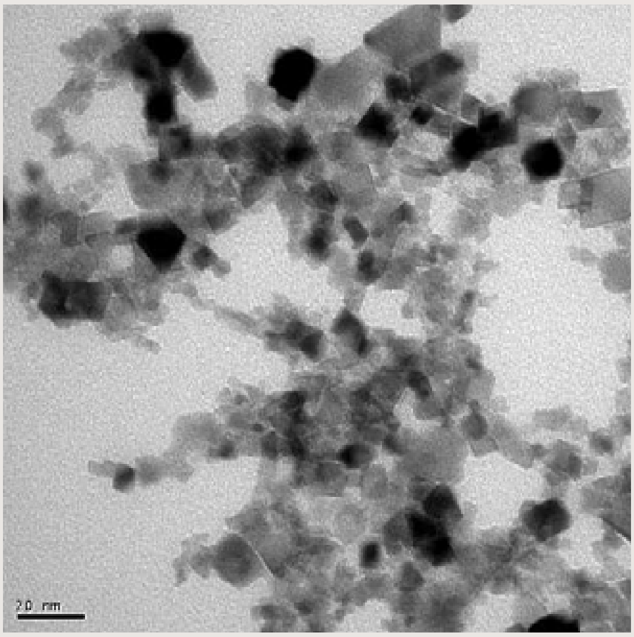
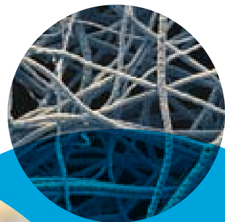
PROSPECT
Global Nanomaterials Safety

Toxicological Review of Nano Cerium Oxide





1 July 2010

IN SUPPORT OF PROSPECT:

Ecotoxicology Test Protocols for Representative Nanomaterials in Support of the OECD Sponsorship Programme

Table of Contents

Cover photo: UMICORE NanoGrain® cerium oxide nano particles (Image courtesy of UMICORE S.A./N.V.)

LIST OF TABLES

LIST OF FIGURES

FOREWORD/PREFACE

This document is a summary of the literature review on the toxicity and specifically ecotoxicology of nanoparticles in general and nano-cerium oxide in particular. It is to be submitted as the first deliverable of the PROSPEcT Project.

The PROSPEcT Project * is the UK's contribution to the OECD Sponsorship Programme † to examine the environmental safety of nanomaterials in accordance with the agreed OECD WPMN *'Guidance Manual for Sponsors of the OECD Sponsorship Programme for the Testing of Manufacture Nanomaterials'*. [1] It will provide crucial data to the OECD work, by addressing gaps in the current level of knowledge on the physico-chemical and ecotoxicological properties of these materials, followed by fundamental scientific research leading to establishing scientific test methodologies to study those endpoints that may not be assessed through standard tests used for bulk chemicals.

The first step of the project consists of a thorough literature review, evaluation exercise, and identification of gaps in the current state of knowledge of the physico-chemical and (eco)toxicological properties of both cerium oxide and zinc oxide nanomaterials. The data was gathered, reviewed and evaluated according to its usefulness to the Project, with specific a view to *'addressing'* and/or *'completing'* the endpoints agreed by the OECD WPMN [1]. This review is crucial to maximise the use of any existing data, to determine what further work needs to be done and to avoid any unnecessary testing.

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* **PROSPEcT: Ec**otoxicology **T**est **P**rotocols for **R**epresentative Nanomaterials in Support of the **O**ECD **S**ponsorship **P**rogramme'

† OECD's Working Party on Manufactured Nanomaterials (WPMN) launched a Sponsorship Programme in November 2007. The programme involves OECD member countries, as well as some non-member economies and other stakeholders to pool expertise and to fund the safety testing of specific Manufactured Nanomaterials (MNs). In launching this Sponsorship Programme, the WPMN agreed on a priority list of 14 MNs for testing (based on materials which are in, or close to, commerce). They also agreed a list of endpoints for which they should be tested. Much valuable information on the safety of MNs can be derived by testing a representative set for human health and environmental safety.

1.0 Introduction

Nanotechnology has gained a great deal of public interest due to the needs and applications of nanomaterials in many areas of human endeavours such as industry, agriculture, business, medicine, public health amongst many others. Nanotechnology includes the integration of these nanoscale structures into larger material components and systems, keeping the control and construction of new and improved materials at the nanoscale. [2]

Nanoparticles can be naturally occurring or they can be manufactured, the latter can be classed into several categories including the following: [3]

1. Metal nanomaterials, such as gold and silver nanoparticles
2. Metal oxide nanomaterials, such as titanium dioxide and zinc oxide
3. Carbon nanomaterials such as fullerenes and nanotubes
4. Quantum dots such as cadmium telluride and cadmium selenide

One estimate for the production of engineered nanomaterials was 2000 tonnes in 2004 and increasing to 58,000 tonnes by 2011-2020. [4]

There are various definitions on nanomaterials in the literature and it should be mentioned that the International Standards Organisation has recently developed a technical specification on the terminology and definitions of nano-objects. [5,6] The specification does not define the term nanomaterial, but it describes the term nano-object as a material with one, two or three external dimensions in the nanoscale, with nanoscale being the size range from approximately 1 nm to 100 nm.[‡] Manufactured nanoparticles often exhibit special physico-chemical properties and reactivities due to their small size and controlled composition, structure or surface characteristics which are not present at the larger scale. In particular, nanoparticles possess a much higher specific surface area (SSA) than their larger counterparts of the same material, and the proportion of atoms on the surface versus the interior of the particle is also much larger. These factors can give rise to higher surface reactivity for the same mass of material. [5]

This literature review consists of a literature summary of the current knowledge on the toxicity and specifically ecotoxicology of nanoparticles in general and nano-cerium oxide in particular, followed by an evaluation of the available information with regard to its suitability to be included in the OECD Sponsorship Programme. This summary will be updated periodically to incorporate the latest articles which are thought to be relevant to the project.

