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TOXICOLOGICAL REVIEW

OF

Cerium Oxide and Cerium Compounds

(CAS No. 1306-38-3)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

May 2009

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LIST OF ABBREVIATIONS AND ACRONYMS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMC	benchmark concentration
BMD	benchmark dose
CASRN	Chemical Abstract Service Registry Number
CFU	colony-forming unit
CI	confidence interval
COH	coumarin 7-hydroxylase
CT	computer tomography
CYP	cytochrome P450 (in connection with isozyme abbreviations)
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DCF	dichlorodihydrofluorescein diacetate
DPO	dendriform pulmonary ossification
EDTA	ethylenediamine tetraacetic acid
EGTA	ethylene glycol bis(2-aminoethylether)tetraacetic acid
EPA	Environmental Protection Agency
GD	gestation day
GI	gastrointestinal
GSD	geometric standard deviation
GSH	glutathione
HEC	human equivalent concentration
HEI	Health Effects Institute
i.v.	intravenous
IRIS	Integrated Risk Information System
LC_{50}	median lethal concnetration
LD_X	dose that kills X% of animals
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
MCP	monocyte chemoattractant protein
MDA	malodialdehyde
MMAD	mass median aerodynamic diameter
MT	metallothionein
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OCT	ornithine-carbamyl transferase
PAM	pulmonary alveolar macrophage
PBTK	physiologically based toxicokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
RDDR	regional deposited dose ratio
RfC	reference concentration
RfD	reference dose

SDH	sorbitol dehydrogenase
SOD	superoxide dismutase
UF	uncertainty factor

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to cerium oxide and cerium compounds. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of cerium oxide and cerium compounds.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of cerium oxide and cerium compounds. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values may also be derived for acute ($\leq$24 hours), short-term (>24 hours up to 30 days), and subchronic (30 days up to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are derived from the application of a low-dose extrapolation procedure. The oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for cerium oxide and cerium compounds has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel Reports that were used in the development of this assessment include the following: *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA,

1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)*, Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2005c), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through June 2007.

2. CHEMICAL AND PHYSICAL INFORMATION

Cerium is a member of the lanthanide series of metals and is the most abundant of the rare-earth elements in the earth's crust (average concentration of 50 ppm) (Hedrick, 2004). Elemental cerium is an iron-gray, ductile, malleable metal (O'Neil, 2001). Cerium metal is very reactive and is a strong oxidizing agent that is stabilized when associated with an oxygen ligand (Kilbourn, 2003). When present in compounds, cerium exists in both the trivalent state (Ce^{3+}, cerous) and the tetravalent state (Ce^{4+}, ceric) (Kilbourn, 2003; Reinhardt and Winkler, 2002). Chemical structures and selected chemical and physical properties of cerium and cerium compounds are listed in Table 2-1.

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 (Lide, 2005; Kilbourn, 2003). 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 (forming CeO_2) followed by variable solubility filtration (Reinhardt and Winkler, 2002). Cerium salts can be prepared by liquid-liquid extraction from rare-earth cerium-containing solutions. Cerium metal is prepared by reacting CeF_3 with an excess of calcium at approximately 900°C (Kilbourn, 2003). It can also be obtained by the fused-salt electrolysis of a mixture of cerium chlorides and fluorides (Reinhardt and Winkler, 2002).

Cerium is most heavily used in the form of mischmetal for metallurgical purposes (Kilbourn, 2003; Reinhardt and Winkler, 1996). Cerium is the major component of mischmetal (50–75% by weight for the most common grades), a commercial mixture of metallic light lanthanides prepared by the electrolysis of mixed lanthanide chlorides and fluorides obtained from bastanite or monazite (Kilbourn, 2003; Reinhardt and Winkler, 2002). 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 (Reinhardt and Winkler, 2002). Mischmetal has been used in the manufacture of steel to improve shape control, reduce hot shortness, and increase heat and oxidation resistance. It can be added to cast iron to improve ductility, toughness, and microstructure. Mischmetal is also used in the manufacture of cerium-iron alloy lighter flints (Kilbourn, 2003; Reinhardt and Winkler, 2002).

