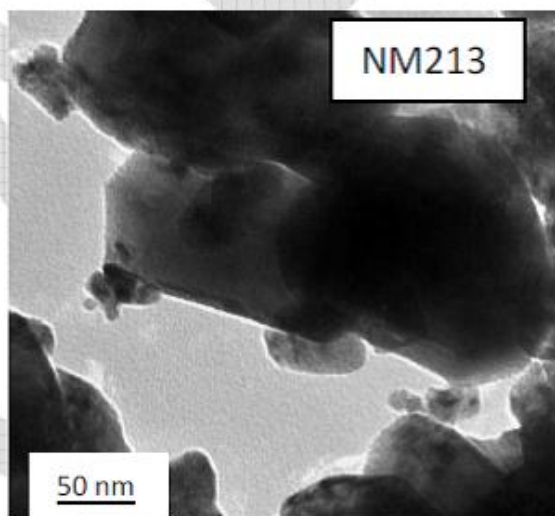
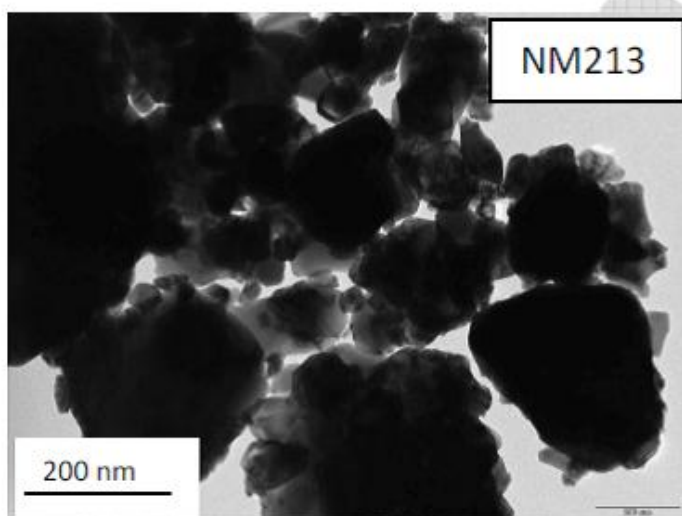
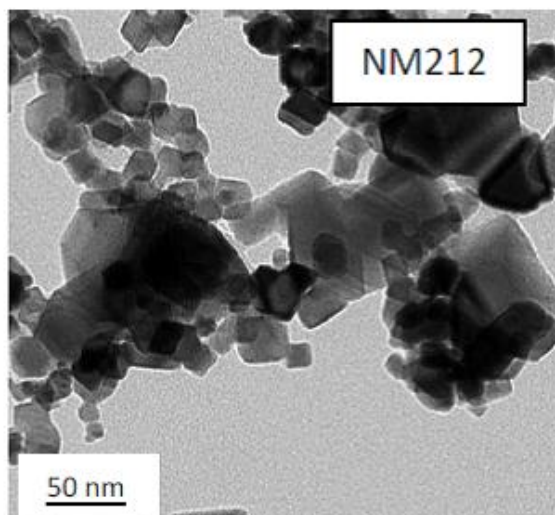
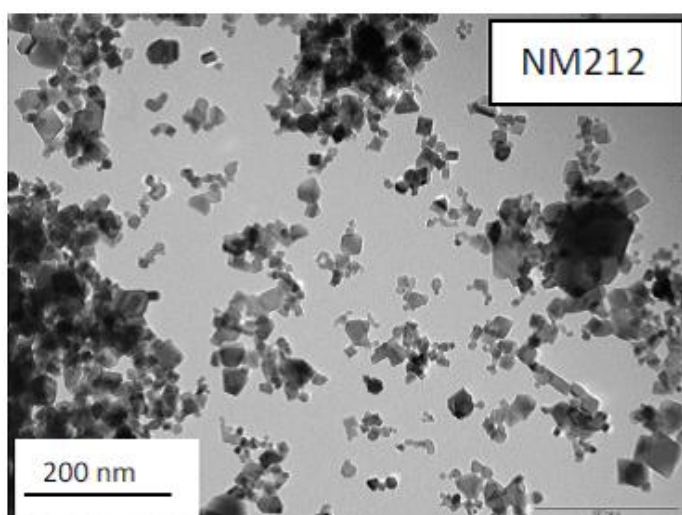
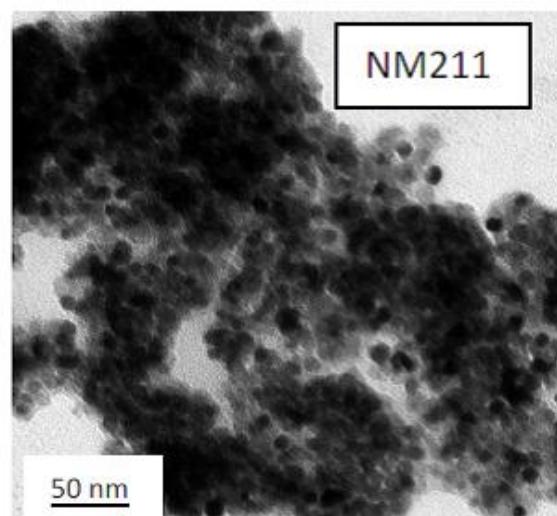
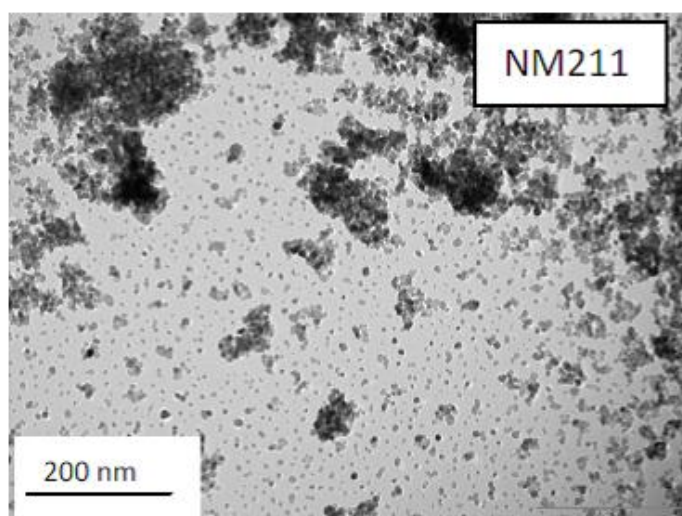
Figure shows typical TEM images of the CeO₂ samples, NM211, NM212 and NM213, at two different magnifications. TEM images are shown in the attached study report.

Attached full study report

Attached full study report

NM_211_ANNEX_A05_OECD CeO₂ Appearance TEM CSIRO Australia.docx / 665.9 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Illustration (picture/graph)



Applicant's summary and conclusion

Conclusions

The TEM image of NM-211 at lower magnification shows particles with roughly the same sizes, and generally located within agglomerated/aggregated structures. The TEM image at higher magnification shows that primary particles are near spherical (rather than polyhedral) with regular morphology and have little variation in size. Generally, the particles have an aspect ratio close to 1.

The TEM image of NM212 at lower magnification shows particles with a large variation in size, and these appear to be less agglomerated than NM211. The TEM image at higher magnification indicates that primary particles are polyhedral with irregular morphology and have a non-homogenous size distribution. The TEM image of NM213 at lower magnification shows large particles with similar sizes.

The TEM image of NM213 at higher magnification shows the sample has a bi-modal particle-size distribution with large, polyhedral, irregularly-shaped particles (150-200nm) associated with smaller particles (dimensions up to approximately 50nm).

Endpoint study record: 2012-03-02_Australia-Deakin_appearance_TEM

Administrative Data

Study result type experimental result

Reliability 2 (reliable with restrictions)

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	T Tsuzuki, R He, R Chaudhary	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Principles of method if other than guideline

Transmission Electron Microscopy (TEM)

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Any other information on materials and methods incl. tables

Instrument: JEOL JEM-2100

Sample preparation: TEM specimen was prepared by evaporating a drop of the nanoparticle dispersion on a carbon-coated specimen grid.

Measurement parameters: electron beam energy 200 kV

Results and discussion

Colour

Light yellow

Overall remarks, attachments

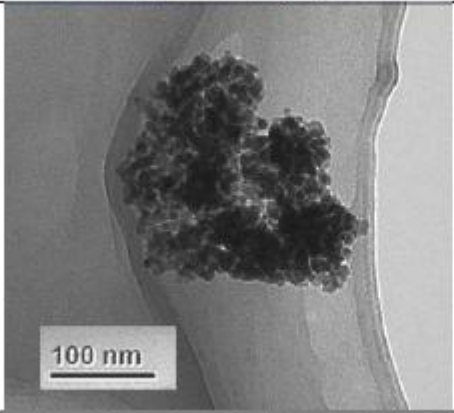
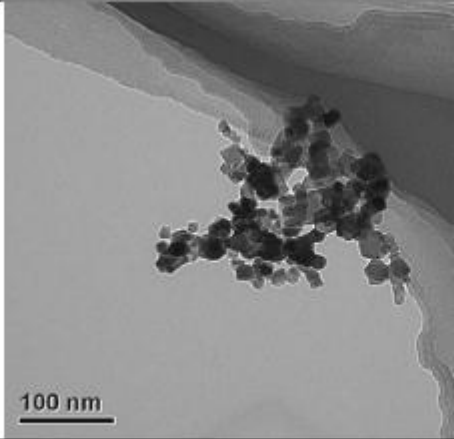
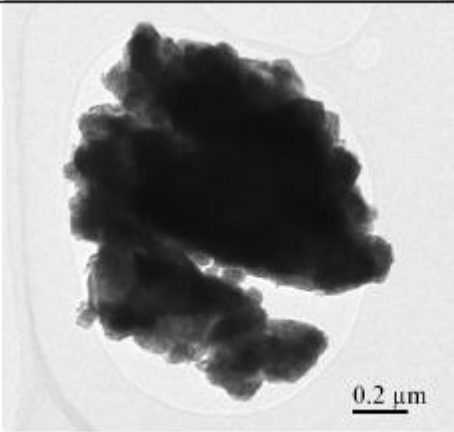
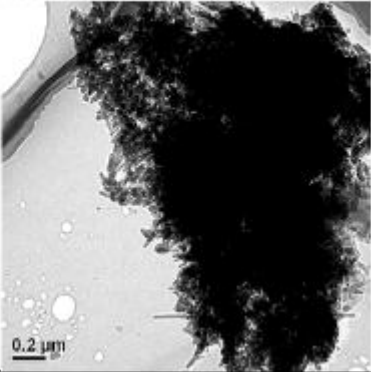
Remarks on results including tables and figures

Primary particles appear to be near-spherical. Some agglomerations evident. TEM images in the attached document.

Attached full study report

Attached full study report
NM_211_ANNEX_A06_OECD CeO2 TEM Australia Deakin.pdf / 535.73 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Illustration (picture/graph)

Sample ID	Electron micrograph	Remark
NM211		Near spherical primary particles.
NM212		- Geometrical shaped primary particles. - Some neck formation evident between primary particles.
NM213		- Irregular shapes, - Very wide size distribution. - Non-uniform morphologies. 

Applicant's summary and conclusion**Conclusions**

NM-211: Near spherical primary particles

NM-212: Geometrical shaped primary particles. Some neck formation evident between primary particles

NM-213: Irregular shapes, very wide size distribution, non-uniform morphologies

4.2 Melting point/freezing point

4.3 Boiling point

4.4 Density

4.5 Particle size distribution (Granulometry)

Endpoint study record: 2010-06-17_NPL for PROSPECT_Particle size, size distribution_SEM

Administrative Data

Study result type experimental study planned
Reliability 4 (not assignable)
Rationale for reliability incl. deficiencies Preliminary results from scoop sampled bulk material

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of distribution

other: SEM

Principles of method if other than guideline

Scanning Electron Microscopy (SEM). For analysis of the “as received” nanoparticle powder, a small scoop of the sample of the nanoparticle powder was sprinkled over a SEM carbon adhesive discs; one side of the carbon disc was placed securely on a metal stub, whilst the other side was exposed to the nanoparticle powder. Excess powder on top of the disc was removed by gently tapping the stub on its side until an even (light) coating of powder on the surface was apparent. Scanning electron microscope SEM images were recorded obtained using a Carl Zeiss Supra 40 field emission scanning electron microscope from Carl Zeiss, in which the optimal spatial resolution of the microscope was a few nanometres. In-Lens detector images were acquired at an accelerating voltage of 15 kV, a working distance of ≈ 3 mm, and a tilt angle 0° .

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
EC number	215-150-4

Test material form

nanomaterial

Details on test material

NM-211, original bulk sample

Any other information on materials and methods incl. tables

Instrument: Carl Zeiss Supra 40 field emission scanning electron microscope

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A07_Cyprus2010v2.pdf / 318.37 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	
NM_211_ANNEX_A08_FinalVersionSustainableDevPlanning2.pdf / 1.21 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	

Endpoint study record: 2010-06-17_NPL for PROSPECT_Particle size, size distribution_DLS**Administrative Data**

Study result type experimental study planned

Reliability other:

Rationale for reliability incl. deficiencies Preliminary testing using scoop sample from original bulk powder

Materials and methods**Principles of method if other than guideline**

Dynamic Light Scattering (DLS)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM212, NM-213

Test material form

nanomaterial

Details on test material

State of test material: Dispersion

Any other information on materials and methods incl. tables

Hydrodynamic size (z-average mean) measurements were obtained using a Zetasizer Nano ZS (Malvern Instruments, UK) equipped with a 633 nm laser. A reference standard (polystyrene, latex bead, nominal size of 100 nm, NIST traceable) was used to qualify the performance of the instrument. Sample analysis involved filling of a disposable capillary cell (DTS1060, Malvern). The advantage of using this cell is that zeta-potential measurements can be taken immediately after acquiring the DLS measurement. Prior to

their use, these cells were thoroughly cleaned with ethanol and de-ionised water, as recommended by the instrument vendor. Individual cell was then filled with the appropriate sample and flushed before re-filling; measurement was carried out on the second filling. Malvern Instrument's Dispersion Technology software (Version 4.0) was used for data analysis. For particle size it is the z-average diameter (the mean hydrodynamic diameter) that is reported.

Instruments: Zetasizer Nano ZS (Malvern Instruments, UK) equipped with a 633 nm laser

Calibration: A reference standard (polystyrene, latex bead, nominal size of 100 nm, NIST traceable) was used to qualify the performance of the instrument

Overall remarks, attachments

Remarks on results including tables and figures

Experimentation still to be completed

Attached background material

Attached document	Remarks
NM_211_ANNEX_A07_Cyprus2010v2.pdf / 318.37 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	
NM_211_ANNEX_A08_FinalVersionSustainableDevPlanning2.pdf / 1.21 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	

Endpoint study record: 2010-06-17_NPL for PROSPECT_Particle size, size distribution_SMPS

Administrative Data

Study result type experimental study planned

Reliability other:

Rationale for reliability incl. deficiencies Preliminary results. Scoop sampled from original bulk powder.

Materials and methods

Principles of method if other than guideline

Scanning Mobility Particle Sizing (SMPS)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213
EC number	212-222-5

Test material form

nanomaterial

Details on test material

Dry bulk, original bulk sample

Any other information on materials and methods incl. tables

The sample was aerosolised using a Fluidised Bed Aerosol Generator. These aerosolised particles were then put through a Scanning Mobility Particle Sizer. Data was taken from a stable 51-hour segment of the sampling time.

Instruments: TSI Scanning Mobilty Particle Analyser (SMPS 3080 with a 3081 LDMA and a 3022A CPC) TSI Fluidised Bed Aerosol Generator (FBAG)

Calibration: Condensation Particle Counter internally calibrated

Overall remarks, attachments**Remarks on results including tables and figures**

Experiments not yet completed

Endpoint study record: **2012-03-02_Australia_CSIRO_Particle_Size_TEM_Feret**

Administrative Data

Study result type experimental result

Reliability other:

Rationale for reliability incl. deficiencies Sizes of 100 partcils measured, as representative of total sample

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Principles of method if other than guideline**

Transmission Electron Microscopy (TEM)

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Details on test material

State of test material: Dry bulk

Any other information on materials and methods incl. tables

Carbon-coated grids (copper, 300 mesh) were glow discharged in nitrogen for 30 seconds to render them hydrophilic. Samples were dispersed by briefly sonicating a few mg of the material in approximately 20 µl ethanol to form a milky dispersion. 5 µl of dispersion was applied to the freshly glow-discharged grids. After 2 mins adsorption time, excess dispersion was wicked off using filter paper (Whatman 541) and the grids were air-dried for 15 minutes. Grids were examined using a Tecnai 12 TEM (FEI, Eindhoven, Netherlands) operating at 120 kV, and micrographs were recorded using an Olympus Megaview III CCD camera (Tokyo, Japan) running AnalySiS imaging software (Olympus) at a variety of magnifications chosen to show particle morphology (magnifications eg 100 000x - 360 000x). Feret's diameter can be defined as the "maximum calliper length" i.e. the longest distance between any two points along the selection boundary. Feret's diameter was determined by measuring 100 individual particles in TEM images.

Instruments: Tecnai 12 TEM (FEI, Eindhoven, Netherlands) operating at 120 kV. Micrographs were recorded using an Olympus Megaview III CCD camera (Tokyo, Japan) running AnalySiS imaging software (Olympus)

Results and discussions

Particle size

Percentile	Mean	St. dev.	Remarks
	ca. 12 nm		Feret's diameter for NM-211 is 12 (+/-3) nm.
	ca. 29 nm		Feret's diameter for NM-212 is 29 (+/-19) nm.
	ca. 67 nm		Feret's diameter for NM-213 is 67 (+/-64) nm.

Any other information on results incl. tables

Comparison of crystallite/particle sizes in samples NM211, NM212 and NM213, as determined from XRD analyses, TEM images and BET surface area measurements:

	D _{R-XRD} (nm)	D _{S-XRD} (nm)	D _{Feret TEM} (nm)	D _{Circ TEM} (nm)	D _{SA BET} (nm)	Average of all methods (nm)
NM211	14	10	12 ± 3	10 ± 3	12	11.6
NM212	57	20	29 ± 19	24 ± 16	28	31.6
NM213	170	28	67±64	54 ± 53	164	96.6

Overall remarks, attachments

Remarks on results including tables and figures

The attached study report compares the Feret diameter, as determined by measurement of particle sizes in TEM images, with crystallite and particle sizes determined by other methods.

All methods indicate that the particle sizes are in the same order as sizes provided by the suppliers. Based on the average value from all methods, NM-211 is the smallest (12 nm), NM-212 larger (32 nm) and NM-213 the largest (97 nm). The difference between the measured and supplier-supplied particle size is large for NM-213 (measured 97 nm, compared with supplied value of <5 µm), while the differences are small for NM-211 (12 nm compared with 10 nm) and NM-212 (32 nm compared with 20-25 nm). Of the three samples analysed, NM-211 has the smallest size distribution, with the particles likely to be single crystals.

The sizes of particles determined by different methods were generally in the order of

$$D_{R-XRD} > D_{SA\ BET} > D_{Feret\ TEM} > D_{Circ\ TEM} > D_{S-XRD}$$

D_{S-XRD} was smallest because Scherrer's formula ignores the contributions of width broadening due to

strain and instrument effects. If these contributions are non-zero, the crystallite sizes could be larger than those predicted by the Scherrer formula, as revealed by D_{R-XRD} . The real states of particles can be described as singlets, agglomerates or aggregates. Agglomerates are primary particles held by weak Van der Waals force that can be overcome if sufficient energy is provided. Aggregates are primary particles held together by strong chemical bonds that cannot be separated by conventional methods. $D_{\text{Ferret TEM}}$ provides the most reliable assessment of aggregate size, because aggregates are not broken up by ultrasonication during sample preparation whereas agglomerates can be. $D_{\text{Circ TEM}}$ provides a smaller value than $D_{\text{Ferret TEM}}$ since this method treats all particles as spheres; it is not the most suitable method for analysing samples containing particles with a broad or multimodal size distribution like NM212 and NM213. D_{SABET} was calculated from measurements of specific surface area and assumed that all particles are non-porous and have the same spherical shape and size.

Attached full study report

Attached full study report									
NM_211_ANNEX_A09_OECD CeO2 crystallite size CSIRO Australia.docx / 28.83 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1									

Endpoint study record: 2012-03-02_Australia_CSIRO_Particle_Size_TEM_Equiv_Circular_Diameter

Administrative Data

Study result type experimental result

Reliability other:

Rationale for reliability incl. deficiencies Sizes of 100 particles measured, as representative of total sample.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of distribution

other: TEM

Principles of method if other than guideline

Transmission Electron Microscopy

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

State of test material: Dry bulk

Any other information on materials and methods incl. tables

Carbon-coated grids (copper, 300 mesh) were glow discharged in nitrogen for 30 seconds to render them hydrophilic. Samples were dispersed by briefly sonicating a few mg of the material in approximately 20 µl ethanol to form a milky dispersion. 5 µl of dispersion was applied to the freshly glow-discharged grids. After 2 mins adsorption time, excess dispersion was wicked off using filter paper (Whatman 541) and the grids were air-dried for 15 minutes. Grids were examined using a Tecnai 12 TEM (FEI, Eindhoven, Netherlands) operating at 120 kV, and micrographs were recorded using an Olympus Megaview III CCD camera (Tokyo, Japan) running AnalySiS imaging software (Olympus) at a variety of magnifications chosen to show particle morphology (magnifications eg 100 000x - 360 000x). The equivalent circular diameter (D_{circ TEM}) is an estimate of the diameter of a circle that has the same area as that of the projected particle. The equivalent circular diameter was determined by measuring 100 individual particles in TEM images.

Instruments: Tecnai 12 TEM (FEI, Eindhoven, Netherlands) operating at 120 kV. Micrographs were recorded using an Olympus Megaview III CCD camera (Tokyo, Japan) running AnalySiS imaging software (Olympus).

Results and discussions**Particle size**

Percentile	Mean	St. dev.	Remarks
	ca. 10 nm		The equivalent circular diameter for NM-211 is 10 (+/-3) nm
	ca. 24		The equivalent circular diameter for NM-212 is 24 (+/-16) nm
	ca. 54 nm		The equivalent circular diameter for NM-213 is 54 (+/-53) nm

Any other information on results incl. tables

Comparison of crystallite/particle sizes in samples NM211, NM212 and NM213, as determined from XRD analyses, TEM images and BET surface area measurements.

	D _{R-XRD} (nm)	D _{S-XRD} (nm)	D _{Feret TEM} (nm)	D _{Circ TEM} (nm)	D _{SA BET} (nm)	Average of all methods (nm)
NM211	14	10	12 ± 3	10 ± 3	12	11.6
NM212	57	20	29 ± 19	24 ± 16	28	31.6
NM213	170	28	67±64	54 ± 53	164	96.6

Overall remarks, attachments

Remarks on results including tables and figures

The attached study report compares the equivalent circular diameter, as determined by measurement of particle sizes in TEM images, with crystallite and particle sizes determined by other methods.

All methods indicate that the particle sizes are in the same order as sizes provided by the suppliers. Based on the average value from all methods, NM-211 is the smallest (12 nm), NM-212 larger (32 nm) and NM-213 the largest (97 nm). The difference between the measured and supplier-supplied particle size is large for NM-213 (measured 97 nm, compared with supplied value of <50 nm), while the differences are small for NM-211 (12 nm compared with 10 nm) and NM-212 (32 nm compared with 20-25 nm). Of the three samples analysed, NM-211 has the smallest size distribution, with the particles likely to be single crystals.

The sizes of particles determined by different methods were generally in the order of

$$D_{R-XRD} > D_{SA\text{ BET}} > D_{Feret\text{ TEM}} > D_{Circ\text{ TEM}} > D_{S-XRD}$$

D_{S-XRD} was smallest because Scherrer's formula ignores the contributions of width broadening due to strain and instrument effects. If these contributions are non-zero, the crystallite sizes could be larger than those predicted by the Scherrer formula, as revealed by D_{R-XRD} . The real states of particles can be described as singlets, agglomerates or aggregates. Agglomerates are primary particles held by weak Van der Waals force that can be overcome if sufficient energy is provided. Aggregates are primary particles held together by strong chemical bonds that cannot be separated by conventional methods. $D_{Feret\text{ TEM}}$ provides the most reliable assessment of aggregate size, because aggregates are not broken up by ultrasonication during sample preparation whereas agglomerates can be. $D_{Circ\text{ TEM}}$ provides a smaller value than $D_{Feret\text{ TEM}}$ since this method treats all particles as spheres; it is not the most suitable method for analysing samples containing particles with a broad or multimodal size distribution like NM212 and NM213. D_{SABET} was calculated from measurements of specific surface area and assumed that all particles are non-porous and have the same spherical shape and size.

Attached full study report

Attached full study report									
NM_211_ANNEX_A09_OECD CeO2 crystallite size CSIRO Australia.docx / 28.83 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1									

Endpoint study record: 2012-03-04_Australia_NMI_Particle_Size_TEM_Feret

Administrative Data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	V.A. Coleman	2012							

Materials and methods

Type of distribution

other: Transmission Electron Microscope (TEM)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM211, NM-212

Test material form

nanomaterial

Details on test material

State of test material: TEM grid prepared from aqueous suspension

Any other information on materials and methods incl. tables

Transmission electron microscope (TEM) images were generated on a Philips CM120 Biofilter (Eindhoven, NL) TEM at an accelerating voltage of 120 kV. The images were acquired in bright field with an Gatan Imaging Filter (GIF) (1024×1024 pixels) using Gatan DigitalMicrograph software. The samples were fabricated on thin carbon films deposited on a lacey carbon support film on top of a copper mesh grid. The samples were imaged at eucentric height over a range of magnifications, from 6400×–71000×. A representative TEM image of each sample imaged at 71000× is given below. Image J software (v1.42) was used for analysis of the digital TEM images. Outlines of 100 particles were traced manually, and the corresponding areas, and Feret diameters, were determined by the software. The areas were used to determine equivalent circle diameters, i.e. diameters of circles with the same area as the projected particle images. The values are given with their standard deviation (based on 100 measurements).

Instruments: Philips CM120 Biofilter (Eindhoven, NL) TEM at an accelerating voltage of 120 kV

Results and discussions**Particle size**

Percentile	Mean	St. dev.	Remarks
D50	ca. 13 nm	3.2	NM-211, Standard deviation based on 100 measurements
D50	ca. 27.3 nm	13.6	NM-212. Standard deviation based on 100 measurements

Any other information on results incl. tables

	Feret Diameter (nm)	Equivalent Circle Diameter (nm)
NM211	13.0 ± 3.2	10.7 ± 2.6
NM212	27.3 ± 13.6	22.4 ± 10.9
NM213	Not reported	Not reported

NM213 was not suitable for TEM analysis because the size of most of the primary particles was too large for accurate analysis by TEM. It is worth noting, however, that NM213 did contain a non-negligible proportion of particulates with primary size <100nm.

Overall remarks, attachments**Remarks on results including tables and figures**

See attached report for representative image.

Attached full study report

Attached full study report
NM_211_ANNEX_A10_Transmission electron microscope - Primary particle size distribution.doc / 375 KB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

NM211 is size and shape homogeneous with an average Feret diameter of 13.0+/-3.2 nm.
 NM212 has shape and size dispersity, with an average Feret diameter of 27.3+/-13.6 nm.

Endpoint study record: 2012-03-04_Australia_NMI_Particle_Size_TEM_Equiv_Circ_Diam

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	V.A. Coleman	2012							

Materials and methods**Type of distribution**

other: TEM

Test materials**Test material form**

nanomaterial

Details on test material

State of test material: TEM grid prepared from aqueous dispersion

Any other information on materials and methods incl. tables

Transmission electron microscope (TEM) images were generated on a Philips CM120 Biofilter (Eindhoven, NL) TEM at an accelerating voltage of 120 kV. The images were acquired in bright field with an Gatan Imaging Filter (GIF) (1024×1024 pixels) using Gatan DigitalMicrograph software. The samples were fabricated on thin carbon films deposited on a lacey carbon support film on top of a copper mesh grid. The samples were imaged at eucentric height over a range of magnifications, from 6400×–71000×. A representative TEM image of each sample imaged at 71000× is given below. Image J software (v1.42) was used for analysis of the digital TEM images. Outlines of 100 particles were traced manually, and the corresponding areas, and Feret diameters, were determined by the software. The areas were used to determine equivalent circle diameters, i.e. diameters of circles with the same area as the projected particle images. The values are given with their standard deviation (based on 100 measurements).
 Instruments: Philips CM120 Biofilter (Eindhoven, NL) TEM at an accelerating voltage of 120 kV

Results and discussions

Particle size

Percentile	Mean	St. dev.	Remarks
D50	ca. 10.7 nm	2.6	NM-211. Standard deviation based on 100 measurements
D50	ca. 22.4 nm	10.9	NM-212. Standard deviation based on 100 measurements

Any other information on results incl. tables

	Feret Diameter (nm)	Equivalent Circle Diameter (nm)
NM211	13.0 ± 3.2	10.7 ± 2.6
NM212	27.3 ± 13.6	22.4 ± 10.9
NM213	Not reported	Not reported

NM213 was not suitable for TEM analysis because the size of most of the primary particles was too large for accurate analysis by TEM. It is worth noting, however, that NM213 did contain a non-negligible proportion of particulates with primary size <100nm.

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A10_Transmission electron microscope - Primary particle size distribution.doc / 377.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion

Conclusions

NM211 is size and shape homogeneous with an average equivalent circle diameter of 10.7+/-2.6 nm. NM212 has shape and size dispersity, with an average equivalent circle diameter of 22.4+/-10.9 nm.

Endpoint study record: 2010-06-17_NPL for PROSPECT_Particle size, size distribution_TEM

Administrative Data

Study result type	experimental result
Reliability	other:
Rationale for reliability incl. deficiencies	Needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of distribution

other: Transmission Electron Microscopy (TEM)

Principles of method if other than guideline

TEM

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
EC number	215-150-4

Test material form

nanomaterial

Details on test material

State of test material: Dry bulk

Any other information on materials and methods incl. tables

Nanomaterial sample dispersion of 50 mg/L was made in accordance to the OECD recommended protocol for sample dispersion. The dispersion was allowed to settle out in order to remove the larger particles naturally as it sediments by gravity. The purpose of this sedimentation was to remove large micron size particles; TEM sample preparation is crucial and particles up to ~ 200 nm thick is required for acquiring successful transmission images. On day 2, there was clear visible evidence of sedimentation events of the larger micron size particles having taken place. 2 microliter aliquots were extracted from the top layer (an opaque but slightly transparent layer). The sample was placed on TEM grids and allowed to air dry for 10 minutes; grids are formvar/carbon on 400 mesh copper (purchased from Agar Scientific UK). Grids were used as supplied with no further modification. All images were acquired using a Hitachi 2300A instrument operated at 200 kV. The instrument is based at the University of Surrey, Guildford and analysis was carried out by NPL personnel. An adequate magnification was chosen for image acquisition e.g. for the estimation of primary particle mean diameter. The shape and limits of the primary particles should become apparent. TEM micrographs were analysed by manually tracing contours of primary particles on to a transparency sheet. The transparency sheet was scanned for further image analysis using ImageJ software, which automatically calculated particle diameter dimensions. The mean primary particle size was quoted with the corresponding standard deviation; note that the s.d. here represents the broadness of the size distribution (not error).

Instruments: Hitachi 2300A

Results and discussions

Particle size

Percentile	Mean	St. dev.	Remarks
	ca. 10.2 nm	3.9	NM-211. Number of particles: 212
	ca. 18.1 nm	11.3	NM-212. Number of particles: 236
	ca. 81.2 nm	46	NM-213. Number of particles: 14

Any other information on results incl. tables

NM-211: This has an average of ~ 10.2 nm with s.d. of 3.9 nm; no of particles 212.

NM-212: This has an average of ~ 18.1 nm with s.d. of 11.3 nm; no of particles 236.

NM-213: This has an average of ~ 81.2 nm with s.d. of 46.0 nm; no of particles 14.

Overall remarks, attachments**Remarks on results including tables and figures**

NM-211 is Sample G in attached report from NPL

NM-212 is Sample A in attached report from NPL

NM-213 is Sample F in attached report from NPL

Attached full study report

Attached full study report									
NM_211_ANNEX_A11_	TEM	data	from	NPL.doc	/	1.39	MB	(application/octet-stream):	
ENV/JM/MONO/(2015)8/ANN1									

Endpoint study record: 2010-12-03_NPL for PROSPECT_Particle size, size distribution_CPS**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies Preliminary results. Scoop sampled from original bulk powder.

Materials and methods**Type of distribution**

other: Ultracentrifuge

Principles of method if other than guideline

Ultracentrifuge

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

4 sample media tested: DI, Fish Media, Seawater, Daphnia media

Any other information on materials and methods incl. tables

Particle size distribution by centrifugal sedimentation was acquired using CPS Disc Centrifuge Model DC 20000 instrument (Analytik Ltd, UK). At the start of the method, the centrifuge was brought up to speed by partially filling the disc with a sucrose gradient fluid and dodecane cap fluid. The purpose of the

gradient fluid was to stabilise the sedimentation; the purpose of the cap fluid was to maintain the gradient inside the disc. The disc centrifuge was then allowed to equilibrate at 6000 rpm for 1 hour; this gradient will be stable and used within the next 6 hours. 0.2 ml of the nanoparticle sample (50 mg/L) was injected into the disc; a calibration standard was injected after every three samples. Analysis was run against a calibration standard, NIST traceable standard, PVC 0.377 micron. The Disc Centrifuge Control System software (CPS Instruments Inc.) was used to acquire and process the data.

Instruments:

CPS Disc Centrifuge Model DC 20000 instrument (Analytik Ltd, UK)

Calibration:

Analysis was run against a calibration standard, NIST traceable standard, PVC 0.377 micron.

Results and discussions

Any other information on results incl. tables

CPS disc centrifugal sedimentation results: a) equivalent spherical particle diameter as measured by CPS centrifugal sedimentation; the mean and $\pm$ SD of 3 replicates are shown

Sample Name	DI water (nm)	Fish medium (nm)	Seawater (nm)	Daphnia (nm)
NM-212	135 $\pm$ 4	164 $\pm$ 8	187.6 $\pm$ 1.7	146 $\pm$ 5
NM-213	570 $\pm$ 80	530 $\pm$ 30	650 $\pm$ 80	630 $\pm$ 40
NM-211	340 $\pm$ 50	380 $\pm$ 50	520 $\pm$ 90	400 $\pm$ 30

The corresponding D_{10} , D_{50} , D_{90} values (oversize percentiles) from the averaged CPS measurements

Sample Name	DI water (nm)	Fish medium (nm)	Seawater (nm)	Daphnia (nm)
NM-212	D_{10} 185 $\pm$ 2 D_{50} 109 $\pm$ 3 D_{90} 73 $\pm$ 3	D_{10} 226 $\pm$ 9 D_{50} 134 $\pm$ 6 D_{90} 89 $\pm$ 5	D_{10} 521 $\pm$ 3 D_{50} 149.8 $\pm$ 0.7 D_{90} 98.3 $\pm$ 1.5	D_{10} 200 $\pm$ 5 D_{50} 90 $\pm$ 60 D_{90} 81 $\pm$ 2
NM-213	D_{10} 1110 $\pm$ 150 D_{50} 510 $\pm$ 90 D_{90} 158 $\pm$ 12	D_{10} 1060 $\pm$ 60 D_{50} 470 $\pm$ 40 D_{90} 138 $\pm$ 6	D_{10} 1160 $\pm$ 120 D_{50} 590 $\pm$ 70 D_{90} 210 $\pm$ 20	D_{10} 1210 $\pm$ 60 D_{50} 570 $\pm$ 40 D_{90} 163 $\pm$ 13
NM-211	D_{10} 810 $\pm$ 160 D_{50} 202 $\pm$ 17 D_{90} 130 $\pm$ 60	D_{10} 900 $\pm$ 200 D_{50} 231 $\pm$ 17 D_{90} 113 $\pm$ 4	D_{10} 900 $\pm$ 500 D_{50} 400 $\pm$ 110 D_{90} 163 $\pm$ 14	D_{10} 980 $\pm$ 80 D_{50} 230 $\pm$ 20 D_{90} 108 $\pm$ 3

Overall remarks, attachments

Remarks on results including tables and figures

The above tables show the CPS disc centrifugal sedimentation results, with Table a) showing the equivalent spherical mean particle diameter and Table b) the corresponding D_{10} , D_{50} , D_{90} values (oversize percentiles).

D_{10} , D_{50} , D_{90} values are often used to describe the particle size distribution of the sample. If D_{10} = 1225 nm, then this means that that 10 mass % of the particles will have particle diameter of 1225 nm or larger. The results show that the largest mean particle size exists when the NMs are dispersed in seawater; this is reflected on the particle mean size as well as the corresponding D_{90} values. Results also show that the smallest particle size exists when the NMs are dispersed in DI water. This suggests that larger agglomerates exist in the ecotox media, with seawater being the worst i.e. largest agglomerates found in seawater.

Attached full study report

Attached full study report
NM_211_ANNEX_A12_PARTICLE SIZE MEASUREMENTS BY CPS DISC CENTRIFUGE.doc / 58.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1
NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2011-10-03_NPL for PROSPECT_Particle size, size distribution_SMPS**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Materials and methods**Type of distribution**

other: Scanning Mobility Particle Sizing (SMPS)

Principles of method if other than guideline

Scanning Mobility Particle Sizing (SMPS)

Test materials**Test material identity**

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Any other information on materials and methods incl. tables

A NANEUM PA100 was used to produce an aerosol from the dry powder sample. A Scanning Mobility Particle Sizer (TSI 3080 SMPS), consisting of a DMA and CPC system, was used to determine the particle size distribution. The Differential Mobility Analyser (DMA) within the SMPS was calibrated using reference material polystyrene latex beads from NIST. The Condensation Particle Counters (CPC) within the SMPS setup were calibrated according to NPL's UKAS accredited (ISO 17025) procedure, using an internally calibrated Faraday Cup Electrometer and soot generator (model CAST 2). The SMPS was set to record at 4-minute intervals; at least 6 SMPS scans of 200 seconds each were used for analysis. The data was processed using TSI Aerosol Instrument Management (AIM) software, in which the mean size distribution from the stable time segment was estimated. The size distribution was also analysed using an in-house curvefitting program (as implemented in a recent SMPS intercomparison at METAS). Instruments: TSI Scanning Mobilty Particle Analyser (SMPS 3080 with a 3081 LDMA and a 3775 CPC) Stand alone TSI Condensation Particle Counter 3022a (used to correct for any source fluctuations) Calibration: Condensation Particle Counter internally calibrated to UKAS ISO 17025

Results and discussions

Any other information on results incl. tables

Table shows the Geometric Mean particle sizes and the Geometric Standard Deviation of the different aerosolised PROSPECt powders as measured by the SMPS. The SMPS size distributions were corrected for any source drift using the particle number concentration data from a stand alone Condensation Particle Counter. A log-normal curve was fitted to the size distribution data plots to calculate the Geometric Mean and Geometric Standard Deviation

Sample Name	Geometric Mean Particle Size	Geometric Standard Deviation of log-normal
NM-212	359nm	1.66
NM-213	233nm	1.63
NM-211	225nm	1.77

Overall remarks, attachments

Attached full study report

Attached full study report							
NM_211_ANNEX_A13_NPL	SMPS	Data.doc	/	119	KB	(application/octet-stream):	
ENV/JM/MONO/(2015)8/ANN1							
NM_211_ANNEX_A04_FINALINTERIM	REPORT2010corrected.pdf	/	1.05	MB	(application/octet-stream):		
ENV/JM/MONO/(2015)8/ANN1							

Endpoint study record: 2011-10-05_NPL for PROSPECt.Particle size distribution.TEM

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Materials and methods

Type of distribution

other: Transmission Electron Microscopy (TEM)

Principles of method if other than guideline

TEM

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Any other information on materials and methods incl. tables

A small amount of the nanoparticle sample (10 mg) placed into a clean glass container and a 3ml volume of ethanol added. The suspension is mixed by agitating by hand. From the suspension a small (3 ml) droplet is placed on a holey carbon film coated TEM grid and allowed to dry. NOTE: Ethanol is preferred to water as it more volatile and a lower surface energy. This prevents a 'slow' moving liquid/air interace, which can displace weakly attached particles. All images will be acquired using a Hitachi 2300A

instrument operated at 200 kV. The instrument is based at the University of Surrey, Guildford and analysis will be carried out by NPL personnel. An adequate magnification was chosen for image acquisition e.g. for the estimation of primary particle mean diameter. The shape and limits of the primary particles should become apparent. TEM micrographs were analysed by manually tracing contours of primary particles using a tablet PC (Dell XT2). The mean primary particle size was quoted with the corresponding standard deviation.

Instruments: Hitachi 2300A

Calibration: Catalase crystals and latticed spacings

Results and discussions

Any other information on results incl. tables

Sample	Mean Feret Diameter (nm)	Standard Deviation
NM-212	26.9	16.4
NM-213	112.4	74.5
NM-211	10.2	3.6

The TEM results above show that the mean primary particle sizes of the PROSPECt samples range from 10.2 nm to 112.4 nm. Although some similarities in the trend between SEM particle size and TEM particle size information can be observed between the different particle type, in general the TEM results are smaller than results obtained from SEM.

Overall remarks, attachments

Attached background material

Attached document	Remarks
NM_211_ANNEX_A80_010811 TEM data sheet v2.doc / 2.15 MB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN2	

Endpoint study record: 2012-05-16-Particle size, size distribution_DLS_Spain

Administrative Data

Study result type experimental result Study period 14 days
 Reliability other: needs to be evaluated and set by expert researchers group
 Rationale for reliability incl. deficiencies Study in two solvents: Milli Q water and cell culture media (EMEM)

Materials and methods

Type of distribution

other: Dynamic Light Scattering (DLS)

Principles of method if other than guideline

PROSPECt Protocol

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211

Test material form

nanomaterial

Any other information on materials and methods incl. tables

PROSPeCT Protocol has been used for the preparation of aqueous and media suspension of Cerium dioxide nanoparticles in six concentrations. Their aggregation/agglomeration has been measured by DLS, and their stability has been followed up for 14 days and 3 days.

Instruments: Size measurements were performed using dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS ZEN 3600 at 298 K. Samples were irradiated with red light (HeNe laser, wavelength $\lambda = 632.8$ nm), and the intensity fluctuations of scattered light (detected at a back scattering angle of 173°) were analyzed to obtain an autocorrelation function.

Calibration: A reference standard (polystyrene nominal size of 100 nm) was used to quality the performance of instrument

Results and discussions**Any other information on results incl. tables**

Table. Size distribution by Intensity of NM-211 in media.

c (mg/L) in media	0 h		24 h		72 h	
	d (nm)	StdDev	d (nm)	StdDev	d (nm)	StdDev
100	330.47	18.92	312.27	7.15	301.27	8.24
50	358.30	87.60	299.03	7.63	284.93	20.07
25	280.35	10.68	286.25	27.51	338.90	71.42
12.5	261.30	21.07	381.50	160.23	302.83	56.21
6.25	308.60	31.08	430.95	195.94	266.97	80.19

The particle size distribution of Cerium dioxide in water showed, that agglomerates of the particles range between approximately 450 nm to 200 nm with the exception of NM-211 sized particles at concentrations of 100 mg/L and 50 mg/L, to be significantly larger. Measurement of size distribution at lower concentrations proved to be not possible since no repeatable results were acquired. The measurements showed that the size distribution *in media* is comparable to the size distribution in water.