[‡] NOTE: Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties, size limits are considered approximate. The definition takes into consideration the formation of agglomerates and aggregates, in which nano-objects can be original source particles that are termed primary particles.

Most of the articles used in this review are dispersed across a range of disciplines and focussed primarily on *in vitro* testing and often do not specify an exposure pathway. There is little research on consumer products, particularly on their environmental fate and most research is on the basic nanomaterials. [7] It should be noted however, that most attention has thus far been devoted to the toxicology and health implications of manufactured nanoparticles (MNP) while the behaviour of MNP in the environment and their ecotoxicology has been less reviewed. [4]

2.0 Chemical and physical information

Cerium is a member of the lanthanide series of metals and is the most abundant in the earth's crust of the rare-earth elements. [8] Elemental cerium is an iron-gray, ductile, malleable metal. [9] Cerium metal is very reactive and is a strong oxidizing agent that is stabilized when associated with an oxygen ligand. [10] When present in compounds, cerium exists in both the trivalent state (Ce^{3+}, cerous) and the tetravalent state (Ce^{4+}, ceric). [9,11] Selected chemical and physical properties of cerium and cerium oxide are listed in Table 1.

Name	**Cerium**	**Cerium oxide**
CAS No.	7440-45-1	1306-38-3
Synonyms		Cerium dioxide; ceria; cerium(IV) oxide
Molecular weight	140.116	172.11
Molecular formula	Ce	CeO_2
Form	Iron-gray, ductile, malleable metal	Pale-yellow, heavy powder (white when pure); commercial product is brown
Melting point	798 °C; boiling point = 3443 °C	2400 °C
Density	6.770 g/cm^3	7.65 g/cm^3
Water solubility	Decomposes slowly with cold water and rapidly with hot water	Insoluble in water
Other solubility	Soluble in dilute mineral acids	Insoluble in dilute acid

Table 1. Physical properties of cerium and cerium oxide

Cerium is found in nature along with other lanthanide elements in the minerals alanite, bastanite, monazite, cerite, and samarskite; however, only bastanite and monazite are important sources commercially. [9,12] Because of its unique stability in the tetravalent state (other lanthanides are stable in only the trivalent state), cerium can be separated out from the other rare-earth elements through oxidation followed by variable solubility filtration. [10] Cerium metal is prepared by reacting CeF_3 with an excess of calcium at approximately 900 °C. [9] Cerium can also be obtained by the fused-salt electrolysis of a mixture of cerium chlorides and fluorides. [10]

Cerium oxide nanoparticles, meanwhile, have been produced using many different preparation methods such as sol-gel, thermal decomposition, solvothermal oxidation, microemulsion methods, flame spray pyrolysis and microwave-assisted solvothermal process. [2] A synthetic method has recently been reported of being capable of producing homogeneous cerium oxide nanoparticles of 2 nm in average size by merely mixing cerium sulphate and ammonia solution at room temperature. [13]

3.0 Commercial uses of cerium and cerium oxide

Cerium is most heavily used in the form of Mischmetal § for metallurgical purposes and is the major component of Mischmetal (50–75% by weight for the most common grades), a commercial mixture of metallic light lanthanides. [9,10,14] Mischmetal reacts with the impurities found in metals to form solid compounds, thereby reducing the effect of these impurities on the properties of the metal. [10] Mischmetal is also used in the manufacture of cerium-iron alloy lighter flints.

Cerium oxide is used as a polishing agent for glass mirrors, plate glass, television tubes, ophthalmic lenses, and precision optics. [9,10,15] Cerium oxide is also used as a glass constituent to prevent solarization and discoloration. [10] Cerium oxide is also used in emission control systems in automobile engines as a diesel fuel-borne catalyst to reduce particulate matter emissions. [12,16] Exposure to commercially used cerium compounds is most likely through exposure to cerium oxide.

The industrialised world currently uses a huge amount of fossil fuel of which a large portion is used in transportation. The combustion of hydrocarbons results in the formation of carbon dioxide and water vapour with the release of thermal energy, which can be transformed into useful work. [17,18] Incomplete combustion generates a variety of pollutants including unburnt hydrocarbons and soot, which is a particular problem with diesel combustion. [19]

There is some evidence that soot is the second most effective atmospheric global warming pollutant and that a reduction in soot emissions might be a relatively easy 'win' in the battle against global warming. [16,20] Products that reduce the formation of CO_2 and soot while reducing the fossil fuel consumption, potentially provide huge benefit to the consumer, in reduced fuel bills, and the environment, in the reduction in greenhouse gas formation.

Some rare earth oxides, such as cerium oxide, have the ability to donate and store oxygen from their crystal lattices depending on a variety of environmental factors. One of these factors is the oxygen level in the atmosphere surrounding the cerium oxide; in an oxygen-lean environment, cerium oxide will act as a catalyst, donating oxygen and assisting in the combustion of various species, such as hydrocarbons and soot. Thus the introduction of cerium oxide into the engine catalyses the combustion process, reducing the amount of fuel used and also reducing the pollutants emitted from the engine during the combustion process. [16]

Cerium oxide can also aid in the burn-off of soot collected on diesel particulate filters and as a result enhance the efficiency of these filters. A limited number of short-

§ Mischmetal (from German: Mischmetall - "mixed metal") is an alloy of rare earth elements in various naturally-occurring proportions.

term diesel engine tests have confirmed that cerium (20 to 100 ppm in the fuel), used in combination with a particulate filter, substantially decreases both particle mass (> 90%) and number (99%) concentrations in the emissions exhaust. [15]

There is also evidence for an increase in catalytic activity of cerium oxide as the particle size decreases, rendering a nanoparticle form of the material attractive from a fundamental property point of view. [21] Studies have shown that adding cerium oxide nanoparticles to fuel reduces pressure within the engine cylinder and hence reduces the NO_x emissions and prolongs combustion, which leads to a reduction in unburnt hydrocarbons (by up to 15%) and a decrease in fuel consumption of between 5 to 9%. It also decreases the emission of combustion-derived nanoparticles (CDNP).