Table 2-1. Physical properties of cerium and selected cerium compounds

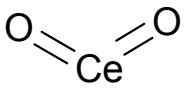
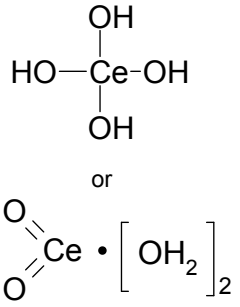
Name	Cerium	Cerium oxide	Hydrated cerium oxide
CASRN	7440-45-1	1306-38-3	12014-56-1 (hydroxide) 23322-64-7 (hydrate)
Synonyms		Cerium dioxide; ceria; ceric oxide; cerium(IV) oxide	Hydrated ceric oxide; cerium hydrate; ceric hydroxide; cerium(IV) hydroxide; cerium perhydroxide; cerium tetrahydroxide
Structure			
Molecular weight	140.116	172.11	208.148
Molecular formula	Ce	CeO_2	$CeO_2 \cdot 2H_2O$
Form	Iron-gray, ductile, malleable metal	Pale-yellow, heavy powder (white when pure); commercial product is brown	The hydroxide precipitate is amorphous and on drying, converts to hydrated ceric oxide; whitish powder when pure.
Melting point	798°C; boiling point = 3443°C	2400°C	Not available
Density	6.770 g/cm^3	7.65 g/cm^3	Not available
Water solubility	Decomposes slowly with cold water and rapidly with hot water	Insoluble in water	Insoluble in water
Other solubility	Soluble in dilute mineral acids	Insoluble in dilute acid	Soluble in concentrated mineral acid

Table 2-1 [continued]. Physical properties of cerium and selected cerium compounds

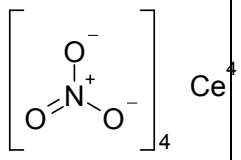
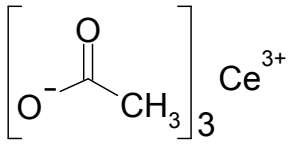
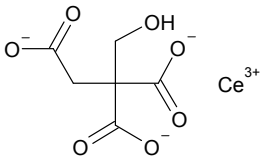
Name	Cerium nitrate	Cerous chloride	Cerous fluoride
CASRN	13093-17-9	7790-86-5	7758-88-5
Synonyms	Cerium tetranitrate; nitric acid, cerium(4+) salt	Cerium(III) chloride; cerium trichloride	Cerium(III) fluoride; cerium trifluoride
Structure			
Molecular weight	388.136 9	246.48	197.11
Molecular formula	$Ce(NO_3)_4$	$CeCl_3$	CeF_3
Form	Not available	White crystals; fine powder	Hexagonal crystals or powder
Melting point	Not available	817°C	1430°C
Density	Not available	3.97 g/cm^3	6.157 g/cm^3
Water solubility	Not available	Soluble in water	Insoluble in water
Other solubility	Not available	Soluble in ethanol	Soluble in acids (monohydrate)

Table 2-1 [continued]. Physical properties of cerium and selected cerium compounds

Name	Cerous acetate	Cerous citrate
CASRN	537-00-8	512-24-3
Synonyms	Cerium(III) acetate; cerium triacetate; acetic acid; cerium(3+) salt	Cerium citrate; cerium(III) citrate; cerium(3+) 2-hydroxypropane-1,2,3-tricarboxylate
Structure		
Molecular weight	317.251	329.219
Molecular formula	$Ce(C_2H_3O_2)_3$	$C_6H_8O_7.Ce$
Form	Not available	Not available
Melting point	Not available	Not available
Density	Not available	Not available
Water solubility	Not available	Not available
Other solubility	Not available	Not available

Sources: ChemIDplus (2006); Lide (2005); Lewis (2001); O'Neil (2001).