The dispersions appear to be stable in the time of study.

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A79_Particle size distribution Antaria.docx / 337.77 KB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN2	

Endpoint study record: 2012-05-16-Particle size, size distribution_TEM-Spain**Administrative Data**

Study result type experimental result

Materials and methods**Type of distribution**

other: Transmission Electron Microscopy (TEM)

Principles of method if other than guideline

TEM. All samples for transmission electron microscopy were prepared by ultra-sonication (5-10 min). After few drops of a suspension was allowed to evaporate on the copper grid at room temperature

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

State of test material: Dry bulk

Any other information on materials and methods incl. tables

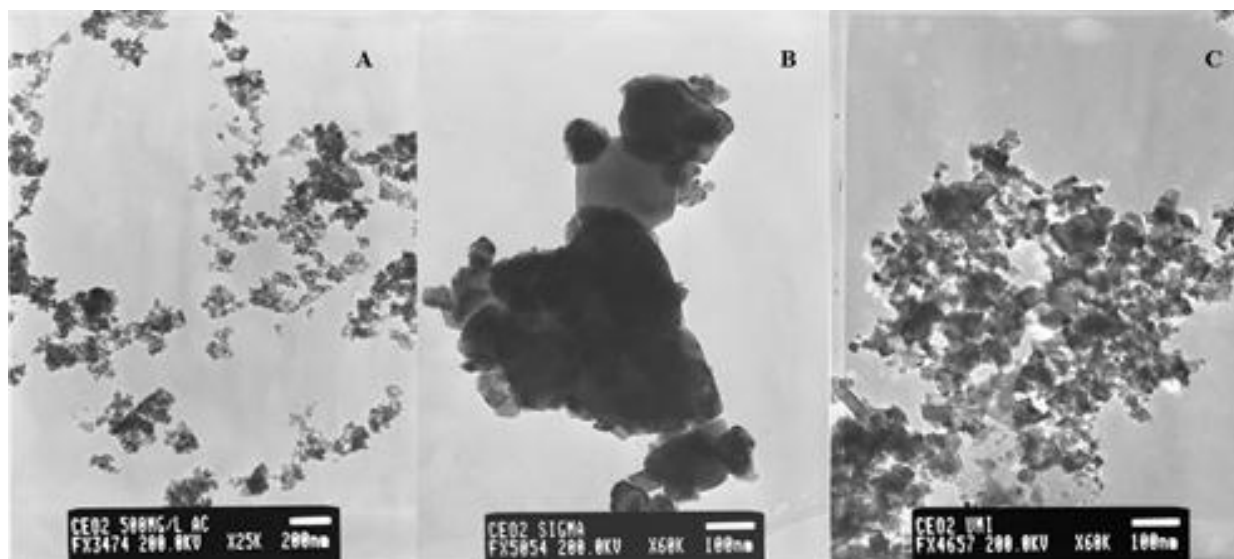
Instruments: The size and morphology of particles were examined under a transmission electron microscope (JEOL, model 2000FX) operating at 200 KV

Results and discussions**Any other information on results incl. tables**

TEM pictures of the three CeO₂ particles in water were taken and aggregates were visible for all three particles in water (Fig. 1). The measurement of the pristine particle size through TEM images showed for the NM 213 particles that these are for the most part in the nano range below 100 nm (mean 81 ± 47 nm, median 65 nm, $75\% \leq 98$ nm), an order of magnitude smaller than expected by the statement of the supplier. The same measurement gave a particle size of 8 ± 2 nm and 16.5 ± 6 nm for the NM 211 and NM 212 particles, respectively, which corresponds to the size stated by the supplier.

Overall remarks, attachments**Attached full study report**

Attached full study report				
NM_211_ANNEX_A81_TEM1.docx	/	1.49	MB	(application/octet-stream):
ENV/JM/MONO/(2015)8/ANN1				

Illustration (picture/graph)**4.6 Vapour pressure****4.7 Partition coefficient****4.8 Water solubility**

Endpoint study record: 2010-10-22_NPL for PROSPECT_Water solubility, hydrophilicity, dispersibility_TURBIDITY

Administrative Data

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Tested with bulk powder

Materials and methods**Type of method**

other: Turbidity and half-live measurements

Principles of method if other than guideline

Turbidity measurements of dispersion stabilities in Di water, Fish medium, seawater and daphnia medium.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

Bulk powder

Any other information on materials and methods incl. tables

Turbidity was measured using HF Scientific – Micro100 RI turbidity meter (Cole-Palmer, UK); this meter has an infrared light source that meets the international standard ISO 7027 for turbidity measurements. The meter was calibrated on standards, which are based on AMCO-AEPA-1 microspheres; these standards are traceable to standard formazin suspension. Standard values of 1000, 10 and 0.02 NTU were used to calibrate the meter. Prior to use, the meter was allowed to warm up for 30 minutes. Sample cuvettes (HF Scientific (USA)) were used to hold the sample. Note that glass thickness may vary from cuvette to cuvette and within the same cuvette. Hence, individual vials were indexed; indexing of the cuvette entails finding the point of the cuvette that light passes through that gives the lowest reading; once indexed the holder can be marked accordingly. Prior to their use, cuvettes were cleaned, in accordance to manufacturer's instructions. This involved washing the interior and exterior of the cuvette with a detergent (2% Hellmanex in DI water); it was then rinsed several times in distilled water before finally rinsing in DI water. The cuvette was further rinsed with the sample two times before filling (30ml) and analysed. The cuvette was placed into the meter and signal allowed to settle before taking readings.

Results and discussions

Any other information on results incl. tables

Table. Dispersion stability of PROSPeCT powders as measured by their corresponding “half lives” (the time it takes for particle concentration to reduce by half, as determined from turbidity values).

Sample Name	DI water (min)	Fish media (min)	Seawater (min)	Daphnia media (min)
NM-212	2676	282	288	252
NM-213	432	348	294	294
NM-211	780	438	534	600

Table shows the corresponding “half-lives” of the PROSPeCT powders when dispersed in the various media. The concept of “half lives” has been put forward in the OECD guidelines on NM testing and this value is an indication of dispersion stability through time i.e. the larger the half life value the longer it takes for the concentration to reduce by half and thus the more stable the dispersion. Results show that overall NMs are most stable when dispersed in DI water and least stable when in an ecotox media. Also, when dispersed in DI water, NM-213 CeO₂ results show that it is the least stable (as reflected by the small half life value) relative to the other PROSPeCT NMs; this dispersion instability is also reflected by the rather small corresponding zeta-potential results. Interestingly, it is apparent that for NM-213 CeO₂, the half-life results (Table) show that dispersion in DI water was more stable compared to fish medium. However, this finding was not consistent with the corresponding zeta-potential results, in which the dispersion was more stable in fish medium (i.e. -22 mV) when compared to DI water (i.e. -7 mV). The reason for this discrepancy lies on the fact that dispersion stability was measured in two different ways i.e. through the measurement of interparticle force (zeta-potential) or through analyzing the stability via sedimentation measurements (turbidity with time). The former measurement will solely be governed by the electric properties of solid surface in contact with liquid which will subsequently contribute towards sedimentation rate; the latter measurement will not only be determined by the zeta-potential value but also by other factors e.g. particle size (in which the larger particles will be expected to sediment faster).

Overall remarks, attachments**Attached full study report****Attached full study report**

NM_211_ANNEX_A03_DISPERSION STABILITY AS DETERMINED BY TURBIDITY MEASUREMENTS.doc / 35.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-02_Australia_CSIRO_Dissolution**Administrative Data**

Study result type experimental result

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Cornelis et al.	2012	Dissolution of NM-211 in artificial soil solutions at varying pH		Adelaide, SA		CSIRO		

Data access

data submitter is data owner

Data protection claimed

yes

Materials and methods**Type of method**

flask method

Principles of method if other than guideline

Dissolution of NM-211 was examined in an artificial soil solution at pH 4, 7 and 9 (see attached dissolution method).

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211

Test material form

nanomaterial

Any other information on materials and methods incl. tables

The dissolution experiments occurred in artificial soil solution (Table 1) of which the ionic composition is typical of that found in Australian soil porewater (McLaughlin et al., 1997). The solution pH was buffered to 4, 7 or 9 throughout the dissolution study (Figure 1). The NM-211 stock solution was added with 20 ml of artificial soil solution (final concentration of $250 \mu\text{g Ce L}^{-1}$) into 50 ml centrifuge tubes. The solution pH was maintained at 4, 7, or 9 using perchloric acid (HClO_4 , 0.1 M) or sodium hydroxide (0.1 M) and buffered using 0.1 mM sodium acetate (NaCH_3COO), 0.1 mM 2-(N-morpholino) ethanesulfonic acid sodium salt (MES), or 0.1 mM sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) solutions, respectively. The solutions were shaken on an end-over-end shaker for 0, 24h and 7d. The dissolution of bulk CeO_2 was examined by weigh ~ 0.01 g of bulk CeO_2 (Aldrich) powder into 50 ml centrifuge tubes and adding 20 ml of artificial soil solution at pH 4, 7 and 9. At individual time periods, 2 ml of solution was removed and added into 1kDa ultra filtration devices (UF) (Pall-Gellman). The UF devices were centrifuged at 4000g for 15 min and total Ce concentrations in filtrates determined using inductively coupled plasma-mass spectrometry (Agilent 7500ce). The dissolution of NM-211 in each pH solution and time period was expressed as a percentage of the total Ce added.

Table. Composition of artificial soil solution used during dissolution experiments.

Component	Concentration (mM)
Ca	6.22
Mg	6.00
K	9.55
Sulfate	6.00
Phosphate	0.02
Nitrate	22.00

Results and discussions

Any other information on results incl. tables

Nanoparticle	Time (day)	pH	Dissolution (%)
NM-211	0	4	< 0.03
	1	4	< 0.03
	7	4	< 0.03
	0	7	< 0.03
	1	7	< 0.03
	7	7	< 0.03
	0	9	< 0.03
	1	9	< 0.03
	7	9	< 0.03
Bulk CeO ₂	0	4	< 0.000008
	1	4	< 0.000008
	7	4	< 0.000008
	0	7	< 0.000008
	1	7	< 0.000008
	7	7	< 0.000008
	0	9	< 0.000008
	1	9	< 0.000008
	7	9	< 0.000008

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A14_NanoHub_NM-211_dissolution_method.docx / 102.02 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1
NM_211_ANNEX_A15_NnanoHub_NM-211_dissolution_data.docx / 11.7 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-02_Australia_CSIRO_Solubility

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Angel et al.	2012	Solubility of NM-211		Sydney, NSW		CSIRO		

Data access

data submitter has Letter of Access

Data protection claimed

yes

Materials and methods**Type of method**

flask method

Principles of method if other than guideline

The dissolution of NM-211 in synthetic softwater medium without EDTA and buffered at pH 6.5 ± 0.1 with 2 mM piperazine-N,N'-bis(ethanesulfonic acid) (PIPES: Sigma-Aldrich) (US EPA, 1994) was determined using the equilibrium dialysis technique described by Rogers et al., (2010). Cole Parmer Spectra/Por 7 dialysis membranes with a molecular weight cut-off of 1000 Dalton (~1 nm nominal pore size) and 45 mm diameter were cut into 9 cm lengths with a Teflon-coated razor blade and washed thoroughly in Milli-Q water. The dialysis cells were filled with 10 mL Milli-Q water and sealed with acid washed (1% v/v HNO₃) plastic dialysis clips. A concentrated suspension of cerium oxide was prepared for each type of nanoparticle by accurately weighing 0.12 g CeO₂ into polycarbonate vials, pipetting 10 mL deionised water into the vial, and sonicating for 30 minutes. The suspension was then shaken vigorously and quantitatively transferred into 3 L of synthetic softwater to achieve 40 mg/L CeO₂ in solution. Triplicate tanks were utilised in dialysis tests so that three replicates were sampled at each time-point. The dialysis cells were added to the test solution and continually stirred under constant light and temperature (24°C) conditions for 72 hours. The total volume of the dialysis cells was kept to below 5% of the test solution in order to minimise dilution effects as dissolved cerium diffused into the dialysis cells. A cell was removed from each triplicate tank at each sampling time and an aliquot of solution was removed from the cells by pipette. A 5 mL volume of the external solution was also withdrawn using a syringe and filtered through a 0.1 µm filter (Pall) at each time point to measure the cerium in this fraction. Total cerium was measured at the start and end of the experiment only. The samples were acidified to 0.5% v/v HNO₃ (Merck tracepur) and the cerium concentration measured by ICP-MS (Agilent 7500ce).

Test materials**Test material identity**

Identifier	Identity
other: OECD Sponsorship Program	NM-211

Test material form

nanomaterial

Overall remarks, attachments**Remarks on results including tables and figures**

Sample ID	Dispersion Medium	pH	Solubility
NM-211	US EPA Medium	6.5	<1 µg/L

Attached background material

Attached document	Remarks
NM_211_ANNEX_A16_Rogers et al 2010.pdf / 395.58 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	

Endpoint study record: 2010-12-09_NPL for PROSPECT_Water solubility**Administrative Data**

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Bulk powder used. Powder not subsampled by spinning riffler.

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Type of method**

flask method

Principles of method if other than guideline

Dissolution to the mass proportion of the NM in colloidal suspension that has lost its particulate character i.e. from a particle form to ionic or molecular form i.e. measuring the ion concentration over a specific period of time. This involved a sample extraction followed by analysis i.e. extraction in the removal of the NM particles and subsequent collection of supernatant using ICP-MS; the series of data below ties in with the calorimetric testing.

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on methods

Dispersion was performed in accordance to the recommended PROSPECT video. Concentrations of 50 mg/L was made up for each sample; a total volume of 1 L was made up and stored in clean media (1 L) bottles at room temperature. After day 2, the bottles were stored in the fridge. Several extractions from the 1 L sample was made over a period of 22 days; prior to extraction, the bottles were gently agitated (this was done by hand) to allow proper mixing to ensure homogeneity. The extracted sample (~ 50 ml) was then subjected to a three-step process in order to remove particles and to extract the resultant supernatant. This step was done immediately after extraction. First step, involved the extraction of aggregates/agglomerates using filtration method, through a Millipore Express PES membrane, 0.1 µm pore size filter (Fisher, UK) under vacuum. In the second step, the resultant filtrant was centrifuged (Centrifuge 5430, Eppendorf, UK) (7500 rpm for one hour). Finally, the extraction of the clear supernatant was carried out by using Peri-Star Pro peristaltic pump (World Precision Instruments, UK); this was done carefully (so as to not disturb the pellet). Less than half of the supernatant was collected.

The resultant supernatant was then stored in the freezer for analysis using ICP-MS; this work was subcontracted and performed by a personnel in LGC (Laboratory Government Chemist, UK). The ICP-MS analysis was carried out using an Agilent 7500ce ICP-MS Octopole Reaction System, operating in standard (no collision cell gas) mode for Cerium (Ce) and Helium mode for Zinc (Zn). The instrument is UKAS accredited and was set up following standard operating procedure (SOP) INS/A1-0013. The samples were defrosted and equilibrated to room temperature, then shaken, to ensure homogeneity. An aliquot of 0.2g – 0.23g was taken from each sample and digested in a CEM Discover microwave, SOP INS/A1-0014, using a mixture of HNO₃/H₂O₂. The digested samples were then diluted to 5g prior to analysis. The samples were digested and analysed over a period of 5 days. Validation was carried out following SOP INS/A1-0015, this includes spiked recoveries and replicate analyses. The limit of detection (LoD) and limit of quantitation (LoQ) are given in the table below (Table 1). The estimated uncertainty at 95% confidence (k=2) is 12% for Zn and 20% for Ce. Results below the LoQ are likely to have a higher uncertainty. The results for all samples in ng/g as received are detailed in the following tables

Any other information on materials and methods incl. tables

Table 1. Limit of detection (LoD) and Limit of quantification (LoQ) for Zn and Ce.

	Ce concentration (ng g ⁻¹)
LoD	0.2
LoQ	0.8

Results and discussions

Details on results

The Ce ion concentration given here is after 14 days. The first 2 days were at room temperature and then the samples were placed in a fridge at 4 degrees.

Any other information on results incl. tables

Table. Data showing the ICP-MS test results for cerium. The ICP-MS measurement was used to evaluate PROSPeCT NMs (extracted supernatant) when dispersed in one of four different media: a) DI water b) fish medium c) daphnia medium d) seawater, over time.

TYPE of media	DAY	Cerium concentration of the supernatant extracted (ng g ⁻¹)		
		NM-212	NM-213	NM-211
DI water	2	2.49	sample missing	1.67
	6	2.9	1.88	1.83
	9	1.94	1.05	2.06
	14	5.08	1.79	1.87
	21	1.59	1.33	2.7
Fish	2	1.09	1.55	1
	6	1.72	2.61	1.75
	9	2.22	3.96	2.37
	14	1.57	1.42	1.9
	21	1.58	1.35	3.1
Daphnia	2	1.91	1.78	sample missing
	6	2.02	1.93	1.68
	9	1.7	1.43	sample missing
	14	1.68	1.92	1.62
	21	1.64	3.45	22.4

Seawater	2	1.92	2.01	1.22
	6	3.95	5.76	4.83
	9	1.88	3.17	4.76
	14	1.97	6.09	5.92
	21	2.19	2.52	9.23

Table. Data showing the values of the corresponding media blanks

Media blanks	Cerium concentration (ng g ⁻¹)
DI water	1.38
Fish medium	1.5
Seawater medium	1.57
Daphnia medium	1.43

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1
NM_211_ANNEX_A18_AdditionalDissolutionDataICP-MS.doc / 106.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

4.9 Solubility in organic solvents / fat solubility

4.10 Surface tension

4.11 Flash point

4.12 Auto flammability

4.13 Flammability

4.14 Explosiveness

4.15 Oxidising properties

4.16 Oxidation reduction potential

Endpoint study record: 2010-12-10_NPL for PROSPECT_Oxidation reduction potential

Administrative Data

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Preliminary work. Method under development.

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Principles of method if other than guideline**

Redox potential were measured using an ORP Oakton® Waterproof ORP Testr®, purchased from Cole Palmer UK; this in effect measures the potential difference across two electrodes i.e. a Pt electrode against a double junction Ag/AgCl reference electrode. The electrode was used in accordance to the manufacturer's instructions. Prior to use the electrode was pre-condition in clean tap water for 30 minutes before rinsing in distilled water. When making measurements, the electrode was carefully placed in a vial containing the sample; there must be sufficient liquid sample to cover the sensing element. The electrode was carefully stirred a little and then placed in a fixed position, slightly above the bottom of the container. The signal output was allowed to settle for 5 minutes before a reading i.e. the "field potential" was noted. After measurement, the electrode was cleaned with tap water and final rinse was with distilled water, after which further measurements can be made. When not in use, the electrode was stored in a solution of Oakton® electrode storage solution, as recommended by the manufacturer.

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Results and discussions**Any other information on results incl. tables**

Table. Redox potential of liquid media blanks only

MEDIA BLANKS ONLY	Redox potential (mV)	pH
DI water	405	N/A
Daphnia	425	7.94
Fish water	418	7.34
Seawater	384	8.75

Table. Redox potential of NM dispersion in various liquid media, the value quoted is relative to the standard hydrogen reference electrode; values quoted in mV.

	NM-212	NM-213	NM-211
DI water	416	422	414
Fish media	439	430	436
Daphnia media	415	429	426
Seawater	384	382	387

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1
NM_211_ANNEX_A19_ Redox Potential from NPL.rtf / 46.56 KB (application/rtf): ENV/JM/MONO/(2015)8/ANN1

4.17 Stability in organic solvents and identity of relevant degradation products

4.18 Storage stability and reactivity towards container material

4.19 Stability: thermal, sunlight, metals

Endpoint study record: 2012-03-02_Australia_CSIRO_Thermal Stability_DTA/TGA

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated & set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Principles of method if other than guideline

Thermal properties of the CeO₂ samples were analysed using a Setaram Evolution Differential Thermal Analysis /Thermogravimetric (DTA/TG) thermal analyser with a carrier gas of air or argon, and using a heating ramp rate of 20°C/min to a maximum temperature of 1100°C. Volatile species were simultaneously analysed by mass spectrometry. species of interest were those with atomic mass units of 2, 12, 16, 17, 18, 28, 44, 48, and 64.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Results and discussions

For thermal stability study

Test substance thermally stable

yes

Thermal degradation products

yes

Identity of thermal degradation products

No.	Identifier	Identity
#1	other:	Carbon dioxide
#2	other:	water

For study on stability to sunlight

Test substance stable to sunlight

not determined

For study on stability to metals

Test substance stable to metals / metal ions

not determined

Any other information on results incl. tables

Percentage change in weights of CeO₂ samples when heated in air or argon, as determined by DTA/TG. A negative number indicates weight loss with increasing temperature.

	NM211	NM212	NM213
Weight change under air	-2.86%	-1.0%	+0.1%
Weight change under argon	-2.0%	-1.0%	-2.7%
Difference in weight change between the two treatments	0.86%	0%	2.8%

Overall remarks, attachments**Remarks on results including tables and figures**

Experimental data (DTA/TGA) are in the attached study report.

Attached full study report

Attached full study report
NM_211_ANNEX_A20_OECD CeO2 thermal stability CSIRO Australia.docx / 302.69 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

NM-211 lost 2.9% weight when heated in air and 2.0% weight when heated in argon. As ceria is hygroscopic and can absorb atmospheric CO₂, it was not surprising that traces of both CO₂ and water were associated with the small weight loss. The temperatures at which these losses occurred appeared consistent with those typically associated with physi- and chemi- desorption.

NM212 lost 1% weight when heated in air and in argon. As ceria is hygroscopic and can absorb atmospheric CO₂, it was not surprising that traces of both CO₂ and water were associated with the small weight loss. The temperatures at which these losses occurred appeared consistent with those typically associated with physi- and chemi- desorption.

NM-213 showed almost no weight change when heated in air (a slight weight gain of 0.1%) but lost 2.7% of its weight in argon. It is unclear why this should be so, given the other CeO₂ samples NM211 and NM212 had consistent weight loss behaviours between treatments. A possible explanation for the different behaviour of NM213 may be related to the presence of a higher level of detectable Zn in NM213 compared with NM211 and NM212 (as determined by ICP-AES and XPS analyses) which may promote additional metal-carbonate complexation to pristine ceria and subsequently produce elevated amounts of desorbed/decomposed species. It has been reported that secondary metallic elements in ceria can promote the formation of small amounts of Ce³⁺. If this were the case, it may partly explain why a slight weight gain is observed for NM213 heated under air (containing oxygen).

4.20 pH**4.21 Dissociation constant****4.22 Viscosity****4.23 Additional physico-chemical information*****Endpoint study record: 2010-07-02_NPL for PROSPECT_Additional physico-chemical information_SEM*****Administrative Data**

Study result type experimental study planned

Reliability other: needs to be evaluated and set by expert researchers group

Materials and methods**Endpoint investigated**

other: Scanning Electron Microscopy (SEM)

Principles of method if other than guideline

For analysis of the “as received” nanoparticle powder, a small scoop of the sample of the nanoparticle powder was sprinkled over a SEM carbon adhesive discs; one side of the carbon disc was placed securely on a metal stub, whilst the other side was exposed to the nanoparticle powder. Excess powder on top of the disc was removed by gently tapping the stub on its side until an even (light) coating of powder on the surface was apparent. Scanning electron microscope SEM images were recorded obtained using a Carl Zeiss Supra 40 field emission scanning electron microscope from Carl Zeiss, in which the optimal spatial resolution of the microscope was a few nanometres. In-Lens detector images were acquired at an accelerating voltage of 15 kV, a working distance of ≈ 3 mm, and a tilt angle 0° .

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Overall remarks, attachments**Remarks on results including tables and figures**

Experimentation needed

Endpoint study record: Interference with Biological Reactive Nitrogen Species (US EPA)

Administrative Data

Study result type experimental result Study period (Completed: Expeced Manuscript in Fall 2012)

Materials and methods**Test materials**

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Any other information on materials and methods incl. tables

Analyzed in novel non-cellular or acellular assay to assess their potential to interfere with biological reactive nitrogen species.

Endpoint study record: 2012-02-29_Australia_CSIRO_Chemical composition_ICP-AES

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint investigated

other: elemental analysis of CeO₂ sample

Principles of method if other than guideline

Experiments to determine the elemental composition of the CeO₂ samples were performed in duplicate. Approximately 0.15g of CeO₂ sample was dissolved in a 1:1 HNO₃: H₂O₂ mixture with heating for 30 minutes. The solution was diluted to 100 mL, internal standard Sc was added, and the resultant solution was analysed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (Varian 730 Axial ICP-AES). Certified multi-element solutions were also used to check the accuracy.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Results and discussions

Any other information on results incl. tables

Elemental analyses of three CeO₂ samples, NM211, NM212 and NM213, as determined by ICP-AES. The data are expressed in units of weight% or ppm

Sample Reference	NM-211	NM-212	NM-213
Ce 446.021*	83.09%	81.62%	84.05%
Al 396.152	0.20%	0.20%	0.22%
Ca 422.673	100 ppm	23 ppm	58 ppm
Co 238.892	<2.5ppm	<2.5ppm	<2.5ppm
Cr 267.716	13 ppm	14 ppm	<1.5ppm
Cu 324.754	20 ppm	10 ppm	15 ppm
Fe 259.940	<5ppm	<5ppm	<5ppm
K 769.897	<15 ppm	<15 ppm	<15 ppm
Mg 285.213	<2ppm	<2ppm	<2ppm
Mn 257.610	6.0 ppm	5.8 ppm	6.0 ppm
Na 589.592	64 ppm	20 ppm	45 ppm
Ni 231.604	<20ppm	<20ppm	<20ppm
P 213.618	<90ppm	<90ppm	<90ppm
Pb 283.305	<40ppm	<40ppm	<40ppm
S 181.972	<200ppm	<200ppm	<200ppm
Si 251.611	35 ppm	20 ppm	16 ppm
Sn 283.998	96 ppm	100 ppm	105 ppm
Ti 336.122	8 ppm	6.2 ppm	6.6 ppm
V 311.070	<0.02ppm	<0.02ppm	<0.02ppm
Zn 213.857	23 ppm	80 ppm	305 ppm
Zr 339.198	25 ppm	25 ppm	26 ppm

*The theoretical Ce weight percentage in CeO₂ is 81.4%.

Overall remarks, attachments**Remarks on results including tables and figures**

Data from the elemental analysis of the sample by ICP-AES are given in the attached study report.

Attached full study report**Attached full study report**

NM_211_ANNEX_A21_OECD CeO₂ chemical composition ICPAES CSIRO Australia.docx / 23.36 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

In general the CeO₂ samples were found to have little or no secondary elements present. NM211 and NM213 had slightly higher levels of detectable alkali metals (Ca, Na) than sample NM212. NM212 and NM213 had higher levels of detectable Zn compared with sample NM211.

Endpoint study record: 2010-11-05_NPL for PROSPECT_Homogeneity_XPS**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Endpoint investigated**

other: homogeneity

Principles of method if other than guideline

XPS measurements were obtained in ultra high vacuum using a Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source, which was operated at 15kV and 5mA emission. Photoelectrons from the top few nanometres of the surface were detected in the normal emission direction over an analysis area of approximately 700 x 300 micrometres. Spectra in the range 1400 to -10 eV binding energy and a step size of 1 eV, using a pass energy of 160 eV were acquired from selected areas of each sample. The peak areas were measured after removal of a Tougaard background. The manufacturer's intensity calibration and commonly employed sensitivity factors were used to determine the concentration of the elements present. High resolution narrow scans of some of the peaks of interest were acquired with a step size of 0.1 eV and 20 eV pass energy. (The manufacturer calibrated the intensity calibration over the energy range). The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales. However, the charge neutraliser was used when acquiring the spectra, which shifted the peaks, by several eV. The C 1s hydrocarbon peak (285 eV binding energy) was used to determine the shift for identifying the peaks.

Samples were prepared using carbon adhesive tape to affix them to 1 cm copper squares. Care was taken to cover the tape with the powders as completely as possible but some samples had better coverage than others and in a lot of cases there was a signal detected from the tape as well as the powder itself. The tape contained oxygen and silicon in addition to carbon.

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Any other information on materials and methods incl. tables

Instruments: Kratos AXIS ultra DLD XPS

Results and discussions

Any other information on results incl. tables

Table. XPS element atomic concentrations results of PROSPECT powders; the powders were spread on to an adhesive carbon tape.

Sample Name	C 1s (%)	Ce 3d (%)	O 1s (%)	Si 2s (%)	Zn 2p _{3/2} (%)
NM-212	75.2	2.0	22.9	0.0	0.0
NM-213	66.7	5.6	27.7	0.0	0.0
NM-211	36.3	20.2	43.5	0.0	0.0

Overall remarks, attachments

Attached background material

Attached document	Remarks
NM_211_ANNEX_A01_XPS DATA.doc / 46 KB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN1	

Endpoint study record: *2010-11-19_NPL* *for*
PROSPECT_Homogeneity_XPS_Ratios

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint investigated

other: Homogeneity

Principles of method if other than guideline

XPS measurements were obtained in ultra high vacuum using a Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source, which was operated at 15kV and 5mA emission. Photoelectrons from the top few nanometres of the surface were detected in the normal emission direction over an analysis area of approximately 700 x 300 micrometres. Spectra in the range 1400 to -10 eV binding energy and a step size of 1 eV, using a pass energy of 160 eV were acquired from selected areas of each sample. The peak areas were measured after removal of a Tougaard background. The manufacturer's intensity calibration and commonly employed sensitivity factors were used to determine the concentration of the elements present. High resolution narrow scans of some of the peaks of interest were acquired with a step size of 0.1 eV and 20 eV pass energy. (The manufacturer calibrated the intensity calibration over the energy range). The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales. However, the charge neutraliser was used when acquiring the spectra, which shifted the peaks, by several eV. The C 1s hydrocarbon peak (285 eV binding energy) was used to determine the shift for identifying the peaks. Samples were prepared using carbon adhesive tape to affix them to 1 cm copper squares. Care was taken to cover the tape with the powders as completely as possible but some samples had better coverage than others and in a lot of cases there was a signal detected from the tape as well as the powder itself. The tape contained oxygen and silicon in addition to carbon.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM_212, NM-213

Test material form

nanomaterial

Results and discussions

Any other information on results incl. tables

Table. XPS results of the cerium-based oxide Nanomaterials and the proportion of Ce⁴⁺: Ce³⁺ in the mixture (atomic%)

Reference Material	Sample Name	Supplier	Batch number	Ce ⁴⁺ [CeO ₂] (%)	Ce ³⁺ [Ce ₂ O ₃] (%)
NM-212	A. Nanograin CeO ₂	Umicore, Belgium	CB250#41#03	93.1	6.9
NM-213	F. Micron CeO ₂	Sigma Aldrich, UK	CrA250#40#03	92.0	8.0
NM-211	G. Ceria dry CeO ₂	Antaria, Australia	CA340#13#ICP	94.3	5.7

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A02_XPS_RATIOS FOR CEO2.doc / 30.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion

Conclusions

The XPS results indicate that all cerium-based oxide nanomaterials consist of a mixture of Ce⁴⁺ and Ce³⁺ species: NM-211, NM-212, NM-213 have similar ratios of Ce⁴⁺: Ce³⁺.

Endpoint study record: 2010-12-05 NPL for PROSPECT Stability monitoring_Turbidity

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Endpoint investigated

other: Turbidity

Principles of method if other than guideline

Turbidity was measured using HF Scientific – Micro100 RI turbidity meter (Cole-Palmer, UK); this meter has an infrared light source that meets the international standard ISO 7027 for turbidity measurements. The meter was calibrated on standards, which are based on AMCO-AEPA-1 microspheres; these standards are traceable to standard formazin suspension. Standard values of 1000, 10 and 0.02 NTU were used to calibrate the meter. Prior to use, the meter was allowed to warm up for 30 minutes. Sample cuvettes (HF Scientific (USA)) were used to hold the sample. Note that glass thickness may vary from cuvette to cuvette and within the same cuvette. Hence, individual vials were indexed; indexing of the cuvette entails finding the point of the cuvette that light passes through that gives the lowest reading; once indexed the holder can be marked accordingly. Prior to their use, cuvettes were cleaned, in accordance to manufacturer's instructions. This involved washing the interior and exterior of the cuvette with a detergent (2% Hellmanex in DI water); it was then rinsed several times in distilled water before finally rinsing in DI water. The cuvette was further rinsed with the sample two times before filling (30ml) and analysed. The cuvette was placed into the meter and signal allowed to settle before taking readings.

Results and discussions

Any other information on results incl. tables

Table. Dispersion stability of PROSPECT powders as measured by their corresponding “half lives” (the time it takes for particle concentration to reduce by half, as determined from turbidity values).

Sample Name	DI water (min)	Fish media (min)	Seawater (min)	Daphnia media (min)
NM-212	2676	282	288	252
NM-213	432	348	294	294
G. Ceria dry CeO ₂	780	438	534	600

Overall remarks, attachments

Attached full study report

Attached full study report

NM_211_ANNEX_A03_DISPERSION STABILITY AS DETERMINED BY TURBIDITY MEASUREMENTS.doc / 35.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

4.24 Agglomeration/aggregation

Endpoint study record: 2010-07-02_NPL for
PROSPECT_Agglomeration/aggregation_SEM

Administrative Data

Study result type

experimental result

Reliability

other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies

Preliminary testing. Scoop sampled from bulk powder sample.

Materials and methods

Method

SEM

Details on methods and data evaluation

For analysis of the “as received” nanoparticle powder, a small scoop of the sample of the nanoparticle powder was sprinkled over a SEM carbon adhesive discs; one side of the carbon disc was placed securely on a metal stub, whilst the other side was exposed to the nanoparticle powder. Excess powder on top of the disc was removed by gently tapping the stub on its side until an even (light) coating of powder on the surface was apparent. Scanning electron microscope SEM images were recorded obtained using a Carl Zeiss Supra 40 field emission scanning electron microscope from Carl Zeiss, in which the optimal spatial resolution of the microscope was a few nanometres. In-Lens detector images were acquired at an accelerating voltage of 15 kV, a working distance of ≈ 3 mm, and a tilt angle 0°.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Overall remarks, attachments**Remarks on results incl. tables and figures**

Experimental work needs to be completed

Endpoint study record: 2012-02-29_Australia_CSIRO_Agglomeration/aggregation_SEM

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies A subjective assessment of the agglomeration/aggregation state was made by viewing SEM images of particles in the sample.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Method**

SEM

Details on methods and data evaluation

A Philips XL30 field emission Scanning Electron Microscope (SEM) was used for this study. The optimal spatial resolution of the microscope was from 2-5 nm with varying accelerating voltage from 30 KV to 1 kV. Images of CeO₂ particles were acquired at an accelerating voltage of 5 kV, a working distance of ≈ 10 mm, and a tilt angle 0°. An SEM metal stub was covered with adhesive conducting tape and a small amount of “as received” CeO₂ powder (around 5 mg) was sprinkled over the tape. The surface of the powder sample was flattened with a spatula. Excess powder was removed by gently tapping the stub on its side until a light coating of powder on the surface became apparent. The nanoparticles were thinly sputtered with iridium using a Polaron SC570 sputter coater. Sputtering was conducted under vacuum while passing gas was argon. The coating deposition time was 20 seconds at a plate current of 50mA, giving a coating thickness of approximately 1 nm.

Data gathering**Instruments**

Philips XL30 field emission Scanning Electron Microscope

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Any other information on materials and methods incl. tables

State of test material: Dry bulk

Overall remarks, attachments**Remarks on results incl. tables and figures**

Representative SEM images of samples NM-211, NM-212 and NM-213, showing their agglomerated states, are provided in the attached study report.

Applicant's summary and conclusion**Conclusions**

SEM images reveal that “as received” CeO₂ particles, NM-211, NM-212 and NM-213, are highly agglomerated in the dry state.

Endpoint study record: 2012-02-29_Australia_CSIRO_Agglomeration/aggregation_DLS

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies DLS is commonly used to determine (agglomerated) particle size but the method is best used for homogeneous samples containing monodispersed particles.

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Method**

DLS

Details on methods and data evaluation

Measurements of hydrodynamic size were obtained using a Brookhaven particle size analyzer 90Plus equipped with a 657 nm laser. Reference standards (Duke polystyrene latex, with a nominal size of 100 nm, and NIST RM8013 Au nanoparticles with a nominal size of 60 nm) were used to assess the performance of the instrument. 10 mg “as received” CeO₂ particles were added to a measuring cuvette containing 3 ml of deionised water. The cuvette was placed in an ultrasonic bath, ultrasonicated for 10 seconds and then shaken to ensure the particles were well dispersed before starting the dynamic light scattering measurements. Each size distribution curve and correlation function curve that was generated was based on 10 measurements. Experiments for each sample were performed in triplicate. The temperature was maintained at 25°C. The cuvette was thoroughly washed with deionised water after each experiment.

Data gathering**Instruments**

Brookhaven particle size analyzer 90Plus equipped with a 657 nm laser

Calibration

Reference standards (Duke polystyrene latex, with a nominal size of 100 nm, and NIST RM8013 Au nanoparticles with a nominal size of 60 nm) were used to assess the performance of the instrument.

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - dispersion

Results and discussions**Agglomerate/Aggregate diameter**

Mean diameter	ca. 349 nm
Remarks	NM-213: mean polydispersity 0.300
Mean diameter	ca. 213 nm
Remarks	NM-212: mean polydispersity 0.255
Mean diameter	ca. 293 nm
Remarks	NM-211: mean polydispersity 0.304

Overall remarks, attachments**Remarks on results incl. tables and figures**

Size distribution curves and correlation function curves for the two reference standards and three CeO₂ samples, NM-211, NM-212 and NM-213, are shown in the attached study report.

Attached full study report

Attached full study report NM_211_ANNEX_A22_OECD CeO₂ agglomeration aggregation DLS CSIRO Australia.docx / 224.62 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1 report

Applicant's summary and conclusion**Conclusions**

Duke polystyrene latex, with a nominal particle size of 100 nm, had an experimentally-measured mean hydrodynamic diameter of 98 nm. NIST RM8013 Au nanoparticles, with a nominal particle size of 60 nm, had an experimentally-measured mean hydrodynamic diameter of 61 nm. The measured mean hydrodynamic diameters (and mean polydispersity) were 293nm (0.304) for NM-211, 213nm (0.255) for NM-212, and 349nm (0.300) for NM-213. These diameters are larger than the primary particle sizes as measured by TEM, indicating that particles in each of the CeO₂ dispersions in water are agglomerated.

4.25 Crystalline phase***Endpoint study record: 2012-02-28_Australia_CSIRO_Crystalline_Phase_XRD*****Administrative Data**

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	P. Casey, H. Yin, M. McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Method**

x-ray diffraction (XRD)

Details on methods and data evaluation

A suitable amount of test sample (~0.5g) was packed in a plastic sample holder and a glass slide was used

to flatten the surface. The crystallite phase was determined using a Bruker ASX-D8 X-Ray Diffractometer (XRD) using Cu K(alpha) radiation. The operation current and voltage was 40mA and 40kV, respectively. The scan ranged from 5 degrees to 85 degrees with a step size of 0.02 degrees and a scan speed of 0.40 second/step. The aperture slit size directing the x-ray source was 0.2mm

Data gathering

Instruments

Bruker ASX-D8 X-Ray Diffractometer

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Results and discussions

Crystalline Phase

Common Name CeO₂

Crystal System cubic (cerionite)

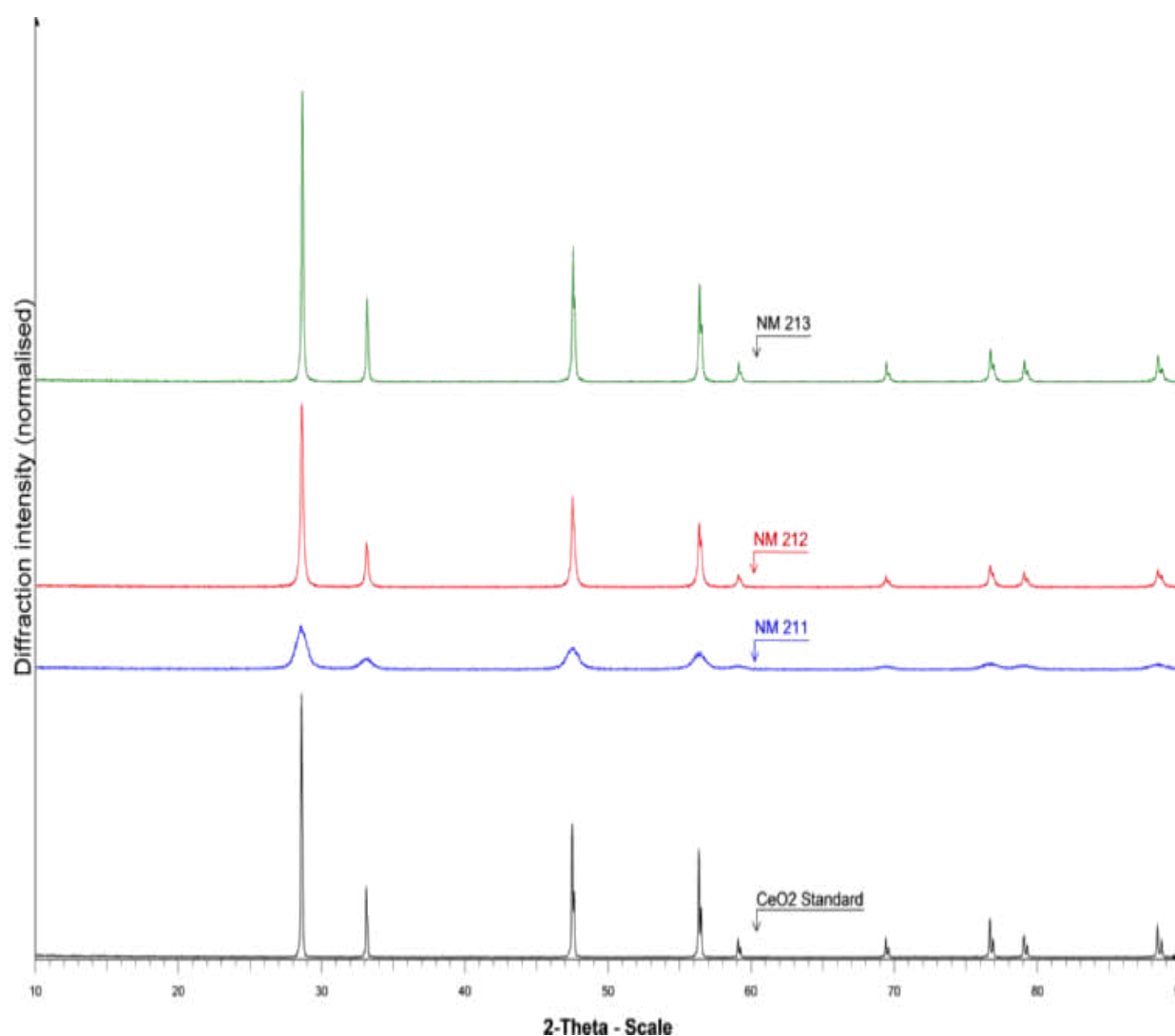
Overall remarks, attachments

Remarks on results including tables and figures

. Figure legend.XRD patterns of NM211, NM212 and NM213 together with the CeO₂cubic cerionite structure reference lines.XRD spectra of NM211, NM212 and NM213, together with that of a CeO₂ standard, are attached.