A recent study of buses in the UK using a cerium-oxide added fuel (Envirox™) concluded that a fuel saving of greater than 6% could be achieved. The tests also showed evidence for a reduction of the amount of soot deposits within the engine cylinders. An eco-efficiency life cycle analysis compared the impact on environmental costs and ecological fingerprint due to the enrichment of diesel fuel with cerium oxide compared against plain diesel. [16] In all cases the environmental impact and costs, and hence the overall eco-efficiency rating was reduced by varying degrees with cerium oxide added to the fuel than with conventional diesel indicating benefit to the consumer and the environment.

Particulate matter (PM) emission is a major component of the ambient air pollution cocktail. Numerous epidemiological studies have demonstrated that both acute and long-term exposure to elevated concentrations of PM with an aerodynamic diameter of less than 10 µm have adverse impacts on cardiac and respiratory health. [22,23] Studies have also shown that the proximity of populations to busy roads, and hence combustion-derived nanoparticles (CDNP), are associated with increased risk of respiratory and allergic complaints. [21,24,25,26,27] Given the evidence for CDNP-induced adverse health effects; it is logical to assume that a reduction in the generation of particles from traffic would have a beneficial effect on health. [22]

The toxic potential of CDNP in PM10 has been extensively reviewed. These studies suggest that whole PM10, PM2.5, and combustion-derived nanoparticles are a hazard to the lung and cardiovascular system through inflammation triggered by the induction of oxidative stress. [22] Heterogeneity in composition and solubility means that the key initiating event of oxidative stress may originate from multiple components such as surfaces, metals, or organics. Hence the potential of PM to drive damaging oxidation reactions and induce oxidative stress reflects the sum of the physical and compositional properties of the particles. Diesel exhaust particles (DEP) represent the most common CDNP in the urban airshed. [22]

While reducing the particulate emissions of vehicles, it is also worth noting that a small amount of cerium is emitted in the emissions exhaust. Studies have shown that the cerium measured in the emissions was primarily in the oxide form. [15] This will ultimately lead to cerium levels in the soil near roads to increase as a result of ambient particle deposition.

Some of the crucial questions that need to be addressed with the use of nanomaterials are: [3]

1. Are nanomaterials more toxic than their non-nano counterparts?
2. Will nanomaterials transform in the environment into more toxic forms?

It is necessary to understand the fate and behaviour of manufactured nanomaterials in the environment. We need to determine:-

1. Whether nanomaterials retain their nominal nanoscale size, original structure and reactivity in environmental systems
2. In addition, is their effect on environmental systems different from that of larger particles of the same material.

4.0 Characterisation of Cerium Oxide

To correlate properties of the nanoparticle to their toxicity potential and ensure that the results are reproducible and meaningful, accurate characterisation of nanoparticles at different stages such as synthesised, as supplied, as administered and after administration is essential. Until the relationships between nanoparticle characteristics and toxicity are fully understood, it will be necessary to ensure that all nanomaterial characteristics that are potentially significant are measured or can be derived in toxicity screening tests. However the determination of every possible characterisation of a nanomaterial is impractical. [28,29]

Furthermore robust characterisation is an essential prerequisite for any investigation to ensure that comparison of results is based on sound science. This characterisation may include measurement of physical-chemical properties such as particle size and distribution, shape, agglomeration state, crystal structure, chemical composition, surface area amongst others. [30] Nanoparticles differ from larger materials in that number of atoms at the surface and their physical properties are different from those of bulk materials. [2] One of the most important attributes in all nanoparticles is their high surface area per unit mass, which results in the surface possessing considerable surface energy. The characterisation of the surface chemistry (reactivity) is also important, since it has been reported that it influences the toxic effects and not the surface area. [31]

Table 2 compares some of the properties of macro-scale (non-nano) and nano-cerium oxide particles, reported in the literature; evidently there is a significant change in the surface area of the particles.

Test	Property	Nano	Bulk
XRD, x-ray diffraction	Crystal form	Cerianite	Cerianite
EDX, energy-dispersive x-ray analysis	Gross elemental analysis	Ce, O	Ce, O
BET, Brunaeur Emmett Teller surface area	Surface area	94.7 m^2/g	2.64 m^2/g
	Mean particle size	9 nm	320 nm
XPS, x-ray photoelectron spectroscopy	Surface chemistry	Ce, O	Ce, O

Table 2. Comparison of properties between bulk and nano-scale cerium oxide. [22,29]

Figure 1 compares the TEM images of nano (on average 9 nm) and bulk cerium oxide particles (on average 320 nm).