Exposure to commercially used cerium compounds is most likely through exposure to cerium (ceric) oxide (CeO_2). It is used either in the pure form or in a concentrate as a polishing agent for glass mirrors, plate glass, television tubes, ophthalmic lenses, and precision optics (Kilbourn, 2003; Reinhardt and Winkler, 2002). Cerium oxide is used as a glass constituent to prevent solarization and discoloration (especially in the faceplates of television screens) (Reinhardt and Winkler, 2002). Cerium oxide is also used in emission control systems in gasoline engines and as a diesel fuel-born catalyst to reduce particulate matter emissions (Health Effects Institute [HEI], 2001; Reinhardt and Winkler, 1996). Cerium nitrate has been used as a topical treatment for burn wounds (Monafo et al., 1976). Major uses for selected cerium compounds are listed in Table 2-2.

Table 2-2. Major uses of selected cerium compounds

Name	CASRN	Use
Cerium oxide	1306-38-3	Polishing and decolorizing glass; opacifier in vitreous enamels and photochromic glasses; heat-resistant alloy coatings; as a cracking catalyst; as a catalyst for automobile emission control; in ceramic coatings; in phosphors; in cathodes; in capacitors; in semiconductors; in refractory oxides; gemstone polishing
Hydrated cerium oxide	23322-64-7	Production of cerium salts and cerium oxide
Cerous chloride	7790-86-5	In the manufacturing of cerium metal and cerium salts; catalyst for the polymerization of olefins
Cerous fluoride	7758-88-5	In the preparation of cerium metal; in arc carbons to increase brilliance

Sources: Kilbourn (2003); HEI (2001); Lewis (2001); O'Neil (2001); Wells and Wells (2001).

Cerium is not expected to exist in elemental form in the environment since it is a reactive metal (Lewis, 2001). Cerium compounds are not expected to volatilize and will exist in the particulate form if released into the air. For cerium compounds that are soluble in water, Ce^{3+} would likely have a pKa close to La^{3+} (8.5) (Wulfsberg, 2000), which indicates that the hydrated Ce^{3+} ion ($[Ce(H_2O)n]^{3+}$) will remain in solution at environmental pHs (4–9). The hydrated Ce^{4+} ion ($[Ce(H_2O)n]^{4+}$) is expected to hydrolyze and polymerize at environmental pH (Cotton et al., 1999) and may precipitate out of solution. In general, metal cations in solution are attracted to the surfaces of soil particles, and the extent of adsorption to soils will depend on the soil characteristics (e.g., pH, mineral content, organic content) (Evans, 1989).

3. TOXICOKINETICS

Many of the studies of the toxicokinetics of cerium were conducted using radioactive cerium. Stable and radioactive cerium are expected to behave in a similar toxicokinetic manner and possess the same chemical properties. Radioactive cerium is a beta-emitter. As such, data from studies using either stable or radioactive cerium are presented below. Data characterizing the toxicokinetics of cerium compounds, such as cerium oxide, are discussed in order to inform the overall database.

3.1. ABSORPTION

3.1.1. Oral Exposure

Studies evaluating the absorption of cerium compounds following oral exposure in humans are not available.

In adult animals, cerium compounds are poorly absorbed following oral exposure, while suckling animals exhibit higher absorption and retention of cerium in the gastrointestinal (GI) tissues. Observed absorption of radioactive cerium salts from the GI tract of adult rats ranged from 0.05% to less than 0.1% of the administered dose (Kostial et al., 1989b; Inaba and Lengemann, 1972; Shiraishi and Ichikawa, 1972). Suckling rats, however, absorbed 40–98% of administered dose, with the youngest rats retaining the largest percentage of the dose (Kostial et al., 1989a, b; Inaba and Lengemann, 1972).