Attached full study report

Attached full study report NM_211_ANNEX_A23_OECD CeO₂ crystalline phase XRD CSIRO Australia.docx / 122.93 KB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN1

Illustration (picture/graph)**Applicant's summary and conclusion****Conclusions**

The only detectable crystallite phase in samples NM211, NM212 and NM 213 is cubic cerionite.

Endpoint study record: 2012-03-01_Australia_Deakin_crystalline phase_XRD**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Takuya Tsuzuki, Rongliang He	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Method**

x-ray diffraction (XRD)

Principles of method if other than guideline

Dry powder (as-received) was packed in a plastic sample holder. The surface was flattened using a glass slide. Power: 40 kV, 30 mA X-rays: Cu-K-alpha Slit width: 2 00 mm Scan step size: 0.02 degree Scan type: continuous Time per step: 0.5 sec

Sampling

Dry powder (as-received) was packed in a plastic sample holder. The surface was flattened using a glass slide.

Data gathering**Instruments**

Panalytical X'Pert Pro MRD

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Any other information on materials and methods incl. tables

Measurement parameters: Power: 40 kV, 30 mA X-rays: Cu-K-alpha Slit width: 2 00 mm Scan step size: 0.02 degree Scan type: continuous Time per step: 0.5 sec

Results and discussions**Crystalline Phase**

Common Name Cerionite

Crystal System cubic

Remarks on results incl. tables

Crystallite size and strain analysis, used Williamson-Hall Method, with an X'Pert High Score Plus Software

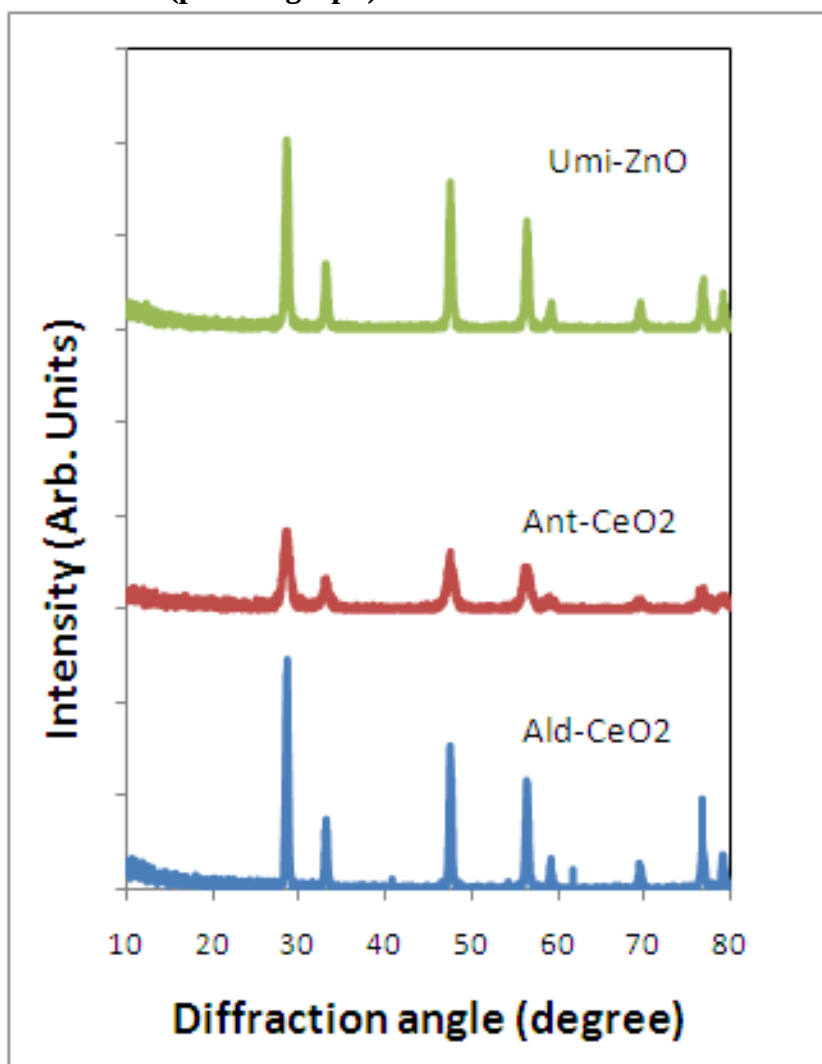
Sample ID	Crystallite size (nm)
NM211	10.2
NM212	18.2
NM213	22.2

Overall remarks, attachments**Remarks on results including tables and figures**

Illustration legend: Diffraction spectra. Cubic cerionite (JCPDS #43 -1002)

Attached full study report

Attached full study report NM_211_ANNEX_A24_OECD CeO2 XRD AUstralia Deakin 2012.pdf / 80.6 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Illustration (picture/graph)

Applicant's summary and conclusion

Conclusions

Only Cerionite phase was detected.

Endpoint study record: 2011-08-11_NPL for PROSPECT_XRD

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Cross-reference to same study

4.26 Crystallite and grain size

Materials and methods

Method

x-ray diffraction (XRD)

Details on methods and data evaluation

X-ray diffraction traces were obtained using a Siemens D5000 diffractometer. This consisted of a theta-theta goniometer and an NPL specimen stage. The X-ray source used for these measurements was the Cu-K α X-ray (40 kV, 30 mA) filtered using a Ni filter that removed the Cu-K β component of the X-ray. The X-ray optics consisted of a 0.6mm anti-scatter slit, a 1mm collimation slit and a 1mm detector slit. The diffraction measurement was conducted using coupled theta-theta drives in standard Bragg-Brentano geometry. The data was collected over a 2-theta range of 5 to 150° using a step size of 0.010° and a count time of 1.5 s/step. The diffracted data was electronically collected and stored on the laboratory PC. Prior to the measurement the X-ray beam was aligned by placing the X-ray source and the detector in line and passing the X-ray beam through a glass slit, the direct beam was attenuated using copper foil placed in front of the detector. Having aligned the two drives and the stage height a standard reference material (corundum) was used to check the alignment over a range of 2-theta values. Having collected the full diffraction trace the data was outsourced to Southampton Chemistry Analytical Solutions (SCAS), for the basic interpretation of the 7 powder diffraction datasets, for the extraction of crystalline phase information. Crystallite size was determined in-house using Scherrer's equation.

The data analyses were performed using the computer program EVA 16.0 (Copyright © Bruker-AXS 1996-2010). The pattern matches were performed using this software linked to the ICDD (International Centre for Diffraction Data) PDF (Powder Diffraction File) database 2005). These database entries are experimentally measured data that have been extracted from the literature.

Data gathering

Instruments

Siemens D5000 diffractometer

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Crystalline Phase**

Common Name	CeO ₂
Crystal System	cubic (Flourite)
Space Group	Fm-3m
Crystallographic planes	a=5.4069(25) Angstrom

Crystallographic composition

Cerium (IV) oxide

Remarks on results incl. tables

Table. Numerical results for the XRD analysis

	Data set	Phase	PDF Card	Ref	Refined unit cell parameters*	I(max) properties	I(max)/Ave bck**
A	NM-212	Cubic CeO ₂	034-0394	1	a = 5.4101(38) Å V = 158.3 Å ³ Fm-3m	Peak (111) Intensity = 3940cps Ave peak background = 241cps FWHM = 0.313°	16.35
F	NM213	Cubic CeO ₂	034-0394	1	a = 5.4108(32) Å V = 158.4 Å ³ Fm-3m	Peak (111) Intensity = 4831cps Ave peak background = 181cps FWHM = 0.208°	26.70
G	NM211	Cubic CeO ₂	034-0394	1	a = 5.4069(25) Å V = 158.1 Å ³ Fm-3m	Peak (111) Intensity = 1567cps Ave peak background = 292cps FWHM = 0.859°	5.37

Table. Crystallite size as determined from the XRD patterns using the Scherrer equation

Sample Name	Crystallite Diameter from XRD / nm
A. NM-212	33.3
F. NM-213	33.3
G. NM-211	10.3

Overall remarks, attachments**Remarks on results including tables and figures**

See attached report

Attached full study report

Attached full study report NM_211_ANNEX_A25_XRD.doc / 1.22 MB (application/octet-stream):
ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

Patterns from the CeO₂ samples show various degrees of line broadening, most likely caused by variations in crystallite size. The crystallite sizes determined from the XRD patterns are around 33.3 nm for NM212 and NM213, NM-211 is much smaller at 10.3nm

4.26 Crystallite and grain size***Endpoint study record: 2010-07-02_NPL for PROSPECT_Crystallite and grain size_XRD*****Administrative Data**

Study result type experimental result
Reliability other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies Preliminary testing using scoop sampled bulk powder

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Method**

x-ray diffraction (XRD)

Data gathering**Instruments**

Siemens D5000 diffractometer, Cu-kalpha radiation (40kV, 30mA)

Calibration

SRM Corundum

Test materials**Test material identity**

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212,NM-213

Test material form

nanomaterial

Results and discussions**Remarks on results including tables and figures**

Scherrer equation used to determine the mean crystallite size

Attached full study report

Attached full study report NM_211_ANNEX_A26_XRD_SIZES.doc / 33.5 KB (application/octet-stream):
ENV/JM/MONO/(2015)8/ANN1

Remarks on results incl. tables

XRD crystallite sizes of PROSPeCT powder; the crystallite sizes were determined using Scherrer's equation.

Sample Name	Crystallite Diameter from XRD/nm
A. NM-212	33.3
F. NM-213	33.3
G. NM-211	10.3

Applicant's summary and conclusion**Conclusions**

It is interesting to compare results of the crystallite size as obtained by X-ray diffraction (XRD) to those of particle size as obtained by SEM imaging. Overall, results show that particle size (as reported from SEM analysis) is much larger than the corresponding reported crystallite size. This is not surprising as a particle (or grain) may be made up of several different crystallites. It is interesting to note however that in the case of Nanograin CeO₂, the crystallite size from XRD (~33 nm) roughly matches the particle size from SEM (~28 nm), which suggests that in this case the grains or particles are composed of single crystals.

Endpoint study record: 2012-03-01_Australia_Deakin_crystallite size_XRD**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Takuya Tsuzuki, Rongliang He	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Method

x-ray diffraction (XRD)

Principles of method if other than guideline

Williamson-Hall Method

Details on methods and data evaluation

Power: 40 kV, 30 mA X-rays: Cu-K-alpha Slit width: 2.00 mm Scan step size: 0.02 degree Scan type: continuous Time per step: 0.5 sec Dry powder (as-received) was packed in a plastic sample holder. The surface was flattened using a glass slide.

Sampling

Dry powder (as-received) was packed in a plastic sample holder. The surface was flattened using a glass slide.

Data gathering

Instruments

Panalytical X'Pert Pro MRD

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Results and discussions

Remarks on results including tables and figures

Crystallite size and strain analysis, used Williamson-Hall Method, with an X'Pert High Score Plus Software.

Sample ID	Crystallite size (NM)
NM211	10.2
NM212	18.2
NM213	22.2

Attached full study report

Attached full study report NM_211_ANNEX_A24_OECD_CeO2_XRD_AUstralia_Deakin_2012.pdf / 80.6 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-02_Australia_CSIRO_Crystallite_Size_XRD_Scherrer

Administrative Data

Study result type experimental result
Reliability other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies Assumptions made on sources of peak broadening in Xray spectrum

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Method

x-ray diffraction (XRD)

Details on methods and data evaluation

A suitable amount of test sample (~0.5g) was packed in a plastic sample holder and a glass slide was used to flatten the surface. Xray data were obtained using a Bruker ASX-D8 X-Ray Diffractometer (XRD) using Cu K(alpha) radiation. The operation current and voltage was 40mA and 40kV, respectively. The scan ranged from 5o to 85o with a step size of 0.02o and a scan speed of 0.40 second/step. The aperture slit size directing the x-ray source was 0.2mm. D S-XRD was calculated using Scherrer's formula. It should be noted that a variety of factors can contribute to the width of a diffraction peak; besides crystallite size, the most important of these usually are inhomogeneous strain and instrumental effects. When Scherrer's formula is applied, it is assumed that all of these other contributions to the peak width are zero.

Data gathering

Instruments

Bruker ASX-D8 X-Ray Diffractometer (XRD) using Cu K(alpha) radiation

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Results and discussions

Remarks on results including tables and figures

Table 2 Comparison of crystallite/particle sizes in samples NM211, NM212 and NM213, as determined from XRD analyses, TEM images and BET surface area measurements

	D_{R-XRD} (nm)	D_{S-XRD} (nm)	$D_{Ferret\ TEM}$ (nm)	$D_{Circ\ TEM}$ (nm)	$D_{SA\ BET}$ (nm)	Average of all methods (nm)
NM211	14	10	12 ± 3	10 ± 3	12	11.6
NM212	57	20	29 ± 19	24 ± 16	28	31.6
NM213	170	28	67 ± 64	54 ± 53	164	96.6

All methods indicate that the particle sizes are in the same order as sizes provided by the suppliers. Based on the average value from all methods, NM-211 is the smallest (12 nm), NM-212 larger (32 nm) and NM-213 the largest (97 nm). The difference between the measured and supplier-supplied particle size is large for NM-213 (measured 97 nm, compared with supplied value of $<5\mu m$), while the differences are small for NM-211 (12 nm compared with 10nm) and NM-212 (32 nm compared with 20-25 nm). Of the three samples analysed, NM-211 has the smallest size distribution, with the particles likely to be single crystals. The sizes of particles determined by different methods were generally in the order of

$$D_{R-XRD} > D_{SA\ BET} > D_{Ferret\ TEM} > D_{Circ\ TEM} > D_{S-XRD}$$

D_{S-XRD} was smallest because Scherrer's formula ignores the contributions of width broadening due to strain and instrument effects. If these contributions are non-zero, the crystallite sizes could be larger than those predicted by the Scherrer formula, as revealed by D_{R-XRD} . The real states of particles can be described as singlets, agglomerates or aggregates. Agglomerates are primary particles held by weak Van der Waals force that can be overcome if sufficient energy is provided. Aggregates are primary particles held together by strong chemical bonds that cannot be separated by conventional methods. $D_{Ferret\ TEM}$ provides the most reliable assessment of aggregate size, because aggregates are not broken up by ultrasonication during sample preparation whereas agglomerates can be. $D_{Circ\ TEM}$ provides a smaller value than $D_{Ferret\ TEM}$ since this method treats all particles as spheres; it is not the most suitable method for analysing samples containing particles with a broad or multimodal size distribution like NM212 and NM213. D_{SABET} was calculated from measurements of specific surface area and assumed that all particles are non-porous and have the same spherical shape and size.

The attached study report compares the crystallite size, as determined by application of Scherrer's formula to XRD data, with crystallite and particle sizes determined by other methods.

Attached full study report

Attached full study report NM_211_ANNEX_A09_OECD CeO2 crystallite size CSIRO Australia.docx /
28.83 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-02_Australia_CSIRO_Crystallite
_Size_XRD_Rietveld

Administrative Data

Study result type

experimental result

Reliability

other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies

Assumptions are made about sources of peak broadening in xray spectrum

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Method

x-ray diffraction (XRD)

Details on methods and data evaluation

A suitable amount of test sample (~0.5g) was packed in a plastic sample holder and a glass slide was used to flatten the surface. Xray data were obtained using a Bruker ASX-D8 X-Ray Diffractometer (XRD) using Cu K(alpha) radiation. The operation current and voltage was 40mA and 40kV, respectively. The scan ranged from 5o to 85o with a step size of 0.02o and a scan speed of 0.40 second/step. The aperture slit size directing the x-ray source was 0.2mm. D R-XRD was calculated using DIFFRACplus TOPAS 4.2 through Rietveld refinement of the diffraction data. Rietveld refinement is a technique that gives the Full Width at Half Maximum (FWHM) of each diffraction peak while taking into account potential broadening contributed from both strain and instrumental effects.

Data gathering

Instruments

Bruker ASX-D8 X-Ray Diffractometer (XRD) using Cu K(alpha) radiation

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Results and discussions

Remarks on results including tables and figures

The attached study report compares the crystallite size, as determined by Rietveld refinement of the XRD data, with crystallite and particle sizes determined by other methods.

Attached full study report

Attached full study report NM_211_ANNEX_A09_OECD CeO2 crystallite size CSIRO Australia.docx / 28.83 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Remarks on results incl. tables

Table 2 Comparison of crystallite/particle sizes in samples NM211, NM212 and NM213, as determined from XRD analyses, TEM images and BET surface area measurements.

	D _{R-XRD} (nm)	D _{S-XRD} (nm)	D _{Feret TEM} (nm)	D _{Circ TEM} (nm)	D _{SA BET} (nm)	Average of all methods (nm)
NM211	14	10	12 ± 3	10 ± 3	12	11.6
NM212	57	20	29 ± 19	24 ± 16	28	31.6
NM213	170	28	67±64	54 ± 53	164	96.6

All methods indicate that the particle sizes are in the same order as sizes provided by the suppliers. Based on the average value from all methods, NM-211 is the smallest (12 nm), NM-212 larger (32 nm) and NM-213 the largest (97 nm). The difference between the measured and supplier-supplied particle size is large for NM-213 (measured 97 nm, compared with supplied value of <5µm), while the differences are small for NM-211 (12 nm compared with 10nm) and NM-212 (32 nm compared with 20-25 nm). Of the three samples analysed, NM-211 has the smallest size distribution, with the particles likely to be single crystals. The sizes of particles determined by different methods were generally in the order of

$D_{R-XRD} > D_{SA\ BET} > D_{Feret\ TEM} > D_{Circ\ TEM} > D_{S-XRD}$

D_{S-XRD} was smallest because Scherrer's formula ignores the contributions of width broadening due to strain and instrument effects. If these contributions are non-zero, the crystallite sizes could be larger than those predicted by the Scherrer formula, as revealed by D_{R-XRD} . The real states of particles can be described as singlets, agglomerates or aggregates. Agglomerates are primary particles held by weak Van der Waals force that can be overcome if sufficient energy is provided. Aggregates are primary particles held together by strong chemical bonds that cannot be separated by conventional methods. $D_{Feret\ TEM}$ provides the most reliable assessment of aggregate size, because aggregates are not broken up by ultrasonication during sample preparation whereas agglomerates can be. $D_{Circ\ TEM}$ provides a smaller value than $D_{Feret\ TEM}$ since this method treats all particles as spheres; it is not the most suitable method for analysing samples containing particles with a broad or multimodal size distribution like NM212 and NM213. D_{SABET} was calculated from measurements of specific surface area and assumed that all particles are non-porous and have the same spherical shape and size.

Endpoint study record: 2010-07-02_NPL for PROSPECT_Crystallite and grain size_XRD

Administrative Data

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Preliminary testing using scoop sampled bulk powder

Materials and methods

Method

x-ray diffraction (XRD)

Details on methods and data evaluation

X-ray diffraction traces were obtained using a Siemens D5000 diffractometer. This consisted of a theta-theta goniometer and an NPL specimen stage. The X-ray source used for these measurements was the Cu-Ka X-ray (40 kV, 30 mA) filtered using a Ni filter that removed the Cu-Kb component of the X-ray. The X-ray optics consisted of a 0.6mm anti scatter slit, a 1mm collimation slit and a 1mm detector slit. The diffraction measurement was conducted using coupled theta-theta drives in standard Bragg-Brentano geometry. The data was collected over a 2-theta range of 5 to 150° using a step size of 0.010° and a count time of 1.5 s/step. The diffracted data was electronically collected and stored on the laboratory PC. Prior to the measurement the X-ray beam was aligned by placing the X-ray source and the detector in line and passing the X-ray beam through a glass slit, the direct beam was attenuated using copper foil placed in front of the detector. Having aligned the two drives and the stage height a standard reference material (corundum) was used to check the alignment over a range of 2-theta values. Having collected the full diffraction trace the Scherrer equation was used to evaluate the crystallite size.

Data gathering

Instruments

Siemens D5000 diffractometer

Calibration

Having aligned the two drives and the stage height a standard reference material (corundum) was used to check the alignment over a range of 2-theta values.

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Any other information on materials and methods incl. tables

Results and discussions

Remarks on results including tables and figures

Scherrer equation size data

Material	Crystallite Diameter (nm)
NM-211	10.3
NM-212	33.3
NM-213	45.5

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream)

Attached full study report NM_211_ANNEX_A28_XRD Preliminary Data from NPL.doc / 503.5 KB (application/octet-stream)

4.27 Specific surface area

Endpoint study record: 2010-06-25_NPL for PROSPECT_specific surface area_BET

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies preliminary study - only scoop sampling was used. A more sensitive balance was used for repeat measurements.

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

BET

Principles of method if other than guideline

BET (Brunauer, Emmett and Teller) Multipoint Specific Surface Area determination

Details on methods and data evaluation

Each sample was degassed overnight at 300°C and under vacuum. An 11-point BET measurement using nitrogen was then taken. This measurement was carried out on two separate aliquots of each powder

Data gathering

Instruments

Autosorb-AS1

Calibration

Manifold calibration using a calibrated quartz rodSSA calibration carried out using BAM CRM BAM-PM-102 and BAM CRM BAM-PM-104

GLP compliance

yes (incl. certificate) (ISO 9277)

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Attached full study report NM_211_ANNEX_A29_NPL BET preliminary data.doc / 641.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Attached full study report NM_211_ANNEX_A30_BET Interim report data.doc / 32.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Remarks on results incl. tables

Material	1 st Aliquot BET SSA (m ² /g)	2 nd Aliquot BET SSA (m ² /g)	Mean BET SSA (m ² /g)
NM-211	65.77±2.15	66.03±2.01	65.9±2.2
NM-212	26.87±0.92	27.49±0.84	27.2±0.9
NM-213	4.25±0.14	4.35±0.14	4.3±0.1

Uncertainty according to GUM (Guidance to the Expression of Uncertainty in Measurement): +/- 4 %

Endpoint study record: 2012-02-29_Australia_CSIRO_Specific_Surface_Area_BET

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies While the specific surface area for the sample was determined by a single experiment, the data agree well with measurements made on the same sample in a different laboratory using a different instrument

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

BET

Details on methods and data evaluation

BET specific surface area was determined using a Micromeritics Tristar II 3020 which uses physical adsorption and capillary condensation principles to obtain information about the surface area and porosity of a solid material. Surface area measurements were acquired with the Micromeritics instrumentation

using an 11-point BET gas adsorption method. Prior to analysis, the powdered sample was transferred to a sample bulb, which was then sealed and de-gassed overnight at 300°C under a high vacuum, and subsequently weighed on a analytical balance in order to determine the sample mass after the degassing step. Then the sample tube containing degassed sample was cooled to 77K (the temperature of liquid nitrogen) and exposed to the analysis gas (nitrogen) at 11 controlled pressures. With each incremental pressure increase, the number of gas molecules adsorbed on the surface increases. The equilibrated pressure (P) is compared to the saturation pressure (P0) and their relative pressure ratio (P/P0) is recorded along with the quantity of gas adsorbed by the sample at each equilibrated pressure.

Data gathering

Instruments

Micromeritics Tristar II 3020

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Overall remarks, attachments

Remarks on results including tables and figures

Table: Specific surface areas of the CeO₂ samples, NM211, NM212, and NM213, obtained by the BET gas adsorption technique, using a Micromeritics instrument.

Sample Name	BET SSA (m ² /g)
NM-211	62.0
NM-212	26.7
NM-213	3.9

The values are consistent with TEM data on particle size.

Results and discussions

Attached full study report

Attached full study report NM_211_ANNEX_A31_OECD CeO₂ specific surface area BET CSIRO Australia.docx / 22.62 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-01_Australia_Deakin_specific_surface_area_BET

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies Only 1 replicate was measured. nonetheless, the data agree with the results from other institutions using different instruments.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Takuya Tsuzuki, Ronglian He	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

BET

Principles of method if other than guideline

Principle: 8-points N₂-gas absorption at 77K

Details on methods and data evaluation

pre-measurement degassing: 150 oC, 1 h under N₂-gas flow Equilibration interval: 10 sec

Data gathering

Instruments

Micromeritics Tristar 3000

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Overall remarks, attachments**Remarks on results including tables and figures**

Table: Results

Sample ID	Sample Mass (g)	Specific Surface Area (m ² /g)	Average pore size (nm)	Pore volume (cm ³ /g)
NM-211	1.0332	62.5 ± 0.3	9.2	0.143325
NM-212	0.5150	23.6 ± 0.3	10.0	0.059087
NM-213	1.4261	3.41 ± 0.05	10.3	0.008833

Note:

Pore volue = total pore volume of pores less than 475.454 Å diameter at P/Po = 0.957701651.

Average pore size was calculated as 4 x (pore volume) /(BET specific surface area) and hence does not necessarily reflect the true state of pore structures.

Results and discussions**Attached full study report**

Attached full study report NM_211_ANNEX_A42_ OECD CeO₂ pore Deakin Australia 2012.pdf / 187.73 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-05_Australia_NMI_Specific_Surface_Area_BET

Administrative Data**Data source****Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	V.A. Coleman	2012							

Materials and methods**Methods**

BET (5-point)

Details on methods and data evaluation

BET Surface Area was determined using a Quantachrome Autosorb IQ system. Surface area measurements were acquired using a 5-point BET gas adsorption method. Prior to analysis, the powdered sample was transferred to a sample bulb, then sealed and subsequently de-gassed overnight at 300°C under a high vacuum. After degassing, the sample was weighed on a analytical balance in order to determine the sample mass. The tube containing the sample was attached to the analysis sector of the instrument, and again evacuated, before being cooled to 77K and exposed to the analysis gas (nitrogen) at 5 controlled pressures. With each incremental pressure increase, the number of gas molecules adsorbed on the surface increases. The equilibrated pressure (P) is compared to the saturation pressure (P₀) and their relative pressure ratio (P/P₀) is recorded along with the quantity of gas adsorbed by the sample at each equilibrated pressure.

Data gathering**Instruments**

Quantachrome Autosorb IQ

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212

Test material form

nanomaterial - powder

Any other information on materials and methods incl. tables

Based on BET surface area, assuming that all the particles have spherical shape and smooth surface and are of the same size, the average equivalent particle size was calculated by

$$D=6000/(\rho \cdot S_w)$$

Where D is the average diameter of a spherical particle; S_w represents the measured surface area of the powder in m^2/g ; and ρ is the particle density. For CeO_2 , ρ is assumed to be 7.215 g/cm^3 .

Overall remarks, attachments**Remarks on results including tables and figures**

Table. Summary

	Surface Area (BET) $m^2 g^{-1}$	Diameter calculated from surface area nm
NM211	67.8	12.3
NM212	28.9	28.8

Endpoint study record: 2011-12-19_NPL for PROSPECT_BET analysis**Administrative Data**

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

BET

Details on methods and data evaluation

Instrumentation A Micromeritics TriStar II (3020) was used for the collection of nitrogen adsorption / desorption isotherm data up to a saturation pressure of approximately 0.995 P/Po. The analysis was

typically conducted to measure 45 adsorption relative pressure points and 23 desorption relative pressure points. Samples were outgassed overnight in vacuo at 300 degC using a Micromeritics VacPrep apparatus prior to analysis. In order to indicate any possible microporous nature of the materials additional relative pressure data were also collected at pressures lower than the usual starting point for analyses using this instrument. These were in the approximate range 0.005 to 0.01 P/Po. Whilst the data reduction methods available are unsuitable for application to the micropore range the characteristic shape of the adsorption isotherm at these low partial pressures would provide a good indication of the presence of micropores in the sample material. The sample tube dead space was measured for each analysis using helium (CP grade) thus providing warm and cold freespace values. Samples requiring only BET surface area analysis were analysed using the same equipment with the application of the same freespace measurement technique. BET surface area was calculated using partial pressures in the nominal range 0.07 to 0.25. Data Reduction. The samples for which the full adsorption/desorption isotherms were analysed graphical plots of the adsorption/desorption isotherm and BET surface area transform plot together with tabulated data for each are presented.

Data gathering

Instruments

Micromeritics TriStar II

Calibration

BAM standard Alumina BAM-pm-104

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Overall remarks, attachments

Remarks on results including tables and figures

Table: Surface Area

Sample	Sample code	Multipoint surface area by Nitrogen (m^2g^{-1})	BET C-value
NM-212	CB250#41#ICP	26.59 ± 0.1467	524
NM-213	CrA250#40	4.10 ± 0.0055	134
NM-211	CA340#13#ICP	66.28 ± 0.0808	113

Glossary:

The BET C-value:

The C-value is part of the characterisation – regardless of the theoretical explanations (relationship to the heat of adsorption, residence time of the adsorbate molecules, etc) the best practical aspect is the relationship to the intercept on the BET plot – the higher the C-value the smaller the intercept. Also, a slight change in the BET slope can lead to a significant change in the intercept (and therefore the C-value) with negligible change in the BET surface area.

Results and discussions

Attached full study report

Attached full study report NM_211_ANNEX_A33_ 010611MCACambridge.doc / 88.5 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-05-16--Specific surface area_BET_Spain

Administrative Data

Study result type experimental result

Materials and methods

Methods

BET

Principles of method if other than guideline

Brunauer-Emmet-Teller surface area measurement and pore size distribution were determined by multipoint nitrogen adsorption using a Beckman Coulter SA3100 device. Prior analysis, samples were vacuum degassed at 120°C for 12 h

Data gathering

Instruments

Beckman Coulter SA3

Results and discussions

Specific Surface Area

Specific Surface Area ca. 69 m²/g

0.5 m²/g

Remarks NM-211

Specific Surface Area ca. 9 m²/g

0.5 m²/g

Remarks NM-213

Specific Surface Area ca. 26 m²/g

0.5 m²/g

Remarks NM-212

Remarks on results incl. tables

Table: Summary

Sample ID	Surface Area (m ² /g)	Standard deviation (m ² /g)
NM-211	69	0.5
NM-212	26	0.5
NM-213	9	0.5

Applicant's summary and conclusion

Conclusions

The surface area of Cerium dioxide measured by BET confirms that NM-211 represents the smallest particle size, followed by NM-212 particles and with NM-213 particles being the largest. The measured values are lower but close to the information provided by the supplier.

4.28 Zeta potential

Endpoint study record: 2010-11-19_NPL for PROSPECT_Zeta potential

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

other: Laser-Doppler-Electrophoresis

Details on methods and data evaluation

Materials were dispersed in four different media; DI water (with or without electrolytes), fish medium, seawater and daphnia medium.

Data gathering

Instruments

Zetasizer Nano ZS (Malvern Instruments, UK) equipped with a 633 nm laser

Calibration

The reference standard (DTS1230, zeta-potential standard from Malvern) was used to qualify the performance of the instrument.

Test materials

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - dispersion

Results and discussions

Remarks on results incl. tables

The mean values of zeta-potential (of six replicates) for different PROSPeCT nanomaterials dispersed in various media at a concentration of 50 mg/L.; *DI water + 5 mM NaCl - this medium was employed to compare with the DI results when in the presence of inert background electrolyte. Values are the mean and ± 1 SD of six replicates.

Nanomaterial Reference	DI water (mV)	DI water + 5mM NaCl* (mV)	Fish medium (mV)	Seawater (mV)	Daphnia medium (mV)
NM-212	33 $\pm$ 2	33.9 $\pm$ 1.7	-11.1 $\pm$ 1.0	N/A	1.2 $\pm$ 0.2
NM-213	-7 $\pm$ 6	-2 $\pm$ 2	-22.3 $\pm$ 0.5	N/A	-15.0 $\pm$ 0.3
NM-211	28 $\pm$ 2	23.0 $\pm$ 1.3	-15.3 $\pm$ 0.6	N/A	-17.4 $\pm$ 0.3

Overall remarks, attachments

Remarks on results including tables and figures

The measured zeta-potential values for the PROSPeCT NMs (50 mg/L) are summarised in the table. Results show that zeta-potential values of NMs when dispersed in seawater cannot be successfully measured (due to high conductivity) and thus displayed as N/A on the table; such unsuccessful measurements were reported in the corresponding “quality report” at the end of the measurement. In general, results indicate high zeta-potential values for NMs that are dispersed either in DI water (or DI water + 5 mM NaCl), and thus confer stability in such media. This is true apart from NM-213 CeO₂ where dispersion in DI resulted in the least stable dispersion. Furthermore, results also show that apart from NM-213 CeO₂, values of zeta-potential measured were lower when the NMs were dispersed in an ecotox media indicating much poorer dispersion stability in such media. Overall, NM-213 CeO₂ has the opposite behaviour i.e. least stable in DI water (and DI water + NaCl) and most stable in fish and daphnia media. Currently, no explanation is available for this behaviour.

Of interest is the apparent charge reversal for NM-212 CeO₂ and NM-211 CeO₂ observed, in going from DI water to fish medium. That is, particles dispersed in DI water exhibit a net positive charge, whereas particles in fish medium generally exhibit a net negative charge, suggesting that one or more of the components in the fish medium are adsorbed on CeO₂ particles causing this effect. This charge reversal is also apparent when NM-211 CeO₂ is dispersed in daphnia medium.

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN1

Attached full study report NM_211_ANNEX_A36_ ZETA-Potential data.doc / 38.5 KB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-01_Australia_CSIRO_zeta_potential**Administrative Data****Study result type** experimental result**Reliability** other: needs to be evaluated & set by expert researchers group**Data source****Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

other: Laser-Doppler-Electrophoresis

Details on methods and data evaluation

Zeta potentials of samples were determined at different pH values (pH=2, 4, 6, 8 and 10) using a Brookhaven particle size analyser 90Plus equipped with a 657 nm laser. 10 mg “as-received” CeO₂ sample was dispersed in a cuvette containing 3 ml DI water, and the pH was adjusted by adding either 0.1M HCl or 0.1M NaOH. The cuvette was placed in an ultrasonic bath for 10 seconds and then shaken manually to ensure good dispersion of particles in the sample. The electrode was inserted into the dispersion and the Zeta potential at each pH was measured 5 times and an average was determined. The temperature of all measurements was maintained at 25 C. The cuvette was thoroughly washed with deionised water after each measurement

Data gathering**Instruments**

Brookhaven particle size analyser 90Plus equipped with a 657 nm laser

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - dispersion

Results and discussions

Remarks on results incl. tables

Table: Zeta potentials for CeO₂ samples dispersed in DI water where the pH was adjusted by adding either 0.1M HCl or 0.1M NaOH.

pH	NM211 Zeta potential mV	std deviation	NM212 Zeta potential mV	std deviation	NM213 Zeta potential mV	std deviation
2.10	34.02	5.75	43.44	1.83	31.94	3.08
4.00	22.92	2.91	25.35	4.95	1.19	2.41
6.20	-17.35	0.98	-27.86	3.09	-34.72	1.51
8.10	-33.1	0.97	-29.63	1.45	-54.88	0.56
10.00	-45.04	0.49	-45.62	1.26	-64.53	1.88

Overall remarks, attachments

Remarks on results including tables and figures

The pH value at which the net surface charge is zero is called the isoelectrical point (IEP). The IEPs of all three CeO₂ samples (5.23, 5.05 and 4.08, for NM211, NM212 and NM213, respectively) are within a pH range of 4-6, which is in general agreement with results reported elsewhere¹. This is consistent with the dissociation of water to H⁺ and OH⁻ on the particle's surface and, where there is no surface coating, the IEP will be due solely to this dissociation. This therefore suggests that there is no specific surface coating on these CeO₂ particles. It is unclear why the IEP of NM213 is slightly lower, but it may be related to the higher observed concentrations of carbon on the surface of NM213, compared with NM211 and NM212, as determined by XPS analyses of these samples.

Attached full study report

Attached full study report NM_211_ANNEX_A37_OECD CeO₂ surface chemistry XPS CSIRO Australia.docx / 23.44 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion

Conclusions

The IEPs of all three CeO₂ samples (5.23, 5.05 and 4.08, for NM211, NM212 and NM213, respectively) are within a pH range of 4-6, which is in general agreement with results reported elsewhere. This is consistent with the dissociation of water to H⁺ and OH⁻ on the particle's surface and, where there is no surface coating, the IEP will be due solely to this dissociation. This therefore suggests that there is no specific surface coating on these CeO₂ particles.

Endpoint study record: 2012-05-16-Zeta potential_Spain

Administrative Data

Study result type	experimental result	Study period	14 days
Reliability	other: needs to be evaluated and set by expert researchers group		
Rationale for reliability incl. deficiencies	solvent: Milli-Q water		

Materials and methods

Methods

other: Laser-Doppler-Electrophoresis

Principles of method if other than guideline

PROSPECT Protocol

Data gathering**Instruments**

Dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS ZEN 3600 at 298 K.

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - dispersion

Applicant's summary and conclusion**Conclusions**

The zeta potential, measured in water, showed for the NM-213 and NM-211 particles a negative zeta potential across all concentrations tested. NM-212 particles showed a positive zeta potential at the two highest concentrations tested (100 mg/L and 50 mg/L) and a negative zeta potential at lower concentrations. The particles showed stable aggregates at the highest concentration tested. A measurement of the zeta potential of NM-213 particles at a very low concentration proved to be impossible.

4.29 Surface chemistry

Endpoint study record: 2010-07-02_NPL for PROSPECT_Surface chemistry_XPS

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies Preliminary testing carried out on scoop sampled bulk powder

Materials and methods**Methods**

XPS

Details on methods and data evaluation

XPS measurements were obtained in ultra high vacuum using a Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source, which was operated at 15kV and 5mA emission. Photoelectrons from the top few nanometres of the surface were detected in the normal emission direction over an analysis area of approximately 700 x 300 micrometres. Spectra in the range

1400 to –10 eV binding energy and a step size of 1 eV, using a pass energy of 160 eV were acquired from selected areas of each sample. The peak areas were measured after removal of a Tougaard background. The manufacturer's intensity calibration and commonly employed sensitivity factors were used to determine the concentration of the elements present. High resolution narrow scans of some of the peaks of interest were acquired with a step size of 0.1 eV and 20 eV pass energy. (The manufacturer calibrated the intensity calibration over the energy range). The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales. However, the charge neutraliser was used when acquiring the spectra, which shifted the peaks, by several eV. The C 1s hydrocarbon peak (285 eV binding energy) was used to determine the shift for identifying the peaks. Samples were prepared using carbon adhesive tape to affix them to 1 cm copper squares. Care was taken to cover the tape with the powders as completely as possible but some samples had better coverage than others and in a lot of cases there was a signal detected from the tape as well as the powder itself. The tape contained oxygen and silicon in addition to carbon.

Data gathering

Instruments

Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source

Calibration

The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales

GLP compliance

yes (ISO 15472)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM_212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Functionalisation

Remarks on results incl. tables

Table: XPS element atomic concentrations results of PROSPeCT powders; the powders were spread on to an adhesive carbon tape.

Sample Name	C 1s (%)	Ce 3d (%)	O 1s (%)	Si 2s (%)	Zn 2p _{3/2} (%)
NM-212	75.2	2.0	22.9	0.0	0.0
NM-213	66.7	5.6	27.7	0.0	0.0
NM-211	36.3	20.2	43.5	0.0	0.0

Overall remarks, attachments

Remarks on results including tables and figures

The elemental composition of the different PROSPeCT powders as measured by XPS is summarised in table, where the elemental concentrations of the elements: carbon (C), cerium (Ce), oxygen (O), silicon

(Si) and zinc (Zn) are shown. As expected, XPS can successfully differentiate between vials containing cerium and vials containing zinc; and it was observed that no cross contamination had occurred between the two types of batches within the detection limit of XPS. As evident from the results, there was a significant contribution of carbon and this can be largely attributed to contamination on the particles. Areas of best coverage were selected for analysis and, using XPS analysis of the carbon tape alone which showed a composition of 74% C, 21% O and 5% Si. From the lack of any significant signal from Si on samples, it was estimated that there was better than 90% coverage within these analysis areas. A different sample preparation procedure could be adopted to separate background carbon signal from that on the particles during XPS measurements.

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Attached full study report NM_211_ANNEX_A38_XPS element concentrations.doc / 38 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2012-03-01_Australia_CSIRO_Surface_Chemistry_XPS

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies Contaminants can be detected by XPS, affecting analysis of data

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

XPS

Principles of method if other than guideline

X-ray photoelectron spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition near the surface of a sample, and the chemical and electronic states of those elements. Spectra are obtained by irradiating the sample with an X-ray beam while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1-10 nm layer of the material being analysed. XPS requires ultra high vacuum (UHV) conditions.

Details on methods and data evaluation

Individual wells of a powder sample holder were filled with “as received” CeO₂ samples (1 well per sample). Samples were analysed using a Kratos HS spectrometer fitted with a monochromated Al K(alpha) source, under standard conditions. The sampling depth was several nm (~10 nm maximum) and the analysis area was ~ 0.3 mm × 0.7 mm. Samples were analysed at 2 different times, approximately 2 months apart.

Data gathering

Instruments

Kratos HS spectrometer fitted with a monochromated Al K(alpha) source

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Functionalisation

Remarks on results incl. tables

Table: Averaged chemical composition of the surface of CeO₂ samples, as determined by XPS measurements taken 2 months apart. Data are given as % atomic concentrations.

	NM 211	NM 212	NM 213
Ce	26.13 ± 1.87	25.73± 0.44	22.17± 0.07
Zn			0.92 ± 0.1
O	42.92 ± 0.32	42.14 ± 0.43	39.21± 1.58
C	30.94 ± 2.2	31.87± 0.93	37.3 ± 1.42

Overall remarks, attachments

Remarks on results including tables and figures

Table presents the average of two data sets obtained 2 months apart. In all samples, cerium, oxygen and carbon were detected. Carbon is unlikely to be an internal component of the CeO₂ samples; rather its presence is likely due to surface contamination or carbon-containing species adsorbed on the surface. NM213 contains more carbon than NM211 and NM212. Zinc is present in sample NM213 but not NM211 or NM212. This latter observation is consistent with data obtained by ICP-AES which showed that NM213 had significantly higher levels of detectable Zn compared with NM211 and NM212. All samples showed good elemental stability over the testing period with consistent levels of Ce, O and C being detected 2 months apart.

Attached full study report

Attached full study report NM_211_ANNEX_A37_OECD CeO₂ surface chemistry XPS CSIRO Australia.docx / 23.44 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion

Conclusions

Cerium, oxygen and carbon were detected in NM211. Carbon is unlikely to be an internal component of the CeO₂ samples; rather its presence is likely due to surface contamination or carbon-containing species adsorbed on the surface. Samples showed good elemental stability over the testing period with consistent levels of Ce, O and C being detected 2 months apart.