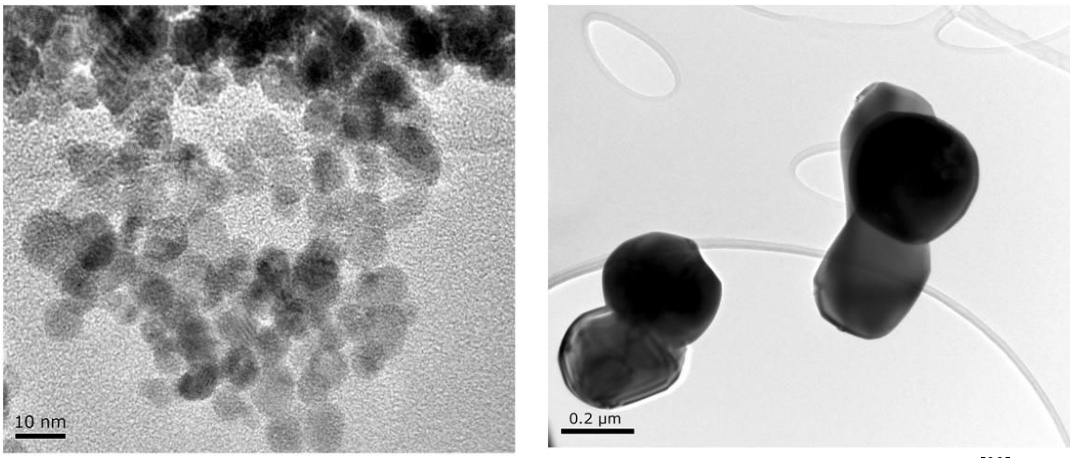

Figure 1. TEM images of nano-cerium oxide (left) and bulk cerium oxide particles (right). [22]

It is also interesting to note that the agglomeration of cerium oxide nanoparticles means that they behave differently in different media. An example of a recent study is shown in Table 3. The values of the zeta potential are also included.

Particle	**Primary particle size**	**Crystal structure**	**Size (nm)**			**Zeta potential (mV)**		
			H_2O	DMEM	BEGM	H_2O	DMEM	BEGM
Cerium Oxide	8 nm	Cubic ceria	2610	323	596	+15	-10	-10

Table 3. Physical characterisation of nano cerium oxide particles in dry, aqueous, and cell culture media. [32]

Some important questions specific to environmental testing of engineered nanoparticles (ENP) include: [33]:-

1. What material should be tested and which form should be studied (*e.g.* coated or not)?
2. What matrix and ENP exposure levels should be studied?
3. How should the ENP be introduced to the study system?
4. What parameters should be monitored during a study?
5. How should the ENP test material and media be characterised before, during and after the testing?

There is a need to define scenarios of exposure. Research into establishing appropriate ecotoxicity test strategies and methods should first define realistic conditions, set appropriate test protocols (including sampling, dosimetry, etc.) and then test the ecotoxicity under the agreed realistic scenarios. [34]

5.0 Toxicology Literature Review

Human Health Effects

An authoritative document collating existing toxicological data from animal and human studies for particulate non-nano cerium salts, including cerium oxide, is available in the Health Effects Institute (HEI) publication. [15]

Only limited studies for specific endpoints of toxicity, in which nano cerium oxide have been investigated and been conducted. [35,36] The two main routes of exposure to cerium-containing particles to the general public are through inhalation and ingestion. Typically, inhaled cerium is cleared from the respiratory tract by different pathways and at different rates depending on the solubility in body fluids. Cerium taken up by ingestion is excreted in the faeces after transiting the digestive tract. [15]

Inhalation of cerium oxide is of more concern than ingestion, since cerium oxide is poorly absorbed by the intestine. Primary targets after inhalation are the lung and the associated lymph nodes. Other organs could also be affected *via* clearance through the blood. The liver, skeleton, spleen and kidney are some of the organs, in which cerium can be distributed after the adsorption into the circulation. Inhaled particles can reach various regions of the respiratory tract depending on the particle size.

Exposure to cerium oxide and other rare earth oxides has been reported for several occupations and has been associated with a pulmonary disease termed 'rare earth pneumoconiosis'. It should be noted that the role of cerium in this disease is not clear.

Cerium compounds are poorly absorbed through the digestive system. Toxicity of cerium delivered orally is therefore not likely to be a major concern. Oxides of cerium are less soluble than other forms of cerium, and therefore it is thought that their acute toxicity may be less, although they may be transformed into more soluble forms once taken into the body. [15]

An important factor in the characterisation of the toxicity of inhaled pollutants is the extent to which the pollutant reaches various parts of the respiratory tract. Particle deposition in the various regions depends on particle size and density and on the subject's breathing patterns and airway geometry. Nanomaterials (also previously known in the literature as *ultrafine particles*) (10 to 100 nm) are typically deposited by diffusion, primarily in the alveolar regions. *Fine particles* (100 nm to 1µm) are deposited by both diffusion and sedimentation. For larger particles (> 1 µm) inertia plays a major role in deposition. Very few particles above 10 µm are inhaled. No data was available on how particle size affects the deposition and fate of cerium in the lower respiratory tract. However, information on other studies suggest that smaller cerium particles (< 0.5 µm in diameter) are more likely to reach the alveolar region and likely to be dissolved due to the large surface area. [15]

The HEI report concludes that - based on the limited data available - toxicity of cerium oxide appears to be small, and cerium oxide might not be of concern when inhaled or ingested at the low levels that would be encountered in the environment from the use of cerium oxide fuel additives. The absence of more complete information precluded fully accessing the possible health effects of using nanoparticulate cerium oxide as a fuel additive.

The safety of cerium added to diesel fuel (Envirox™) was also evaluated in a more recent study by Park *et al.* [2] This was done by addressing the classical paradigm: Risk = Hazard × Exposure.