Four litters of Sprague-Dawley rats (n = 7–9), age 0, 7, 14, or 26 days, were given a single dose of [^{141}Ce]-ceric nitrate of unreported concentration by intragastric dosing (Inaba and Lengemann, 1972). Subsequent, periodic whole-body radioactivity measurements were taken immediately after dosing and periodically thereafter. In another experiment in this study, two litters of 1-day-old newborn rats were given single doses of [^{141}Ce]-ceric nitrate, and one rat from each litter was sacrificed at 1, 3, 5, 7, and 10 days after dosing, after which radioactivity in the GI tract and whole body was measured and macro-autoradiographs of the GI tract were produced.

It was observed that on or about day 16 of life, rats began consuming a solid, grain-based diet and were completely weaned by day 26 (Inaba and Lengemann, 1972). In weanling rats (rats dosed at 26 days of age), only 0.04% of the administered radioactivity remained in the body by 3 days after dosing. However, radioactivity in newborns diminished more slowly, dropping from 98% of administered dose on day 1 to 29% on day 16. The GI tract accounted for nearly 100% of whole-body retention on day 1 and 93% on day 16. After the onset of weaning in the suckling rats dosed as newborns, whole-body radioactivity fell to 3% of administered dose by

day 24, of which only 17% was measured in the GI tract. Autoradiographs of the GI tract from the two litters dosed as 1-day-old newborns suggest rapid transit of radioactivity to the lower small intestine 1 day after exposure. Autoradiography of rats 5 days after dosing (6 days old) suggests that the intestinal radioactivity is restricted to the upper two-thirds of the epithelial villi, which the study authors associated with cerium concentration in the vacuoles.

Kostial et al. (1989b) administered single oral doses of an unreported concentration of [^{141}Ce]-cerous chloride by intragastric dosing to 6-day-old and 6–8-week-old rats (strain unreported) to investigate whether distribution of cerium (and other metals) in the GI tract differed in suckling versus mature rats. Six-day-old rats were measured for whole-body and gut radioactivity 2, 4, 6, and 12 days after dosing, while adult rats were measured 2 days after dosing. Orally administered cerium chloride was more readily retained in the whole body, gut, and carcass of suckling rats than in older rats. The ileum was the main site for cerium chloride accumulation in the suckling rats following oral administration, while the stomach and large intestine were the main sites for cerium chloride accumulation in the 6–8-week-old rats. Whole-body radioactivity 6 days postexposure in 2-week-old suckling rats orally dosed with [^{141}Ce] was 40% of the administered dose, of which 95% was found in the gut. However, whole-body radioactivity 6 days postexposure in adult (6–8-week-old) rats was only 0.05%, of which 66% was found in the gut, mostly in the stomach and cecum.

Shiraishi and Ichikawa (1972) administered single oral doses of an unreported concentration of [^{144}Ce]-cerous chloride by intragastric dosing to 0-, 7-, 14-, and 21-day-old juvenile and 100-day-old adult Wistar rats. Cortisone acetate, which alters the morphology of the absorptive epithelium of the small intestine, was injected to a group of 7-day-old rats 4 days prior to [^{144}Ce]-cerous chloride exposure to observe its effect on whole-body retention. Periodically (up to 70 days after dosing), small groups of rats from all age groups were sacrificed and measured for radioactivity in the whole-body, gut, and various excised tissues. The decrease in retention of whole-body radioactivity among suckling rats dosed at 0, 7, and 14 days after birth was approximately 11, 6.5, and 1.5%, respectively, of the administered dose. Weanling and adult rats exhibited a rapid decrease in whole-body radioactivity through 10 days following dosing. At 2 weeks after dosing, the 21- and 100-day-old adults had whole-body radioactivity of 0.08 and 0.018%, respectively, of the administered dose. The intestinal content accounted for most of the retained radioactivity in neonates until the age of weaning. Cortisone acetate treatment resulted in a more rapid loss of the oral dose from young rats, although whether this is due to lower uptake by intestinal cells or a more rapid release to feces is not presently clear. This investigation demonstrated that the whole-body retention of cerium chloride by suckling rats was greater than the retention by weanling and adult rats, and this increased retention by

suckling rats may be due to increased pinocytic activity in the absorptive epithelium of sucklings (Shiraishi and Ichikawa, 1972).