Endpoint study record: 2010-07-02_NPL for PROSPECT_Surface chemistry_XPS_Ce species

Administrative Data

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Preliminary testing carried out on scoop sampled bulk powder

Materials and methods

Methods

XPS

Details on methods and data evaluation

XPS measurements were obtained in ultra high vacuum using a Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source, which was operated at 15kV and 5mA emission. Photoelectrons from the top few nanometres of the surface were detected in the normal emission direction over an analysis area of approximately 700 x 300 micrometres. Spectra in the range 1400 to -10 eV binding energy and a step size of 1 eV, using a pass energy of 160 eV were acquired from selected areas of each sample. The peak areas were measured after removal of a Tougaard background. The manufacturer's intensity calibration and commonly employed sensitivity factors were used to determine the concentration of the elements present. High resolution narrow scans of some of the peaks of interest were acquired with a step size of 0.1 eV and 20 eV pass energy. (The manufacturer calibrated the intensity calibration over the energy range). The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales. However, the charge neutraliser was used when acquiring the spectra, which shifted the peaks, by several eV. The C 1s hydrocarbon peak (285 eV binding energy) was used to determine the shift for identifying the peaks. Samples were prepared using carbon adhesive tape to affix them to 1 cm copper squares. Care was taken to cover the tape with the powders as completely as possible but some samples had better coverage than others and in a lot of cases there was a signal detected from the tape as well as the powder itself. The tape contained oxygen and silicon in addition to carbon.

Data gathering

Instruments

Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source

Calibration

The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales

GLP compliance

yes (ISO 15472)

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Functionalisation****Remarks on results incl. tables**

Table: XPS results of the cerium-based oxide Nanomaterials and the proportion of Ce^{4+} : Ce^{3+} in the mixture.

Sample Name	Ce^{4+} [CeO_2] (%)	Ce^{3+} [Ce_2O_3] (%)
NM-212	93.1	6.9
NM-213	92.0	8.0
NM-211	94.3	5.7

Overall remarks, attachments**Remarks on results including tables and figures**

The values were obtained using a “ten peak fit” method as previously detailed by Zhang and co-workers [5]. In summary this method involved peak fitting the relevant narrow scan spectra and subsequently attributing peaks to Ce^{4+} or Ce^{3+} valence states.

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Attached full study report NM_211_ANNEX_A39_XPS Ce species determination.doc / 33 KB (application/octet-stream):
ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

The XPS results indicate that all cerium-based oxide nanomaterials consist of a mixture of Ce^{4+} and Ce^{3+} species; Nanograin, Micron and Ceria dry have similar ratios of Ce^{4+} : Ce^{3+} .

Endpoint study record: 2011-12-14_NPL for PROSPECT_ToF SIMS

Administrative Data

Data source

Data access

data submitter is data owner

Materials and methods

Methods

other: SIMS ToF SIMS

Principles of method if other than guideline

The ToF-SIMS instrument utilized for this work is an IONTOF (Germany) ToF-SIMSIV. ToF-SIMS analysis was performed with a Bi⁺ cluster primary beam (incident at 45° from the sample normal with an energy of 25 keV) to obtain high mass resolution mass spectra with an imaging resolution of approximately 5 µm. The ion beam is rastered over an area of 500 µm × 500 µm using a 256 × 256 pixel raster. The ion beam current was measured before and after each sample was analysed. The raw data that was recorded consisted of a ToF-SIMS mass spectrum at every pixel. Data analysis was carried out retrospectively from the raw data using ION-TOF SurfaceLab 6.1 software

Sampling

The approach to sample preparation that proved to be the most successful is described below: - 1 cm × 1 cm Si wafer were first cleaned by rinsing with ethanol and acetone and blow drying with N₂. - Double sided conductive carbon tape (Agar Scientific, UK) was cut to size and attached to the Si wafer. - Inside a glove box, excess nanoparticle powder was sprinkled onto the surface of the carbon tape to maximize surface coverage (typically about 170 mg of nanoparticle powder was used). The sample with nanoparticles was then covered with aluminium foil, which was pressed firmly to ensure strong adhesion of the nanoparticles to the tape surface. Loosely surface bound nanoparticles were removed by blowing with an N₂ jet.

Data gathering

Instruments

IONTOF

Calibration

The instrument is periodically checked for repeatability and constancy of the relative intensity scale using ISO 23830:2008. The mass scale is calibrated following ISO/DIS 13084. The spatial scales are calibrated using metal grids with spacings of 25 µm and 125 µm.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial - powder

Results and discussions

Functionalisation

Remarks on results incl. tables

The spectrum of each nanopowder sample was compared against that of the carbon tape. In addition to the peaks characteristic for the carbon tape, the nanopowder samples exhibited peaks that were related to the elemental composition of the nanopowders.

The CeO₂ nanopowder samples appeared relatively similar to each other and were clean, as no peak was detected except for those associated to Ce and CeO and their isotopes with the small addition of the elements C and H, which may arise from trace levels of hydrocarbon contamination from either sample handling, storage or from the adhesive on the carbon tape. Unfortunately, due to the presence of the carbon tape signal (resulting in many strong organic peaks containing the elements C, H and Si), it is not possible to draw a conclusion regarding the identity and quantity of the contamination on the nanoparticle surface.

Overall remarks, attachments

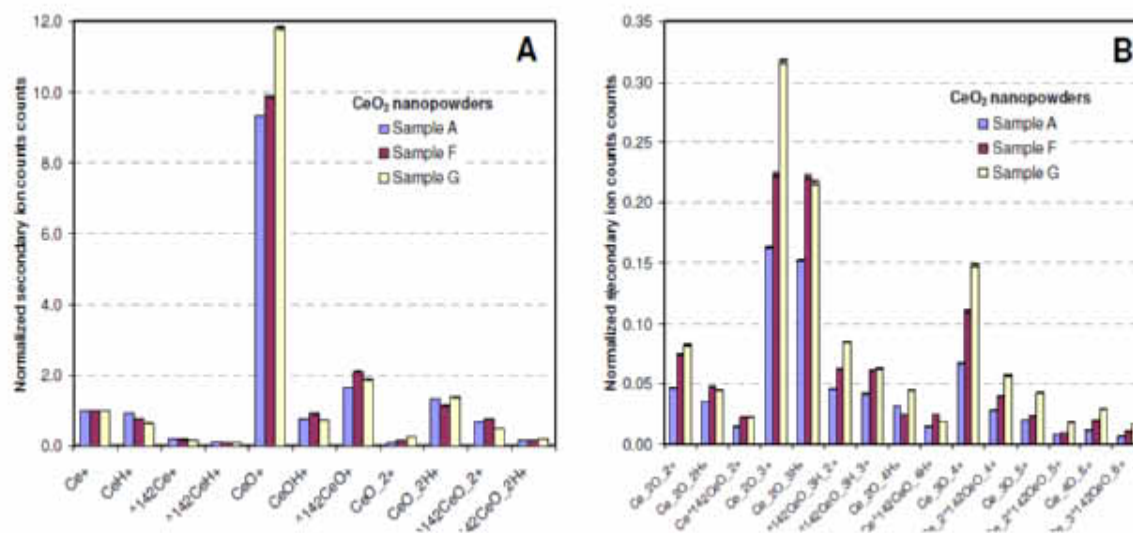
Remarks on results including tables and figures

Figure legend: Normalized secondary ion emission of (A and B) CeO₂ nanopowders. The emission is normalized to the Ce⁺ peak. Bi⁺ beam at 25kV.

Attached full study report

Attached full study report NM_211_ANNEX_A40_Minelli_Characterization of PROSPEC samples by ToF-SIMS.pdf / 265.25 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Illustration (picture/graph)



Applicant's summary and conclusion

Conclusions

The evaluation of sample purity and type of contaminations by ToF-SIMS is challenging, due to the restrictions imposed by sample mounting requirements. The use of the carbon tape limits the extent of the analysis that can be performed on signals related to C and Si elements. A more ideal mounting strategy is that adopted for XPS measurements, where the analysis is performed on a thick nanopowder pellet and therefore 100% of the signal is collected from the nanomaterials. Unfortunately, for reasons that were explained, this approach is unsuitable for ToFSIMS at present. In view of these considerations, we

conclude that XPS technique provides a more informative and quantitative chemical analysis of the PROSPeCT nanopowders.

Endpoint study record: 2011-12-014_NPL for PROSPECT_Surface chemistry_XPS

Administrative Data

Study result type experimental result
Reliability other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies Repeat of data using compressed pellets

Materials and methods

Methods

XPS

Details on methods and data evaluation

XPS measurements were obtained in ultra high vacuum using a Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source, which was operated at 15kV and 5mA emission. Photoelectrons from the top few nanometres of the surface were detected in the normal emission direction over an analysis area of approximately 700 x 300 micrometres. Spectra in the range 1400 to -10 eV binding energy and a step size of 1 eV, using a pass energy of 160 eV were acquired from selected areas of each sample. The peak areas were measured after removal of a Tougaard background. The manufacturer's intensity calibration and commonly employed sensitivity factors were used to determine the concentration of the elements present. High resolution narrow scans of some of the peaks of interest were acquired with a step size of 0.1 eV and 20 eV pass energy. (The manufacturer calibrated the intensity calibration over the energy range). The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales. However, the charge neutraliser was used when acquiring the spectra, which shifted the peaks, by several eV. The C 1s hydrocarbon peak (285 eV binding energy) was used to determine the shift for identifying the peaks. Samples were prepared by compression of the powder to form pellets which were analysed directly.

Data gathering

Instruments

Kratos AXIS Ultra DLD (Kratos Analytical, UK) instrument fitted with a monochromated Al K α source

Calibration

The energy scale was calibrated according to ISO 15472 Surface chemical analysis – X-ray photoelectron spectrometers – Calibration of energy scales

GLP compliance

yes (ISO 15472)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Functionalisation****Remarks on results incl. tables**

Atomic composition

Sample ID	C (%)	Ce (%)	O (%)	Si (%)	Zn (%)
NM-211	35.6	22.4	41.9		
NM-212	33.3	23.8	42.8		
NM-213	35.3	22	42.4		0.2

4.30 Dustiness***Endpoint study record: 2011-25-11_NPL for PROSPECT_Dustiness*****Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated & set by expert researchers group

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

rotating cylinder

Details on methods and data evaluation

The dustiness of the sample powder will be carried out using the rotating drum method specified in the new European standard of the dustiness of bulk samples (EN15051). This device uses two porous size-selective foam stages and a filter to provide information about the dustiness of the powders in terms of the inhalable, thoracic and respirable fractions of the dust dispersed. Three replicate tests of the powder will be carried out to obtain an estimate of the precision of the measurements. The standard also requires simple moisture content measurements to be made for each material, as dustiness has been found to be a function of moisture content. Analysis is carried out at 50±5% relative humidity. For each measurement 35 ml will be tested

Data gathering**GLP compliance**

yes (EN15051)

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Remarks on results incl. tables**

Table: Mean and SD of the dustiness results (in terms of the inhalable, thoracic and respirable fractions of dust dispersed) and moisture content of the PROSPEcT sample powders.

Sample Name	Inhalable fraction (mg kg ⁻¹)		Thoracic fraction(mg kg ⁻¹)		Respirable fraction(mg kg ⁻¹)		Moisture content (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NM-212	2845	244	668	59	66	26	0.4	0.10
NM-213	278	9	80	9	22	10	0.2	0.05
NM-211	2222	202	800	11	86	15	1.1	0.10

Table: The calculated coefficient of variation (COV) values of the results in Table1. The coefficient of variation is the ratio of the standard deviation to the mean. It is a measure of variability in relation to the mean and is used to compare the relative dispersion in one type of data with the relative dispersion in another type of data. The data to be compared may be in the same units, in different units, with the same mean, or with different means.*

Sample Name	Inhalable fraction (mg kg ⁻¹)	Thoracic fraction(mg kg ⁻¹)	Respirable fraction(mg kg ⁻¹)	Moisture content (%)
	COV (%)	COV (%)	COV (%)	COV (%)
NM-212	8.6	8.8	39.6	23
NM-213	3.2	10.9	43.7	23
NM_211	9.1	1.4	16.9	10

*Reference: “Measures of Dispersion – Coefficient of Variation” - <http://jimwright.org/WebEd/u02/we020304.htm>

Table: The dustiness classifications of the sample powders, ranging from Very Low to High.

Sample	Dustiness Classification		
	Inhalable	Thoracic	Respirable
NM-212	Moderate	Moderate	Moderate
NM-213	Low	Low	Low
NM-211	Moderate	Moderate	Moderate

Overall remarks, attachments**Attached full study report**

Attached full study report NM_211_ANNEX_A41_010611HSL_results_only.doc / 68 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

4.31 Porosity***Endpoint study record: 2012-03-02_Australia_CSIRO_porosity_BJH*****Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies suitability of BJH method for analysing adsorption/desorption isotherms of samples consisting of very small nanoparticles?

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, Yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

BET

Details on methods and data evaluation

Porosity was determined simultaneously with surface area using a Micromeritics Tristar II 3020 instrument. The Barrett-Joyner-Halenda (BJH) method for analysing gas adsorption and desorption isotherms was used to determine pore area, specific pore volume and pore size distribution independent of the external area due to the particle size of the sample. The t-plot method is commonly used to determine the external surface area, pore volume and pore surface area in microporous solids.

Data gathering**Instruments**

Micromeritics Tristar II 3020

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Remarks on results incl. tables**

Table: Porosity of the CeO₂ samples, NM211, NM212 and NM213, as obtained by the BET gas adsorption technique.

Sample ID	T-plot External Surface Area (m ² /g)	T-plot Micropore Surface Area (m ² /g)	T-plot Micropore volume (cm ³ /g)	BJH desorption average pore width (4V/A):Å
NM-211	58.1	3.9	0.0015	102.019
NM-212	27.1	0	0	121.736
NM-213	3.37	0.51	0.00022	184.302

Overall remarks, attachments**Remarks on results including tables and figures**

The table presents the external surface area, micropore surface area and volume, and a determination of the average pore width for all CeO₂ samples. All samples have very low or no microporosity. The major contribution to total surface area is from external surfaces and is thus predominantly determined by particle size and shape rather than high internal porosity.

Attached full study report

Attached full study report NM_211_ANNEX_A44_OECD CeO₂ porosity CSIRO Australia.docx / 23.26 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Applicant's summary and conclusion**Conclusions**

NM211 has very low microporosity, but higher than NM212 and NM213. The major contribution to total surface area is from external surfaces and is thus predominantly determined by particle size and shape rather than high internal porosity.

Endpoint study record: 2012-03-01_Australia_Deakin_pore size_BJH**Administrative Data**

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies limited number of replicates

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	T Tsuzuki, R He	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

BET (BJH)

Principles of method if other than guideline

N₂ gas adsorption

Details on methods and data evaluation

Degassing at 150 C for 1 h

Data gathering**Instruments**

Tristar 3000

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Remarks on results incl. tables

Table: Summary of results

Sample ID	Sample Mass (g)	Specific Surface Area (m ² /g)	Average Pore Size (nm)	Pore Volume (cm ³ /g)
NM-211	1.0332	62.5 ± 0.3	9.2	0.143325
NM-212	0.5150	23.6 ± 0.3	10.0	0.059087
NM-213	1.4261	3.41 ± 0.05	10.3	0.008833

Note:

Pore volue = total pore volume of pores less than 475.454 Å diameter at P/Po = 0.957701651.

Average pore size was calculated as 4 x (pore volume) /(BET specific surface area) and hence does not necessarily reflect the true state of pore structures.

Overall remarks, attachments

Attached full study report

Attached full study report NM_211_ANNEX_A42_ OECD CeO₂ pore Deakin Australia 2012.pdf / 187.73 KB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

Endpoint study record: 2011-12-19_NPL for PROSPECT_Porosity

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

BET

Details on methods and data evaluation

A Micromeritics TriStar II (3020) was used for the collection of nitrogen adsorption / desorption isotherm data up to a saturation pressure of approximately 0.995 P/Po. The analysis was typically conducted to measure 45 adsorption relative pressure points and 23 desorption relative pressure points. Samples were outgassed overnight in vacuo at 300 degC using a Micromeritics VacPrep apparatus prior to analysis. In order to indicate any possible microporous nature of the materials additional relative pressure data were also collected at pressures lower than the usual starting point for analyses using this instrument. These were in the approximate range 0.005 to 0.01 P/Po. Whilst the data reduction methods available are unsuitable for application to the micropore range the characteristic shape of the adsorption isotherm at these low partial pressures would provide a good indication of the presence of micropores in the sample material. The sample tube dead space was measured for each analysis using helium (CP grade) thus providing warm and cold freespace values. Samples requiring only BET surface area analysis were analysed using the same equipment with the application of the same freespace measurement technique.

BET surface area was calculated using partial pressures in the nominal range 0.07 to 0.25.

Data gathering

Instruments

Micromeritics TriStar II

Calibration

BAM Standard - Alumina BAM-PM-104BAM Standard - Alumina BAM-PM-102

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Remarks on results incl. tables

Remarks on results incl. tables

Table: Porosity

Sample	Porosity (cm ³ /g)	s.d.
NM-212	0.118098	
NM-213	0.021829	
NM-211	0.207229	

The pore size distribution is presented as pore size by volume and area from the adsorption isotherm using the BJH method. The lower limit of BJH calculations in terms of pore size (by diameter) is extended below the typical value in order to highlight any possible microporous nature of the materials. The pore size distribution data presented in the BJH reports is applied to a maximum of 1000Å, although data below approximately 20Å should be considered only as a guide to the full porous nature of the materials.

The total pore volume of the materials is calculated from the volume of nitrogen adsorbed at the maximum relative pressure obtained on the adsorption branch of the isotherm and is detailed on the summary report.

Overall remarks, attachments

Remarks on results including tables and figures

The BJH (Barrett, Joyner & Halenda) method is one of many calculation methods for pore size distribution – BJH is commonly used and we apply this as a default procedure unless requested otherwise. It uses the adsorption branch of the isotherm to calculate the pore size distribution.

Attached full study report

Attached full study report NM_211_ANNEX_A33_ 010611MCACambridge.doc / 88.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1

4.32 Pour density

Endpoint study record: 2011-12-13_NPL for PROSPECT_pour density

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Method

other: Tapped density

Principles of method if other than guideline

Using a Copley JV2000, a known mass of the dry sample was placed into a measuring cylinder to a recorded volume and 'tapped' by mechanically raising and lowering by a set distance until a consistent volume was reached, which corresponds to the maximum packing density of the material.

Data gathering

Instruments

Copley JV2000

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Remarks on results incl. tables

Table: Summary of tapped density

Sample	Bulk (g/cm ³)	Density	Tapped (g/cm ³)	Density	Carr Index
NM-211	1.121		1.404		20.171
NM-213	1.304		1.523		14.374
NM-212	0.507		0.630		19.626

The smaller the Carr Index the better the flow properties. For example 5-15 indicates excellent, 12-16 good, 18-21 fair and 23-28 poor flow.

Table: Results of tapped density measurements

Sample	Mass (g)	Volume Before (cm ³)	Volume After (cm ³)	Bulk Density (g/cm ³)	Tapped Density (g/cm ³)	Carr Index
Ceria Dry CeO ₂	69.5	62	49.5	1.121	1.404	20.171
Micron CeO ₂	70.3	54	46.25	1.304	1.523	14.374
Nanograin CeO ₂	27.1	53.5	43	0.507	0.630	19.626

Overall remarks, attachments

Attached full study report

Attached full study report NM_211_ANNEX_A27_pour density.doc / 45.5 KB (application/octet-stream):
ENV/JM/MONO/(2015)8/ANN1

4.33 Photocatalytic activity

Endpoint study record: 2010-12-05 NPL for PROSPECT Photocatalytic activity.001 Free radical formation

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated & set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

other: UV-vis spectroscopy

Principles of method if other than guideline

Radical formation under photocatalytic conditions. The photocatalytic activity of materials refers to their ability to create electron-hole pairs under light irradiation, which then generate reactive oxygen species (ROS) such as free radicals (e.g. superoxide radicals, hydroxyl radicals, etc.), hydrogen peroxide, singlet oxygen, etc. ROS lifetime is generally below the millisecond range, which makes their detection challenging. One strategy to detect ROS formation is to use scavenging substances that get oxidised by ROS into stable chemical compound whose concentration can be detected. A method for assessing the general oxidative activity of the NMs under irradiation is by using potassium iodide (KI) test. The

oxidation of iodide ions results in the production of tri-iodide ions, whose concentration can be assessed by measuring optical absorbance at 352 nm. UV-vis spectroscopy for the detection of tri-iodide ions

Details on methods and data evaluation

KI test under irradiated conditions

A 5 M KI (Sigma, St. Louis, MO) solution in ultra-pure water was freshly prepared; shaking and vortexing was preferred to sonication to dissolve KI. KI solution was added to the samples of NMs as received after dispersion (50mg/L), to obtain a typically 1 mL volume sample, with 0.1M KI. 6 x 3 samples were prepared for each NM/media combination. Additionally, 6 x 3 samples containing 0.1 M KI only and 50mg/L Anatase NMs (Anatase Nanopowder, Sigma) for each media were prepared as negative and positive controls respectively; 6 NM samples plus controls were prepared and assessed in total. All samples were contained in individual 2mL microcentrifuge tubes. Samples were irradiated under a 1kW Solar Simulator (Newport Corporation, Stratford, CT). The instrument possesses a personal wavelength correction TM Certificate by Newport. The irradiance of the Solar Simulator was measured to be 1000 Wm⁻² using an optical power/energy meter (Newport, model 842-PE). Irradiation was performed on groups of 40 microcentrifuge tubes. The tubes were placed vertically under the centre of the lamp of the solar simulator, on an in-house made polystyrene holder, their cups having been removed. The samples were subjected to 10min periods of irradiation, followed by 5min period of non-irradiation to reduce sample overheating. After each 10 min period, 1x3 samples for each NM/media combination and controls were removed from the irradiations. Samples irradiated for 0 min, 10 min, 20 min, 30 min, 40 min and 60 min were collected for each NM/media combination and controls. The samples containing NMs were centrifuged at 20800 rcf for 15 min and 800 µL of supernatant was collected in a new micro-centrifuge tube and then analysed using UV-visible spectroscopy (see protocol below).

UV-visible spectroscopy for KI test

The UV-visible spectrum (absorbance scans from 300 nm to 500 nm) was acquired for samples that were irradiated for 60 minutes. Optical absorbance at 352 nm was acquired for all samples. Absorption spectra were acquired with a Lambda 850 UV-Vis spectrometer supported by UV Winlab software [Version 5.1.5] (Perkin Elmer, Waltham, MA). The instrument wavelength calibration was checked using Holmium glass standards (Serial # 9393, Starna Scientific, Hainault, UK). For the reference channel of the spectrophotometer a matched cell containing the corresponding dispersing media (with no nanoparticles) was used. Absorption spectra were acquired on samples that have been irradiated for 60 minutes. Absorbance scans from 300nm to 500nm were performed, using a slit width of 2 nm and a scan rate of 50 nm/min. After each sample, the cuvette was cleaned with a 2% solution of Hellmanex detergent, rinsed with pure water and ethanol and then blow-dried. Optical absorbance at 352nm was performed using a plate-reader Victor3 1420 multilabel counter (Perkin Elmer), supported by Wallac 1420 software (Perkin Elmer). 300µL of each sample (supernatant after centrifugation) was placed in the wells of a 96-well plate. Only the wells of rows 2 to 6 and columns 1 to 10 were used, as they had the same level of noise. The absorption at 352nm was measured using a 0.1s measurement time. Measured absorption values were displayed on a 0 a.u. to 2 a.u. scale.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Remarks on results incl. tables

Figure legend: Absorbance readings at 352 nm, of PROSPEcT NMs in 4 different media (DI water, seawater, daphnia and fish media) after being irradiated with solar simulator at 1000 W/m², for 60 minutes. Anatase NM dispersed in the four different media was used as positive control; media with no NMs were used as a negative control. The values are normalized to the absorption measured for the negative control in DI water.

Sample A - Nanograin CeO₂ is NM-212. Sample F - Micron CeO₂ is NM-213, Sample G - Ceria Dry is NM-211.

Figure compares the absorption measured at 352 nm for all the NM samples in 4 different media after 60 min of total irradiation; the corresponding negative control (i.e. media with no NMs) are also shown. Results show that there was a certain level of tri-iodide (I₃⁻) measured in the irradiated sample containing media only. Interestingly, tri-iodide was suppressed in seawater and may be attributed to a higher concentration of ions (potentially with some scavenging capacity either to ROS species or to electron (or holes) at the NM surface) in this media. As expected, results for Anatase (TiO₂), being the most active photocatalytic material, show a much higher rate of tri-iodide formation than the corresponding PROSPEcT NMs. In particular, the absorbance signal was highest in DI water, with the lack of ionic species in the media. Again, when in seawater, the absorbance signal was reduced (as in the corresponding blank i.e. seawater with no Anatase). There are several possible explanations for this:

- a) Presence of scavengers in solution, as previously described.
- b) Enhanced aggregation/sedimentation of the NMs in seawater media compared to other media.

Out of all the PROSPEcT NMs, we see NM-212 (Nanograin CeO₂) following a similar trend to Anatase, in having the largest absorbance signal in DI water and the smallest when in seawater. Z-COTE ZnO is interesting, in that it does not follow a similar pattern observed with Anatase and NM-212 (Nanograin CeO₂). With Z-COTE ZnO, the absorbance signal is much higher in seawater than when dispersed in the other three media. At present we offer no explanation for this observation. With the other PROSPEcT NMs, the absorbance signals were within a similar range to that of the corresponding irradiated blank. Samples that were kept in the dark exhibited no absorption peak at 352 nm.

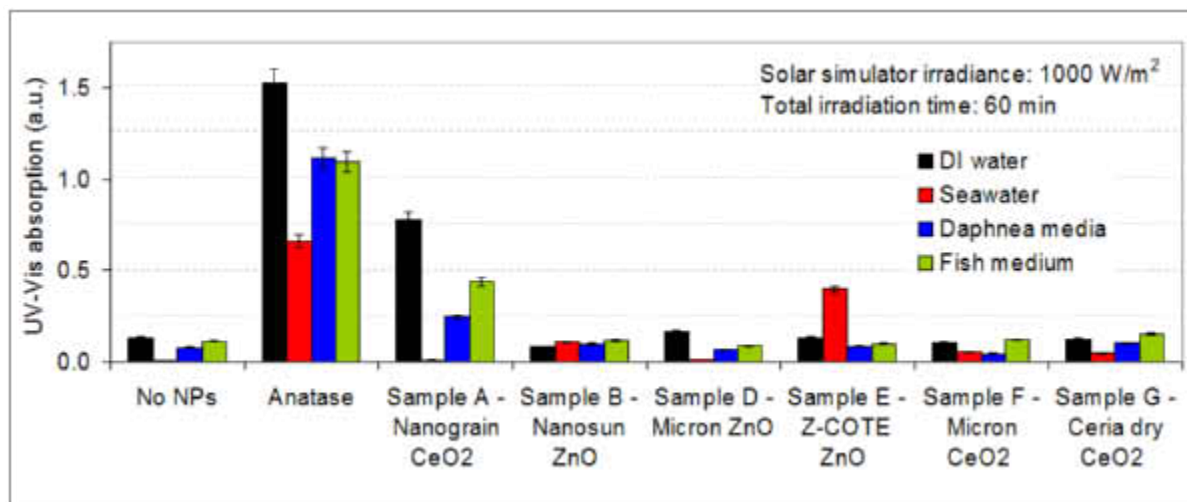
Lastly, a UV-visible plate reader was used to follow the cumulative production of I₃⁻ with varying irradiation time; this was quantified by measuring absorption at 352 nm. In summary, results show that absorbance signal generally increases with irradiation time and this can be attributed to the increase in the amount of ROS being generated. Again, our findings are consistent with previous observations, in that:

- a) Anatase gave the highest absorbance reading
- b) NM-212 (Nanograin CeO₂) gave a similar trend to Anatase, i.e. largest absorbance reading in DI water and lowest when in seawater

Overall remarks, attachments

Attached full study report

Attached full study report NM_211_ANNEX_A44_FREE RADICAL FORMATION UNDER PHOTOCATALYTIC CONDITIONS.doc / 78 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2

Illustration (picture/graph)**Endpoint study record: 2012-03-02_Australia_Deakin_Photocatalytic activity****Administrative Data**

Study result type

experimental result

Reliability

other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies

Low number of replicates

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	T Tsuzuki, J Wang	2012			Deakin University				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Methods**

other: UV-Vis

Principles of method if other than guideline

Degradation of Rhodamine-B dye under simulated sunlight in the presence of nanomaterials

Details on methods and data evaluation

For each measurement, 0.012g of the powder sample was added into 100 ml of Rhodamine B (RhB) aqueous solution having the concentration of 0.0096g/L. The suspension was placed in a quartz beaker with a quartz watch glass as a lid. The suspension was then stirred in the dark for 1 h to ensure the

establishment of adsorption and desorption equilibrium of RhB on the particle surface. Subsequently the suspension was irradiated with simulated sunlight using an Atlas Suntest XLS+ instrument (equipped with a 150 W xenon lamp) with a flux of 300 W m^{-2} while continuously stirred. The temperature of the solution was regulated to 37 °C (air cooling). At given intervals, 3 mL of the suspension was extracted and then centrifuged at 6000 rpm for 10 min to separate the nanoparticles from the supernatant. UV-Vis absorbance spectra of the supernatant were measured with a Varian Cary 3E spectrophotometer. The intensity of the optical adsorption peak around 554 nm was used to monitor the rate of dye degradation. Each of the photocatalytic activity tests was repeated three times. Overall rates of the reactive dye degradation were calculated assuming the first-order kinetics. It is assumed that extraction of a small quantity of sample (3 mL each x max 6) does not alter the UV exposure conditions of the remaining sample.

Data gathering

Instruments

Varian Cary 3E UVVis spectrophotometer: Atlas Suntest CPS+ instrument equipped with a 150 W Xenon lamp.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Photocatalytic Activity equation/description

Table: Photocatalytic rate constant

Sample ID	Rate Constant	Ratio between the rate constant
NM-211	0.0056	0.57
NM-212	0.0225	2.3
NM-213	0.0098	1.0

Overall remarks, attachments

Attached full study report

Attached full study report NM_211_ANNEX_A45_OECD CeO2 Photocatalysis Australia Deakin.pdf / 236.65 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2

Applicant's summary and conclusion

Conclusions

It was found that the photocatalytic activity was the highest for NM212 and the lowest for NM211 (Table). The rate constant did not show strong correlation with specific surface area. Although NM213 is supposed to be a non-nano material, the photocatalytic activity was higher than MN211. When the photoactivity rates are normalised with specific surface area values, NM213 showed the highest photocatalytic activity per unit surface area. The results could be explained as surface defects acting as

charge recombination sites to prevent photocatalysis and NM211 has the highest surface defects while NM212 has geometrical morphology with low surface defects

Endpoint study record: 2012-03-06_Australia_CSIRO_Photocatalytic_Activity

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Casey, yin, McCall	2012			CSIRO				

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Methods

other:

Principles of method if other than guideline

Degradation of 1,1-diphenyl-2-picrylhydrazyl (DPPH) dye in the presence of nanomaterial, detected by UV-Vis spectroscopy.

Details on methods and data evaluation

Photocatalytic activity of the CeO₂ samples was determined by monitoring the degradation of 1,1-diphenyl-2-picrylhydrazyl (DPPH), following the procedure described by Dransfield et al (2000). To quantify the photo-reactivity, the absorbance at 520 nm (the wavelength of maximum absorbance for the purple DPPH radical) was monitored. Two 62.5ml mixtures of 1:1 Mineral Oil White Light (Aldrich): Caprylic Capric C8/C10 Triglyceride (MOTG) were prepared. CeO₂ (0.031g) was added to one mixture, and DPPH (0.0052g) to the other. Each was magnetically stirred for 1.5 hours in a beaker covered on all sides with Al foil. Then the two mixtures were combined, poured into a crystallising dish (135mm diameter x 23mm height) covered on all sides with foil, and stirred for 5 minutes. Before exposure to UV (t=0), 3ml of the solution was withdrawn and its UV-Vis absorption spectrum was measured using a Cary 5G UV-Vis NIR spectrophotometer. The 3ml sample was returned to the solution. The solution was then exposed to UV using a pre-warmed Spectroline UV lamp (BIB150 P/FA 365 nm, 150W concentrated spot bulb, lamp diameter=110mm) placed 12cm from the CeO₂/dye mixture. Samples were taken at various times and absorbance at 520nm was measured. The equipment was designed with a sliding shield separating the sample and UV lamp so that between exposures the lamp remained on, thus avoiding variations in lamp intensity. G. Dransfield, P.J. Guest, P.L. Lyth, D.J. McGarvey, T.G. Truscott, J. (2000) Photochem. Photobiol. B 59 147.

Data gathering**Instruments**

Cary 5G UV-Vis NIR spectrophotometer Spectroline UV lamp (BIB150 P/FA 365 nm, 150W concentrated spot bulb, diameter=110mm)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions**Remarks on results incl. tables**

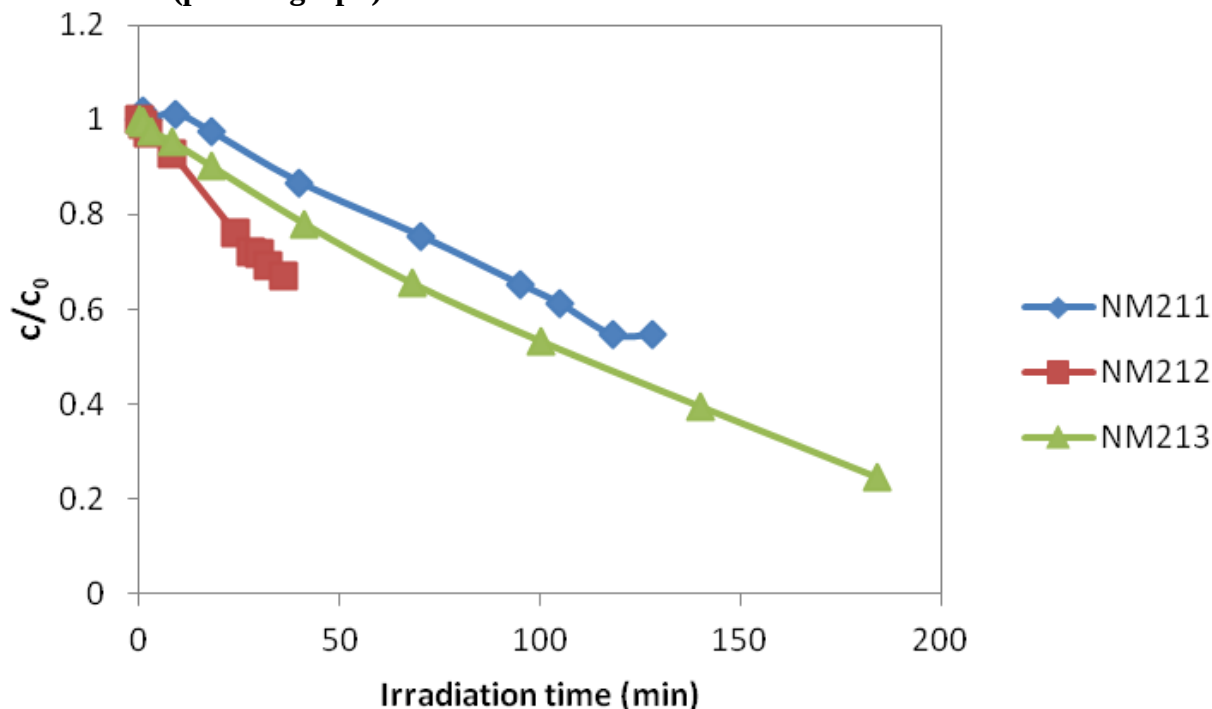
Table: Percentage decay at 30 minutes, and rate constant for decay, of the absorbance at 520nm of DPPH used as a probe to determine the photocatalytic activity of CeO₂ samples NM211, NM212 and NM213.

	DPPH as probe	
	% decay at 30 min	Rate constant (min ⁻¹)
NM211	8.3%	0.005
NM212	33.3%	0.014
NM213	16.7%	0.006

Figure legend: Dependence of absorbance ratio (c/c_0) on UV-irradiation time for the dye DPPH in a CeO₂-containing dispersion; c_0 is the absorbance at time zero.

Overall remarks, attachments**Attached full study report**

Attached full study report NM_211_ANNEX_A46_OECD CeO₂ photocatalytic activity CSIRO Australia.docx / 52.14 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Illustration (picture/graph)**Applicant's summary and conclusion****Conclusions**

Theoretically, photocatalytic activity should decrease with increasing particle size (or decreasing surface area) because recombination processes dominate at larger sizes. However, the expected correlation between surface area and photocatalytic activity does not appear to be supported by the data obtained for NM-211, NM212 and NM213. NM211 has less photocatalytic activity than its larger sized counterparts NM212 and NM213, in the system used here.

4.34 Radical formation potential***Endpoint study record: 2010-12-09_NPL for PROSPECT_Radical formation potential_Photocatalytic conditions*****Administrative Data**

Study result type	experimental result
Reliability	other: needs to be evaluated and set by expert researchers group
Rationale for reliability incl. deficiencies	Bulk powder used. Powder not subsampled by spinning riffler.

Materials and methods**Principles of method if other than guideline**

The photocatalytic activity of materials refers to their ability to create electron-hole pairs under light irradiation, which then generate reactive oxygen species (ROS) such as free radicals (e.g. superoxide radicals, hydroxyl radicals, etc.), hydrogen peroxide, singlet oxygen, etc.

Details on methods and data evaluation

- Irradiation was performed on groups of 40 microcentrifuge tubes. The tubes were placed vertically under the centre of the lamp of the solar simulator, on an in-house made polystyrene holder, their cups having been removed.
- The samples were subjected to 10min periods of irradiation, followed by 5min period of non-irradiation to reduce sample overheating.
- After each 10 min period, 1x3 samples for each NP/media combination and controls were removed from the irradiations.
- Samples irradiated for 0 min, 10 min, 20 min, 30 min, 40 min and 60 min were collected for each NP/media combination and controls.
- The samples containing NPs were centrifuged at 20800 rcf for 15 min and 800 mL of supernatant was collected in a new microcentrifuge tube.

Data gathering

Instruments

1kW Solar Simulator (Newport Corporation, Stratford, CT) Lambda 850 UV-Vis spectrometer Plate-reader Victor 1420 multilabel counter (Perkin Elmer)

Calibration

The instrument wavelength calibration was checked using Holmium glass standards (Serial # 9393, Starna Scientific, Hainault, UK)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material Identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial - powder

Results and discussions

Remarks on results incl. tables

Figure legend: Absorbance readings at 352 nm, of PROSPEC T NMs in 4 different media (DI water, seawater, daphnia and fish media) after being irradiated with solar simulator at 1000 W/m², for 60 minutes. Anatase NM dispersed in the four different media was used as positive control; media with no NMs were used as a negative control. The values are normalized to the absorption measured for the negative control in DI water.

Sample A - Nanograin CeO₂ is NM-212. Sample F - Micron CeO₂ is NM-213, Sample G - Ceria Dry is NM-211.

Figure compares the absorption measured at 352 nm for all the NM samples in 4 different media after 60 min of total irradiation; the corresponding negative control (i.e. media with no NMs) are also shown. Results show that there was a certain level of tri-iodide (I₃⁻) measured in the irradiated sample containing media only. Interestingly, tri-iodide was suppressed in seawater and may be attributed to a higher concentration of ions (potentially with some scavenging capacity either to ROS species or to electron (or

holes) at the NM surface) in this media. As expected, results for Anatase (TiO_2), being the most active photocatalytic material, show a much higher rate of tri-iodide formation than the corresponding PROSPEC T NMs. In particular, the absorbance signal was highest in DI water, with the lack of ionic species in the media. Again, when in seawater, the absorbance signal was reduced (as in the corresponding blank i.e. seawater with no Anatase). There are several possible explanations for this:

a) Presence of scavengers in solution, as previously described.

b) Enhanced aggregation/sedimentation of the NMs in seawater media compared to other media.

Out of all the PROSPEC T NMs, we see NM-212 (Nanograin CeO_2) following a similar trend to Anatase, in having the largest absorbance signal in DI water and the smallest when in seawater. Z-COTE ZnO is interesting, in that it does not follow a similar pattern observed with Anatase and NM-212 (Nanograin CeO_2). With Z-COTE ZnO, the absorbance signal is much higher in seawater than when dispersed in the other three media. At present we offer no explanation for this observation. With the other PROSPEC T NMs, the absorbance signals were within a similar range to that of the corresponding irradiated blank. Samples that were kept in the dark exhibited no absorption peak at 352 nm.

Lastly, a UV-visible plate reader was used to follow the cumulative production of I_3^- with varying irradiation time; this was quantified by measuring absorption at 352 nm. In summary, results show that absorbance signal generally increases with irradiation time and this can be attributed to the increase in the amount of ROS being generated. Again, our findings are consistent with previous observations, in that:

a) Anatase gave the highest absorbance reading

b) NM-212 (Nanograin CeO_2) gave a similar trend to Anatase, i.e largest absorbance reading in DI water and lowest when in seawater

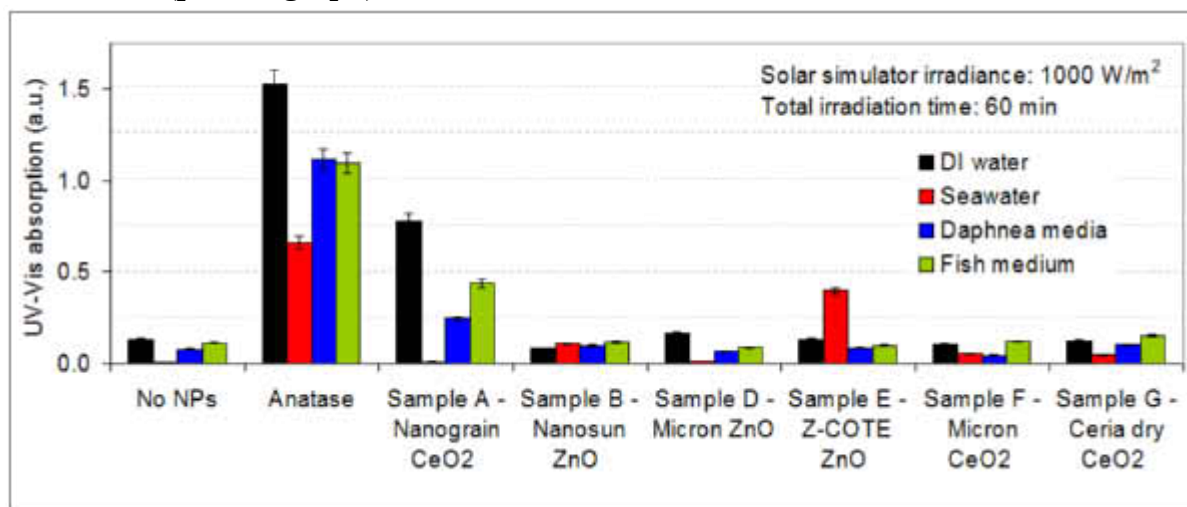
Overall remarks, attachments

Attached full study report

Attached full study report NM_211_ANNEX_A04_FINALINTERIM REPORT2010corrected.pdf / 1.05 MB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Attached full study report NM_211_ANNEX_A44_FREE RADICAL FORMATION UNDER PHOTOCATALYTIC CONDITIONS.doc / 78 KB (application/octet-stream): : ENV/JM/MONO/(2015)8/ANN2

Illustration (picture/graph)



5. ENVIRONMENTAL FATE AND PATHWAYS

5.1 Stability

5.2 Biodegradation

5.2.1 Biodegradation in water: screening tests

5.2.2 Biodegradation in water and sediment: simulation tests

Endpoint study record: 20120309_Switzerland_OECD303A_simulation

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Kottelat et al.	2010	Elimination of CeO ₂ nanoparticles in activated sludge units according to guideline OECD 303 A		BMG Engineering AG		FOEN		

Data access

data published

Data protection claimed

yes, but willing to share

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 303 A (Simulation Test - Aerobic Sewage Treatment. A: Activated Sludge Units)	yes
according to	other guideline: OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	no

Principles of method if other than guideline

Nanoparticles were separately fed into the aeration chamber. Toxicity of the nanoparticles on the sludge was separately investigated. Outlet concentration of cerium dioxide was measured by inductively coupled plasma optical emission spectrometry, ICP OES (including a centrifugation step to differentiate the state of agglomeration)

Test materials**Details on test material**

The WWTP elimination of the test material was measured and compared to the same material with a coating of a biodegradable or a non-biodegradable surfactant.