The data collected demonstrated that - for the exposure levels measured - the estimated internal dose for a referential human in a chronic exposure situation is much lower than the no-observed-effect level (NOEL) in the *in vitro* toxicity studies. Exposure to nano-size cerium oxide as a result of the addition of Envirox™ to diesel fuel at the current levels of exposure in ambient air was unlikely to lead to pulmonary oxidative stress and inflammation, which are the precursors for respiratory and cardiac health problems. [22]

Some of the results obtained in this study are shown below. Table 4 shows the general toxicity assays performed. The results in Table 4 show there is no discernable difference in toxicity, which - in any event - is low, when nanoparticulate and non-nano cerium oxide are compared in a limited number of *in vitro* assays representing an initial screening strategy for adverse health effects.

Assay performed	Nano cerium oxide	Bulk cerium oxide
EpiDerm skin irritation	Unlikely to be a skin irritant [50 mg: mean irritation potential of 0.01]	Unlikely to be a skin irritant [50 mg: mean irritation potential <0.03]
Cytotoxicity assay (L929 cells)	Non-cytotoxic [1 cm^2 area of powder placed on agar overlay]	Non-cytotoxic [1 cm^2 area of powder placed on agar overlay]
Mutagenicity—bacterial cells (Ames test)	Nonmutagenic [50–5000 µg/plate]	Non-mutagenic [50–5000 µg/plate]

Table 4: Results of general toxicity assays. [22]

Table 5 and Table 8 compare the potential biological impact of engine emissions from both Envirox™-added diesel fuel and a reference diesel fuel.

Biological endpoint	Standard diesel fuel	Standard diesel fuel + Envirox
ATP	Dose-related decrease; SS at highest emission [a] concentration	No SS variation observed; no impairment cell viability
GSH	Decrease conc. at highest emission concentration	Decrease conc. at highest emission concentration
Catalase	No SS alteration in levels observed	SS increase observed at highest emission concentration [b]
GPX (total)	No adverse effects observed	No adverse effects observed
Nucleosome numbers	Tendency for increase although no SS alteration in levels observed	Tendency for increase although no SS alteration in levels observed
TNF-alpha	Dose response decrease; 40% at highest emission concentration	~15% decrease but no dose response observed
SOD, total SOD-Mn	No adverse effects observed	No adverse effects observed

Table 5: Effects of emissions from a standard diesel engine compared with those from Envirox™-treated diesel. [21]

Note. SS, statistically significant [p = .05].

a Lung slices were exposed to a continuous flow of 2%, 10%, and 20% dilutions in air of raw engine emissions for 3 h in continuous flow-through chambers.

b Biological/physiological relevance of this observation of limited increase in oxidant activity is questionable.

The effect of nanoparticulate cerium oxide aerosol generated as a result of using nano-cerium oxide in diesel was studied using an organotypic culture of lung slices. The study concluded that no impact on lung tissue biological parameters was observed with one exception, which was an increased catalyse activity which was not associated with a concomitant loss in cell viability. It therefore concluded that a very low prospective risk is associated with the expected dissemination of cerium oxide in the atmosphere. [37]

Ecotox Review

Accurately assessing the environmental risks posed by nanoparticles requires using effective quantitative analytical methods to determine their mobility, reactivity, ecotoxicity and persistency, many which have still to be developed. [38] Whether a substance can be hazardous depends not only on its toxicity, but also on its likelihood of ever coming into contact with living cells. Assessing the stability of nanoparticles in the environment entails evaluating their ability to aggregate or interact with other particles. One other major factor is the ability of the environment to modify nanoparticles through the action of light, oxidants or microorganisms.

Nanoparticles may have an impact in the environment *via* one or more of the following mechanisms:

- A direct effect on biota (toxicity)
- Changes in the bioavailability of toxins or nutrients
- Indirect effects resulting from their interaction with natural organic compounds
- Changes in the environmental microstructures

Effectively monitoring nanoparticles in the environment requires the ability to detect environmentally relevant concentrations, which fall in the nanogram per litre or the picogram per litre range. It is also crucially important to prevent the potential interference of natural nanoparticles present in environmental samples. [37] Currently, there is no information on background concentrations and the physical-chemical form of nanoparticles in the environment due to the limitations in separation and analytical methodologies. [2]

In toxicity studies of nanoparticles, different research groups have used different cell lines, culturing conditions, and incubation times; therefore, it is difficult to compare results from different research groups and determine whether the cytotoxicity observed is physiologically relevant. [3]

Some results from ecotoxicological studies show that certain nanoparticles have effects on organisms under environmental conditions, though mostly at elevated concentrations. The next step towards the assessment of the risks of nanoparticles in the environment should be to estimate the exposure of the different nanoparticles. [4]

Park *et al.* describe an initial *in vitro* preliminary screening strategy to examine the potential for human health hazards and the environmental effects following exposure to nanoparticulate cerium oxide. The tests conducted included non-nanoparticulate cerium oxide as a reference material for comparison with nano cerium oxide. [29] Daphnia magna immobilisation studies indicate that there were no toxic effects observed at any time period during this test. The 48-hour EC_{50} for nano and non-nano cerium oxide to Daphnia magna based on nominal test concentrations was greater than 100% (v/v) saturated solution and correspondingly the No Observed Effect Concentration (NOEC) was 100% (v/v) saturated solution. The activated sludge respiration inhibition study demonstrated that the 3-hour EC_{50} for inhibition of respiration of activated sewage sludge bacteria for both nano cerium oxide and non-nano cerium oxide test materials was greater than 1000 mg/l. There was no effect on the respiration of activated sewage sludge following a 3-hour exposure to either of the test materials at 1000 mg/l *i.e.* No Observed Effect Concentration (NOEC) = 1000 mg/l.