Yorkshire piglets treated with [^{144}Ce]-cerous chloride by gavage on the first or fourth day after birth and sacrificed 4 or 18 or 3 or 21 days, respectively, after dosing absorbed 2.5–8% of the administered dose (Mraz and Eisele, 1977). Absorption was threefold greater in piglets treated at 1 day of age versus those treated at 4 days of age. Body content of cerium chloride did not differ significantly between piglets sacrificed at the earlier dates and those sacrificed later, indicating that absorption was almost complete within 3–4 days.

Eisele et al. (1980) gave single gavage doses of either [^{144}Ce]-cerous chloride or [^{144}Ce]-cerous citrate (concentration unreported) to 0–6- or 6–24-hour-old C_3H mice and Sprague-Dawley rats and to 6–24-hour-old Yorkshire piglets. Radioactivity levels were measured in the GI tract and other tissues, including the remaining carcass, at days 1, 5, 7, 9, 12, 15, 17, 19, and 21. In the 0–6-hour-old mice, a high of 31% of the administered dose of cerium chloride was retained in the body 9 days following exposure. At 21 days post administration, the amount of pooled cerium citrate and chloride retained was approximately 25%. The mice dosed 0–6 hours after birth retained more cerium in the GI tract throughout the 21-day observation period than the mice dosed 6–24 hours after birth. The 0–6- and 6–24-hour-old rats exhibited absorption of 9–10% of administered cerium 21 days after exposure. In the Yorkshire piglets, the absorbed dose did not differ significantly over the 21-day observation period.

Two studies in adult rats also reported low absorption of soluble cerium following oral exposures. Durbin et al. (1956) administered single [^{144}Ce]-ceric nitrate (unreported concentrations) intramuscular and intragastric doses to adult female Sprague-Dawley rats. The rats dosed intramuscularly were sacrificed at post-administration days 1, 4, 64, and 256, while the intragastric-dosed rats were sacrificed 4 days after administration. Less than 0.1% of the intragastric-administered dose was absorbed from the GI tract.

Stineman et al. (1978) administered single intragastric [^{141}Ce]-cerous chloride doses of 1000 mg/kg (lethal to 5% of the animals [LD_5]) and 1163 (LD_{25}) mg/kg to 6–8-week-old male Swiss ICR mice and sacrificed them at 4 hours or at 1, 3, or 7 days later. No measurements were made of whole-body radioactivity; however, 97–99% of radioactivity in the 12 sampled tissues was found in the gut (stomach + duodenum).

In young (suckling) animals, soluble cerium appears to be retained in intestinal cells, particularly in the ileum, possibly resulting in a much greater absorption than in adult animals (Kostial et al., 1989a, b; Inaba and Lengemann, 1972). However, cerium retained in intestinal cells has been demonstrated to be unavailable systemically (Inaba and Lengemann, 1972). The high gut retention of cerium in young animals may be associated with high pinocytotic activity

of the newborn intestinal cells (Kostial et al., 1989b). Glucocorticoids, such as methylprednisolone, stimulate production of endogenous corticosteroids, which cause precocious gut closure (decreased pinocytosis) and maturation of the metal absorptive process (Kargacin and Landeka, 1990). Administration of methylprednisolone in conjunction with artificial feeding of [^{141}Ce]-cerous chloride in cows' milk to 4-day-old suckling rats (strain not reported) resulted in a nearly 40-fold reduction in the amount of cerium chloride detected in gut tissue (Kargacin and Landeka, 1990). This finding suggests that the pinocytotic activity of the intestinal cells contributed to the differences. Similarly, injection with cortisone acetate resulted in a more rapid loss of an oral dose to feces of young rats (Shiraishi and Ichikawa, 1972), although whether this is due to lower uptake by intestinal cells or a more rapid release is not presently clear.