Study design**Oxygen conditions**

aerobic

Inoculum or test system

activated sludge (adaptation not specified)

Details on source and properties of surface water

synthetic wastewater as recommended in OECD TG 303 A

Details on inoculum

Activated sludge from the aeration tank of the wastewater treatment plant ARA Werdhölzli, CH-8048 Zurich, Switzerland, was used for the testing. No acute toxicity to activated sludge microorganisms due to the presence of CeO₂ nanoparticles was measured, this test was performed separately according to OECD Guideline 209.

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A47_Robust summary STP_Draft_100629.pdf / 200.71 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2	

Attached full study report

Attached full study report
NM_211_ANNEX_A48_Limbach_removal oxide nanoparticles_2008.pdf / 1.87 MB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2
NM_211_ANNEX_A49_Limbach_sup_inf_removal of oxide nanoparticles_2008.pdf / 882.38 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Applicant's summary and conclusion**Conclusions**

While a majority of the nanoparticles without coating could be captured through adhesion onto the biosolid, a significant fraction of the coated nanoparticles escaped the clearing system, and up to 6wt% of cerium oxide was found in the exit stream of the model plant.

5.2.3 Biodegradation in soil**5.2.4 Mode of degradation in actual use**

5.3 Bioaccumulation

5.3.1 Bioaccumulation: aquatic / sediment

Endpoint study record: 2011_09_30_Imperial College for PROSPECT_Isotopes_Ceria NP & Ce Materials Characterisation

Administrative Data

Study result type experimental result

Reliability other: needs to be evaluated and set by expert researchers group

Data source

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Principles of method if other than guideline

Particles dissolved in 0.1M nitric acid

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on analytical methods

Multi-collector inductively coupled plasma mass spectrometry. Standard-sample bracketing data correction.

Overall remarks, attachments

Remarks on results including tables and figures

Attached full study report

Attached full study report
NM_211_ANNEX_A50_Stable Isotope Data for Ceria Nanoparticles.xls / 871.5 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Applicant's summary and conclusion**Conclusions**

NM-211 is isotopically indistinguishable from natural materials and organisms. Isotopically labelled engineered nanoparticles required for tracing.

NM-212 is isotopically indistinguishable from natural material and organisms. Isotopically labelled engineered nanoparticles required for tracing.

NM-213 is isotopically indistinguishable from natural material and organisms. Isotopically labelled engineered nanoparticles required for tracing.

Endpoint study record: 2011_09_30_Imperial College for PROSPECT_Isotopes_Tracing_Report

Administrative Data**Overall remarks, attachments****Attached full study report**

Attached full study report
NM_211_ANNEX_A51_Ce Isotopic Tracing PROSPECT Report.pdf / 965.87 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Applicant's summary and conclusion**Conclusions**

Labelling with ^{140}Ce will be most suitable for the majority of applications, as this isotope is relatively inexpensive but can still provide good detection sensitivities. In order to achieve the best sensitivities, it will be useful to couple use of labelled $^{140}\text{CeO}_2$ nanoparticles, with high precision isotope analyses, as can be provided by MC-ICPMS. If extremely high tracing sensitivities are required, it may be useful to employ CeO_2 nanomaterials labelled with ^{142}Ce , but enriched ^{142}Ce is about 10 times more costly than ^{140}Ce labels.

5.3.2 Bioaccumulation: terrestrial**5.4 Transport and distribution****5.4.1 Adsorption / desorption**

Endpoint study record: 2012-03-02_Australia_CSIRO_Retention

Administrative Data

Study result type experimental result

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Cornelis et al	2012	Retention on NM-211 in soils		Adelaide, SA	00001	CSIRO		

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Study type**

other: Retention

Media

soil/sewage sludge

Type of method

batch equilibrium method

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 106 (Adsorption - Desorption Using a Batch Equilibrium Method)	yes

Principles of method if other than guideline

Retention of NM-211 and NM-212 was examined in five soils with varying physical and chemical characteristics from Australia (see attached partitioning and retention methods).

Test materials**Test material identity**

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212

Results and discussions**Results: Batch equilibrium or other method****Any other information on results incl. tables**

Table. NM-211 K_d and K_r values

Soils	Bulk CeO ₂	Soluble Ce(III)	Soluble Ce(IV)	NM-211
	K _d (L kg ⁻¹)	K _d (L kg ⁻¹)	K _d (L kg ⁻¹)	K _r (L kg ⁻¹)
Mt Compass	6.2 ± 0.14	2.4 ± 0.02	2.4 ± 0.03	1.09 ± 0.08
Ingham	8.1 ± 0.09	> 3.4	3.4 ± 0.07	1.94 ± 0.03
Emerald Black	8.1 ± 0.08	> 3.7	> 3.7	3.52 ± 0.01
Bute	8.1 ± 0.06	> 3.4	> 3.4	1.32 ± 0.06
Pt Kenny	8.2 ± 0.13	> 3.4	> 3.4	1.91 ± 0.06

Table NM-212 Kd and Kr values

Soils	Bulk CeO ₂	Soluble Ce(III)	Soluble Ce(IV)	NM-211
	K _d (L kg ⁻¹)	K _d (L kg ⁻¹)	K _d (L kg ⁻¹)	K _r (L kg ⁻¹)
Mt Compass	6.2 ± 0.14	2.4 ± 0.02	2.4 ± 0.03	0.86 ± 0.01
Ingham	8.1 ± 0.09	> 3.4	3.4 ± 0.07	1.73 ± 0.07
Emerald Black	8.1 ± 0.08	> 3.7	> 3.7	2.60 ± 0.03
Bute	8.1 ± 0.06	> 3.4	> 3.4	0.89 ± 0.08
Pt Kenny	8.2 ± 0.13	> 3.4	> 3.4	0.56 ± 0.09

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A53_Cornelis et al.2010.pdf / 486.08 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2	

Attached full study report

Attached full study report
NM_211_ANNEX_A54_Kd and Kr value data_NM-211.docx / 12.16 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2
NM_211_ANNEX_A55_NanoHub_NM-211_Kd and Kr methods.docx / 70.04 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Endpoint study record: 2012-03-09_Switzerland_FOEN_retention**Administrative Data**

Study result type experimental result

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Kottelat et al	2010	Adsorption of NM-211 to soils – Method development and screening of adsorption behaviour of NM-211 to three different soils		BMG engineering AG		FOEN		

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Study type

other: Retention

Media

soil

Type of method

batch equilibrium method

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 106 (Adsorption - Desorption Using a Batch Equilibrium Method)	yes

Principles of method if other than guideline

Due to the instability of the NM-211 and NM-212 suspensions observed during the tests, the adsorption was calculated as the percentage of substance adsorbed on the soil related to the quantity present in a reference sample. The calculation of distribution coefficients (K_d) or organic carbon normalized adsorption coefficients (K_{OC}) were not performed because no verification that equilibrium conditions were reached was done.

Results and discussions

Results: Batch equilibrium or other method

Any other information on results incl. tables

Sample ID	Soil	[Ce] in Test Sample (mg/ml)	[Ce] in control Supernatant (mg/l)	Adsorption to soil (%)
NM-211	01 -A	24.6	25.1 **	2**
NM-211	05 -G	28.1	30.6	8
NM-211	06 -A	28.2	30.5	7
NM-212	01 -A	14.2*	17.3	18
NM-212	05 -G	10.1	11.5	13
NM-212	06 -A	10.7	13.8	22

*1 replicate instead of 2.
 ** large uncertainty due to the difference in the duplicate [Ce] measurements in the Control Supernatant.

Overall remarks, attachments

Attached full study report

Attached full study report
NM_211_ANNEX_A56_OECD_CeO2_Adsorption to soil_BMG_draft_100705.pdf / 193.9 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

Applicant's summary and conclusion

Conclusions

According to the results of the screening test presented here, the adsorption to soils is probably less relevant for the behaviour and fate of CeO₂ nanoparticles in the environment than aggregation. This conclusion should however be verified for different soil: solution ratios, natural and synthetic

soil models (for instance sand or CaCO_3), and contact time as well as for lower nanoparticle concentrations.

Although no significant adsorption to soil could be observed in the study, it has to be pointed out that CeO_2 nanoparticles can be retained in soil by mechanisms other than adsorption, such as the filtration of agglomerates, for instance. The batch adsorption test OECD 106 is not designed to answer these issues, but the retention of nanoparticles by soils could probably be investigated using soil columns as described in guideline OECD 312 guideline. This method seems more adapted than OECD 106 to answer the issues on the environmental fate and behaviour of CeO_2 and probably many other insoluble nanoparticles.

6. ECOTOXICOLOGICAL INFORMATION

6.1 Aquatic toxicity

6.1.1 Short-term toxicity to fish

Endpoint study record: 2012.03.06_Zebrafish testing _CeO2 _

Administrative Data

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: 212	

Test materials

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211

Details on test material

Principal nanomaterial name: Cerium (IV) Oxide (Antaria) CAS Number: CAS 1306-38-3, EINECS 215-150-4 Structural formula/ molecular structure: CeO_2 , cubic Composition of nanomaterial being tested: Purity: > 99.5 wt% CeO_2 Basic morphology: Nanostructured powder Description of surface chemistry: Neither coated nor modified. Major commercial uses: Catalysts and chemomechanical polishing Known catalytic activity: None Method of production: Precipitation

Test organisms

Test organisms (species)

Danio rerio

Details on test organisms

petri dishes were used for exposures and the eggs/embryos/fish were scored every day

Study design

Total exposure duration

192 h

Test conditions**Any other information on materials and methods incl. tables**

Appropriate amounts of CeO₂ particles were weighed in a 20 ml vial. A few drops of exposure media was added to create a thick paste by stirring the suspension with a glass rod. Particles were transferred to exposure medium and vials were washed thoroughly. A probe sonicator was used to sonicate the suspension for 20s at 90% amplitude. Suspensions were stirred for 1 h at approx. 900rpm with a magnetic stirrer. Exposure dilutions were made during stirring. Unused suspensions were kept in closed bottles wrapped in aluminium foil and stirred for 1 h before reusing.

Results and discussions**Details on results**

See attached document

Overall remarks, attachments**Attached full study report****Attached full study report**

NM_211_ANNEX_A57_NM 211 Zebrafish experimental results.docx / 73.86 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2

6.1.2 Long-term toxicity to fish**6.1.3 Short-term toxicity to aquatic invertebrates*****Endpoint study record: 2012.02.22_Acute toxicity Daphnia magna _CeO₂*****Administrative Data**

Purpose flag supporting study

Study result type experimental result

Materials and methods**Test guideline**

Qualifier	Guideline	Deviations
according to	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

Principal nanomaterial name: Cerium (IV) Oxide (Antaria) CAS Number: CAS 1306-38-3, EINECS 215-150-4 Structural formula/ molecular structure: CeO₂, cubic Composition of nanomaterial being tested: Purity: > 99.5 wt% CeO₂ Basic morphology: Nanostructured powder Description of surface chemistry: Neither coated nor modified. Major commercial uses: Catalysts and chemomechanical polishing Known catalytic activity: None Method of production: Precipitation

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Daphnia Clon F (Barata and Baird, 2000)

Study design

Water media type

saltwater

Total exposure duration

48 h

Test conditions

Hardness

ASTM reconstituted Hard water

Test temperature

20 ± 1 °C

pH

range 7.8 to 8.1

Dissolved oxygen

95-100% of oxygen saturation levels

Salinity

350-410 uS/cm conductivity

Details on test conditions

All tests were initiated with < 24 h old neonates obtained from third to the sixth brood of gravid females. The tests were conducted in reconstituted hard ASTM water. The animals were not fed during the exposure. All tests were performed in an orbital incubator set to 3 rpm, to minimize sedimentation of NPs during exposure. The pH value, dissolved oxygen concentration (DO) and conductivity of the test medium were assessed at the beginning and end of the tests. DO was measured using a polarographic oxygen electrode coupled to a CyberScan DO 300/3001 EUTECH model meter (Lab Process Distributions, Alella, Barcelona, Spain), pH and conductivity were measured using an epoxy-body combination electrode, coupled to a Crison micro pH 2001 and conductivity meter (Crison Instruments S.A., Alella, Barcelona, Spain), calibrated with standard pH and conductivity buffer solutions (Sigma, Madrid, Spain).

Any other information on materials and methods incl. tables

Three replicates of 10 animals per concentration were exposed in 20 ml of particle suspension in ASTM hard water in 20 ml glass bottles. Mortality was assessed daily by observing mobility of the animals and gentle agitation of immobile animals. The exposure concentrations used were 2000 mg/L, 1500 mg/L, 750 mg/L, 500 mg/L and 250 mg/L.

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
48 h	EC10 (EC50)	400 — 1814 mg/L	nominal	test mat.	mobility	

Details on results

A dose dependent mortality can be observed after 48 h. EC 50 Lower 95 % CI/Higher 95 % CI EC 10 Lower 95 % CI/Higher 95 % CI 1391/2936 400 mg/ml 224/541

Reported statistics and error estimates

The percentage of immobile individuals was used as endpoint, transformed into probits and used to determine LC50s (Finney 1971). In acute tests mortality data was transformed into probits and 48 h LC50 and its 95% CI determined using conventional regression procedures. Tests were analysed using the IBM SPSS 19.0 software. All results were considered significant if $p < 0.05$.

Any other information on results incl. tables

Table. Respective EC 50, EC 10 and lower and higher 95% CI for the three CeO₂, NM 213 (micronized Sigma), NM 211 (ANTARIA) and NM 212 (UMICORE) following 48 h exposure to CeO₂.

	EC 50	Lower 95 % CI	Higher 95 % CI	EC 10	Lower 95 % CI	Higher 95 % CI
Sigma (NM 213)	1333	1252	1428	731	646	804
Antaria (NM 211)	1814	1391	2936	400	224	541
Umicore (NM 212)	1060	926	1209	547	407	659

See attached background material

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A58_Daphnia Testing results.docx / 50.1 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2	

Applicant's summary and conclusion**Conclusions**

No mortality was observed after 24 h of exposure to the three CeO₂ particles. However, a dose dependent mortality can be observed for all three particles after 48 h

6.1.4 Long-term toxicity to aquatic invertebrates

Endpoint study record: 2012.02.21_Chronic toxicity Daphnia magna Survival and reproduction _CeO2

Administrative Data

Study result type experimental result

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 211 (Daphnia magna Reproduction Test)	

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Details on test material

Principal nanomaterial name: Cerium (IV) Oxide (Antaria)

CAS Number: CAS 1306-38-3, EINECS 215-150-4

Structural formula/ molecular structure: CeO₂, cubic

Composition of nanomaterial being tested: Purity: > 99.5 wt% CeO₂

Basic morphology: Nanostructured powder

Description of surface chemistry: Neither coated nor modified.

Major commercial uses: Catalysts and chemomechanical polishing

Known catalytic activity: None

Method of production: PrecipitationSupplier Antaria

Test organisms

Test organisms (species)

Daphnia magna

Details on test organisms

Daphnia magna clone F as previously described (Barata and Baird, 2000). All tests were initiated with < 24 h old neonates obtained from third to the sixth brood of gravid females. Individual cultures were fed daily with *Chorella vulgaris* (50,000 cells/ml, respectively, corresponding to 1.8 µg C/ml)

Study design

Test type

semi-static

Water media type

saltwater

Total exposure duration

21 d

Test conditions**Hardness**

Reconstituted hard ASTM water

Test temperature

20 ± 1 °C

pH

range 7.8 to 8.1

Dissolved oxygen

95-100% of oxygen saturation levels

Salinity

350-410 uS/cm conductivity

Details on test conditions

Reproduction experiments were conducted with algae present (*Chlorella vulgaris*) at 50,000 cells/ml. The photoperiod was set to 14 h light: 10 h dark cycle and temperature to 20 ± 1 °C. Chronic tests were conducted in semi-static conditions replacing the medium every 24 h. In those tests animals were cultured individually in 120 mL of medium using 3, 10 and 30 mg/l of NM as exposure concentrations. Ten animals were exposed per treatment. During tests survival and reproduction were monitored until the release of the fourth brood (21-24 days at 20 °C).

Any other information on materials and methods incl. tables

Chronic tests were conducted in semi-static conditions replacing the medium every 24 h. In those tests animals were cultured individually in 120 mL of medium using 3, 10 and 30 mg/l of NM as exposure concentrations. During tests survival and reproduction were monitored until the release of the fourth brood (21-24 days at 20 °C). Age specific survival and reproduction rates were then used to estimate population growth rates using the Lotka equation (eq 1) (see attached document). Lowest effect concentration (LOEC) Treatment differences against controls in length, cumulative fecundity and population growth rates were determined using one way ANOVA and comparing treatment effects against controls followed by one site post hoc Dunnett's test.

Results and discussions**Effect concentrations**

Duration	Endpoint	Effect conc.	Nominal/Measured	Conc. based on	Basis for effect	Remarks (e.g. 95% CL)
21 d	LOEC	30 mg/L	nominal	test mat.		

Details on results

See attached background document During the reproduction tests survival at the end of exposures was marginally or unaffected (Table 2). Age at first reproduction was significantly ($P < 0.05$) delayed ($df = 9$, $\chi^2 = 46.1$) following exposure to 3 and 10 mg/l of NM211

Any other information on results incl. tables

During the reproduction tests survival at the end of exposures was marginally or unaffected (Table 2). Age at first reproduction was significantly ($P < 0.05$) delayed ($df = 9$, $\chi^2 = 46.1$) following exposure to 3 and 10 mg/l of NM211

Table Survival (%) at day 21 and age at first reproduction (Mean SE) of *D. magna* individuals across treatments. * significantly ($P < 0.05$) different from control (Ct) following one way ANOVA and Dunnett's test.

	Exposure concentration (mg/L)	Survival (%)	N	Age (mean)	SE	
Ct		100	10	10.6	0.3	
NM 213	3	90	10	10.9	0.5	
	10	90	10	10.5	0.2	
	30	100	10	11.7	0.5	
NM 211	3	100	9	13.6	1.1	*
	10	90	10	15.6	1.1	*
	30	80	5	21.8	0.2	*
NM 212	3	90	10	10.9	0.5	
	10	100	10	13.1	0.8	*
	30	90	10	14.2	0.7	*

Table: LOEC values for *Daphnia magna* exposure to CeO₂

	NM-213	NM-211	NM-212
21 -d reproduction (LOEC)	>30 mg/l	30 mg/l	30 mg/l

Overall remarks, attachments**Remarks on results including tables and figures**

NM may also aggregate with edible food alga particles and hence decrease food ratios to *Daphnia* individuals. Previous studies have indicated that growth and reproduction of *D. magna* individuals is very sensitive to decreasing food levels in the water column ((Barata and Baird, 1998)). Future research should be conducted to test these effects.

Attached background material

Attached document	Remarks
NM_211_ANNEX_A59_Daphnia magna Chronic reproduction and survival test results.docx / 44.7 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A60_ Statistical analysis Lotka equation.docx / 29.72 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN2	

6.1.5 Toxicity to aquatic algae and cyanobacteria

Endpoint study record: 2012-03-02_Australia_CSIRO_Toxicity to aquatic algae

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
	Agnel et al	2012	Toxicity of NM-211 to P.subcapitata		Sydney NSW	00001	CSIRO		

Data access

data submitter has Letter of Access

Data protection claimed

yes

Materials and methods

Principles of method if other than guideline

The toxicity of NM-211, NM-212, NM-213 was assessed using *Psuedokircheriella subcapitata* (ATCC, Maryland, USA). The alga was cultured axenically in US Environmental Protection Agency (USEPA) synthetic softwater on a 24 hour light cycle (Philips TL 40W cool white fluorescent lighting, 70 $\mu\text{mol photons/m}^2/\text{s}$) at 24°C (US EPA, 1994). Tests were performed using the same conditions in the same medium without EDTA and buffered at pH 6.5 $\pm$ 0.1 with 2 mM piperazine-N,N'-bis(ethanesulfonic acid) (PIPES: Sigma-Aldrich) (Rogers et al., 2010). Six millilitres of synthetic softwater was dispensed into 30 mL borosilicate glass mini-vials, pre-coated with a silanizing solution (Coatasil, Ajax Finechem) to reduce adsorption of dissolved cerium to the flask walls. Additional mini-vials were prepared at each concentration for pH measurement at the beginning and end of the test. A concentrated spiking solution of NM-211 was prepared by accurately weighing 0.1 g NM-211 into a polycarbonate vial, pipetting 10 mL deionised water into the vial to achieve 10 g/L, and sonicating for 30 minutes. Different volumes were then spiked to achieve a range of concentrations in toxicity test vials. Exponentially-growing cells of *P. subcapitata* were harvested by centrifuging (700 x g, 7 min) and washed three times with the test medium. Each minivial was inoculated with a known concentration of pre-washed cells to give an initial cell density of 2 – 4 x 10⁴ cells/mL. Algal cell counts were measured daily over the 72 h test using a 4-colour BD-FACSCalibur™ (Becton Dickinson BioSciences, San Jose, CA) flow cytometer as previously described (Rogers et al., 2010). Algal growth rate 72-h IC50 values were calculated using Linear Interpolation Method (ICP) analysis (ToxCalc Version 5.0.23C, Tidepool Software McKinleyville, CA).

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211, NM-212, NM-213

Test material form

nanomaterial

Test organisms**Test organisms (species)**

Pseudokirchnerella subcapitata

Study design**Total exposure duration**

72 h

Test conditions**Test temperature**

24 C

pH

6.5

Results and discussions**Any other information on results incl. tables**

Table

Sample ID	Dispersion Medium	pH	IC50 (a)
NM-211	US EPA Medium	6.5	26 (21 -31) mg/l (b)
NM-212	US EPA Medium	6.5	9.8 (9.2 -10.7) mg/l (b)
NM-213	US EPA Medium	6.5	85 (67 -107) mg/l (b)

(a) The concentration that elicited 50 % growth rate inhibition relative to controls

(b) 95 % confidence interval

Overall remarks, attachments**Attached background material**

Attached document	Remarks
NM_211_ANNEX_A16_Rogers et al 2010.pdf / 395.58 KB (application/octet-stream) : ENV/JM/MONO/(2015)8/ANN1	

6.2 Sediment toxicity**6.3 Terrestrial toxicity****6.4 Biological effects monitoring****6.5 Biotransformation and kinetics****6.6 Additional ecotoxicological information**

7. TOXICOLOGICAL INFORMATION

7.1 Toxicokinetics, metabolism and distribution

7.2 Acute Toxicity

7.2.1 Acute toxicity: oral

7.2.2 Acute toxicity: inhalation

Endpoint study record: *Acute toxicity: inhalation_CeO2 NanoCare materials_5day inhalation study*

Administrative Data

Study result type experimental result **Study period** 2006-2009

Reliability other: needs to be evaluated & set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NanoCare project partners	2009	NanoCare Health related Aspects of Nanomaterials Scientific Report (http://www.nanopartikel.info/files/content/dana/Dokumente/NanoCare/Publikationen/NanoCare_Final_Report)	ISBN: 978-3-89746-108-6					

Data access

data published

Data protection claimed

yes, but willing to share

Materials and methods

Test type

standard acute method

Limit test

no

Principles of method if other than guideline

In order to investigate the potential effects of CeO₂ nanomaterials upon inhalation, a 5-day inhalation study was performed. A short-term inhalation study was developed as the standard inhalation method to assess the toxicity of aerosols from nanomaterials. A short-term study is an adequate and

sufficient method to determine the toxicity of inhaled particles based on the hypothesis, that the main effects in the lung are inflammation and / or cytotoxicity. Male Wistar rats were exposed to CeO₂ nanomaterials (CeO₂, 70 nm, BASF and 5%-Al doped CeO₂, Evonik) for 6 hours per day on 5 consecutive days. Concurrent control groups were exposed to conditioned air. Animals were sacrificed either shortly after the last exposure or after a recovery period. Cell proliferation was examined at study day 5, directly after the last exposure. Analysis of BALF and histological examinations were performed 3 days after the last exposure. The CeO₂ material was tested at target concentrations of 0.5, 2.5 and 10 mg/m³. The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material form

nanomaterial

Details on test material

The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂. In order to investigate the potential effects of CeO₂ nanomaterials upon inhalation, in the NanoCare project 2 different CeO₂ nanomaterials (CeO₂, 70 nm, BASF and 5%-Al doped CeO₂, Evonik) were used in a short term inhalation study. For further material information please refer to the attachment below. NanoCare No. Material Powder Primary particle size [nm] Remarks Provider 3.5 CeO₂ X 70 - BASF details on the test materials investigated within the NanoCare project:- 4.24 agglomeration/aggregation: method: In Air: Test for agglomerate stability according to the publication by Stahlmecke et al. in J. Nanopart Res (2009) 11:1625 –1635 and Nanocare Final Scientific Report for 5 modifications of CeO₂ In Liquid: Analytical Ultracentrifugation (AUC), in review: Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, ACS Nano-4.8 Water Solubility/Dispersibility: protocol: Initial concentration 10 mg/ml, 24h agitation at 300 rpm; centrifugation and supernatant analysis - 4.25 Crystalline phase: method: XRD-4.31 Dustiness: Test for powder dustiness in accordance to EN 15051. The continuous drop method, modified to adjust for nanoscale particle measurements, was employed. Published in: Nanocare Final Scientific Report for 5 modifications of CeO₂- representative TEM picture: in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano), see also attached material information sheet below-4.28 Specific surface area: BET BASF SE 33 m²/g-4.29 zeta potential: method: measurements with Zetasizer Nano ZS from Malvern Instruments (DLS and Zeta potential) //results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano) -4.30 Surface chemistry: measurements with XPS and TOF-SIMS//results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano)

Test animals

Species

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions

Exposure to CeO₂ nanomaterials (CeO₂, 70 nm, BASF and 5%-Al doped CeO₂, Evonik) for 6 hours per day on 5 consecutive days. Concurrent control groups were exposed to conditioned air. Age at supply: about 7 weeks; start of preflow: about 8 weeks; start of exposure: about 9 weeks

Administration / exposure**Route of administration**

inhalation: dust

Type of inhalation exposure

nose/head only

Vehicle

other: unchanged (no vehicle)

Details on inhalation exposure

For further information on test material administration please refer to the SOP attached below.

Duration of exposure

6 h Remarks on 5 consecutive days

Concentrations

The CeO₂ materials were tested at target concentrations of 0.5, 2.5 and 10 mg/m³.

No. of animals per sex per dose

Test groups: Lavage: 5 animals per concentration and time point; Pathology and histopathology: 3 animals per concentration and time point; Lung burden: 3 animals per concentration and time point

Control animals

yes

Details on study design

The parameter to be examined and the exact time points of the examinations are presented in the figure below: Study day 1 2 3 4 5 6 7 8 9-27 28x x x x R R R R E L E+Lx: head/nose exposure; R: recovery period; E: lung burden, gross necropsy, organ weights, preservation of organs and tissues for histopathology; L: broncho alveolar lavage (BAL). For further information on test method please refer to the SOP attached below.

Results and discussions**Other findings**

Organ burden: The organ burden was evaluated three days after exposure of CeO₂ (70 nm, BASF material): Test materials were only detected in lungs and lung associated lymph nodes. In the liver, kidney, spleen and basal brain with olfactory bulb, no nanomaterial could be detected (detection limit for Ce 0.37 µg/tissue). Mean values of organ burden of CeO₂ in the lung were 51.9 µg (target concentration of 0.5 mg/m³), 165.6 µg (target concentration of 2.5 mg/m³) and 417.2 µg (target concentration of 10 mg/m³), respectively (mediastinal lymph nodes for all target concentrations: < 0.37 µg). BALF parameters: Changes of selected BALF parameters (LDH = Lactate dehydrogenase, ALP = Alkaline phosphatase, LYMPH = lymphocytes, NAG = NAcetylamino glucosaminidase, GGT = γ-

Glutamyltransferase, MPH = Macrophage, PMN = Polymorph nuclear neutrophilic granulocytes) were found upon inhalation of all concentrations of both tested CeO₂ nanomaterials. Severity of changes increased with raised concentration. Inflammation markers were increased even at the lowest concentration (0.5 mg/cm³).

Overall remarks, attachments

Remarks on results including tables and figures

The project NanoCare was funded by the Germany ministry of education and research within the programme NanoCare1 and comprised of 15 partners from industry, universities and research institutes (running from 2006 through 2009). In NanoCare 10 different synthetic nanomaterials in altogether 31 modifications (size, surface modification, pH) were investigated with regard to the potential impacts of nanomaterials on human health. For this aim new applications and measurement methods were developed and several in vivo and in vitro methods were employed. Two different CeO₂ nanomaterials (CeO₂, 70 nm, BASF and 5% Al-doped CeO₂, Evonik) were used in 5-day inhalation studies, in which lung doses of approximately 0.05 mg, 0.17 mg and 0.42 mg were found for CeO₂ BASF material. Signs of inflammation such as increased PMN counts occurred after 3 days at the lowest concentration, pointing to a high biologic activity. First results showed that effects of doped CeO₂ on differential cell counts were even more pronounced.

Attached background material

Attached document	Remarks
NM_211_ANNEX_A61_material information sheets NanoCare CeO ₂ .pdf / 1.28 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN1	
NM_211_ANNEX_A62_SOP_Inhalation.pdf / 48.55 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	

Endpoint study record: Acute toxicity: inhalation_CeO₂ NanoCare materials_intratracheal installation

Administrative Data

Study result type experimental result **Study period** 2006-2009
Reliability other: needs to be evaluated and set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NanoCare project partners	2009	NanoCare Health related Aspects of Nanomaterials Scientific Report (http://www.nanopartikel.info/files/content/dana/Dokumente/NanoCare/Publikationen/NanoCare_Final_Re)	ISBN: 978-3-89746-108-6					

Data access

data published

Data protection claimed

yes, but willing to share

Materials and methods**Test type**

other: Intratracheal installation

Limit test

no

Principles of method if other than guideline

In order to investigate the potential effects of CeO₂ nanomaterials upon inhalation, intratracheal installation studies were performed. The intratracheal instillation (ITI) of particles is a method to test for adverse toxic effects of respirable particles. In contrast to the weeks-lasting deposition of particulate matter necessary to achieve an appropriate lung burden during inhalation experiments, responses of the lung are challenged by a single bolus application of defined doses. It should be pointed out that as an indispensable precondition the ITI exposure must be given in the sub-overload domain, which is determined by the volumetric load of the present alveolar macrophages in the experimental animal. Though the ITI is often regarded as nonphysiological because the applied particles are deposited in a more centralized manner, the technique appears advantageous if precise dosing and/or short observation times are required, e.g. in case of comparative in vitro studies. Investigated endpoints were BALF parameters (differential cell counts, protein, fibronectin and TNF α activity), as well as histological examinations of the lung to determine lung fibrosis (Sirius Red staining), proliferation (Ki67 antigen), mutation rate (p-53 protein), and DNA adducts (8-oxo-Guanine). The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂.

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

no

Test material form

nanomaterial

Details on test material

The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂. In order to investigate the potential effects of CeO₂ nanomaterials upon intratracheal installation, in the NanoCare project 2 different CeO₂ nanomaterials (CeO₂, 14 nm, pH 6.2, Evonik and 5%-Al doped CeO₂, Evonik) were used. For further material information please refer to the attachment below. NanoCare No. Material Powder Primary particle size [nm] Remarks Provider
 3.1 CeO₂-A X 14 pH 6.2 Evonik
 details on the test materials investigated within the NanoCare project:-
 4.24 agglomeration/aggregation: method: In Air: Test for agglomerate stability according to the publication by Stahlmecke et al. in J. Nanopart Res (2009) 11:1625 –1635 and Nanocare Final Scientific Report for 5 modifications of CeO₂
 In Liquid: Analytical Ultracentrifugation (AUC), in review: Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, ACS Nano-4.8 Water Solubility/Dispersibility: protocol: Initial concentration 10 mg/ml, 24h agitation at 300 rpm; centrifugation and supernatant analysis - 4.25 Crystalline phase: method: XRD-4.31 Dustiness: Test for powder dustiness in accordance to EN 15051. The continuous drop method, modified to adjust for

nanoscale particle measurements, was employed. Published in: Nanocare Final Scientific Report for 5 modifications of CeO₂- representative TEM picture: in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano), see also attached material information sheet below-4.28 Specific surface area: BET Evonik A: 63m²/g-4.29 zeta potential: method: measurements with Zetasizer Nano ZS from Malvern Instruments (DLS and Zeta potential) //results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano) -4.30 Surface chemistry: measurements with XPS and TOF-SIMS//results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano)

Test animals

Species

rat

Strain

Wistar

Sex

female

Details on test animals and environmental conditions

CeO₂-A (14 nm, Evonik) or Al-doped CeO₂ (5%, Evonik) were intratracheally instilled into the lungs of female Wistar rats (200-250 g) by a single bolus application of defined doses. Animals were kept at a 12 hour light-dark-cycle. Food and water were accessible ad libitum. Particles were suspended in the instillation fluid, vortexed and suspended by brief ultrasonication shortly before application. Negative controls received instillation fluid; quartz DQ12 (1.2 mg per rat lung), a strongly fibrogenic dust, was used as positive control.

Administration / exposure

Route of administration

other: intratracheal installation

Type of inhalation exposure

other:

Vehicle

other: unchanged (no vehicle)

Duration of exposure

Remarks single bolus application

Concentrations

First set of experiments (CeO₂-A (14nm, Evonik) and 5% doped CeO₂ (Evonik)): 0.6 and 4.8 mg per lung
second set of experiments (5% doped CeO₂ (Evonik)): 0.6, 1.2, 2.4 and 4.8 mg per rat lung

No. of animals per sex per dose

10 animals per group and time point

Control animals

yes

Details on study design

Negative controls received instillation fluid; quartz DQ12 (1.2 mg per rat lung), a strongly fibrogenic dust, was used as positive control. A first set of experiments with a low and a high dose of particles (0.6 and 4.8 mg per lung) using 10 animals per group and time point was performed. After 3 days, 5 animals were sacrificed for BALF analysis (differential cell counts, protein, fibronectin and TNF α activity); the remaining animals were used for histological examination of the lung to determine lung fibrosis (Sirius Red staining), proliferation (Ki67 antigen), mutation rate (p-53 protein), and DNA adducts (8-oxo-Guanin). In a second set of experiments, 5% Al-doped CeO₂ was selected with a multi-dose and multi-time approach and was intratracheally instilled into the lung of femal Wistar rats at doses of 0.6, 1.2, 2.4 and 4.8 mg per rat lung. Effects of this material were tested after observation periods of 21 and 90 days. Observed endpoints were BALF parameter (total cell count, number of macrophages, polymorph nuclear cells, total Protein (Lowry method), Fibronectin (ELISA) relative to DQ12 control) and histological examination (Ki67, proliferating cells relative to DQ12 control).

Results and discussions

Other findings

First set of experiments with a low and a high dose of particles (0.6 and 4.8 mg per lung) and 3 days of observation time: CeO₂ materials induced a dose-dependent increase of inflammatory cells (PMN, polymorphonuclear neutrophils) of BALF protein, fibronectin and epithelial cell proliferation. It is important to note that none of the materials exceeded the inflammatory potential of the positive control quartz DQ12 (1.2 mg/ lung), even when applied at 4-fold higher concentration. Second set of experiments using 5% Al-doped CeO₂ at doses of 0.6, 1.2, 2.4 and 4.8 mg per rat lung and observation periods of 21 and 90 days: The doped CeO₂ elicited strong effects after instillation even at a concentration of 0.6 mg and after 21 days. Interestingly, there was no dose-dependency at this time point. However, after 90 days dose-dependency was detected for nearly all parameters (for parameters please refer to "details on study design") investigated. This type of CeO₂ showed a comparatively high toxicity since its NOEL was below the lowermost concentration of 0.6 mg instilled per lung. Even 0.6 mg CeO₂ elicited severe focal epithelial damage indicated also by enhanced protein release and, after 21 days, increased levels of protein and fibronectin in BALF which exceeded those of the time-matched quartz DQ12 value (1.2 mg per lung were used as positive control). After 90 days and at concentrations larger than 0.6 mg all inflammation markers were still elevated. Histological analysis (Sirius Red staining) showed progressive fibrosis diffusely scattered throughout the lung parenchyma. These findings exemplify that observation periods of 90 days are necessary to adequately describe adverse effects of particles in lung. Furthermore, the surface reactivity of a given nanoparticle may be involved into biological processes in a complex and unforeseeable manner and thus deserves particular attention.

Overall remarks, attachments

Remarks on results including tables and figures

The project NanoCare was funded by the Germany ministry of education and research within the programme NanoCare1 and comprised of 15 partners from industry, universities and research institutes (running from 2006 through 2009). In NanoCare 10 different synthetic nanomaterials in altogether 31 modifications (size, surface modification, pH) were investigated with regard to the potential impacts of nanomaterials on human health. For this aim new applications and measurement methods were developed and several in vivo and in vitro methods were employed. In the instillation study the Al-doped modification of CeO₂ was used due to better dispersion characteristics. This type of CeO₂ showed a comparatively high toxicity since its NOEL was below the lowermost concentration of 0.6 mg instilled per lung. Even 0.6 mg CeO₂ elicited severe focal epithelial damage indicated also by enhanced protein

release and, after 21 days, increased levels of protein and fibronectin in BALF which exceeded those of the time-matched quartz DQ12 value (1.2 mg per lung were used as positive control). After 90 days and at concentrations larger than 0.6 mg all inflammation markers were still elevated. Histological analysis (Sirius Red staining) showed progressive fibrosis diffusely scattered throughout the lung parenchyma.

Attached background material

Attached document	Remarks
NM_211_ANNEX_A61_material information sheets NanoCare CeO2.pdf / 1.28 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	

7.2.3 Acute toxicity: dermal

7.2.4 Acute toxicity: other routes

7.3 Irritation / corrosion

7.4 Sensitisation

7.5 Repeated dose toxicity

7.5.1 Repeated dose toxicity: oral

7.5.2 Repeated dose toxicity: inhalation

Endpoint study record: 2011-08-04 RIVM Subacute inhalation toxicity study with Ceria Dry CeO2 in rats

Administrative Data

Study result type experimental result Study period 10 February 2010 - 15 April 2010

Reliability other: needs to be evaluated and set by expert researchers group

Rationale for reliability incl. deficiencies GLP-study

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	W.R. Pels Rijcken, I.L. Tuinman, H. Muijser	2011	A sub-acute (28-day) inhalation toxicity study with Ceria Dry CeO2 in rats		TNO Triskalion	V8781	RIVM, Bilthoven, The Netherlands		2011-07-29

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Test type**

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	yes

Principles of method if other than guideline

The following deviations from the study plan occurred:- Instead of 50 liters, the nose-only inhalation chamber had a volume of ca. 75 L (exposed animals) or 66 L (control animals).- The exposure units for the control and exposed groups both included three rodent tube sections, instead of two (control group) or four (exposed group) sections.- Number concentrations in the exposure chamber were measured daily with a CPC in addition to gravimetric analysis.- Lungs were rinsed twice with a volume of 26.7 ml saline per kg body weight instead of with a single volume. The actual volume was calculated using the group mean body weight per sex instead of individually per animal. The supernatant of the first volume was used for biochemical determinations. The two cell pellets were each resuspended in 0.25 mL and combined.- The duties of Mr M. Vermeulen were delegated during his absence from 12 June to 5 July 2010 to Mr F. van Schaik.- The cerium content of blood and organs except the lungs were measured with ICP-MS (inductively coupled plasma mass spectrometry), a more sensitive method than ICP-AES (inductively coupled absorption emission spectrometry).- The lungs of the animals of the intermediate groups were not histopathologically investigated. The lungs of the recovery group were histopathologically investigated.- Urine samples were stored at $\leq -18^{\circ}\text{C}$. In 4.6.13 of the study plan it was erroneously stated that urine samples would be stored at -20°C .- In the animal rooms, minor deviations from the optimal range for relative humidity (45-65%) were observed during the study period.- Inadvertently, weekly cascade impactor measurements were not performed during the study. Instead a cascade impactor measurement was performed (in duplicate) at a later date. - On 22 February 2010, the animals of group 2 were exposed inadvertently for a period of 35 minutes instead of 40 minutes. - In one animal of group 4 blood was sampled for kinetics 48 hours (instead of 66 h) after the 20th exposure. These deviations are considered not to have affected the validity of the study.

GLP compliance

yes (incl. certificate)

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

yes

Test material form

nanomaterial

Details on test materialName of test material (as cited in study report): Ceria Dry CeO₂- Other name : Ceric oxide, Ceria- Molecular formula: CeO₂

- Molecular weight: 172.11
- Smiles notation: - InChI:
- Structural formula:
- Substance type:
- Physical state: solid
- Density : 7.13 g/ml at 25°C
- Total quantity : 1020 gram
- Primary particle size: 5-10 nm
- Analytical purity: 99.5% (by weight)
- Impurities (identity and concentrations):
- Composition of test material, percentage of components:
- Isomers composition:- Purity test date:
- Lot/batch No.:- Date of receipt: 4 December 2009
- Expiration date of the lot/batch: 4 December 2010
- Stability under test conditions:
- Storage condition of test material: ambient temperature (15-25°C)
- NPL Ref nos. : CA340#37, CA340#43, CA340#59
- Supplier : Antaria
- TNO reference number: 09015D

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Young adult, male and female Wistar rats (CrI:WI(WU), outbred) were obtained from a colony maintained under SPF-conditions by Charles River Deutschland, Sulzfeld, Germany.
- Age at study initiation: 7-9 weeks
- Weight at study initiation: Mean body weights male animals 227 g and female 167 g
- Fasting period before study:
- Housing: The animals were housed in macrolon cages (3-5 animals/cage separated by group and by sex) with a bedding of wood shavings (Lignocel, Type ¾) and strips of paper (Enviro-dri) as environmental enrichment. During exposure periods, the rats were individually housed in the exposure unit.
- Diet (e.g. ad libitum): Rat & Mouse No. 3 Breeding Diet, RM3; supplier SDS Special Diets Services, Witham, England; batch nos. 7264 (pellets during acclimatization), 7327 and 7388 (powder during study period)
- Water (e.g. ad libitum): domestic mains tap-water suitable for human consumption (quality guidelines according to Dutch legislation based on EC Council Directive 98/83/EC)
- Acclimation period: 5 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 20-24°C
- Humidity (%): 45-65%.