These initial screening studies for environmental effects (acute Daphnia magna toxicity and activated sludge respiration inhibition) demonstrated no differences in biological effects potential between nano and non-nano cerium oxide. However, the paper also recognised that the *in vitro* hazard data reported was limited, and that further work is needed to expand the understanding of the effects of nanoparticulate cerium oxide on entering the environment. [29]

Another study by Zhu *et al.* shows the acute toxicities of nanoparticles to be dose dependent. In their tests, TiO_2, Al_2O_3 and carbon nanomaterials were more toxic than their bulk counterparts. Moreover, Daphnia magna were found to ingest nanomaterials from the test solutions through feeding behaviours, which indicates that the potential ecotoxicities and environmental health effects of these engineered nanoparticles cannot be neglected, especially those that are most likely to be released into the aquatic environments. [39]

Van Hoecke *et al.* recently published an article on fate and effects of nano cerium oxide particles in aquatic toxicity tests. [40] Their study concluded that chronic toxicity was found to increase with decreasing nominal particle diameter and is related to the difference in the surface area. Clustering of the particle aggregates around the algal cells was observed and may have caused toxicity through a local direct effect or local nutrient depletion and/or shading at the cellular level.

Yang *et al.* conducted a study on the distribution and bioavailability of rare earth elements in aquatic microcosm. The study concluded that most rare earth elements were adsorbed by the sediment and might be discharged into water. [41]

Aruoja *et al.* concluded that the shading effect of light to algae growth by nanoparticles was negligible. The study demonstrated that solubility is a key issue in the toxicity of metal containing nanoparticles. [42]

The impact of the use of cerium oxide in the diesel fuel on existing ambient PM levels has also been reported. This was done by atmospheric monitoring at locations in London and Newcastle as well modelling studies on air and soil deposits. [22]

Diesel fuel containing cerium oxide (Envirox^{TM}) was introduced by Stagecoach bus company in its London fleet at the end of 2003, and all buses operated in Newcastle by Stagecoach from early 2005. Table 6 provides a comparison of the effect of Envirox^{TM} on cerium metal content at various UK sites. Full details of the selection of monitoring sites, monitoring methods used, filter archive and particle extractions, and measurement can be found elsewhere. [43,44]

	Site					
	London, Marylebone		**Greenwich, Eltham**		**Newcastle**	
Metal	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Cerium (ng/mg PM10)	14.15 (± 8.40) *n = 12*	14.33 (± 9.03) *n = 12*	9.33 (± 4.62) *n =8*	8.25 (± 5.50) *n=4*	10.42 (± 4.61) *n= 10*	41.83* (± 18.91) *n = 12*

Table 6: Comparison of the effect of introduction of Envirox^{TM} on cerium metal content of extracted PM10 filter samples from various UK sites. [22]

Note: Figures in parentheses are standard deviations; n = number of filters. Asterisk indicates statistically significant increase in cerium concentration post introduction of Envirox™, p < .001.

The concentration of cerium in the PM10 samples were quantified following acid extraction of the filter and are shown in Table 7 (expressed as concentration per cubic metre of ambient air).

	Site					
	London, Marylebone		**Greenwich, Eltham**		**Newcastle**	
Metal	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Cerium (ng/m^3)	0.648 (± 0.375) *n = 12*	0.514 (± 0.281) *n = 12*	0.198 (± 0.190) *n =8*	0.157 (± 0.089) *n=4*	0.145 (± 0.064) *n= 10*	0.612* (± 0.287) *n = 12*

Table 7: Metal concentrations of ambient PM10 at various UK sites pre and post introduction of Envirox^{TM}. [22]

Note: Figures in parentheses are standard deviations; *n* = number of filters. Asterisk indicates statistically significant increase in cerium concentration post introduction of Envirox™, *p* < .001.

There was a significant fourfold increase in cerium concentrations at the Newcastle site following the introduction of Envirox^{TM}. While there were location-dependent differences in PM10 cerium content both pre- and post introduction of Envirox^{TM}, it is worth noting that the PM10 cerium concentration at Newcastle post introduction of Envirox^{TM} was of the same order of magnitude as that seen at the London, Marylebone Road location prior to the introduction of the cerium-based fuel additive. While a statistically significant increase in cerium concentration was observed (see Table 6 and Table 7) in Newcastle following the introduction of Envirox^{TM} to the local bus fleet, this was not accompanied by a corresponding statistically significant increase in PM oxidative activity there or at either of the two London sampling sites post Envirox^{TM} introduction based on ascorbate depletion (data not shown). The study also demonstrated an actual reduction in PM10 oxidative activity, measured as ascorbate depletion, at Newcastle following the introduction of Envirox^{TM} to the bus fleet. [22]

Other measurements of cerium oxide have also been reported previously. Measurements of cerium oxide in ambient PM in the San Francisco Bay area ranged between 1.3 and 5.5 ng/m^3. In the Osaka region, ambient levels of cerium were between 4 (rural) and 11 (urban) ng/m^3. A study in Pasadena, California reported cerium levels of 0.43 ng/m^3 in fine particles (< 1.8 μm) and 0.19 ng/m^3 in ultrafine particles (< 0.097 μm). [15]

Modelling studies based on use of cerium oxide based fuel additive across Europe indicate that the application of exhaust filters in conjunction with cerium added diesel will have a significant effect on the unit emissions of diesel vehicles. It was estimated that the reduction in emissions would vary from 70% to 90%. It was estimated that the introduction of filters will result in the reduction of more than 70,000 tonnes per year of particulate at the expense of not more than 75 tonnes per year of cerium released into the atmosphere. In the worst-case assumption [**] (where all cerium is emitted to the atmosphere) cerium emissions will not exceed 1255 tonnes on an annual basis. [22]

Soil contamination modelling suggests that cerium oxide concentration would vary between 0.32 and 1.12 μg/g depending on the soil depth at a distance of 26 m from the edge of the highway, and for a distance of 96 m away from the soil, cerium oxide concentration was estimated to be between 0.28 and 0.98 μg/g. These results indicate that no major contamination of the soil would be expected and indeed that levels of cerium oxide in soil would be of the same order of magnitude as those found naturally in cities across the world today. [15]

Tiede *et al.* reported that the exposure levels for nanoparticles are significantly lower compared with the concentrations that are typically being used in current ecotoxicity investigations. This means that the results of many of the currently published studies may not represent realistic scenario impacts in the environment. [32]

[**] According to the worst-case approach, all diesel fuel is doped with cerium and cerium is fully emitted to the atmosphere (e.g., due to total filter failure).