3.1.2. Inhalation Exposure

Studies evaluating the deposition or absorption of cerium compounds following inhalation exposure in humans are not available. However, cerium has been detected in the lung tissue and alveolar macrophages of subjects believed to have been exposed to cerium occupationally.

Case reports and a retrospective occupational investigation provide support for the limited absorption of cerium deposited in the lung following inhalation exposure. Transbronchial biopsies in a 60-year-old movie projectionist showed cerium concentrations of 11 μg/g wet weight after 12 years of exposure (Porru et al., 2001). McDonald et al. (1995) demonstrated particulate material (diameter range from <1 μm to 5–10 μm) localized within lung biopsy cells by using a scanning electron microscope. Analysis of bronchioalveolar lavage fluid from a 58-year-old patient exposed to rare earth dusts and asbestos revealed cerium and phosphorus in the alveolar macrophages (Pairon et al., 1995). The cerium particles accounted for 70% of the particles observed in the lung tissue and were also identified in the interstitial macrophages.

Microscopic examination of the tracheobronchial lymph nodes of a movie projectionist of 25 years revealed grey granules in large macrophages, which were characterized as calcium and rare earth elements (cerium, lanthanum, and neodymium) by energy-dispersive analysis of X-rays (Waring and Watling, 1990). Energy-dispersive X-ray also characterized dark particles (diameter of 1 to 6 μm) as cerium, unidentifiable by optical microscopy, in the bronchioalveolar lavage of a 13-year photoengraver.

Lung tissues from a photoengraver exposed to smoke from cored carbon arc lamps for 46 years (Pietra et al., 1985) were found to have cerium concentrations 2,800–207,000 times

higher than those of urine, blood, or nails, suggesting that cerium particles in the lung are poorly mobilized. The concentration of cerium in the lung and lymph nodes of a subject exposed to cerium for 46 years as a photoengraver was 167 and 5 μg/g wet tissue weight, respectively, and 2,400- and 53-times higher, respectively, than the concentration in unexposed control subjects (Vocaturo et al., 1983; Sabbioni et al., 1982).

Pairon et al. (1994) performed a retrospective evaluation of retention of cerium-containing particles in the lungs of workers previously exposed to mineral dusts. Bronchioalveolar lavage and lung tissue samples from mineral dust exposed workers and controls were examined for cerium content. In the seven cases that were judged to have high cerium particle retention (as defined by having lavage fluid or tissue cerium concentrations at least 5 times higher than those of controls), time since last exposure ranged from present to 29 years, with one patient's exposure time not available.

Limited animal data are available regarding total deposition of cerium aerosols within the respiratory tract. Thomas et al. (1972) exposed 4-month-old, mixed sex Holtzman rats to two concentrations (unreported) of aerosolized 1.4 μm median aerodynamic diameter (GSD of 2.0) [^{144}Ce]-ceric hydroxide for 10 minutes. Whole-body radioactivity measurements were used to identify a 28% deposition rate of inhaled cerium aerosol. Boecker and Cuddihy (1974) reported an average deposition of 71% in beagles exposed to [^{144}Ce]-cerous chloride for 4–10 minutes. In both studies, cerium was deposited in the lungs with evidence of absorption from the lung provided by the subsequent detection of cerium in the skeleton, liver, and kidneys.

3.2. DISTRIBUTION

3.2.1. Oral Exposure

Although cerium appears to be poorly absorbed from the GI tract, the bone and liver were the organs with the highest cerium levels in rats following oral gavage of cerium chloride (Shiraishi and Ichikawa, 1972). The concentration of cerium in the kidney, liver, lung, and spleen of male ICR mice was significantly elevated relative to controls following 6 and 12 weeks of oral exposure to 20 or 200 ppm cerium chloride (Kawagoe et al., 2005). The lung and spleen contained the highest cerium concentrations in male ICR mice.