- Air changes (per hr): 10
- Photoperiod (hrs dark / hrs light): 12/12IN-LIFE DATES: From: 10 February 2010 To: 18 March 2010

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

nose only

Vehicle

other: unchanged (no vehicle)

Details on inhalation exposure

GENERATION OF TEST ATMOSPHERE / CHAMBER DESCRIPTION

- Exposure apparatus: The animals were exposed to the test atmosphere in nose-only exposure units consisting of a cylindrical column, surrounded by a transparent hood. The test atmosphere was introduced at the top of the central column, and was exhausted at the bottom of the ca. 75 L (exposed groups) or 66 L (control group) exposure unit. Each column included three* rodent tube sections of 20 ports each for animal exposure. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. Empty ports were used for test atmosphere sampling and measurement of temperature and relative humidity. The remaining ports were closed. Only the nose of the rats did protrude into the interior of the column.
- Method of holding animals in test chamber: the rats were individually housed in the exposure unit. The rats of the various groups were placed in Battelle tubes
- Source and rate of air:
- Method of conditioning air:
- System of generating particulates/aerosols: The inhalation equipment is designed to expose the rats to a continuous supply of fresh test atmosphere. To generate the test atmosphere, the test material was aerosolized using a dust feeder, a venturi and a jetmill. The generated test atmosphere was directed to the top inlet of the exposure unit and at the bottom of the chambers the test atmospheres was exhausted.
- Temperature, humidity, pressure in air chamber: $22 \pm 2^{\circ}\text{C}$, 30-70%
- Air flow rate: The air flow for the control group was 45.8 L/min. The air flow for the exposed groups was approximately 90 L/min.
- Air change rate:
- Method of particle size determination: Particle size was measured daily by an SMPS (TSI Inc, Shoreview MN, USA) and an APS (TSI Inc, Shoreview MN, USA). Inadvertently, weekly cascade impactor (Anderson, 11-stage) measurements were not performed during the study. Instead a cascade impactor measurement was performed (in duplicate) at a later date.

TEST ATMOSPHERE

- Brief description of analytical method used: Mass concentrations were monitored using a Dusttrak aerosol monitor (TSI Inc, Shoreview MN, USA). The registrations were used as a monitor only, were not made available digitally, and are therefore not reported.
Number concentrations were measured for at least 45 minutes per day with a CPC (condensation particle counter; TSI 3022A).
- Samples taken from breathing zone: yes, the actual concentrations of the test material in the atmospheres were measured at least three times daily by gravimetric analysis.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

CONCENTRATION ANALYSES

The average mass concentrations ($\pm$ standard deviation) for groups 2, 3 and 4 as measured by gravimetric analysis were 10.9 ($\pm$ 1.8), 10.6 ($\pm$ 1.3) and 10.8 ($\pm$ 0.8) mg/m³, respectively. Equivalent 6 hour exposure concentrations are 1.2, 3.5 and 10.8 mg/m³, respectively.

AIR FLOWS

The air flow for the control group was 45.8 L/min. The air flow for the exposed groups was approximately 90 L/min. Analysis of the number concentration using a CPC showed an average particle concentration ($\pm$ standard deviation) of 1.75×10^6 ($\pm$ 0.38×10^6) particles/cm³

PARTICLE SIZE MEASUREMENTS

Particle size as measured in duplicate by cascade impaction after the end of the exposure period was 1.2 and 1.0 μ m (MMAD, mass median aerodynamic diameter). The corresponding geometric standard deviation of the distribution of particle sizes was 2.7 and 3.8, respectively. According to the APS measurements the MMAD (mass median aerodynamic diameter) was 1.0 μ m with a geometrical standard deviation (GSD) of the distribution of 1.8 and the NMAD (number median aerodynamic diameter) was 0.77 μ m with a GSD of 1.3. The SMPS measures the mobility diameter of the smallest particles, i.e. 0.015 μ m to 0.5 μ m. The SMPS measurements of this size range show an MMD (mass median diameter) of 0.28 μ m and a NMD (number median diameter) of 0.21 μ m.

PARTICLE SHAPE

Figures 1.1 and 1.2 show SEM photographs of the test material on glass fibers of the filter. The material consists of aggregates with primary particles of roughly 10-15 nm. Very small single particles are also visible. BET surface area of the Ceria Dry CeO₂ used for this study was 63.95 ± 0.30 m²/g. For N spherical particles with a radius R the mass is $\frac{4}{3} \pi N R^3$, in which sm is the specific mass (7.13 10⁶ g/m³). The surface area is $4 \pi N R^2$. Hence the (surface area) / mass is $4 \pi N R^2 / (\frac{4}{3} \pi N R^3) = 3/(sm R)$. Therefore, $R = 3/(63.95 \times 7.13 \times 10^6) = 0.0066 \times 10^{-6}$ m, or 6.6 nm. This is consistent with a primary particle size of 13 nm.

Duration of treatment / exposure

5 days/week during a 28 day period

Frequency of treatment

20 exposure days

MMAD / GSD

1.0 - 1.2 μ m GSD 2.7 - 3.8

No. of animals per sex per dose

Group	Group	Duration	CeO ₂	Number animals	Number animals	No. description	daily exposure
(particles/cm ³)	(main study)	(recovery study)	1	Control	6 hours	0 (clean air)	5 ♂ and 5 ♀
Low	40 min	1.5×10^6	5 ♂ and 5 ♀	3	Mid	2 hours	1.5×10^6
and 5 ♀	5 ♂ and 5 ♀			4	High	6 hours	1.5×10^6
				5 ♂ and 5 ♀			

Details on study design

Dose selection rationale:

- Rationale for animal assignment (if not random):
- Rationale for selecting satellite groups:
- Post-exposure recovery period in satellite groups:
- Section schedule rationale (if not random):

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS:

YesTime schedule: Animals were observed daily in the morning hours by cage-side observations and, if necessary, handled to detect signs of toxicity. A group-wise observation was made halfway through each exposure day. On working days, all cages were checked again in the afternoon, especially for dead or moribund animals. In weekends and on public holidays only one check per day was carried out. All abnormalities, signs of ill health, reaction to exposure and mortality were recorded.

DETAILED CLINICAL OBSERVATIONS:

YesTime schedule: Animals were observed daily in the morning hours by cage-side observations and, if necessary, handled to detect signs of toxicity. A group-wise observation was made halfway through each exposure day. On working days, all cages were checked again in the afternoon, especially for dead or moribund animals. In weekends and on public holidays only one check per day was carried out. All abnormalities, signs of ill health, reaction to exposure and mortality were recorded.

BODY WEIGHT: Yes

- Time schedule for examinations: The body weight of each main and recovery study animal was recorded one day before the start of exposure (day -1), prior to exposure on the first day (day 0) and weekly thereafter. All animals were weighed on the day before overnight fasting and on their scheduled sacrifice date in order to calculate the correct organ to body weight ratios. In addition, body weights were also determined in the selected males immediately before blood sampling for kinetics. The body weight of the males of the kinetic group was recorded once before start of the study (day -1) together with the main and recovery animals and on the day(s) of blood sampling and/or sacrifice. The latter body weights were determined immediately before blood sampling, but after any exposure on that day.

FOOD CONSUMPTION:

Food consumption of animals was measured per cage by weighing the feeders. The consumption was measured over three 7-day periods, starting on the day of first exposure, followed by a 6-day period for the animals of the main groups. Food consumption of the animals of the recovery groups was measured over seven 7-day periods, starting on the day of first exposure, followed by a 6-day period.

FOOD EFFICIENCY: NOWATER CONSUMPTION:

NoOPHTHALMOSCOPIC EXAMINATION:

NoHAEMATOLOGY:

Yes Haematology was conducted at the end of the treatment period on all five animals/sex/group of the main groups. Blood samples were taken from the abdominal aorta of overnight fasted rats whilst under pentobarbital anaesthesia at necropsy. K3-EDTA was used as anticoagulant. In each sample the following determinations were carried out according to the methods:- haemoglobin- packed cell volume- red blood cell count- reticulocytes - total white blood cell count- differential white blood cell count- prothrombin time- thrombocyte count. The following parameters will be calculated:- mean corpuscular volume (MCV)- mean corpuscular haemoglobin (MCH)- mean corpuscular haemoglobin concentration (MCHC). Since some of these parameters showed a treatment-related change at the end of the exposure period of the main

groups, the haematology parameters were also determined for all animals of the recovery study at the end of the recovery period.- Time schedule for collection of blood: at the end of the treatment period - Anaesthetic used for blood collection: Yes, pentobarbital- Animals fasted: Yes, 1 night- How many animals: 5/sex/group

CLINICAL CHEMISTRY:

Yes. Clinical chemistry was conducted at the end of the exposure period on all animals of the main study at the same time blood samples for haematology were collected. The blood was collected in heparinized plastic tubes and plasma was prepared by centrifugation. The following standard measurements were made in the plasma:alkaline phosphatase activity (ALP)aspartate aminotransferase activity (ASAT)alanine aminotransferase activity (ALAT)gamma glutamyl transferase activity (GGT) total proteinalbuminratio albumin to globulinureacreatininefasting glucosebilirubin totalcholesteroltriglyceridesphospholipidscalcium (Ca)sodium (Na)potassium (K)chloride (Cl)inorganic phosphateBecause of the absence of treatment related changes at the end of the exposure period, clinical chemistry was not conducted in recovery animals at the end of the recovery period.In addition to the standard parameters mentioned above, the following fibrinolysis parameters were measured:- Von Willebrand factor (vWF)- Plasminogen activator inhibitor-1 (PAI-1)- FibrinogenVon Willebrand Factor (vWF) and plasminogen activator inhibitor 1 (PAI-1) were analyzed using Multiplex analysis; a bead-based ELISA on multiple analytes (see Annex 8 for details).Fibrinogen was analyzed using a highly sensitive two-site enzyme linked immunoassay.- Time schedule for collection of blood: at the end of the treatment period- Animals fasted: Yes, 1 night- How many animals: 5/sex/group

URINALYSIS:

Yes. Oxidative stress measurements in urineOn the day before scheduled necropsy, urine was collected overnight (approximately 16 hours) from all main and recovery group rats. During urine collection the animals were housed individually and were deprived of food, but water was available. Urine volume and creatinine content were measured to standardize concentrations in urine. Thereafter, urine samples were stored (at <- 18°C) until determination of 8-hydroxy-2'-deoxyguanosine as a representative oxidative stress parameter.8-hydroxy-2'-deoxyguanosine (8-oxodG) in urine was analyzed by diluting the sample with formic acid, containing internal standard (8-oxodG-15N5). After this dilution, 8-oxodG was determined using UPLC-MS/MS.- Time schedule for collection of urine: at the end of the treatment period on the day before necropsy- Metabolism cages used for collection of urine: - Animals fasted: Yes , 16 hours

NEUROBEHAVIOURAL EXAMINATION: No

BRONCHOALVEOLAR LAVAGE AND MEASUREMENTS

At necropsy, the lungs were lavaged according to a standardized method. In short: the right half of the lungs (after binding off the left lung lobe) were rinsed twice with a volume of 26.7 ml saline per kg body weight (calculated using the group mean body weight per sex). Each volume was injected and withdrawn three times before collection of the lavage fluid. The two aliquots of lavage fluid, containing the lung lining fluid and cells, were collected in separate tubes and retained on ice until further processing. The bronchoalveolar lavage cells were separated from the supernatant by centrifugation (250xG) for 5 minutes at 4°C. The supernatant from the first lung lavage was used for biochemical determinations, whereas that of the second lavage was discarded. Each cell pellet was resuspended in 0.25 mL saline, combined in one tube per animal and subsequently used for determination of viability and total and differential white blood cell numbers.Biochemical determinationsIn the supernatant of the lavage fluid, total protein, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), N-acetylglucosaminidase (NAG), gamma-glutamyltransferase (γ -GT) and superoxide dismutase (SOD) were determined according to the methods listed in Annex 4. In addition, malondialdehyde (MDA) in bronchoalveolar lavage fluid (BALF) was analyzed by derivatizing MDA with thiobarbituric acid and extracting the formed product with n-butanol. The MDA-derivate was determined using HPLC with fluorescence detection (FLD). Details are presented

in Annex 8. Cellular determinations Total white blood cell numbers were counted using an automated haematology analyser (K-800, Sysmex, Toa, Kobe, Japan). The percentages of viable cells were determined using an acridine orange / ethidium bromide staining method in combination with fluorescent microscopic evaluation. Differential white blood cells were determined by counting 200 cells under the microscope.

Sacrifice and pathology

GROSS PATHOLOGY: Yes

Gross examination At scheduled necropsy, the animals were killed in such a sequence that the average time of killing was approximately the same for each group. The animals of the main study were killed on the day after the last exposure. The animals of the recovery groups were killed at the end of the 28-day observation period following the last exposure. Animals were killed by exsanguination from the abdominal aorta under pentobarbital anaesthesia and then examined grossly for pathological changes. The brain was removed intact, weighed and immediately frozen in liquid nitrogen and stored at $< -18^{\circ}\text{C}$ thereafter. The brains were transferred to the sponsor for further analysis. The analysis and reporting of the results are the responsibility of the sponsor and are not part of the present study. Samples of the following organs and tissues were preserved in a neutral aqueous phosphate-buffered 4 per cent solution of formaldehyde (10% solution of formalin). The lungs (after weighing) were infused with the fixative under ca. 15 cm water pressure to insure fixation. The bolded organs were trimmed and weighed wet as soon as possible after dissection to avoid drying. Adrenals Heart Kidneys Larynx (3 levels, 1 level to include the base of the epiglottis) Liver Lung (left lobe at three levels, including main bronchi) Tracheobronchial lymph nodes Nasopharyngeal tissues (6 levels) Spleen Trachea (2 levels including 1 longitudinal section through the carina and 1 transverse section) All gross lesions

HISTOPATHOLOGY: Yes

For histopathological examination, the tissues to be examined were embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin. All preserved tissues of all animals of groups 1 and 4 of the main study were examined histopathologically (by light microscopy). In addition, all relevant gross lesions observed in rats of the intermediate concentration groups were examined microscopically. The nose was examined at 6 levels (Woutersen et al., 1994), the larynx at 3 levels, the trachea at 3 levels (including the bifurcation), and the left lung lobe at 3 levels. Some mild treatment-related changes were observed in the lungs in animals of group 4. Hence, examination of the lungs was performed on recovery groups 1 and 4 but not on animals of groups 2 and 3 of the main study.

Statistics

The statistical procedures for analysis of data are described below.

- Body weight data, clinical pathology data measured on continuous or semi-continuous scales, and organ weights: data were analysed using one-way analysis of variance (Anova), after checking for homogeneity of variance (Bartlett test) and normality of data distribution (Shapiro-Wilks test). If variances were not homogeneous or data not normally distributed, the data were stepwise log or rank transformed prior to the Anova. If the Anova yielded a significant effect ($p < 0.05$), intergroup comparisons with the control group were made by Dunnett's multiple comparison test.
- Food consumption: no statistics were applied on food intake (only one cage per sex).
- Incidences of histopathological changes: Fisher's exact probability test. Arithmetic means and standard errors of the means or standard deviations are given in the reports of continuous and semi-continuous data. Tests were performed as two-sided tests with results taken as significant where the probability of the results was $p < 0.05$ (*) or $p < 0.01$ (**). Because numerous variables were subjected to statistical analysis, the overall false positive rate (Type I errors) was greater than suggested by a probability level of 0.05. Therefore, the final interpretation of results were based not only on statistical analysis but also on other considerations such as dose-response relationships and whether the results were significant in the light of

other biological and pathological findings.

Results and discussions

Results of examinations

Clinical signs and mortality

yes

Body weight and weight gain

yes

Food consumption

yes

Food efficiency

not examined

Water consumption

not examined

Ophthalmoscopic examination

not examined

Haematology

yes

Clinical chemistry

yes

Urinalysis

yes

Neurobehaviour

not examined

Organ weights

yes

Gross pathology

yes

Histopathology: non-neoplastic

yes

Details on results**CLINICAL SIGNS AND MORTALITY**

There were no clinical signs observed in any of the animals in any of the dose groups or mortality during the 4 weeks exposure period and the 4 weeks recovery period that were considered to be related to treatment.

BODY WEIGHT AND WEIGHT GAIN

Normal growth was observed in the male and female rats of all dose groups in the course of the study. Group mean body weights were comparable and no statistical significant differences were observed between the dose groups during the exposure and recovery period.

FOOD CONSUMPTION

Overall food consumption was considered similar among the groups in both sexes during the exposure as well as the recovery period.

FOOD EFFICIENCY

not examined

WATER CONSUMPTION

not examined

OPHTHALMOSCOPIC EXAMINATION

not examined

HAEMATOLOGY

Main groups: At the end of the exposure period, the red blood cell and coagulation parameter values in the Ceria Dry CeO₂ exposed animals were similar and no statistically significant differences were observed when compared with those in the control animals. No changes were observed in the total number of white blood cells, but a few changes were apparent in differential white blood cell counts in Ceria Dry CeO₂ exposed animals compared to control animals:- A dose related increase in the absolute and relative number of neutrophils in males, achieving levels of statistical significance for the relative number of neutrophils in the mid and high dose males only. A statistically significantly increased absolute number of neutrophils in the low and mid dose females. The absolute number of neutrophils in the high dose females was also increased, but did not reach a level of statistical significance when compared to the controls.- A dose related decrease in the relative number of lymphocytes in males, likely the result of the increase in relative number of neutrophils in these animals, achieving levels of statistical significance in the mid and high dose males. The relative number of lymphocytes was also decreased in females, but no dose response relationship and statistical significances were apparent. Recovery groups: At the end of the recovery period, no changes were observed in the red blood cell and coagulation parameters and total and differential white blood cell counts that could be related to treatment. The statistical significance noted for the calculated parameter MCHC in the high dose males was considered to have occurred by chance, since the group mean value for MCHC was considered similar to that in controls and was not associated with changes in the values for haemoglobin and packed cell volume in these males. The differences in the number of reticulocytes between the high dose males and females and their respective controls were considered to be incidental findings as the group mean values for reticulocytes in control and high dose animals were within the range observed in this study. A single statistically significant difference was observed in the absolute number of basophils between the high dose females and the controls. This difference was also considered to have occurred incidentally, as the group mean values for basophils in both the controls and high dose females were within the range observed in this study. Therefore, no toxicological significance was attached to the statistically significant differences noted in the

haematology parameters in the recovery animals.

CLINICAL CHEMISTRY

Main study: No changes were observed in any of the clinical chemistry parameters measured at the end of the exposure period that could be related to treatment. A single statistically significant difference was observed for albumin between the high dose males and the controls. Since the group mean value for albumin in the high dose males was only slightly higher than that in the controls and was within the normal range in rats of this strain and age, the statistical significance was considered to have occurred by chance. Therefore, no toxicological significance was attached to this finding. Recovery study: Clinical chemistry was not conducted in recovery animals at the end of the recovery period with respect to the standard parameters, because treatment related findings were not observed in any of the standard clinical chemistry parameters at the end of the exposure period. Significant differences in the non-standard parameters fibrinogen, PAI-1 and vWF between control and high dose group were not seen

URINALYSIS

Overnight urine was collected to measure hydroxydeoxyguanosine as a parameter for DNA damage repair. Creatinine was measured to control for the amount of urine produced. A large variation was observed between the individual animals in the volume of the overnight collected urine. In some cases, the urine volume may have been increased by water spillage by the animal while drinking from the bottle. As expected, the creatinine concentration varied approximately inversely with urine volume. The variability of the concentration of hydroxydeoxyguanosine in urine decreased appreciably when normalized with respect to urinary creatinine. Significant differences between groups were not seen in the main study or in the recovery study.

NEUROBEHAVIOUR

not examined

BRONCHOALVEOLAR LAVAGE

- Total and differential cell counts Main groups: in control animals, the monocytes/macrophages accounted for approximately 99% of the total number of cells recovered from the lungs by lavage. The neutrophils, lymphocytes and eosinophils were present in only very low numbers and varied between 0 and 1.2 % of the total number of cells. In Ceria Dry CeO₂ exposed animals, the total number of cells recovered after lavage of the lungs showed a dose related increase in both males and females, achieving statistical significance in the high dose males and mid and high dose females when compared to controls. The cell viability, however, was not affected by exposure to Ceria Dry CeO₂ and was between 90-95% in all dose groups. The increases in total numbers of cells were caused by increases in absolute numbers of monocytes/macrophages, neutrophils and lymphocytes. The eosinophils, however, seemed unaffected and were almost undetectable in all dose groups. The (relative) increases were not the same for each cell type and also resulted in differences in the relative cell counts between the exposed and control groups. Higher doses of Ceria Dry CeO₂ resulted in increases in the relative number of neutrophils (up to and, to a lesser extent, of lymphocytes and a concurrent decrease in the relative number of monocytes/macrophages. These changes in relative number of cells reached levels of statistical significance for all three cell types in all male and female rats exposed to Ceria Dry CeO₂, except for the lymphocytes in the low dose females. Recovery groups: At the end of the recovery period, the difference in the total number of cells in the lavage fluid between the control and high dose groups had decreased in both sexes when compared to the difference at the end of the exposure period. The decrease in difference was not only due to a recovery from the increased total numbers of cells in the high dose animals, but also due to higher total numbers of cells in the lungs of the controls. The viability of the cells in the lavage fluid was approximately 95%, in both the controls and high dose group animals and was considered similar to the cell viability determined at the end of the exposure period. The increase in total number of cells in the control animals was not accompanied by changes in the proportional presence of different cell types. The percentages of the

various cell types present after the 4-week recovery period were similar to those immediately after the exposure period. In spite of the increase in total cell count, the monocytes/macrophages still accounted for approximately 99% of the cells present in the lavage fluid, whereas the neutrophils, lymphocytes and eosinophils were present in (very) low numbers. In the high dose animals, the absolute numbers of monocytes/macrophages, neutrophils and lymphocytes were decreased at the end of the recovery period when compared to immediately after the exposure period. The numbers of monocytes/macrophages were comparable between high dose and control animals, whereas the numbers of neutrophils and lymphocytes were statistically significantly higher in the high dose males and females when compared to controls at the end of the recovery period. As a result, the relative counts of monocytes/macrophages were still significantly decreased and the relative counts of neutrophils and lymphocytes were still significantly increased, however, less than at the end of the exposure period. - Biochemical determinations Exposure period: The enzyme levels of ALP, GGT, LDH and NAG and protein concentration showed dose related increases in the supernatant of the lavage fluid from the lungs of Ceria Dry CeO₂ expose animals. The group mean values for ALP and protein were statistically significantly different at all dose levels in males and females when compared to controls. Statistically significant differences were also observed for GGT and LDH at all dose levels in females and in males in the mid and high dose groups. The group mean values for NAG were statistically significantly different in the mid and high dose females and high dose males only. The magnitude of the increases in ALP, LDH and NAG were comparable between males and females, whereas slightly higher increases were observed in females in group mean values of GGT and protein compared to the males. Although its variability was appreciable, MDA was significantly increased in male and female animals of the high dose main group. A low group mean value for SOD was observed in high dose females, achieving a level of statistical significance when compared to controls. Relatively high levels of SOD were observed in a few individual males and females among control and Ceria Dry CeO₂ exposed groups, thus affecting the mean values for SOD in these groups. In the absence of a relation to treatment of these findings, they were considered incidental findings and of no toxicological significance. Recovery period: At the end of the 4-week recovery period, the group mean values for ALP, LDH, NAG and protein in the high dose animals were all decreased compared to the values at the end of the exposure period but were still (slightly) higher than in the respective controls. The group mean value for GGT in males was also decreased, but this value in females appeared to be increased at the end of the recovery period. However, the group mean value for GGT in females was affected by an incidental high value in female no 53. The GGT values in the other females of this group were all lower than those observed at the end of the exposure period, indicating that the tendency to a decrease of the GGT levels during the recovery period was also found in high dose females. Statistically significant differences were apparent for the group mean values of ALP and protein for both sexes and for GGT and LDH in high dose females when compared to the controls. MDA seems decreased in male and female animals of the high concentration group, but the decrease was not statistically significant. Large variations were observed in the group mean values for SOD. Since these group mean values were affected by incidental high values for SOD in individual animals (similarly as at the end of the exposure period) no toxicological significance was attached to the differences in SOD levels observed at the end of the recovery period.

ORGAN WEIGHTS

Main study: At the end of the exposure period, dose related increased lung weights in Ceria Dry CeO₂ exposed males and females were observed, achieving levels of statistical significance for the absolute weights in mid and high dose animals and for those relative to body weight in the mid and high dose males and females of all dose groups. No further statistically significant differences were found in organ weights of Ceria Dry CeO₂ exposed animals and controls. Recovery study : At the end of the recovery period, the lung weights of the high dose animals were still increased when compared to those in controls, achieving levels of statistical significance for the absolute as well as the relative weights. However, the magnitude of the differences in lung weights between high dose and control animals at the end of the recovery period was less than at the end of the exposure period. The statistically significant differences

apparent for the group mean weights of the heart (absolute in males and relative in females), kidneys (relative in females) and liver and spleen (relative in males) were considered to be incidental findings and of no toxicological significance.

GROSS PATHOLOGY

- Macroscopic findings Main study: The other gross findings were unremarkable, because they occurred in one or two animals. Recovery study: Macroscopic observation at necropsy revealed pale ('white') and enlarged tracheobronchial lymph nodes in one male and three females of the high-concentration group. One saline control male exhibited a red-coloured tracheobronchial lymph node. Other gross changes were not observed

- Microscopic findings Main study: Microscopic examination revealed exposure-related findings in the lungs and tracheobronchial lymph nodes. Lungs. Brown/greenish particles were observed throughout the lungs of the high-concentration animals, as aggregates and in the cytoplasm of (alveolar) macrophages. In addition, the incidence of slight to moderate accumulations of alveolar macrophages was statistically significantly increased in the high-concentration group. One female exhibited moderate focal alveolitis; granuloma and cholesterol clefts; because of their single occurrence these findings could not be related to the exposure. Tracheobronchial lymph nodes. Accumulations or aggregates of brown/greenish particles were observed in the lymph nodes of the high-concentration animals, presumably within macrophages. All other histopathological findings were considered to be unremarkable. Recovery study: Microscopic examination revealed exposure-related findings in the lungs, and tracheobronchial lymph nodes. Lungs: Patchy accumulation of macrophages containing brown/greenish aggregates were observed throughout the lungs of the high-concentration animals. In addition, increased septal cellularity was observed and the presence of brown/greenish particles in the alveoli. Tracheobronchial lymph nodes: Accumulation of macrophage aggregates containing brown/greenish aggregates were observed in the lymph nodes of the high-concentration groups.

Overall remarks, attachments

Attached full study report

Attached full study report					
NM_211_ANNEX_A63_V8781_final.pdf	/	3.54	MB	(application/octet-stream)	:
ENV/JM/MONO/(2015)8/ANN3					

Applicant's summary and conclusion

Conclusions

The microscopic findings of an increased number of macrophages involved in the clearance of particles from the respiratory tract are considered a physiological reaction and not an adverse effect. Nevertheless, the increased neutrophil content and increased biochemical parameters in broncho-alveolar lavage fluid indicated that exposure to Ceria Dry CeO₂ resulted in inflammation in the lungs at all dose levels. The inflammation, although at a lower level, was still present after a recovery period of 28 days. Hence, the no-observed-adverse-effect level was below the exposure level of 10.9 mg/m³ during 40 minutes/day (equivalent 6-hour exposure concentration is 1.2 mg/m³).

Executive summary

The inhalation toxicity of Ceria Dry CeO₂ was investigated in a sub-acute study in Wistar rats. The main study consisted of four groups of 5 male and 5 female rats each exposed by inhalation to target concentrations of aerosols of 0 (control) or to a number concentration equivalent to the number concentration resulting from a mass concentration of 50 mg/m³ in the earlier study 8779 with cerium oxide micro-sized powder. Animals were exposed on 20 working days in a 28 day period. To vary the dose, animals of the low, mid and high dose groups were exposed for 40 minutes, 2 hours or 6 hours, respectively. Animals were sacrificed the day after the last exposure day. To study the reversibility of the

toxicity, recovery groups also consisting of 5 animals/sex/group were exposed to the control condition and the 6 hours/day (high dose) condition in the same period and kept thereafter for a 4 week recovery period before sacrifice. To study the toxicokinetics of cerium oxide, 12 male animals were sacrificed after exposure for a different number of days to the high dose condition to investigate the content of cerium in a selection of organs. The content of cerium was also investigated in blood obtained from these animals at varying time-points and from animals of the mid and low dose groups. To evaluate the toxicity, data on clinical observations, body weight, food consumption, haematology, clinical chemistry, gross examination at necropsy, organ weight and histopathology of a selection of organs and tissues were examined. The lobes of the right lung were lavaged to determine differential white blood cell count and a selection of biochemical parameters. The mean actual mass concentrations of Ceria Dry CeO₂ for the animals of the low, mid and high dose groups were 10.9 ($\pm$ 1.8; 40 minutes/day), 10.6 ($\pm$ 1.3; 2 h/day) and 10.8 ($\pm$ 0.8; 6 hours/day) mg/m³, respectively. Equivalent 6 hour exposure concentrations are 1.2, 3.5 and 10.8 mg/m³, respectively. The mass median aerodynamic particle size (MMAD) of the particles in the test atmosphere was 1.0 and 1.2 μ m and the corresponding geometric standard deviation of the distribution of particle sizes (GSD) was 2.7 and 3.8. Treatment related clinical signs were not seen. Group differences with respect to body weight or food consumption were not observed. Changes in red blood cell parameters were not seen. The total number of white blood cells was not changed. However, the absolute and relative number of neutrophils were increased and the relative amount of lymphocytes was decreased in male animals of the mid and high dose groups. The absolute number of neutrophils was increased in female animals of the mid and low dose groups. These effects at the end of the exposure period were no longer seen at the end of the recovery period. Other exposure-related effects were not seen. Treatment related changes in clinical chemistry parameters were not seen at the end of the exposure period. Therefore, clinical chemistry was not performed in the animals of the recovery study. Group differences with respect to the concentration of hydroxydeoxyguanosine relative to urinary creatinine were not seen at the end of the treatment period or at the end of the recovery period. The total number of cells was significantly increased in broncho-alveolar lavage fluid (BALf) obtained from male and female animals of the high dose group and females of the mid dose group. This was due to increases in the number of monocytes/macrophages (high dose group), neutrophils (mid and high dose groups and males of the low dose group) and lymphocytes (mid and high dose groups). At the end of the recovery period, the relative and absolute numbers of neutrophils and lymphocytes were increased and the relative number of monocytes/macrophages was decreased in the high dose group but effects were less pronounced. Biochemical determinations of ALP, GGT, LDH, NAG and total protein showed dose dependent increases in these parameters, which were significant in male animals down to the low dose group for ALP and total protein and in female animals down to the low dose group for ALP, GGT, LDH and total protein. MDA was significantly increased in the animals of the high dose group. At the end of the recovery period ALP and total protein were still significantly increased for male animals of the high dose group and ALP, GGT, LDH and total protein were still significantly increased for female animals of the high dose group though at lower levels. At the end of the treatment period, absolute and relative weights of the left lung were increased in male and female animals of the mid and high dose groups and relative weight of the left lung was increased in female animals of the low dose group. At the end of the recovery period absolute and relative weights of the left lung were still increased in male animals of the high dose group but to a lesser degree. Macroscopic examination at necropsy revealed lungs with a gray appearance in three females of the high-concentration group. Microscopic examination of a selection of organs and tissues of the animals of the main control and high dose groups revealed exposure-related findings in the lungs, trachea/bronchi, larynx and tracheobronchial lymph nodes. Brown/greenish particles were observed throughout the lungs of the high-concentration animals, as aggregates and in the cytoplasm of (alveolar) macrophages. In addition, the incidence of slight to moderate accumulations of alveolar macrophages was statistically significantly increased in the high-concentration group. Accumulations or aggregates of brown/greenish particles were observed in the lymph nodes of the high-concentration groups, presumably within macrophages. Microscopic examination of organs and tissues of the animals of the recovery groups revealed exposure related findings in the lungs and tracheobronchial

lymph nodes in the high-dose animals. Patchy accumulation of macrophages containing brown/greenish aggregates were observed throughout the lungs. In addition, increased septal cellularity and the presence of brown/greenish particles in the alveoli were observed. In the tracheobronchial lymph nodes of these animals accumulation of macrophage aggregates containing brown/greenish aggregates were observed.

Endpoint study record: 2012-03-07 RIVM Kinetics of inhaled nano & micro cerium oxide

Administrative Data

Study result type experimental result

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	L. Geraets, A.G. Oomen, J.D. Schroeter, V.A.Coleman, F.R. Cassee	2012	Tissue Distribution of Inhaled Micro- and Nano-sized Cerium Oxide Particles in Rats: Results from a 28-day Exposure Study		TNO Triskalion	draft	RIVM Bilthoven, The Netherlands		

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material form

nanomaterial

Details on test material

Three different sizes of test material were used: SIZE <5000 nm

- Name of test material (as cited in study report): cerium oxide micro-sized powder
- Other names : Cerium(IV) oxide , Ceric oxide, Ceria
- Molecular formula : CeO_2
- Molecular weight : 172.11
- Smiles notation :- InChI :
- Structural formula :
- Substance type :
- Physical state : solid
- Density : 7.13 g/ml at 25°C
- Total quantity : 750 gram
- Particle size : < 5µm
- Analytical purity : 99,5%
- Impurities (identity and concentrations) :
- Composition of test material, percentage of components:
- Isomers composition :
- Purity test date :
- Lot/batch No. :
- Date of receipt : 9-12-2009
- Expiration date of the lot/batch : 4-12-2010
- Stability under test conditions :
- Storage condition of test material : ambient (15-25 °C)
- NPL Ref nos. : CrA250#11, CrA250#24, CrA250#31
- Supplier: Sigma-Aldrich Company Ltd.
- TNO reference number : 09015BSIZE 40 nm
- Name of test material (as cited in study report): Nanograin Ceria
- Other names : Ceric oxide, Ceria
- Molecular formula: CeO_2
- Molecular weight : 172.11
- Smiles notation :
- InChI:- Structural formula:
- Substance type:
- Physical state: solid
- Density : 7.13 g/ml at 25°C
- Total quantity : 1000 gram
- Primary particle size: 40 nm
- Analytical purity: 99.95%
- Impurities (identity and concentrations):
- Composition of test material, percentage of components:
- Isomers composition:
- Purity test date:
- Lot/batch No.:
- Date of receipt : 9-12-2009
- Expiration date of the lot/batch: 4-12-2010
- Stability under test conditions:
- Storage condition of test material: ambient (15-25 °C)

- NPL Ref nos. : CB250#27, CB250#45, CB250#46, CB250#47
- Supplier : Umicore
- TNO reference number: 09015CSIZE 5-10 nm
- Name of test material (as cited in study report): Ceria Dry CeO₂
- Other name : Ceric oxide, Ceria- Molecular formula: CeO₂
- Molecular weight: 172.11
- Smiles notation:
- InChI:
- Structural formula:
- Substance type:
- Physical state: solid
- Density : 7.13 g/ml at 25°C
- Total quantity : 1020 gram
- Primary particle size: 5-10 nm
- Analytical purity: 99.5% (by weight)
- Impurities (identity and concentrations):
- Composition of test material, percentage of components:
- Isomers composition:
- Purity test date:
- Lot/batch No.:
- Date of receipt : 4 December 2009
- Expiration date of the lot/batch: 4 December 2010
- Stability under test conditions:
- Storage condition of test material: ambient temperature (15-25°C)
- NPL Ref nos. : CA340#37, CA340#43, CA340#59
- Supplier : Antaria
- TNO reference number: 09015D

Test animals

Species

rat

Strain

Wistar

Sex

male

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Charles River (Sulzfeld, Germany)- Age at study initiation: 6 weeks- Weight at study initiation: 212 +/- 14 g- Fasting period before study: no- Housing: in standard laboratory cages (2 or 5 animals per cage) under controlled conditions of temperature, humidity, and light. During exposure periods, the rats were individually housed in the exposure unit. The experiments were conducted at TNO Triskelion (Zeist, The Netherlands) under a protocol approved by the Ethics Committee for Animal Experiments of TNO (Zeist, The Netherlands).- Diet: ad libitum during the experiments, except during exposure unit- Water: ad libitum during the experiments, except during exposure unit- Acclimation period: 5 days

ENVIRONMENTAL CONDITIONS

- Temperature (°C):

- Humidity (%):
 - Air changes (per hr):
 - Photoperiod (hrs dark / hrs light):
- IN-LIFE DATES: From: To:

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

nose only

Details on inhalation exposure

The experiments were conducted at TNO Triskelion (Zeist, The Netherlands) under a protocol approved by the Ethics Committee for Animal Experiments of TNO (Zeist, The Netherlands).

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

TEST SCHEME

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

No. of animals per sex per dose

3 per group

Details on study design

EXPERIMENTAL PROTOCOL

A 28-day inhalation study was performed in rats. Rats (3 per group) were exposed (6 hours per day) by inhalation to cerium oxide in nose-only exposure units consisting of a cylindrical column, surrounded by a transparent hood. The test atmosphere was introduced at the top of the central column and was exhausted at the bottom of the ~50 L exposure unit. Each column included four rodent tube sections 5 of 20 ports each for animal exposure. The animals were secured in plastic animal holders (Battelle) and positioned radially through the outer cylinder around the central column. Only the nose of the rats protruded into the interior of the column. The animals were placed in the exposure unit after stabilization of the test atmosphere. Particle number concentrations in the test atmosphere were targeted to be the same for each cerium oxide type.

TEST ATMOSPHERE

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

Examinations

Other examinations

BLOOD AND TISSUE SAMPLING

During the study, blood samples were taken at various time points after a single or repeated exposure. Blood sampling was performed in most cases well within the first hour after exposure, but also at post-exposure periods of up to 67 hours. Blood was sampled by orbital puncture under O₂/CO₂ anaesthesia in EDTA-containing tubes and frozen (below -18 °C) for analysis of cerium. For tissue sampling, rats were sacrificed while under ether anaesthesia, and tissues (lung, liver, spleen, kidney, testis, epididymis and brain) were removed and frozen (below -18 °C) for cerium analysis.

CERIUM ANALYSES IN BLOOD AND TISSUE

Blood samples were digested with suprapure nitric acid. Total cerium content was then measured by inductively coupled plasma mass spectrometry (ICP-MS, Element 2, Thermo Scientific, USA). Tissue samples were digested with a mixture of suprapure nitric, sulfuric and perchloric acids (2:1:1). After oxidation the mineral acids were evaporated and the residue was taken up with suprapure hydrochloric acid. Total cerium content in the lung tissue samples was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Iris Intrepid, Thermo Scientific, USA), whilst total cerium content in the other tissue samples was measured by ICPMS.

DEPOSITION MODEL CALCULATIONS

In order to estimate the dose deposited in the rat lung upon inhalation of cerium oxide, the Multiple-Path Particle Dosimetry Model (MPPD v 2.11; <http://www.ara.com/products/mppd.htm>) was used. The deposition fractions in the head, tracheobronchial and pulmonary region were calculated. We used the default parameters of the model for rats, i.e. a forced respiratory capacity of 4 mL, head volume of 0.42 mL, nasal breathing, tidal volume of 2.1 mL and a breathing frequency of 102 min⁻¹. The inspiratory fraction was 0.5 and no pause was entered. Calculations were done using the measured mass median aerodynamic diameter (MMAD), count median diameter (CMD), geometric standard deviation (GSD), the mass concentration and a density of 7.65 g·cm⁻³. In addition, more detailed calculations were performed to estimate deposition in the olfactory epithelium, since deposited particles may directly translocate to the brain, bypassing the blood brain barrier. A detailed, anatomically accurate computational fluid dynamics (CFD) model of the rat nasal passages was used for these calculations. The CFD model was originally developed by Kimbell et al. (1997) and was subsequently smoothed using medical imaging software to provide a more realistic anatomic form according to Schroeter et al. (2012). Particle sizes from 1 nm to 5 µm were simulated and nasal and olfactory deposition fractions were predicted for each particle size.

Statistics

DATA EVALUATION AND STATISTICAL ANALYSIS

The measured cerium tissue levels were converted into cerium oxide tissue levels by multiplying the former with the ratio of the molecular masses of cerium oxide and cerium (i.e. 1.23), thus assuming that the deposited and distributed cerium oxide is still present as particles. As the mass concentrations of the different cerium oxide types were not similar, cerium oxide tissue concentrations were normalized for the total inhaled dose in order to study particle-dependent differences in tissue concentrations. First, the absolute amount of cerium oxide per tissue was calculated using organ weights. No information on organ weights of the rats in this study was available, data on organ weights from a different rat study were used. Second, the total inhaled dose of cerium oxide was calculated using the following formula: tidal volume × breathing frequency × exposure concentration × exposure duration (analogous to parameter settings of the MPPD model; the tidal volume was 2.10 mL and breathing frequency was 102 min⁻¹). Finally, the absolute amounts of cerium oxide in tissue were then normalized by expressing these amounts as percentage of the total inhaled dose cerium oxide (i.e. 4.24, 1.54 and 0.83 mg cerium oxide for Sigma-

Aldrich, Umicore and Antaria particles respectively). Results are presented as mean values $\pm$ standard deviation unless otherwise indicated. The cerium oxide tissue concentration data were not normally distributed. The nonparametric Mann-Whitney U test was used to evaluate the effect of particle size on cerium oxide tissue content, to study potential tissue-dependent differences in cerium oxide levels and to assess the potential effect of number of exposures and recovery period on the cerium oxide tissue levels. Two-tailed P values of 0.05 or less were considered statistically significant.

Results and discussions

Results of examinations

Details on results

For tables and figures see attachment full study paper.

CHARACTERISTICS OF TEST ATMOSPHERE AND DOSE

The physical characteristics of the aerosols are summarized in Table 2. The mean mass concentration for the different exposure protocols were 55.00, 19.95 and 10.79 mg·m⁻³ for Sigma-Aldrich, Umicore and Antaria cerium oxide particles respectively. The actual concentrations of the test material in the atmosphere were measured at least 6 times daily by gravimetric analysis. The total air flow through the unit was monitored at least 3 times a day and was at least 1 L·min⁻¹ for each rat. The air entering the unit was controlled at 22 ± 2 °C and the relative humidity was targeted between 30 and 70 %. TEM-analysis and SEM-analysis were used to verify the manufacturer's nominal sizes of <5000, 40 and 5-10 nm. Figure 1A-B-C presents TEM-images from the bulk material, acquired at a magnification of 41000 $\times$ (scale bar is 200 nm). The Sigma- Aldrich sample contained very large particles (>500 nm), and overall was unsuitable for size-characterization by TEM. It is worth noting, however, that together with the larger particles, there was a non-negligible proportion of sub-100 nm sized particles present in this sample, with some particles as small as ~10 nm visible. These small particles appeared to "decorate" the large particles present in the sample. Overall the sample appeared to have an extremely broad size distribution. TEM analysis of the Umicore sample revealed crystals of polydisperse size and shape, with a mean Feret diameter of 27.3 ± 13.6 nm (100 particles). The Antaria sample revealed small aggregate clusters of primary particles with much greater size- and shape homogeneity. A mean Feret diameter of 13.0 ± 3.2 nm (100 particles) was determined for this sample. SEM-analysis showed actual primary particle sizes of 615.3 ± 430.5 , 28.4 ± 10.4 and 44.9 ± 14.6 nm for Sigma-Aldrich, Umicore and Antaria particles respectively. Both TEM and SEM-analysis revealed that the difference between the two nanoparticles was smaller than anticipated. The mean particle size determined from TEM measurements shows good equivalence with the average particle size calculated from the BET surface area measurements. Although the nano- and micro- cerium oxides differ in primary particle size (i.e. size of a single particle), the aerodynamic diameter for all three particles is similar, indicating that in particular the Umicore and Antaria samples were present in the aerosol as aggregates. Figure 1D-E-F presents SEM-images of the three different cerium oxide particles sampled from the test atmosphere during the inhalation study on a glass fiber filter.