Some studies have also demonstrated the reduction of PM emissions. [22,36] Table 8 indicates that the engine running with cerium oxide added diesel fuel (Envirox^{TM}) had emissions with a slightly increased mean mobility diameter (MAD) and a 20% decrease in particle number (PM) over the fuel without the additive. This led to a decrease in PM volume fraction of ~8 % and hence a reduction in PM mass of 8%.

	No addition to diesel fuel, aerosol (w/v)			ENVIROX^{TM} addition to diesel fuel, aerosol, w/v		
Parameter	2%	10%	20%	2%	10%	20%
Particle number per cm^3	78,414	395,031	845,706	58,014	316,907	653,745
MAD (nm)	69.7	74.1	76.4	75.7	80.0	82.0
Volume.fraction	9.83E-11	5.73E-10	1.27E-09	9.21E-11	5.48E-10	1.18E-09

Table 8: Comparative raw exhaust emission data. [22]

Current regulations do not deal with the disposal of nanomaterials since there is a lack of experimental data. This appears to be problematic since it is not clear what happens when inorganic nanomaterials are dispersed in the environment or brought into public sewage treatments plants. [14] A study using a model wastewater treatment plant for the removal of oxide nanoparticles showed that, while a majority of the nanoparticles would be captured through the adhesion to clearing sludge, a significant fraction of the engineered nanoparticles would escape the wastewater plant's clearing system. Up to 6 wt% of the model compound which was cerium oxide was found in the exit stream of the model plant.

The study revealed the influence of surface charge and the addition of dispersion stabilising surfactants as routinely used in the preparation of nanoparticle derived products. A detailed investigation of the agglomeration of oxide nanoparticles in wastewater streams revealed a high stabilization of the particles against the clearance (adsorption on the bacteria from the sludge). This finding suggests a need to investigate nanoparticle clearance in more detail and demonstrates the complex interactions between the dissolved species and the nanoparticles within the continuously changing environment of the clearing sludge.

Thill *et al.* studied the impact of a model water dispersion of 7 nm cerium oxide particles on Gram-negative bacteria (Escherichia coli). [45] The study concluded that a direct spatial contact has to be made in order to provoke cytotoxicity of cerium oxide nanoparticles on E.coli.

A review by Handy *et al.* suggests that manufactured NPs are already in the aquatic environment and that wild fish populations are being exposed to these new materials though no measurements of NP exposure or accumulation have been made in wild fish populations. Much of the available data are on freshwater fish and there is a need to collect data on marine and estuarine species, and on flatfish (sediment dwelling species) and so an understanding of the effects of nanoparticle physico-chemistry is essential to compare the likely effects in seawater compared with those identified in freshwater. [46] Nanomaterials will tend to aggregate in water and it could be considered more ecologically relevant to use the naturally aggregated nanomaterials for regulatory testing. [33]

6.0 Relevance to the OECD Sponsorship Programme.

The OECD Sponsorship Programme involves OECD member countries, as well as some non-member economies and other stakeholders to pool expertise and to fund the safety testing of specific Manufactured Nanomaterials (MNs). The programme ultimately will provide valuable and relevant information on the safety of MNs and the methods used to assess their safety by appropriately testing a representative set of MNs for identified human health and environmental safety endpoints. The Working Party for Manufactured Nanomaterials (WPMN) has determined that the programme is intended to develop the data that will improve the understanding of MNs, and, if possible, to understand what information may be generalised across different MNs or classes of MN. [1]

The programme contains of two phases:

Phase 1

In Phase 1, the WPMN has invited WPMN participants to volunteer to sponsor the testing of one or more of the MNs on the list of representative MN. Sponsors [††] have been asked to prepare a Dossier Development Plan (DDP) for the testing of the MN. The DDP will be reviewed by the WPMN. Based upon the reviewed DDP, Sponsors would complete Phase 1 by addressing endpoints appropriate for the material. The scope of Phase 1 is to provide a dataset by addressing the endpoints listed in Section 6.1 Endpoints for testing nanomaterials. This includes, where appropriate, the utilisation of existing relevant information, the generation of new information or the rational why the information is not needed. Also specified under each endpoint in the Guidance Manual are the terms 'address' [‡‡] and 'completed', [§§] which provides that all dossiers will contain the identified endpoint information. [1]

Where it is not feasible or not appropriate to develop test data for an endpoint, a rationale for not testing must be provided. Phase 1 is exploratory in nature as the testing methodology and strategy may need to be developed and might evolve during Phase 1 testing. As far as possible and appropriate, a full dataset shall be generated for each MN independent from decision logic based on risk management considerations. The sum of datasets generated by Phase 1 testing together with the methodology developed and experiences gained will help to understand properties specific to the nanoscale features of MNs and to identify data to be developed in Phase 2 testing.

Phase 2

The scope of Phase 2 is to address additional endpoints that are necessary to gain understanding of the hazard potential of the respective sponsored MN. The

[††] A Sponsor assumes responsibility for conducting or coordinating all of the testing determined to be appropriate and feasible to address the endpoints of Phase 1 for a listed MN.