Manoubi et al. (1998) gave a single intragastric dose of stable cerium nitrate (20 mg/mL) to Wistar rats. Three hours after dosing, cerium was found in the lysosomes of the duodenal villosity but not in the liver or spleen. In 1-day-old Sprague-Dawley rats given a single intragastric dose of [^{141}Ce]-ceric nitrate of unreported concentration, Inaba and Lengermann (1972) found cerium to be localized centrally, likely in the vacuoles, within epithelial cells of the small intestine.

Cerium is capable of crossing the placenta and entering the fetal circulation in mice, but the amounts found in the uterus and placenta were generally less than 5% of the maternal body burden and decreased rapidly with increased time after exposure (Naharin et al., 1969). Fetal body burdens in rodents were generally less than 1% of the initial maternal body burden after either injected or oral administration (Levack et al., 2002; Inaba et al., 1992; Naharin et al., 1969). Small amounts of injected cerium were also found in the maternal milk of mice (Naharin et al., 1974), although at a small proportion (<0.01%) of the maternal body burden.

3.2.2. Inhalation Exposure

As poorly soluble particles, cerium particles behave like other airborne particles, depositing within the respiratory tract based on aerodynamic character (Schulz et al., 2000). Hamsters inhaling [^{144}Ce]-cerium oxide aerosols with particle activity median diameters of 0.11 and 0.06 μm exhibited lung burdens of 3.6% at 5 hours and 50% at 3 hours after exposure, respectively, of initial body burden (Kanapilly and Luna, 1975).

Once deposited in the lung, insoluble cerium compounds may dissolve slowly, as evidenced by the low percentage of cerium found in other tissues. In an investigation of the toxicity of insoluble cerium, Hahn et al. (2001) exposed beagles to aerosolized [^{144}Ce]-fused aluminosilicate particles for 2–48 minutes and collected a variety of tissues (tissues studied not reported) at death (up to 6,205 days following exposure). Cerium was dissolved from the lung into the systemic circulation and observed in the liver, skeleton, and tracheobronchial lymph nodes. Hahn et al. (2001) found between 1.0 and 10% of the initial lung burden of [^{144}Ce]-fused aluminosilicate particle aerosol in the liver and skeleton of beagles observed for 800 days following 2–48-minute inhalation exposures. Hahn et al. (1999) also observed [^{144}Ce]-fused aluminosilicate particle translocation to the tracheobronchial lymph nodes following acute inhalation of the particles. Lundgren et al. (1992) exposed adult F344/Crl rats to [^{144}Ce]-cerium oxide for 5–50 minutes or to bimonthly exposures of 25 minutes for 1 year; rats were sacrificed at 1 hour and 3, 7, 14, 28, 56, 112, 224, 448, 560, and 672 days after exposure. The lungs, heart, liver, spleen, kidney, and skeleton (remaining carcass) were measured for cerium. Cerium was detected in the liver and skeleton in increasing percentages of body burden with respect to time, while cerium was not detected in the spleen and kidneys.

More soluble forms of cerium (e.g., cerium citrate) may be systemically absorbed more easily from the lung due to the increased solubility of the compound. Morgan et al. (1970) exposed Swiss mice to aerosols of [^{144}Ce] in the form of chloride, citrate, or fused clay, with activity median diameters from 1.3 to 2.75 μm, in unreported concentrations or durations. While the initial body burden of all forms decreased rapidly during the first 2 weeks, likely due to

mucociliary elimination to the GI tract, the remaining lung burden for the relatively insoluble fused clay remained higher than the chloride or citrate for the duration of the study (130 days). Conversely, the liver burdens of the citrate and chloride forms remained higher than the fused clay by about an order of magnitude. Further, as lung burdens of the chloride and citrate forms decreased, the bone burdens of these forms increased. The bone burden for the fused clay form, like the liver, was about an order of magnitude lower than the citrate or chloride forms.