TISSUE CONTENT

Lung, liver, spleen, kidney, testis, epididymis, brain and blood were analyzed for the presence of cerium. After single and repeated exposure, cerium could be detected in all investigated organs, although substantial higher levels were measured in the lungs. Cerium tissue levels in control rats were very low and were very close to and partly below the tissue-specific detection limits (data not shown). Cerium concentration in blood after single or repeated exposure was below the detection limit (< 5 μ g·kg⁻¹) for most samples (data not shown). Figure 2 shows the cerium oxide tissue amounts after single inhalation exposure expressed as percentage of the total inhaled dose. Though small differences in pulmonary deposition efficiencies of cerium oxide were observed, no obvious size dependent differences in the

pulmonary deposition of cerium oxide particles occurred. The percentage of inhaled cerium oxide measured in lung tissue for Umicore particles (5.6%) was significantly lower ($P=0.05$) than the lung content for Sigma-Aldrich efficiently deposited in the lung as compared to the Sigma-Aldrich particles and the Antaria particles. Cerium oxide detected in the extrapulmonary tissues was less than 0.2% of the inhaled dose for all cerium oxide particles. Unlike the deposition in the lung, the extrapulmonary cerium oxide tissue levels for Umicore particles were slightly (though not statistically significant) increased as compared to the extrapulmonary tissue levels of rats exposed to Sigma-Aldrich and Antaria particles, indicating a moderately better distribution to extrapulmonary organs.

TISSUE DISTRIBUTION

In order to study tissue-dependent differences in cerium oxide concentrations, tissue cerium oxide concentrations were expressed per kg tissue after single (Figure 3A) and repeated exposures (Figure 3B and C) and after the recovery period (Figure 3D). For Sigma-Aldrich particles, cerium oxide levels in lung tissue were significantly higher than the other investigated tissues, and were at least 1000× higher than the extrapulmonary tissue levels. After single exposure, cerium oxide particles were distributed to the extrapulmonary tissues with an order of concentration of epididymis > brain > spleen > liver > kidney > testis. After repeated exposures, the differences in distribution were less clear. For Umicore particles, the highest cerium levels were also found in lung tissue. After a single exposure, cerium oxide particles were distributed to the extrapulmonary tissues with an order of concentration of epididymis > brain > liver = spleen > kidney = testis. After multiple exposures, differences in distribution to the various extrapulmonary organs were less, though testis levels remained the lowest. Lung cerium oxide levels were also the highest of all investigated tissues for the Antaria particles. Except for a slightly reduced distribution to testis, epididymis and brain, no clear differences in distribution pattern to the various extrapulmonary organs were observed after single and repeated exposures.

TISSUE ACCUMULATION AND ELIMINATION

In figure 4, the cerium oxide tissue levels are normalized for the tissue levels after single exposure in order to evaluate potential accumulation after repeated exposure. Repeated exposure to all cerium oxide particle-types was found to result in significant increasing cerium oxide concentrations, indicating that accumulation occurred. This was most evident and significant in lung tissue. Increasing the number of repeated exposures was found to result in higher fold of tissue accumulation for rats exposed to Sigma-Aldrich and Umicore, indicating that steady state was not yet reached after 11 exposures. Particularly, significant increases in cerium oxide lung (Sigma-Aldrich and Umicore) and liver (Umicore) levels could be observed. In an additional experimental group of rats, cerium oxide tissue levels were measured 48 hours (Antaria) or 72 hours (Sigma-Aldrich and Umicore) after finishing the last exposure in order to study elimination of cerium oxide. As observed in figure 4, the elimination of Sigma-Aldrich and Umicore cerium oxide particles from the investigated tissues was slow, with, in almost all cases, insignificant amounts of cerium oxide eliminated in 72 hours. Rats exposed to Sigma-Aldrich cerium oxide particles showed significant decreases in brain cerium levels. Umicore-exposed rats showed significant decreases in lung and liver cerium levels after recovery. Elimination of Antaria cerium oxide particles appeared to be slower.

MODELING OF LUNG DEPOSITION

The deposition fractions estimated with MPPD are presented in Table 3 for MMAD, CMD and primary particle size (as measured with SEM-analysis). The cerium oxide particles are expected to be mainly deposited in the head region, and solely a small part (10 %) is expected to be deposited in the pulmonary region. Based on MMAD, the smallest aggregates resulted in the highest deposition fraction in the pulmonary region, though the differences were very small: 7, 8 and 9 % of the inhaled Sigma-Aldrich, Umicore and Antaria cerium oxide particles, respectively, were expected to be deposited in the lung. Model calculations indicate that should the diameter in the aerosol be equal to the primary particle size, significant differences would have been expected between the nano-sized particles (28 and 45 nm, based

on SEM-measurements) and the micro-sized particles (615 nm based on SEM-measurements). MPPD calculates a pulmonary deposition of around 30% for these nano-sized particles and only 7 % for the micro-sized particles. Similar results are obtained for tracheabroncheolar deposition, which is in the order of 7-10 % and 3.5 % for nano-sized and micro-sized particles, respectively. Additional olfactory deposition calculations showed that the most efficient olfactory deposition is expected for particles with much smaller diameter (i.e. approximately 7 nm) as compared to the aggregates of the cerium oxide particles as studied in the present study (data not shown). Though the deposition in the head region was estimated to be approximately 33-52 % (i.e. 73-89 % of the total deposition), the olfactory deposition of the three cerium oxide particles is expected to be very low.

Overall remarks, attachments

Remarks on results including tables and figures

For tables and figures see attachment full study paper. The present study describes the tissue distribution and elimination of cerium oxide nano- and micro-particles of different primary particle size in rats. Animals were exposed by inhalation to cerium oxide. Inhalation of cerium oxide 5 aerosol provided a relevant and natural route of exposure, as exposure in occupational settings is mainly via inhalation.

DIFFERENCES BETWEEN CERIUM OXIDES TESTED

Despite the fact that the micro- and nano- cerium oxide particles clearly differed in primary particle size, the size distributions of the aerosol generated for the inhalation exposure were very similar due to aggregation of the primary particles. This was confirmed by electron microscopy. An aerosolization regime that mimics what can occur due to (re)suspension of cerium oxide in air at a workplace, for example, was intentionally applied. Lung deposition of airborne particles is highly dependent on the aerodynamic diameter (Carvalho et al., 2011). Since, in the present study, the three types of cerium oxide did not differ substantially in the MMAD or CMD, it seems safe to assume that the deposition efficiency and rate and the distribution in the upper respiratory tract, trachea, conducting airways and alveoli are more or less similar, as was also estimated by the MPPD deposition model. Measurement of the cerium oxide content of lung tissue confirmed this assumption. Though small differences in pulmonary deposition efficiencies of cerium oxide were observed, no consistent relationship between the primary particle size or MMAD and the pulmonary dose was noted. These findings do not support the hypothesis that the BET surface area is a good predictor of either the pulmonary or extra-pulmonary dose or for adverse effects (unpublished findings). As aggregates may break up upon contact with body fluids, it is remarkable that the extrapulmonary distribution was more or less similar for the three samples examined in this study. This may indicate that 1) the aggregates are not broken up by body fluids and remain intact during distribution, 2) the nanoparticles and microparticles distribute similarly, 3) the particles dissolve and behave as molecules, 4) or 5) the fraction distributed to other organs aside from the lung is too small to show the differences clearly. Solubility tests conducted with the three cerium oxide particles showed very low solubility, and thus the third interpretation of the distribution data can be ruled out. Currently, no information is available on the condition of the cerium oxide in the tissues (i.e. if it is present as aggregates or single particles).

DISTRIBUTION OF CERIUM OXIDE

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

total amount of cerium measured in the tissues was far below the inhaled dose. This might be explained by

- 1) the selection of investigated organs for cerium analysis (i.e. not all tissues were investigated),
- 2) possible elimination of inhaled cerium via exhalation during, or shortly after the exposure period and
- 3) rapid elimination of deposited cerium oxide via the mucociliary escalator of the bronchia and conducting airways.

Remarkably high levels were detected in brain, epididymis and testis, indicating that further attention should be paid to potential central nervous system effects and reprotoxic effects.

Potential important routes responsible for extrapulmonary tissue distribution are:

- 1) translocation to the lymphatic and circulatory system or
- 2) activity of the mucociliary escalator and subsequent oral exposure.

The high cerium oxide levels in the brain could have furthermore been a consequence of deposition of cerium oxide in the olfactory epithelium in the nose and subsequent translocation via the olfactory nerve. This effect has previously been described for other materials, both soluble and insoluble (Henriksson et al., 1999; Dorman et al., 2002; Oberdorster et al., 2004; Elder et al., 2006). Additional modeling of the deposition showed that, though the deposition in the head region is relatively high (deposition fractions of 0.33-0.52, corresponding to 73-89% of the total deposition), the olfactory deposition of the three cerium oxide particles is expected to be minimal. Though translocation via the olfactory nerve is possible, this route should not contribute extensively to the relatively high levels of cerium oxides observed in the brain as compared to particles with a smaller diameter. Distribution across protecting membranes such as the blood cerebrospinal fluid barrier, blood brain barrier and blood testis barrier might be an additional explanation for the relatively high brain and testis levels.

KINETIC PROFILE OF NANOPARTICLES VS CERIUM OXIDE

Extrapulmonary and extensive systemic distribution to liver, spleen, kidney, testis and brain was previously observed after intratracheal instillation and intravenous administration of nano cerium oxide (He et al., 2010; Yokel et al., 2009; Hardas et al., 2010; Dan et al., 2011; Yokel et al., 2011). These studies and the present study are generally thus in agreement with the broad distribution of cerium oxide particles. The present study is the first to employ the most relevant exposure route (inhalation) and to show that cerium oxide after inhalation may become systemically bioavailable. Extrapulmonary distribution has also been demonstrated after inhalation of other nanoparticles, including: elemental carbon (Oberdorster et al., 2002), iridium (Kreyling et al., 2002), ferric oxide (Zhu et al., 2009), fullerenes C₆₀ (Shinohara et al., 2010) and gold (Yu et al., 2007). Although the nanoparticle concentrations were always highest in lung tissue, nanoparticles were also recovered from other extrapulmonary tissues such as liver, spleen, kidney, heart and brain after inhalation exposure (Kreyling et al., 2002; Oberdorster et al., 2002; Yu et al., 2007; Zhu et al., 2009; Shinohara et al., 2010).

SYSTEMIC EXPOSURE VIA THE ORAL ROUTE

Potential exposure via the oral route following inhalation exposure of particles (e.g. via activity of the mucociliary escalator mediated by macrophages) might also contribute to extrapulmonary tissue deposition. The deposition model used in this work estimated that the deposition of cerium oxide in the head region would be high (i.e. 73-89% of the total deposition). This suggests that oral exposure might be an important contributor to systemic tissue deposition. A recent rat in vivo study by He et al. (2010) showed that after intratracheal instillation of cerium oxide nanoparticles (primary particle size: 6.6 ± 0.9 nm, BET surface area: $86.85 \text{ m}^2 \cdot \text{g}^{-1}$) approximately 25 % of the given intratracheal dose was cleared via the faeces, pointing towards particle transport from the lung to the GI tract (He et al., 2010). Furthermore, approximately 90 % of the oral dose was excreted in faeces in the first day post-exposure and nearly 100 % of the given oral dose was excreted in faeces after three days post-exposure (He et al., 2010). This suggests a limited GI absorption of cerium oxide nanoparticles. However, absorption and subsequent excretion via bile and faeces could also result in faecal excretion. Hence, in the present study exposure via the oral route following the mucociliary escalator may also be possible. Analysis of cerium in the faeces

after inhalation of cerium oxide could thus aid in clarification of this exposure route. The contribution from the oral route can be quantified if the oral bioavailability of cerium oxide and the faecal cerium content is known.

ELIMINATION

In the present study, the elimination half-life could not be reliably determined. At 48 hours and 72 hours exposure there was no significant reduction of cerium oxide in either lung tissue or other investigated tissues, indicating a long elimination half-life, which was also recently demonstrated by He et al. (2010). Additionally, significant accumulation of cerium oxide in tissues was observed after repeated exposure in the present study, which was also recently reported following repeated intravenous and intraperitoneal administration of cerium oxide particles (Hirst et al., 2011). In addition, elimination from the body of intravenously administered 30 nm cerium oxide in a different rat study was found to be < 1% of the total dose during the first two weeks post-exposure (Yokel et al., 2011). The negligible elimination and subsequent accumulation of cerium oxide particles observed after repeated exposure therefore highlight the importance of assessing the potential toxicity after chronic exposure to this material.

LIMITATIONS

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

Attached full study report

Attached full study report
NM_211_ANNEX_A64_manuscript ceriumoxide kinetiek.pdf / 704.99 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN4

Applicant's summary and conclusion

Conclusions

Conclusions and recommendations In the present study, rats were exposed via inhalation to cerium oxide particles with similar aerodynamic sizes but varying primary particle size. It can be concluded that after exposure, the cerium oxide particles were mostly retained in lung tissue. Distribution of the inhaled cerium oxide particles to various extrapulmonary tissues was also observed. Though there were small differences in the pulmonary deposition efficiencies of the three cerium oxide materials, no clear effect of the primary particle size or surface area on pulmonary deposition and extrapulmonary tissue distribution was observed. Repeated exposure resulted in significant accumulation of the particles in both the pulmonary as well as the extrapulmonary tissues. Furthermore, tissue clearance of the particles was slow, and insignificant amounts of cerium oxide were eliminated in 48–72 hours. A lack of strong correlation between primary particle size and pulmonary deposition or extrapulmonary distribution is likely to be explained by the presence of aggregates rather than single nano-sized particles in the test atmosphere resulting in similar aerodynamic diameter of the micro- and nano- cerium oxide but with different surface characteristics, though this latter is also known to affect particle distribution. At present, no work has been undertaken to establish whether the cerium oxide measured in tissues is present as aggregates or single particles. Such information would provide insight into the differences and similarities between micro- and nano cerium oxide. The present results provide a solid foundation for further studies on the fate

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

Executive summary

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

Endpoint study record: 2012-02-27 RIVM Effects of subacute inhalation of diesel exhaust with ceriumoxide in mice

Administrative Data

Study result type experimental result **Study period** 23-11-2009 / 4-1-2010

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	F.R. Cassee, A.Campbell, J.F.Boere, S.G. McLean, R. Duffin, P. Krystek, I.Gosens, M.R. Miller	2012	The biological effects of subacute inhalation of diesel exhaust following addition of cerium oxide nanoparticles in atherosclerosis-prone mice		RIVM Centre for Environmental Health Dep. Inhalation Toxicology	draft	RIVM, Bilthoven, The Netherlands		

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Test type**

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
according to	OECD Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day)	

Test materials**Identity of test material same as for substance defined in section 1 (if not read-across)**

no

Test material form

nanomaterial

Details on test material

Name of test material (as cited in study report): Envirox

- Molecular formula (if other than submission substance): CeO₂
- Molecular weight (if other than submission substance): 172,12
- Smiles notation: CeO₂- InChI: 1/Ce.2O/rCeO₂/c2-1-3
- Substance type:
- Physical state: liquid
- Colour: clear (yellow/orange)
- Odour: hydrocarbon
- Analytical purity:
- Impurities (identity and concentrations):
- Composition of test material, percentage of components: hydrocarbon > 90%; CeO₂ < 10%
- Boiling point: 200-250 C
- Flash Point: > 77 C
- Explosion/Flammable limits: 0,5 - 7,0% (v)
- Auto ignition temperature: 251 C
- Pour point: - 39 C
- Water solubility: < 0,10% 9wt) (immiscible)
- Relative density: 0,798@15,6 C
- Viscosity: 1,68 cSt @ 40 C; 2,16 cSt @ 25 C
- Vapour density: (Air=1): 6,2 @ 101kPa
- Vapour pressure (20 C) 0,023 kPa (0,17 mm Hg)
- Evaporation rate (n-butyl acetate=1): 0,1
- Isomers composition:
- Purity test date:

- Lot/batch No.:
- Expiration date of the lot/batch:
- Radiochemical purity (if radiolabelling):
- Specific activity (if radiolabelling):
- Locations of the label (if radiolabelling):
- Expiration date of radiochemical substance (if radiolabelling):
- Stability under test conditions:
- Storage condition of test material: Material is stable under normal conditions. Avoid heat, sparks, open flames and other ignition sources. Do not store in direct sunlight
- Other:

Test animals

Species

mouse

Strain

other: ApoE^{-/-} mice (B6.129P2-Apoetm1Unc N11; Taconic, Denmark)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS & EXPERIMENTAL DESIGN

ApoE^{-/-} mice (B6.129P2-Apoetm1Unc N11; 5 males and 5 females per group; Taconic, Denmark) were fed a 'Western' diet high in fat (21% fat; ABdiets, The Netherlands) ad libitum for 7 weeks from an age of 11 weeks until the end of the study. After three weeks of feeding (age 14 weeks), mice were exposed (nose only) to filtered air or diluted exhaust originating from an engine fuelled with standard diesel with (DCeE) or without (DE) cerium oxide, 5 days/week for 28 days. Preliminary experiments showed that pre-exposure, mice will have developed only modest atherosclerotic lesions in the aortic arch and vessels branching from this point. To assess the dose-dependence of the exposures, three different lengths of exposure (20, 60 or 180 minutes) of a single concentration of exhaust (see below) were used to provide three 'dose' levels within each exposure type as described in the OECD TG412 guidelines. Animals were sacrificed 3 days after the last exposure, with the exception of "recovery groups" which were sacrificed 14 days later (17 days after last exposure) to explore the reversibility or possible delayed effects of exposure (high dose groups only). Where possible, experimental variation was limited by randomisation of treatment and analysis (e.g. allocation of animals to treatment groups, alternating the time of day for the exposure to DE or DCeE and introducing a random assignment into the sectioning and blood-taking schedule).

Administration / exposure

Route of administration

inhalation: aerosol

Type of inhalation exposure

nose only

Vehicle

other: diesel

Details on inhalation exposure**GENERATION AND CHARACTERIZATION OF THE TEST ATMOSPHERE**

An Ingersol Rand (4IRD5AE) engine (run at 1500 rpm with a generator load of 35KW) fuelled with low sulfur (10 ppm) diesel (EN590, purchased at a regular gas station in the Netherlands; CAS number 68334-30-5) with or without 9 ppm nanosized cerium oxide was used to generate exhaust for exposures. A cerium oxide suspension “Envirox”, (2.5% Ce in non-flammable aliphatic hydrocarbon solvent (Exxsol D-80), Oxonica, Haddenham Bucks, UK,) was mixed into the regular fuel in barrels of 100 L that were used in the engine. The exhaust was captured and diluted with humidified compressed air to obtain a particle concentration of 1.7 mg/m³ for the DE exposure. This level was based on our previous studies in mice that did not cause severe pathological effects (Hiramatsu et al., 2005). Identical engine parameters were used for the DCeE exposure. The dilution of the engine exhaust was adjusted to get equal mass concentrations for both fuel types at a given exposure day (i.e. with and without cerium oxide). Although cerium oxide did slightly affect the mass emissions of the engine, the major influence came from the ambient temperature. Continuous mass recordings were made using a TEOM 1400a (Rupprecht & Patashnik, Albany NY, USA) and time-integrated mass concentrations were determined gravimetrically using particles collected on 47 mm Teflon filters. Particle number concentrations were measured with a condensation nucleus counter (CPC 3022A, TSI Inc, St Paul MN, USA; logfile 10sec interval) and the surface area was measured using a Nanoparticle Surface Area Monitor (NSAM model 3550, TSI Inc, St Paul MN, USA). Carbon monoxide and nitrogen oxides were detected with on-line analyzers (Envitec model 300E, Teledyne, City of Industry CA, USA and model 42W, Thermo Electron Corporation, Hopkinton MA, USA, respectively). Aerodynamic particle size distributions were recorded using a scanning mobility analyser (SMPS model 3080 with CPC 3785, TSI Inc, St Paul MN, USA). Cerium content and particle size was measured by high resolution inductively coupled plasma mass spectrometry (HR-ICPMS, Thermo Fisher, Germany), following digestion in nitric acid and hydrogen peroxide at 100°C for 1 h. Based on possible interferences, Ce was measured as ¹⁴⁰Ce in medium resolution mode. An external calibration with internal standard correction was applied. The procedure is comparable to other applied analysis within this field (e.g. Ulrich and Wichser 2003). Transmission electron microscopy (TEM; TECNAI F30ST electron microscope, operated at 300kV and with a high angle annular dark field detector) and local energy dispersive X-ray (EDX) analysis were also used to assess particle size and to detect cerium oxide in soot.

Analytical verification of doses or concentrations

yes

Duration of treatment / exposure

28 days

Frequency of treatment

5 days/week, for 4 weeks

Details on study design

Exposure by inhalation to diluted exhaust (1.7 mg/m³, 20, 60 or 180 min, 5 days/week, for 4 weeks), from an engine using standard diesel fuel (DE) or the same diesel fuel containing 9 ppm cerium oxide nanoparticles (DCeE).

Examinations***Sacrifice and pathology***

GROSS PATHOLOGY: Yes

HISTOPATHOLOGY: Yes

Other examinations

Changes in hematological indices, clinical chemistry, atherosclerotic burden, tissue levels of inflammatory cytokines and pathology of the major organs were assessed.

PATHOLOGY

One half of the lung was preserved on ice to determine cerium content of the lung. The remaining lung was infused with fixative (4% solution of formaldehyde in neutral aqueous phosphate-buffered) under water pressure for 1 h to insure fixation and expanded lung structure. Bronchoalveolar lavage was not performed in order to maintain the fine structure of the lungs for histological analysis. All other tissues were preserved in a neutral aqueous phosphate-buffered 4% formaldehyde. Fine dissection was used to examine pathological changes in the adrenals, brain, heart, kidneys, liver, lung (left lobe at three levels, including main bronchi), nasopharyngeal tissues (at least 4 levels, one level including the nasopharyngeal duct and the nasal associated lymphoid tissue (Woutersen et al. 1994)), spleen, testes and thymus.

HEMATOLOGY AND CLINICAL CHEMISTRY

Hematology and cell differentials were conducted in EDTA-anticoagulated blood taken one day after the last exposure via an orbita puncture as described in Gerlofs-Nijland et al. (2010b) with volume adjustments for mice. The following parameters were measured: white and red blood cell concentrations, hemoglobin concentration, platelet concentrations, the mean platelet volume, hematocrit value, mean corpuscular volume, mean cell hemoglobin, mean cell hemoglobin concentration, red blood cell distribution width, mean platelet component and hemoglobin distribution width. Immediately after sacrifice, plasma was obtained from heparinised blood samples to measure albumin (assesses nutritional status), alkaline phosphatase activity (ALP; increase is associated with hepatobiliary and bone disorders), alanine aminotransferase activity (ALT; liver disease), calcium (parathyroid diseases, a variety of bone diseases, chronic renal failure), glucose (diabetes mellitus, adrenal gland aberrations), inorganic phosphate (PHOS; renal disease), urea (renal disease), aspartate aminotransferase activity (AST; liver, heart, muscle, brain, and kidney tissue damage), gamma glutamyl transferase activity (GGT; liver disease), total protein (TP; dehydration or cancer of blood cells), total bilirubin (TBIL; hemolysis or liver disease), creatinine (kidney function), phospholipids (PLIP; lipid maintenance) and triglycerides (TRIG; lipid maintenance).

ASSESSMENT OF ATHEROSCLEROSIS

The brachiocephalic artery was fixed in formalin, embedded in wax and histologically sectioned every 100 µm (3 sections/slide, 4 replicates per 100 µm) from the first complete ring until the last, with on average 6 serial sections (range: 2-12 sections) per vessel. Slides were stained with United States Trichrome (UST), a structural stain that highlights the composition of atherosclerotic plaques clearly (Hadoke et al. 1995). Cross-sectional area of the plaque was measured and standardized as a percentage of the area of the vascular media (It was not possible to perfuse fix vessels, therefore, plaques were not expressed as a percentage of the lumen). The mean plaque size of all sections was calculated to provide a single score of atherosclerotic burden throughout the brachiocephalic artery of each animal. Maximum plaque size for a single section of the brachiocephalic was also recorded and used as an additional method to compare plaque size between groups. The presence of buried fibrous caps is taken as a general marker of plaque complexity either from the ongoing development of a single plaque or the merging of two separate sites of plaque growth (Rosenfeld et al. 2000). However, it has also been suggested that buried fibrous caps represent the growth of a new plaque over a site of a previous plaque rupture (Jackson et al. 2007; Johnson & Jackson 2001). These possibilities were considered by counting the number of potentially distinct plaques within an artery (either existing separately or adjoining with a clear fibrous divide indicating the merging of two separate plaques) and the number of buried fibrous caps within each section, whereby a buried fibrous cap was defined as “a length of fibrous/cellular matter that completely bisects a lipid-rich regions of two overlying plaque sections”. A single section from each artery was chosen for analysis of plaque composition, specifically lipid cavities (enclosed regions of plaque,

unstained with UST; Johnson et al. 2005) and macrophage content (MAC-2 immunohistochemistry). For macrophage (MAC2) staining, a rat anti-mouse antibody was used for the primary antibody (1/12000; CL8942AP, VH Bio, Gateshead, UK) with rat IgG (1/12000; I-400, Vector Labs, Peterborough, UK) as a negative control, followed with a goat anti-rat IgG biotinylated secondary antibody (BA-9400, Vector Labs). Slides were then incubated with extravidin-peroxidase solution (1/200), followed by avidin biotin complex (ABC; PK-4001, Vector Laboratories, Peterborough, UK) before detection of MAC-2 with 0.05% 3,3'-diaminobenzidine (DAB; SK-4100; Vector Labs, Peterborough, UK). Nuclei were counterstained with Harris' hematoxylin. Sections were photographed and images were imported into Adobe Photoshop v11.0, and a colour range was selected from three randomly chosen positively stained sections, which was then used to identify positively stained plaque components from all subsequent slides.

CYTOKINE ASSESSMENT IN THE BRAIN

Half of the brain was placed in 4% paraformaldehyde for immunohistochemical analysis. The remaining half was immediately frozen and further subdivided into the cerebellum & brain stem (referred to as "cereb") and other regions (referred to as "brain"). Cytoplasmic fractions were prepared (Lahiri & Ge 2000) and analysed for levels of tumor necrosis factor alpha (TNF-*) and interleukin-1beta (IL-1 β) using immunoassay kits (Invitrogen, Carlsbad, California).

Statistics

DATA ANALYSIS & STATISTICS

Arithmetic means+standard error of the mean are reported in the text and on figures, whereas +standard deviation is given in the tables. Body and organ weight, clinical chemistry and hematology data were analysed using two-way analysis of variance (ANOVA). If variances were not homogeneous or data not normally distributed, the data were stepwise log or rank transformed prior to the ANOVA. Histopathological data was analyzed using Fisher's exact probability test. Cytokines in the CNS and atherosclerotic plaque data were analysed by one-way ANOVA or unpaired t-test as appropriate. $P < 0.05$ was regarded as statistically significant.

Results and discussions

Results of examinations

Haematology

yes

Clinical chemistry

yes

Details on results

For tables and figures see attachment full study paper.

EXPOSURE CHARACTERIZATION

The overall exposure characteristics of both exhaust types are shown in Table 1. The concentrations indicated in Table 1 are those attained after dilution, i.e. the levels that were used to expose the animals. There was no difference in the mass of particulate matter between the two exhaust types, however, there was a 30% reduction in average particle number in exhaust from cerium oxide enriched diesel ($P=0.011$) that equated to an ~10% reduction in particle deposition on the tracheobroncheolar surface of the lung ($P=0.04$). An opposite trend was noted for the gases, which exhibited a slight increase of 6%, 9% and 8% for CO, NO and NO_x, respectively. The data obtained from this study do not allow clear conclusions to be drawn on the fuel efficiency for both fuel types. Atmospheric conditions during the 4th week (outdoor

temperatures fell to $\sim 0^{\circ}\text{C}$ where the engine was situated) resulted in different engine operating conditions and different exhaust composition (24% larger median particle size, 33% lower particle numbers, 23% reduced particle surface area and 35-55% lower CO/NO_x concentration), however, these changes were observed in both the DE and DCeE groups (data not shown). Prior to adding to the fuel, the most abundant particle size in the ENVIROX cerium oxide additive was 15.6 ± 0.2 nm. A few large particles with sizes ~ 200 nm were seen infrequently. The particle size (mass mean diameter) of diesel exhaust particles were similar for DE and DCeE groups (Table 1). TEM analysis of loaded filters from DCeE exposures identified numerous types of particles consisting of a wide range of elements (K, Na, Cl, Mg, Si, Al, O, FeO_x, TiO_x, AlO_x, Ni, Cr, Fe, Ca, S, Ba, Sn). Small nanoparticles were observed within the clusters of soot particles that yielded EDX spectra with C and Ce peaks (Fig. 1). Filters from DCeE exposures contained 5.5 ± 4.0 μg Ce/mg loading of soot in comparison to 0.2 ± 0.1 μg Ce/mg of soot from DE exposures. Unexposed collection filters contained insignificant levels of Ce (<10 ng abs; $n=3$).

ASSESSMENT OF BIOLOGICAL EFFECTS: SEX DIFFERENCES, RECOVERY GROUPS AND DOSE EFFECTS

With the exception of body and organ weight, biological responses, were similar in male and female mice. Therefore, for most parameters, the data is pooled to attain an $n=10$. Biological responses to the two exposure types were also similar in mice sacrificed 17 days after the last exposure (data not shown), suggesting that there were no delayed response to the exposure within this time frame. In general there was no clear dose-response in mice receiving either 20, 60 or 180 mins of exposure. Therefore, unless otherwise stated, only the responses to 180 min exposure are reported in key experiments.

BODY AND ORGAN WEIGHT AND CERIUM CONTENT

The average organ (thymus, heart, left lung, brain, liver, kidney, adrenal gland, spleen, testis) and body weights of male or female mice was not affected in a dose dependent manner by either exposure (data not shown). The cerium and cerium oxide levels in lung tissue were 1.06 ± 0.07 for males and 1.30 ± 0.09 $\mu\text{g}/\text{gram}$ for females in DCeE exposed mice, whereas the levels in the DE group were below the detection limit (<0.02 $\mu\text{g}/\text{gram}$ observed at necropsy (Table 2). Microscopic examination of the lungs revealed greater number of macrophages laden with dark-brown pigment in the alveolar and bronchiolar lumina (and occasionally in the interstitium) of DE-exposed mice. There was a similar frequency of these observations in animals exposed to the highest concentration of DCeE. Cells with a golden-brown pigment were very occasionally observed in the spleen and the brain, especially the meninges (Table 2), although, incidences were similar in mice receiving filtered air, suggesting this is an endogenous pigment e.g. melanin. There was no evidence of restructuring of the pulmonary tissue (e.g. fibrotic thickening of the airways and cuffing of alveolar blood vessels) in response to either exposure.

HEMATOLOGY & CLINICAL CHEMISTRY

A 4-week exposure to DE had little effect on blood cell differentials, hemoglobin concentration, hematocrit, platelets and mean corpuscular volume (Table 3) or associated haematological indices (see Methods, data not shown). There were no statistically significant differences between the DE and DCeE groups for any of the hematological indices studied. There were small, but significant, differences in several of the clinical chemistry parameters between the exposure types (Table 4). Albumin, ALP & TP levels were slightly greater in the DE group than the DCeE group at all doses, whereas ALT levels were lower in the DE group than the DCeE group. Blood calcium levels also showed significant differences between the DE and DCeE groups, although a dose response relationship was not evident.

A THEROSCLEROSIS

Overall, there was a trend that 'standard' diesel exhaust increased the atherosclerotic burden, with an $\sim 35\%$ increase in plaque size compared to control (Fig 2a). This effect was not seen in the DCeE group. However, these differences did not achieve statistical significance ($P=0.12$ for Control vs DE; $P=0.10$ for Control vs DCeE). No dose related effect was seen and similar findings were observed if the data on the

maximum plaque size (rather than burden throughout the artery) was used (data not shown). Histological analysis of plaques showed lesions rich in foam cells, cholesterol crystals and regions that were dense in fibro-elastic matrix and smooth muscle cells. All sections were assessed for the number of individual plaques within a single section (Fig 2b(i)) and, where this was observed, as two overlying plaques separated by a buried fibrous layer (Fig 2b(ii)). The number of plaques in a single section varied from one to five, with a mean of 1.8 ± 0.1 adjoining plaques and 0.6 ± 0.1 buried fibrous layers in air-treated ApoE^{-/-} mice. There was a significantly greater number of adjoining plaques (2.3 ± 0.4 plaques, $P=0.049$) and buried fibrous layers (1.2 ± 0.4 buried fibrous layers, $P=0.019$) in mice exposed to high DE, but not in DCeE-treated mice (Fig 2c). Exposure to DE or DCeE (high dose only) had no significant effect on either lipid or macrophage/foam cell content of plaques (Fig 3).

PROINFLAMMATORY CYTOKINES IN THE CNS, SPLEEN AND LIVER

When compared to exposure to DE, there was an increase in the levels of the proinflammatory cytokine, TNF- α , in the cereb fraction of the brain after exposure to DCeE ($P=0.05$; Fig 4). Levels of IL-1 β were also increased after exposure to DCeE, although, this did not reach statistical significance ($P=0.10$). There was no change in the levels of these proinflammatory cytokines in the spleen, however, in liver tissue a decrease in the levels of both proinflammatory cytokines was observed after DCeE exposure compared to DE exposure ($P=0.04$; Fig 4).

Overall remarks, attachments

Remarks on results including tables and figures

For tables and figures see attachment full study paper.

EXPOSURE CHARACTERISTICS

The use of CeO₂ as a diesel fuel additive changed the physicochemical composition of the exhaust, reducing the total number of particles and, to a lesser extent, the total particle surface area predicted to be deposited in the tracheobronchial region. The decrease in particle numbers was accompanied by an increase in NO_x and CO levels, however, these alterations were small and non-significant. In the present study we maintained a fixed mass concentration for the DE exposure. Switching to cerium-spiked fuel may have a more dramatic reduction in particle numbers if the running conditions of the engine are kept constant rather than the mass of PM in the exhaust. Similarly, it is unlikely that levels of gaseous co-pollutants will increase when simply switching to cerium enriched fuel. Improved combustion may result in higher CO₂ levels, however, this was not measured in the present study and may require attention from an environmental perspective. Sajith et al. (2009) reported that the addition of 80 ppm CeO₂ (~9 fold higher than the concentration of cerium used in the present study) resulted in a doubling of the CO production, and marked reductions in the levels of NO_x. In the present study we used an engine under a fixed load, whereas it is notable that Sajith et al. initially used an idling engine and when higher engine loads were used the changes in gases became less evident. Thus, different engines, engine load and fuel composition will influence the exhaust characteristics, and therefore, the potential biological consequences caused by exposure to these emissions. Interestingly, the particle size distribution in the diesel engine exhaust was unchanged by addition of cerium to the fuel, yet despite particle mass remaining constant, the overall surface area of the particulate was reduced. These characteristics suggest that the net effect of using CeO₂ is the formation of more condensed particle agglomerates in the exhaust. Cerium itself could be detected in the exhaust as 1-3 nm (geometric diameter) spherical CeO₂ nanoparticles captured within or on the surface of agglomerates of diesel exhaust soot. Therefore, the toxicity of CeO₂ will depend on the complex formed in exhaust (e.g. the availability of the cerium on the outer surface of the diesel exhaust agglomerates) and this may differ from CeO₂ aerosolized from a dry powder, which has formed the basis of risk-assessment studies in the past (Park et al. 2008; Cassee et al., 2011), or applied via intratracheal instillation into the lung (Ma et al. 2011).

GENERAL TOXICOLOGY

Exposure to nano-sized CeO₂ at the current levels in ambient air have been suggested to be unlikely to lead to pulmonary oxidative stress and inflammation (Park et al. 2008; Ma et al. 2011). These events are considered potential precursors for respiratory and cardiac health problems. However, these predictions have been made from the results of *in vitro* assays and cell culture experiments. Our study is conducted in an *in vivo* disease model using a biologically relevant route of exposure with realistic exposure atmosphere (albeit higher than can be expected to occur in urban environments) to accurately assess the toxicity of airborne cerium and the effect of cerium on the properties of engine exhausts. The overall evaluation of the effects of a 4-week diluted engine exhaust exposure reassuringly revealed very little adverse biological changes for either DE or DCE, and consequently, no apparent dose-response relationships. While there were significant differences between the DE and DCE groups for a select number of parameters, the changes observed were small and did not appear to be related to the dose of exhaust. Additionally, these changes might be transient, since 17 days after the final exposure, the effects of the highest dose of exhaust were no longer detected. Furthermore, the clinical consequences of these modest effects on individual parameters may be limited, since the changes are mostly within the range of reference values (Fox et al. 2007) and in some cases in the opposite direction than that associated with various diseases. The modest effects of both exhaust types is surprising since previous studies in our own lab (Van Berlo et al. 2010; Gerlofs-Nijland et al. 2010a), as well as by others (Kobayashi et al. 2000; Sunil et al. 2009), revealed that similar exposures to DE usually results in detectable adverse effects (e.g. hyperresponsiveness and inflammation). Bronchoalveolar fluid was not collected in order to maintain the structural integrity of the lungs for histological analysis. Subsequently, we were unable to accurately quantify levels of lung inflammation induced by DE exposure. Nevertheless, histological analysis indicated that DE exposure induced macrophage infiltration into the alveolar and bronchiolar lumina, and the interstitium of the lungs, verifying that the exposure levels used were sufficient to induce an inflammatory response in the lungs. Furthermore, numerous macrophages contained a dark brown pigment, suggesting that they were laden with diesel exhaust particles, confirming that particles themselves penetrated deep into the alveoli. Previous studies using prolonged exposures to DE focused on either animal models of allergy (Ichinose et al. 2003; Miyabara et al. 1998; Ferraj et al. 2010) or bacterial infections (Hiramatsu et al. 2005). In these studies 4 or 8 week exposures to DE with similar or higher PM levels (2-3 mg/m³) had little effect on pulmonary cytokines, chemokines, pathology of the airways, or acute murine mycobacterial infection. Some effects only became evident after 2 months (Hiramatsu et al. 2005), suggesting mice are capable of coping with relatively long periods of exposure. Sunil and colleagues (2009) suggest that young mice can adapt to DE-induced pulmonary changes, perhaps due to the relatively high pulmonary clearance rates of particles in rodents (Ferraj et al. 2010) and this may apply to our study presented here. Finally, another possible explanation for the limited effects of DE in the present study is that a newer engine has been used under a load, whereas previous studies used older engines running under idling conditions. The former results in relatively low particle emissions compared to the gaseous fraction and substantially lower levels of organic carbon (including harmful polycyclic aromatic hydrocarbons) in the particulate (Maricq et al. 2007). While the current study demonstrates that the hazard of the emission from newer engines and fuels may be lower than that reported in studies using older technologies or idling engines, several specific biological parameters were influenced by diesel exhaust (discussed below), highlighting that the exhaust emissions may still have relevant health effects.

A THEROSCLEROTIC EFFECTS OF EXPOSURES

There is a large body of evidence showing that levels of particulates within the air are associated with an increase in the size and development of atherosclerotic plaques (Araujo & Nel 2008; Sun et al. 2010). The proatherosclerotic effect of diesel exhaust, specifically, has not been studied in man. However, a limited number of studies in ApoE^{-/-} mice have suggested that diesel exhaust promotes the growth of atherosclerotic plaques (Campen et al. 2010; Bai et al. 2011). In this study, we found a trend towards an increase in plaque size in animals exposed to DE that was not apparent in DCE-exposed animals. This trend adds further weight to the findings suggesting that DE exposure may accelerate proatherosclerotic

formation. Furthermore, our results also suggest that the particulate fraction of DE, rather than the gaseous component, may mediate the proatherosclerotic effect of DE. This is because exposure to DCeE, which contained a greater proportion of the gases believed to mediate the harmful actions of combustion-derived emissions (e.g. CO, NO_x) did not potentiate proatherosclerotic changes. We reiterate again that the effects seen fell just short of statistical significance. The size and composition of plaques varied considerably between animals, perhaps not surprisingly due to the multi-faceted nature of this disease process. Therefore, in hindsight, greater numbers of animals may have been needed to account for this variability and obtain statistical significance. In this regard, we feel at present it would be unwise to speculate on these findings. However, should the proatherosclerotic effects of DE be a true phenomena, it would be interesting to establish a mechanism for the effects in the absence of a marked inflammatory response. Several biological messengers that were not measured in this study may underlie the process. These include tissue factor (Sun et al. 2008), upregulation of adhesion molecules (Yatera et al. 2008), matrix metalloproteinases (Campen et al. 2010), NAD (P)H oxidase (Ying et al. 2009), or altered function of high-density lipoprotein (Araujo et al. 2008). In the current study, after exposure, levels of IL-1 β and TNF- α were modulated in the liver raising the possibility that organ-specific changes in certain proinflammatory cytokines may mediate the effects of diesel exhaust. Whether similar changes occur in vascular tissue, and to what extent this influences atherosclerosis, is currently under investigation. Plaque growth alone does not account for the pathology of atherosclerosis in man and plaque size needs to be considered in parallel to plaque vulnerability to rupture that underlies the clinical consequences of atherothrombosis. Plaques that are rich in lipids and inflammatory cells are believed to be more susceptible to rupture. Neither DE or DCeE exposure altered the percentage of plaque composed of lipids or macrophages/foam cells. The brachiocephalic artery was chosen for an in-depth analysis of plaque development as complex plaques with fibrous layers form at this site and it has been suggested that this represents a site of a previous plaque rupture (Jackson et al. 2007). There was a significant increase in the occurrence of buried fibrous layers in plaques from DE-treated mice, that was not seen in the DCeE-exposed group. The ongoing debate (Jackson et al. 2007; Jackson 2007; Rosenfeld et al. 2008) as to whether buried fibrous layers represent the formation of a new plaque over a site of plaque rupture, or is merely a feature of the ongoing development of a single plaque cannot currently be answered with this model. Nevertheless, the trend towards a greater size of plaque with a more complicated structural phenotype, suggests that diesel exhaust inhalation promotes atherosclerotic burden, and that these effects may be prevented by the use of cerium-spiked fuels.