[‡‡] Possible ways to 'address' an endpoint are: 'not relevant', 'not measurable', 'read-across' and 'data provided'.

[§§] Data must be provided for endpoints indicated as 'this element must be completed' (*i.e.* 'completed' = 'data provided') combined data provided by Phase 1 and Phase 2 testing will allow, but not necessarily be entirely sufficient for, application to risk assessment paradigms as considered for specific sponsored MN applications (given that adequate exposure data are available). Phase 2 would also be where aspects such as life cycle of MNs and evaluation of by-products of the use of nanomaterials could be considered in greater depth and specificity than may be possible in Phase 1. Thus, Phase 2 testing may be guided by risk related decision logic.

6.1 Endpoints for testing nanomaterials

	Status (to be completed/addressed)	Input from Literature
Nanomaterial Information/Identification		
• *Nanomaterial name*	All of the endpoints must be completed	Yes
• *CAS Number*		Yes
• *Structural formula/molecular structure*		Yes
• *Composition of nanomaterial being tested (including degree of purity, known impurities or additives)*		None
• *Basic morphology*		Yes
• *Description of surface chemistry (e.g., coating or modification)*		None
• *Major commercial uses*		Yes
• *Known catalytic activity*		None
• *Method of production (e.g., precipitation, gas phase)*		Yes
Physical-chemical Properties and Material Characterization		
• *Agglomeration/aggregation*	Must be Addressed	Some data available
• *Water Solubility/Dispersibility*	Must be Completed	None
• *Crystalline phase*	Must be Completed	None
• *Dustiness*	Must be Addressed	None
• *Crystallite size*	Must be Addressed	Some data available
• *Representative Electron Microscopy (TEM) picture(s)*	Must be Addressed	Yes
• *Particle size distribution – dry and in relevant media*	Must be Completed	Some data available
• *Specific surface area*	Must be Completed	Yes
• *Zeta potential (surface charge)*	Must be Completed	Yes
• *Surface chemistry*	Must be Completed	None
• *Photocatalytic activity*	Must be Addressed	None
• *Pour density*	Must be Addressed	None
• *Porosity*	Must be Addressed	None
• *Octanol-water partition coefficient*	Must be Addressed	None
• *Redox potential*	Must be Addressed	None
• *Radical formation potential*	Must be Addressed	None
• *Other relevant Physical-Chemical Properties and Material*	Must be Addressed	

Characterization information (where available)		
Environmental Fate		
• *Dispersion stability in water*	Must be Addressed	None
• *Biotic degradability*	Must be Addressed	Not applicable
• *Identification of degradation product(s)*	Must be Addressed	Not applicable
• *Further testing of degradation product(s) as required*	Must be Addressed	Not applicable
• *Abiotic Degradability and Fate*	Must be Addressed	None
• *Adsorption-Desorption*	Must be Addressed	None
• *Adsorption to soil or sediment*	Must be Addressed	Some data available
• *Bioaccumulation potential*	Must be Addressed	None
• *Other relevant environmental fate information (when available)*	Must be Addressed	
Environmental Toxicology		
• *Effects on pelagic species (short term/long term)*	Must be Addressed[***]	None
• *Effects on sediment species (short term/long term)*	Must be Addressed[***]	None
• *Effects on soil species (short term/long term)*	Must be Addressed	None
• *Effects on terrestrial species*	Must be Addressed	None
• *Effects on microorganisms*	Must be Addressed	None
• *Effects on activated sludge at WWTP*	Must be Addressed[***]	None
• *Other relevant information (when available)*	Must be Addressed[***]	
Mammalian Toxicology		
• *Pharmacokinetics/Toxicokinetics (ADME)*	Must be Addressed	None
• *Acute toxicity*	Must be Addressed	Some data available
• *Repeated dose toxicity*	Must be Addressed	None
• *Chronic toxicity*	Must be Addressed[***]	Some data available
• *Reproductive toxicity*	Must be Addressed[***]	Some data available
• *Developmental toxicity*	Must be Addressed[***]	None
• *Genetic toxicity*	Must be Addressed[***]	None
• *Experience with human exposure*	Must be Addressed[***]	None
• *Other relevant test data*	Must be Addressed[***]	
Material Safety • *Flammability* • *Explosivity* • *Incompatibility*		

Even though some data is available through the literature, major gaps exist in the list of endpoints being tested. Due to the different nanomaterial samples that are being investigated for PROSPEcT and the Sponsorship Programme, it will be necessary to perform all of the listed endpoints where ever applicable.

[***] The status of the endpoint is being clarified by the OECD.

7.0 Conclusion

(Eco)toxicology testing of engineered nanoparticles requires the development of agreed testing protocols and guidelines to allow for the comparison and interpretation of data from the studies. In order to achieve more comparable and reproducible data, reference materials need to be agreed upon, and standards may have to be developed. [32] However, it is still not clear at which concentrations and in what form MNs will be released into the environment and so it is difficult to devise experiments to simulate what might happen when nanoparticles are released into soil or the aquatic environment. It is extremely important therefore to establish and agree the criteria for such tests at the beginning of this project.

This literature review illustrates that the existing literature on nano cerium oxide does not provide the required level of comparability and reproducability for any of the endpoints agreed by the OECD WPMN Sponsorship Programme. In particular the use of the same nanomaterial from the same batch, stored under controlled conditions and tested under agreed sample-handling protocol represent important steps in obtaining viable data on nano cerium oxide, and making this data ultimately comparable to the data obtained from the other nanomaterials within the OECD WPMN Sponsorship Programme.

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