CNS EFFECTS OF EXPOSURE

The potential adverse effects of inhaled nanoparticles are not limited to the cardiopulmonary system, but may also affect the CNS. Enhanced neuroinflammation and an altered blood-brain barrier (BBB) has been observed in children and young adults living in cities with high air pollution (Calderón-Garcidueñas et al. 2008). TNF- α and IL-1 are the main proinflammatory cytokines in the brain and chronic neuroinflammation can amplify the generation of these factors. Neuroinflammatory events are associated with the pathology of a number of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Tilleux & Hermans, 2007). Exposure to PM may potentiate the pathogenesis of these disorders by enhancing neuroinflammation (Campbell, 2004). We have previously demonstrated that subacute exposure to DE is associated with increased levels of these cytokines in the striatum of rats (Gerlofs-Nijland et al. 2010a) and in ApoE^{-/-} mice exposed to PM derived from Los Angeles freeways (Campbell et al. 2009). In the present study, standard DE exposure was not accompanied by changes in these cytokines, most likely due to the reasons discussed above. Interestingly, in the DCeE exposed group the levels of TNF- α were increased in the cerebellum and brain stem when compared to the DE-exposed samples. The absence of Apolipoprotein E compromises the integrity of the BBB (Methia et al. 2001), perhaps allowing CeO₂ particles to access the brain parenchyma and induce a local inflammatory response. In normal rats, CeO₂ nanoparticles do not bypass the BBB although they have the capacity to alter the levels of the antioxidant enzyme catalase in the hippocampus and cerebellum (Hardas et al. 2010). Therefore, it appears that the direct entry of CeO₂ into the brain parenchyma is not necessary for

modulation of CNS responses. To what extent the elevation in the cytokines may predispose the brain to adverse effects requires further investigation.

LIMITATIONS

The present study design (e.g. use of engine under load, newer engine, moderate exposure dose) was used to take into account OECD guidelines as well as reflect real-world scenarios to some extent, rather than use conditions where we were assured to see clear toxicological effects. Consequently, standard DE had limited detrimental actions and, unfortunately, it is difficult to fully assess the magnitude or preventative effects of the DCeE. At present there are no rodent studies that demonstrate adverse effects of exhaust from CeO₂-spiked diesel fuel. This most likely reflects the limited data available on the biological actions of this fuel additive rather than an inherent problem with rodent models. Nevertheless, it is reassuring that, with the exception of the change in proinflammatory cytokines in the cerebellum and brain stem region of the CNS, subacute exposure to DCeE, at CeO₂ levels above that likely to be reached following dilution in ambient air, did not result in other harmful effects. Direct extrapolation of our results into human risk are challenging and will need to consider the biopersistence of CeO₂ in the body and in the environment. Nevertheless, the limited systemic effects of cerium-spiked diesel exhaust in mice, suggests that controlled studies evaluating the effects of environmentally-relevant levels of this exhaust should be feasible in human subjects.

SUMMARY AND CONCLUSION

In summary, very few detrimental effects were observed following 4 week DCeE exposure providing data for establishing a lowest as well as no observed adverse effect level. Changes in the physicochemical composition of diesel engine exhaust when using the additive cerium oxide may limit the proatherosclerotic effects associated with regular diesel fuel. Further toxicological studies on the use of cerium containing fuel additives are required, especially in regards to the possibility of potential adverse effects in the CNS. However, the general lack of toxicological consequences in this initial study are promising and offer some hope that addition of cerium to diesel fuel may limit the cardiorespiratory effects of combustion-derived nanoparticles.

Attached full study report

Attached full study report

NM_211_ANNEX_A82_Cerium oxide and diesel exhaust toxicity draft RIVM.pdf / 2.04 MB
(application/octet-stream): ENV/JM/MONO/(2015)8/ANN5

Applicant's summary and conclusion

Conclusions

These results imply that addition of CeO₂ nanoparticles to fuel decreases the number of particles in exhaust and may reduce atherosclerotic burden associated with exposure to standard diesel fuel. From the extensive assessment of biological parameters performed, the only concerning effect of cerium addition was a slightly raised level of cytokines in a region of the central nervous system. Overall, the use of cerium as a fuel additive may be a potentially useful way to limit the health effects of vehicle exhaust. However further testing is required to ensure that such an approach is not associated with a chronic inflammatory response which may eventually cause long-term health effects.

Executive summary

Background: Cerium oxide (CeO₂) nanoparticles improve the burning efficiency of fuel. However, little is known about health impacts of altered emissions from the vehicles. Methods: Atherosclerosis-prone apolipoprotein E knockout (ApoE) mice were exposed by inhalation to diluted exhaust (1.7 mg/m³, 20, 60 or 180 mm, 5 days/week, for 4 weeks), from an engine using standard diesel fuel (DE) or the same diesel fuel containing 9 ppm cerium oxide nanoparticles (DCeE). Changes in hematological indices, clinical

chemistry, atherosclerotic burden, tissue levels of inflammatory cytokines and pathology of the major organs were assessed. Results: Addition of CeO₂ to fuel resulted in a reduction of the number (30%) and surface area (10%) of the particles in the exhaust, whereas the gaseous co-pollutants were increased (6-8%) There was however, a trend towards an increased size and complexity of the atherosclerotic plaques following DE exposure, which was not evident in the DCeE group. There were no clear signs of altered hematological or pathological changes induced by either treatment. However, levels of proinflammatory cytokines were modulated in a brain region and liver following DCeE exposure. Conclusions: These results imply that addition of CeO₂ nanoparticles to fuel decreases the number of particles in exhaust and may reduce atherosclerotic burden associated with exposure to standard diesel fuel. From the extensive assessment of biological parameters performed. the only concerning effect of cerium addition was a slightly raised level of cytokines in a region of the central nervous system. Overall, the use of cerium as a fuel additive may be a potentially useful way to limit the health effects of vehicle exhaust. However further testing is required to ensure that such an approach is not associated with a chronic inflammatory response which may eventually cause longterm health effects. Key words: cerium oxide. diesel exhaust. atherosclerosis. inhalation toxicology, fuel additive, particle. central nervous system, nanomaterial. nanoparticles, neuroinflammation.

7.5.3 Repeated dose toxicity: dermal

7.5.4 Repeated dose toxicity: other routes

7.6 Genetic toxicity

7.7 Carcinogenicity

7.8 Toxicity to reproduction

7.9 Specific investigations

7.10 Exposure related observations in humans

7.11 Toxic effects on livestock and pets

7.12 Additional toxicological information

Endpoint study record: Cytotoxicity in HepG2 Human Hepatoblastoma Cell Line (US EPA)

Administrative Data

Study result type experimental result

Materials and methods

Test guideline

Qualifier	Guideline	Deviations
according to	other guideline: Attagene	

Test materials**Test material form**

nanomaterial

Details on test material

nano-CeO₂ uncoated 15 - 30 nm NanoAmor 1406RE; nano-CeO₂ uncoated 70 -105 nm Alfa Aesar 44758

Overall remarks, attachments**Remarks on results including tables and figures**

Nano CeO₂ not cytotoxic up to 100 ug/ml;

Endpoint study record: In vitro toxicological information_CeO₂ NanoCare materials_primary cells**Administrative Data**

Study result type experimental result **Study period** 2006-2009

Reliability other: needs to be evaluated and set by expert researchers group

Data source**Reference**

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NanoCare project partners	2009	NanoCare Health related Aspects of Nanomaterials Scientific Report (http://www.nanopartikel.info/files/content/dana/Dokumente/NanoCare/Publikationen/NanoCare_Final_Report)	ISBN: 978-3-89746-108-6					

Data access

data published

Data protection claimed

yes, but willing to share

Materials and methods**Type of information**

In this study, the responses of primary rat alveolar macrophages after exposure to several CeO₂ nanomaterials of different physical-chemical properties (different pH, Al-doped material) were determined in vitro in order to bridge the in vitro and in vivo systems and rat inhalation models applied in the NanoCare project. For this aim freshly isolated alveolar macrophages of guinea pigs were used to investigate their reaction on respirable particles. The assay concept is based on the finding that macrophages lavaged from the lungs of healthy donor animals retain most of their native properties such as extensive phagocytosis of particles and generation of mediators such as tumour necrosis factor (TNF).

Adaptations of alveolar macrophages to their micro-environment inside the lung alveolus, which might influence the reaction to particles, are most likely preserved making primary alveolar macrophages a unique cell model. Investigated endpoints were ROS production, release of LDH and glucuronidase as indicators of plasma membrane integrity or damage to phagolysosomes, respectively, as well as production of the tumour necrosis factor (TNF). Tested CeO₂ materials were 2 materials (CeO₂-A and – D) of 14 nm but of different pH (6.2 and 3.4) and one Al-doped CeO₂ (5%) from Evonik. Quartz and corundum were used as positive and negative controls, respectively. The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂.

Sample preparation/conditioning protocol for preparing suspensions please refer to the SOPs above

Method

Any other information on materials and methods incl. tables see attached material information sheet of NanoCare

Principles of method if other than guideline

investigation of different in vitro toxicological endpoints using freshly isolated rat alveolar macrophages (SOPs attached) - for investigated endpoints see below

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

no

Test material form

nanomaterial

Details on test material

The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂. In the NanoCare project 5 different CeO₂ nanomaterials of different sizes were investigated for potential cytotoxic effects in in vitro tests systems. 2 of them were used to investigate the responses of primary rat alveolar macrophages after exposure (please refer to the table below). In addition 5%-Al doped CeO₂ material was investigated in selected studies. For further material information please refer to the attachment below.

NanoCare No.	Material	Powder	Primary particle size [nm]	Remarks	Provider
3.1	CeO ₂ -A	X	14	pH 6.2	Evonik
3.4	CeO ₂ -D	X	14	pH 3.4	Evonik

details on the test materials investigated within the NanoCare project:-

- 4.24 agglomeration/aggregation: method: In Air: Test for agglomerate stability according to the publication by Stahlmecke et al. in J. Nanopart Res (2009) 11:1625 –1635 and Nanocare Final Scientific Report for 5 modifications of CeO₂
- In Liquid: Analytical Ultracentrifugation (AUC), in review: Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, ACS Nano- 4.8
- Water Solubility/Dispersibility: protocol: Initial concentration 10 mg/ml, 24h agitation at 300 rpm; centrifugation and supernatant analysis - 4.25
- Crystalline phase: method: XRD-4.31
- Dustiness: Test for powder dustiness in accordance to EN 15051. The continuous drop method, modified to adjust for nanoscale particle measurements, was employed. Published in: Nanocare Final Scientific Report for 5 modifications of CeO₂- representative TEM picture: in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano), see also attached material information sheet below-4.28
- Specific surface area: BET Evonik A: 63m²/g, Evonik D: 63 m²/g-4.29
- zeta potential: method: measurements with Zetasizer Nano ZS from Malvern Instruments (DLS and Zeta potential) //results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano) -4.30
- Surface chemistry: measurements with XPS and TOF-

SIMS//results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano)

Method

Any other information on materials and methods incl. tables

Describe the scientific and technical basis of the test method What biological/cellular model is the method based on? Freshly isolated alveolar macrophages of guinea pigs What biological endpoints/responses does this method address? Tumour necrosis factor (TNF): cytokine involved in systemic inflammation, able to induce apoptotic cell deathRelease of glucuronidase: damage to phagolysosomesRelease of lactate dehydrogenase (LDH): plasma membrane integrityPMA (Phorbol 12-myristate 13-acetate) induced ROS production: functional cell damage What specific mechanisms associated with the biological response are targeted? see above What methods/techniques are used for endpoints/responses determination? please refer to the attached SOPs Are there potential technical limitations of this method for testing nanomaterials? In the NanoCare project, the nanoparticles have been characterised in advance regarding their dispersion and interactions in physiological media, their sterility, their endotoxin concentration, and their possible interference with the test systems (see also authos` s publication: Current in vitro methods in nanoparticle risk assessment: Limitations and challenges Kroll A, Pillukat MH, Hahn D, Schnekenburger J. 2009European Journal of Pharmaceutics and Biopharmaceutics, Volume 72, Issue 2, June 2009, Pages 370-377). Describe the role of the method in context of hazard assessment for human health How should the information/results derived from the method be interpreted in relation to an in vivo response/endpoint? The common evaluation of all tests carried out in the NanoCare project has shown that invitro tests are, to a certain extent, able to reflect some of the in vivo results as long as they are directly particle-derived. As to whether in vitro conditions can be translated to the real-life situation was beyond the scope of this study which was to comparatively rank toxic properties of particles without considering durability, associated biopersistence, and/or the organism's long-term response to injury. Describe the Standard Operating Procedure (SOP) SOP description as a template SOP_Preparing_suspensions_with_phagocytic_cellsSOP_Preparing_Suspensions_NanoCareGMZ SOP LDH SOP as attached file

Results and discussions

Any other information on results incl. tables

Alveolar macrophages were lavaged from the lungs of healthy guinea pigs and pre-cultured in 96-well plates in MEM medium before particle-containing medium was added (15, 30, 60 and 120 µg/106 cells, corresponding to 18 -128 µg/cm²). Supernatant was retrieved after 16 hours after 10 min centrifugation such that no particulate matter can interfere with the assays. Functional cell damage was derived from PMA (Phorbol 12-myristate 13-acetate)-induced ROS production 16 hours after application of particles. LDH and glucuronidase were measured colorimetrically. Release of glucuronidase is indicative of damage to phagolysosomes, and this parameter is mostly independent from LDH release. Production of TNF was determined with a bioassay by means of L-929 cells. Quartz and corundum were used as positive and negative controls, respectively. Tested CeO₂ materials were 2 materials (CeO₂-A and -D) of 14 nm but of different pH (6.2 and 3.4) and one Al-doped CeO₂ (5%) from Evonik. All variants of CeO₂ and in particular CeO₂ (Al-doped, 5%, Evonik) showed an increase in cytotoxic potential at concentrations ≥60 pg/cell (LOEL for 14 nm CeO₂ materials is 60-120 pg/cell, for the doped material 30-60 pg/cell). Release of LDH was found upon concentrations of 15-30 pg/cell for CeO₂-A (14nm, pH 6.2) and the Al-doped CeO₂, for CeO₂-D (14 nm/ph 3.4) upon an exposure concentration of 30 pg/cell. Glucuronidase was released upon exposure to all tested CeO₂ (LOELs for CeO₂ materials of 14nm: 60 pg/cell, for the Al-doped CeO₂: 30 pg/cell). Most striking, CeO₂ (14nm, pH 6.2) and the Al-doped CeO₂ induced high amounts of TNFα in a dose dependent manner (LOEL: 15pg/cell).

Overall remarks, attachments

Remarks on results including tables and figures

The project NanoCare was funded by the Germany ministry of education and research within the programme NanoCare1 and comprised of 15 partners from industry, universities and research institutes (running from 2006 through 2009). In NanoCare 10 different synthetic nanomaterials in altogether 31 modifications (size, surface modification, pH) were investigated with regard to the potential impacts of nanomaterials on human health. For this aim new applications and measurement methods were developed and several in vivo and in vitro methods were employed. Three different CeO₂ nanomaterials were investigated with regard to their potential impact on the responses of primary rat alveolar macrophages.

Attached background material

Attached document	Remarks
NM_211_ANNEX_A65_SOP_Preparing_suspensions_with_phagocytic_cells.pdf / 64.1 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A66_GMZ SOP LDH_english.pdf / 85.58 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A67_Preparing_Suspensions_NanoCare.pdf / 40.96 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A61_material information sheets NanoCare CeO ₂ .pdf / 1.28 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	

Endpoint study record: In vitro toxicological information_CeO₂ NanoCare materials_permanent cell lines

Administrative Data

Study result type experimental result **Study period** 2006-2009

Reliability other: needs to be evaluated & set by expert researchers group

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	NanoCare Project Partners	2009	NanoCare Health related Aspects of Nanomaterials Scientific Report (http://www.nanopartikel.info/files/content/dana/Dokumente/NanoCare/Publikationen/NanoCare_Final_Report)	ISBN: 978-3-89746-108-6					

Data access

data published

Data protection claimed

yes, but willing to share

Materials and methods

Type of information

In this study, the responses of a set of cell lines and a coculture system after exposure to several CeO₂ nanomaterials of different physical-chemical properties (e.g. primary particle size) were determined in vitro. Different widely used endpoints such as cell membrane integrity, the intracellular formation of reactive oxygen species (ROS), cellular metabolic activity and the release of the pro-inflammatory mediator IL-8 were addressed. For standardisation, currently available in vitro toxicity methods were carefully evaluated and SOPs were developed. Cell lines derived from different origins (e.g. macrophages, fibroblasts, lung epithelium, kidney epithelium) from different species (human, dog, rat, mouse) are used for in vitro exposure to CeO₂. The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂.

Principles of method if other than guideline

investigation of different in vitro toxicological endpoints using different assays and various permanent cell lines as well as a co-culture system (SOPs attached) - for employed cell lines and investigated endpoints see below

Test materials

Test material form

nanomaterial

Details on test material

The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂. In the NanoCare project 5 different CeO₂ nanomaterials of different sizes were investigated for potential cytotoxic effects in in vitro tests systems (please refer to the table below). For further material information please refer to the attachment below.

NanoCare No.	Material	Powder	Primary particle size [nm]	Remarks	Provider
3.1	CeO ₂ -A	X 14 pH 6.2	Evonik		
3.2	CeO ₂ -B	X 20 pH 5.4	Evonik		
3.3	CeO ₂ -C	X 23 pH 3.4	Evonik		
3.4	CeO ₂ -D	X 14 pH 3.4	Evonik		
3.5	CeO ₂	X 70	BASF	details on the test materials investigated within the NanoCare project:-	

4.24 agglomeration/aggregation: method: In Air: Test for agglomerate stability according to the publication by Stahlmecke et al. in J. Nanopart Res (2009) 11:1625–1635 and Nanocare Final Scientific Report for 5 modifications of CeO₂

In Liquid: Analytical Ultracentrifugation (AUC), in review: Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, ACS Nano-

4.8 Water Solubility/Dispersibility: protocol: Initial concentration 10 mg/ml, 24h agitation at 300 rpm; centrifugation and supernatant analysis -

4.25 Crystalline phase: method: XRD-

4.31 Dustiness: Test for powder dustiness in accordance to EN 15051. The continuous drop method, modified to adjust for nanoscale particle measurements, was employed. Published in: Nanocare Final Scientific Report for 5 modifications of CeO₂-

representative TEM picture: in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano), see also attached material information sheet below-

4.28 Specific surface area: BET Evonik A: 63 m²/g, Evonik B: 44 m²/g, Evonik C: 38 m²/g, Evonik D: 63 m²/g, BASF SE 33 m²/g-

4.29 zeta potential: method: measurements with Zetasizer Nano ZS from Malvern Instruments (DLS and Zeta potential) //results in review (Nanoparticles in blood serum

– atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano) - 4.30 Surface chemistry: measurements with XPS and TOF-SIMS//results in review (Nanoparticles in blood serum – atomic force spectroscopy correlates with protein assays and hydrodynamics, J. Schäfer, C. Schulze, E.E. J. Marxer, U.F. Schäfer, W. Wohlleben, U. Bakowsky, Claus-Michael Lehr, in review ACS Nano)Sample preparation/conditioning protocolfor preparing suspensions please refer to the SOPs above

Method

Any other information on materials and methods incl. tables

Materials and methods Type of information In this study, the responses of a set of cell lines and a coculture system after exposure to several CeO₂ nanomaterials of different physical-chemical properties (e.g. primary particle size) were determined in vitro. Different widely used endpoints such as cell membrane integrity, the intracellular formation of reactive oxygen species (ROS), cellular metabolic activity and the release of the pro-inflammatory mediator IL-8 were addressed. For standardisation, currently available in vitro toxicity methods were carefully evaluated and SOPs were developed. Cell lines derived von different origins (e.g. macrophages, fibroblasts, lung epithelium, kidney epithelium) from different species (human, dog, rat, mouse) are used for in vitro exposure to CeO₂. The used CeO₂ nanomaterials of this study are not identical to the batch of the OECD RMN NM-Series for CeO₂. Principles of method if other than guideline investigation of different in vitro toxicological endpoints using different assays and various permanent cell lines as well as a co-culture system (SOPs attached) - for employed cell lines and investigated endpoints see below Describe the scientific and technical basis of the test method What biological/cellular model is the method based on? permanent cell lines (for cell line and culture media information please refer to the attachment below):-A549 (carcinomic human alveolar basal epithelial cell line) and RAW 264.7 cell line (mouse leukaemic monocyte macrophage cell line) for measuring multiple cytotoxic endpoints-set of 11 different cell lines for evaluating cell type specific sensitivity (representing different routes and different lines of exposure: A549, CaCo2, HaCaT, MDCK, MDCK II, NIH3T3, NRK52E, RAW267, RLE-6TN, CaLu3, T84)-coculture model (A549 cell line and monocyte/macrophage-like cell line Mono-Mac-6 (MM6))The human cell lines were typed by the DSMZ (German Collection of Micro-organisms and Cell Cultures) to confirm their identity. Cell lines were freshly ordered, expanded, and deep frozen in aliquots to serve as standardised stock for the duration of the project. All cells in culture were regularly checked for infection with different mycoplasma species. Cells were maintained at 37°C with 5% CO₂ in a humidified incubator. What biological endpoints/responses does this method address? WST1 assay and MTT assay: cellular metabolic activity, cell viabilityrelease of lactate dehydrogenase (LDH): plasma membrane integrityincrease of 2',7'-dichlorofluorescein (DCF) fluorescence: intracellular formation of reactive oxygen species (ROS) coculture model (A549 cell line and monocyte/macrophage-like cell line Mono-Mac-6 (MM6) with LPS stimulation: proinflammatory effects release of the pro-inflammatory mediator IL-8: pro-inflammatory response What specific mechanisms associated with the biological response are targeted? see above What methods/techniques are used for endpoints/responses determination? please refer to the attached SOPs Are there potential technical limitations of this method for testing nanomaterials? In the NanoCare project, the nanoparticles have been characterised in advance regarding their dispersion and interactions in physiological media, their sterility, their endotoxin concentration, and their possible interference with the test systems (see also author's publication: Current in vitro methods in nanoparticle risk assessment: Limitations and challenges Kroll A, Pillukat MH, Hahn D, Schnekenburger J. 2009 European Journal of Pharmaceutics and Biopharmaceutics, Volume 72, Issue 2, June 2009, Pages 370-377). Describe the role of the method in context of hazard assessment for human health How should the information/results derived from the method be interpreted in relation to an in vivo response/endpoint? The common evaluation of all tests carried out in the NanoCare project has shown that in vitro tests are, to a certain extent, able to reflect some of the in vivo results as long as they are directly particle-derived. As to whether in vitro conditions can be translated to the real-life situation was beyond the scope of this study which was to comparatively rank toxic properties of particles without considering durability, associated

biopersistence, and/or the organism's long-term response to injury. Describe the Standard Operating Procedure (SOP) SOP description as a template SOP_Preparing_Suspensions_NanoCareGMZ SOP DCFGMZ SOP LDHGMZ SOP MTT

Results and discussions

Any other information on results incl. tables

In vitro studies: Standardized Cell lines: Three different experimental set ups were investigated: 1.) Using A549 cell line (carcinomic human alveolar basal epithelial cell line) and RAW 264.7 cell line (Mouse leukaemic monocyte macrophage cell line) and multiple cytotoxic endpoints. Cell lines A549 and RAW 264.7 (for cell line and culture media information see Annex 2) were used in experiments with a set of 5 different CeO₂ materials of different sizes and different pH (material A: 14 nm, pH 6.2, material B: 20 nm, pH 5.4, material C: 23 nm, pH 3.4, material D: 14 nm, pH 3.4, all from Evonik; material BASF: one material from BASF, 70 nm) at 0.5, 5, 25 and 50 µg/cm² for 24, 48 and 72 hours. Multiple cytotoxic endpoints were evaluated: Cell viability, plasma membrane integrity (release of LDH), formation of ROS (upon 1 h of exposure), decrease of Glutathione, expression of biomarkers (HOX-1 and COX-2) and proinflammatory response (secretion of IL-8). A decreasing metabolic activity is an indicator for low cell viability. CeO₂-D decreased metabolic activity (with WST-1 assay) in A549 cells upon exposure of 25 µg/cm² for 24h hours (other tested CeO₂ materials revealed effects upon 72h of exposure). LOELs of 25 µg/cm² for LDH release were observed upon 72h of exposure for all tested materials. The formation of intracellular ROS was measured by monitoring the increasing fluorescence of 2',7'-dichlorofluorescein (DCF) in A549 cells. A LOEL of 6.25 µg/cm² for the generation of ROS was calculated upon 1 hour of exposure of CeO₂-A (other tested CeO₂: LOELs of 25 µg/cm²). The cellular GSH and glutathione disulphide (GSSG) contents were measured after 6 hours of incubation. A decrease was detected upon exposure of CeO₂-B (5 µg/cm², LOELs of other tested CeO₂: 25 µg/cm²). Expression of the anti-oxidative enzymes hemeoxygenase-1 (HO-1) and cyclooxygenase-2 (Cox-2) were investigated after exposure of the murine macrophage cell line RAW264.7 to CeO₂ nanoparticle dispersions. An increase of HO-1 and COX-2 expression was analyzed after 6 hours of incubation. A concentration of 0.1 µg/cm² of CeO₂-B showed an increase of both indicators. CeO₂-A and CeO₂-BASF showed an effect at particle concentration of 1 µg/cm². IL-8 release was measured in order to quantify the pro-inflammatory response of A549 cells to treatment with nanoparticles. Release was measured upon 48 h of exposure of 25 µg/cm² CeO₂-B, -C and D. Secretion of IL-8 was also found upon 72 h of exposure of 5 µg/cm² CeO₂-B. 2.) Using a set of different cell lines These experiments were designed to evaluate the cell type specific sensitivity to CeO₂ exposure. Up to eleven cell lines representing different routes and different lines of exposure were exposed (A549, CaCo2, HaCaT, MDCK, MDCK II, NIH3T3, NRK52E, RAW267.4, RLE-6TN, CaLu3, T84, for cell line and culture media information see Annex 2). Cellular processes such as rapid oxidative stress response, metabolic activity and cell death were determined by standardized LDH release (cell death), DCF (ROS formation) and MTT (metabolic activity) assays using a set of 5 different CeO₂ materials of different sizes and different pH (material A: 14 nm, pH 6.2, B: 20 nm, pH 5.4, C: 23 nm, pH 3.4, D: 14 nm, pH 3.4, all Evonik and one material from BASF, 70 nm). All tests were performed with nanoparticle concentrations of 0.01 µg/cm², 0.1 µg/cm², 1 µg/cm², 5 µg/cm², 10 µg/cm² (SOP available). Cells were used at 3 x 10⁵ cells/well which corresponds at a concentration of 10 µg/cm² to 10.7 pg/cell or 10.7 µg/10⁶ cells (well area 0.32 cm²). None of the CeO₂ materials caused effects on metabolic activity and LDH release upon 24 hours of exposure in all of the cell lines. ROS formation was found for CeO₂-A, -B and -C in several cell lines upon 1 hour exposure of 10 µg/cm². ROS formation on CaCo2 cells was already found upon exposure of 1 µg/cm² of CeO₂-A. 3.) In vitro Testing of Proinflammatory Effects of CeO₂ in Coculture Systems In order to study possible proinflammatory effects of nanoparticles in vitro, a coculture model of A549 and the monocyte/macrophage-like cell line Mono-Mac-6 (MM6) was developed (for cell line and culture media information see Annex 2). This system provides a relatively simple model of the alveolar response to nanoparticle exposure by measuring the release of the inflammatory mediator IL-8 (Interleukin 8) (ELISA). An existing inflammatory state can be simulated in the coculture model by LPS (Lipopolysaccharide) activation (1 ng/ml for 16 h) of the

monocytes/macrophages. The optimum cell culture conditions, controls, stimulation conditions, cell numbers and ratios as well as the most useful inflammatory parameters to be measured were determined previously in a series of studies or selected according to literature, respectively. In the A549/Mono-Mac-6 coculture model, the proinflammatory potential of CeO₂ materials at concentrations of 1 µg/cm² and 10 µg/cm² was determined by measuring the release of the inflammatory mediator IL-8 upon 24 hours of exposure. 3 of the 5 tested CeO₂ did not show any significant effects on IL-8 production in different cell culture types (A549 alone, MM6 alone, MM6 stimulated by LPS, A549 and MM6, A549 and MM6 stimulated by LPS). CeO₂-A and -C revealed slight elevated IL-8 levels at concentrations of 10 µg/cm² in the coculture model of A549 and MM6.

Overall remarks, attachments

Remarks on results including tables and figures

The project NanoCare was funded by the Germany ministry of education and research within the programme NanoCare1 and comprised of 15 partners from industry, universities and research institutes (running from 2006 through 2009). In NanoCare 10 different synthetic nanomaterials in altogether 31 modifications (size, surface modification, pH) were investigated with regard to the potential impacts of nanomaterials on human health. For this aim new applications and measurement methods were developed and several in vivo and in vitro methods were employed. Five different CeO₂ nanomaterials were investigated with regard to their potential impact on cellular processes. For this aim suitable cells/types of cells were exposed to different concentrations of the CeO₂ materials, such as pulmonary phagocytes, epithelial cells and co-cultures.

Attached background material

Attached document	Remarks
NM_211_ANNEX_A67_Preparing_Suspensions_NanoCare.pdf / 40.96 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A68_GMZ SOP DCF english.pdf / 115.75 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A69_GMZ SOP LDH_english.pdf / 85.58 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A70_GMZ SOP MTT_english.pdf / 118.65 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A61_material information sheets NanoCare CeO ₂ .pdf / 1.28 MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A71_NanoCare Cell line and culture media_CeO ₂ .pdf / 277.63 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	

Endpoint study record: 2012.02.23_In vitro toxicological information _CeO₂ _Rat hepatoma cell line (H4IIE) and rainbow trout (Oncorhynchus mykiss) gonadal tissue (RTG-2)

Administrative Data

Study result type experimental result

Data source

Data protection claimed

yes, but willing to share

Materials and methods

Type of information

Cytotoxicity assessment of CeO₂ Np towards a mammalian (Rat hepatoma cell line (H4IIE)) and fish (Rainbow trout (*Oncorhynchus mykiss*) gonadal tissue (RTG-2)) cell line using a variety of toxicity assays. Nominal exposure concentrations were assessed using ICP-MS to determine real exposure concentrations. Cytotoxicity assays were assessed for their reliability and sensitivity in detecting adverse effects due to particle exposure.

Principles of method if other than guideline

Various standard in vitro assays used for chemical toxicity assessment were used in order to investigate the differences in sensitivity and their suitability for testing adverse effects of CeO₂ Np exposure. An investigation into any interference of CeO₂ NPs with assay systems, that would suggest a limitation in the application of these tests, was also determined.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	NM-211

Test material form

nanomaterial

Details on test material

Principal nanomaterial name: Cerium (IV) Oxide (Antaria)

CAS Number: CAS 1306-38-3, EINECS 215-150-4

Structural formula/ molecular structure: CeO₂, cubic

Composition of nanomaterial being tested: Purity: > 99.5 wt% CeO₂

Basic morphology: Nanostructured powder

Description of surface chemistry: Neither coated nor modified.

Major commercial uses: Catalysts and chemomechanical polishing

Known catalytic activity: None

Method of production: Precipitation

Method

Any other information on materials and methods incl. tables

Describe the scientific and technical basis of the test method.

What biological/cellular model is the method based on? Rat hepatoma cell line (H4IIE) Rainbow trout (*Oncorhynchus mykiss*) gonadal tissue (RTG-2)

What biological endpoints/responses does this method address? Cytotoxicity (LDH)-Release of lactate dehydrogenase (MTT) –Cellular metabolic activity (NRU)-Neutral red uptake

What specific mechanisms associated with the biological response are targeted? Although all of those assays have cytotoxicity as an endpoint, they measure different targets of toxicity: Plasma membrane integrity-LDH mitochondrial metabolism-MTT Lysosomal damage-NRU.

What methods/techniques are used for endpoints/responses determination? Optical density determined at a wavelength of 340 nm with a Tecan Genios plate reader. The percentage LDH leakage was calculated by dividing the activity in the supernatant by the total activity (supernatant + cell lysate) and multiplying by 100. (LDH) Optical density at 570 nm using Tecan GENios plate reader (MTT) Optical density at 550 nm

using Tecan GENios plate reader (NRU) Potential nanoparticle interference with endpoint wavelengths was analysed in each assay system using a cell free 96-well plate with respective particle concentrations. Are there potential technical limitations of this method for testing nanomaterials? Concentration dependent interferences of particles with assays as well as possible inaccuracies in the dose metric were assessed and were found to be present and need to be taken into consideration when performing these assays.

Describe the role of the method in context of hazard assessment for human health. How could the information derived from the method be used to refine, reduce or replace an animal test, as specified in recognised testing standards and guidelines (e.g. OECD Test Guidelines)? Results obtained in this study show important differences that are related to the cell type and assay used. By evaluating the sensitivity of the different assays and cell lines, the most sensitive cell line and assay can be selected as the preferred cell line for detecting nanoparticle toxicity (in this study H4IIE and MTT assay). Thus optimising the use of in vitro cell lines in nanoparticle toxicity screening and reducing the use of animal testing as a first line test. What are specific limitations of this test in terms of predicting hazard to human health? Depending on the cell line used toxicity assessment will be organ and cell type specific.

Results and discussions

Any other information on results incl. tables

No evidence of cytotoxicity was detected by the NRU or LDH assay in the H4IIE or RTH-149 cell line. The MTT assay showed a higher sensitivity than the other assays detecting changes in mitochondrial metabolism in H4IIE cells after exposure to CeO₂. Taking real concentrations as measured by ICP-MS the lowest observed effect concentration (LOEC) in H4IIE cells was 0.68 µg/ml after 24 h exposure. In RTG-2 cells there was no observed effect or change in mitochondrial metabolism following 24 h or 72 h exposure.

Overall remarks, attachments

Remarks on results including tables and figures

Verification of the nominal concentration through ICP-MS revealed that there was a large discrepancy between nominal and measured concentration depending on concentration and particle tested (see table attached). This must be considered in future nanoparticle toxicity studies. Also in this study nanoparticle interference with assay systems were revealed. This interference caused an increase in optical density measurement at wavelengths used in assay systems and could have masked a slightly stronger negative effect due to the nanoparticles in the two highest concentrations (see attached document).

Attached background material

Attached document	Remarks
NM_211_ANNEX_A72_SOP_Sample preparation and exposure Protocol CeO ₂ .docx / 46.79 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A73_SOP _Cytotoxicity assay methods.docx / 32.65 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A75_Interference of CeO ₂ with MTT assay absorbance wavelength.docx / 67.74 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A76_EMEM cell culture medium formulations_.doc / 221.5 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	
NM_211_ANNEX_A74_Nominal v real concentrations.docx / 115.54 KB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN2	

Attached full study report

Attached full study report					
NM_211_ANNEX_A77_CeO2	in	vitro.pdf	/	1.44	MB (application/octet-stream): ENV/JM/MONO/(2015)8/ANN6

Endpoint study record: 2012-03-05_Australia_RMIT_In_vitro_tox_Human_skin_&_immune_cells

Administrative Data

Study result type experimental result **Study period** 2010-2012

Data source**Data access**

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods**Type of information**

This report is a collation and summary of the research undertaken by the joint Nanosafety research group at RMIT and Monash Universities, investigating the interaction between various metal oxide nanoparticles (NPs) and cells of immunity and the skin. The work forms part of the Australia consortium effort for the OECD sponsorship programme for the testing of manufactured nanomaterials, under the sub-category of mammalian toxicity tests (in vitro toxicology). This report covers activities associated with the testing of three OECD ceria particulates, and is one of three RMIT documents covering ceria, zinc oxide and silver NP testing. Here we have examined ceria NPs of varied sizes as supplied by the OECD programme, and explored how these particles interact with human immune and skin cells. We have physically characterised the particles under cell exposure conditions, examined cytotoxicity, immune signalling (cytokine production) and generation of reactive oxygen species (oxidative stress).

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
other: OECD Sponsorship Program	MN-211

Test material form

nanomaterial

Details on test material

NPL reference number: CA340#55 Particles were prepared using an identical protocol for all experiments (see appendix 1 of the attached file).

Method

Any other information on materials and methods incl. tables

Describe the scientific and technical basis of the test method

What biological/cellular model is the method based on? Human THP-1 monocytic immune cell line: Both in monocytic and macrophage (derived from phorbol ester stimulation) forms. Human keratinocyte cell line: HaCaT cells. Growth media and conditions: RPMI-1640 + 10% FBS (or 5% FBS for HaCaT cells). 37°C and 5% CO₂. What biological endpoints/responses does this method address? Cryo-TEM: Imaging of NPs in cell culture media with minimum perturbation. Disc Centrifuge Analysis: Gives agglomerate particle size in cell culture media. Cytotoxicity: Measurement of cell viability via the MTS (tetrazolium salt) metabolic assay. Cytokines: Immune modulator secretion measured with a multiplex bead assay system and confirmation via ELISA. Reactive Oxygen: Peroxide (DCF-DA) and superoxide (MitoSOX Red) measurement for generation of reactive oxygen species, leading to cellular oxidative stress. Cells were typically exposed to NPs for 24 hr, unless the assay notes otherwise. The assay protocols are provided in detail in appendix 2 of the attached file. What specific mechanisms associated with the biological response are targeted? See above. What methods/techniques are used for endpoints/responses determination? See above. Are there potential technical limitations of this method for testing nanomaterials?

The assay protocols are provided in detail in appendix 2, and includes discussion of experimental limitations (i.e. for in vitro assays involving nanoparticle exposures and cryo-TEM of nanoparticles in the in vitro exposure media). Experimental limitations on in vitro assays involving nanoparticle exposures: Nanoparticles at concentrations >100 µg/mL may cause turbidity that will affect spectrophotometric assays performed directly in the multiwell plate. In order to control for this effect, additional “cell-free” control wells (containing nanoparticles at each dose level, and all other components of the exposure and assay system, except for the cells) must also be run alongside the test wells in order to measure and correct for the amount of increased background absorbance due to the presence of the nanoparticles. When higher concentrations of nanoparticles are being investigated, this turbidity effect can be reduced by centrifuging the multiwell plate at the end of the exposure period using the highest speed tolerated by the multiwell plate (e.g. 1,107 g for 5 min at 24°C) and then carefully transferring a portion of the supernatant in each well to a new multiwell plate for spectrophotometric measurements. For each new nanoparticle being investigated, it is also necessary to determine whether the nanoparticles themselves directly interact with the assay method or assay kit components (e.g. by binding the detecting chromophore or fluorophore, or quenching the fluorescence of a fluorophore), as such interference can potentially result in false positive or negative results, depending on the assay method employed. Experimental limitations for cryo-TEM of nanoparticles in the in vitro exposure media: It is important to note that while cryo-TEM is an ideal method for visualising the nanoparticle agglomeration state within the in vitro exposure system, there are two issues concerning the upper size limit for agglomerates in cryo-TEM, and therefore an independent method of determining agglomerate size should be used to confirm quantitative measurements (e.g. disk centrifugation), whilst also being aware of the second method’s potential limitations. Issue 1: When the samples are prepared, the nanoparticle suspension is pipetted onto a TEM grid and then blotted with filter paper by hand for a few seconds before being frozen. This process may remove very large agglomerates if they stick to the filter paper, so these don’t end up on the grid at all. Issue 2: For large agglomerates, the ice forms a thicker area around the agglomerate itself due to surface tension. This makes it more difficult to image these larger agglomerates that are multiple microns in diameter because the electrons can’t get through the thick ice. Particles were prepared using an identical protocol for all experiments (see appendix 1 of the attached file).

Results and discussions

Any other information on results incl. tables

Physical Characterisation: Ceria NPs were characterised via cryo-TEM, in order to visualise how each material might aggregate or agglomerate under conditions as close as possible to those found in the in vitro cell environment. Representative images for ceria NPs are shown in the attached file. **Agglomerate distribution of ceria NPs in cell culture media + 10% FBS, measured via disc centrifuge:** NM-211 Mean particle diameter (nm) 50 Range (width at ½ peak height) 25-100 nm **Cytotoxicity:** Cytotoxicity was assessed for ceria NPs in both THP-1 monocytes and macrophages and HaCaT keratinocytes. In all cases ceria was not found to be cytotoxic within our normal test range maximum (100 µg/mL). Extending the test range showed little cytotoxicity in macrophages (at 500 µg/mL), and we were able to show significant cytotoxicity to keratinocytes at 3000 µg/mL. With co-exposure to UVA+B at 0.3 minimal erythral dose (MED), which caused 50% cell death in keratinocytes, ceria was found to be protective against cell death, though this protection was mitigated by the cytotoxicity at extreme doses. These UVA+B exposure data illustrate the UV absorption capacity of ceria at the nanoscale. **Reduction in cell viability, both with and without UVA exposure in human monocytes and macrophages:** for EC20, EC50 and EC80 > 500 µg/mL. **Reduction in cell viability, without UVA exposure in human HaCaT keratinocytes:** EC20 = 405 µg/mL; for EC50 and EC80 > 5000 µg/mL. The attachment shows comparative graphs show the dose-response data expressed as per mass concentration or as a function of particle surface area. **Immune Signaling Responses:** THP-1 macrophages exposed to ceria NPs showed elevated IL-8 immune signalling after exposure to very high concentrations of ceria NPs. Interestingly, UVA co-exposure appeared to abrogate the IL-8 response. In these cells, higher doses of ceria NPs also increased IL-1β levels in the absence or presence of UVA radiation. HaCaT keratinocytes showed no detectable immune signalling responses with ceria. UVA+B exposure itself caused an 8-fold increase in IL-6 production, and co-exposure with ceria NPs were able to reduce UV-induced IL-6 production – likely through the absorption of UV light. **Reactive Oxygen Generation:** Reactive oxygen species (ROS) production was measured via MitoSOX red fluorescence (specific for superoxide) or dichlorofluorescein (DCF, specific for peroxides). When THP-1 macrophages were exposed to ceria NPs, we observed no increase in superoxide production. With UVA co-exposure a small increase in superoxide was observed. DCF measurement in macrophages did not show significant changes in peroxide generation with ceria NP exposure, but with co-exposure to ceria NPs and UVA there was a significant increase at 10 µg/mL that was sustained for NM-211 NPs. HaCaT keratinocytes showed no peroxide ROS when exposed to ceria NPs, but a large increase in peroxide ROS due to UVA+B exposure, which was enhanced at 10 µg/mL with ceria co-exposure. For both cell types we observed a decrease in ROS production with increasing ceria NP concentration, which may be an indication of anti-oxidant properties in the ceria NPs.

Overall remarks, attachments

Remarks on results including tables and figures

These findings suggest that the ceria NPs were well tolerated in human skin and immune cells and were only cytotoxic at very high doses. Ceria NPs, but not bulk ceria, protected skin cells from UVA+B radiation (0.3 MED) which could be due to its UV filtering property at the nanoscale. These NPs did not activate immune signalling in HaCaT skin cells, but at high doses they induced IL-8 and IL-1β in macrophages. Although ceria NPs generated ROS (superoxide and peroxides) in skin and immune cells, these oxidants did not seem to influence cell viability. Detailed data (including comparative tables and figures) are shown in the attached study report.

Attached full study report

Attached full study report

NM_211_ANNEX_A78_2012-03-05_Australia_RMIT_In_vitro_tox_Compiled_OECD_Ceria_Report-final.pdf / 1.31 MB (application/octet-stream); ENV/JM/MONO/(2015)8/ANN2

8. ANALYTICAL METHODS

9. RESIDUES IN FOOD AND FEEDINGSTUFFS

10. EFFECTIVENESS AGAINST TARGET ORGANISMS

11. GUIDANCE ON SAFE USE

12. LITERATURE SEARCH

13. ASSESSMENT REPORTS

14. INFORMATION REQUIREMENTS