Sturbaum et al. (1970) exposed 40 Chinese hamsters via the nose to [^{144}Ce]-cerous chloride aerosol, activity median diameter of 0.83 μm and a GSD of 1.7, for 20 minutes and sacrificed small groups (n = 4) at 2, 8, 16, 28, 64, 128, and 256 days after exposure. Whole-body and tissue (types unreported) measurements of cerium radioactivity were made. The liver and skeleton exhibited between 1 and 10% of the initial body burden throughout the post-administration measurements, while the lung portion of the initial body burden diminished from approximately 20 at two hours to <1% by study's end.

Cerium has been observed to be localized in the cell, particularly in the lysosomes, where it is concentrated and precipitated in an insoluble form in association with phosphorus. Wistar rats were exposed to stable cerium chloride aerosol, mean diameter of 0.1 μm, 5 hours/day for either 5 days or 4 days/week for 4 weeks (Galle et al., 1992; Berry et al., 1989, 1988). Several hours after exposure, cerium was deposited in the lysosomes of alveolar macrophages. The cerium deposits appeared to be in the form of aggregates of fine granules or fine needles that varied in length from 30 to 60 nm, with the longer needles resulting from the 4-week exposure. Cerium was found in the lysosomal fraction of liver centrifugate collected from rats (strain unreported) given an intravenous (i.v.) injection of 1.3 mg/kg [^{141}Ce]-cerous chloride (Wiener-Schmuck et al., 1990). Cerium was also found in the lysosomes of the duodenal villosity, but not in the liver or spleen of Wistar rats following intragastric dosing. Cerium was also localized centrally, likely in the vacuoles, within epithelial cells of the small intestine of Sprague-Dawley rats.

3.3. METABOLISM

As an element, cerium is neither created nor destroyed within the body. The particular cerium compound (e.g., cerium chloride, cerium oxide) may be altered as a result of various chemical reactions within the body, particularly dissolution, but data have not demonstrated a change in the oxidation state of the cerium cation. Exposure to cerium has been shown to change hepatic levels of some cytochrome (CYP) P450 isozymes in a species- and strain-sensitive manner for mice. Salonpää et al. (1992) gave i.v. cerous chloride injections of 2 mg/kg to adult DBA/2 and C57BL/6 mice and observed increases in expression of CYP2A4 and

CYP2A5 in the livers (2 and 3 days after dosing) and in the kidneys (6 hours and 1 day after dosing) of D2 mice but not in B6 mice. Arvela et al. (1991) gave i.v. cerous chloride injections of 0.5, 1, and 2 mg/kg to adult male DBA/2 and C57BL/6 mice and found a greater sensitivity to increased CYP450 expression (isoform not reported) in DBA/2 and C57BL/6 mice 24 hours and 3 days after exposure, respectively. Conversely, Arvela and Karki (1971) observed a 50% reduction, compared to controls, in CYP450 activity in adult Sprague-Dawley rats 3 days after a single i.v. injection of 2 mg/kg cerous chloride. The effect of changes in CYP450 levels on the toxicokinetics or toxicity of cerium, if any, is not known. In addition, the relatively high intravenous bolus doses used in the available studies may not be relevant to oral or inhaled exposure to cerium oxide.

3.4. ELIMINATION

Following inhalation exposure, the initial rapid elimination of insoluble cerium from the body is due primarily to transport up the respiratory tract by the mucociliary escalator and eventual swallowing of the material, as with other poorly soluble particles (Boecker and Cuddihy, 1974). Initial short-term clearance rates range from 35 to 95% of initial cerium body burden, depending on the species tested and length of clearance time investigated. Lundgren et al. (1992) exposed adult F344/Crl rats to [^{144}Ce]-cerium oxide aerosol for 5–50 minutes, with clearance of approximately 90% of the initial body burden by 7 days. Kanapilly and Luna (1975) exposed hamsters to [^{144}Ce]-cerium oxide aerosols with particle activity median aerodynamic diameters of 0.11 and 0.06 μm and observed decreases in initial body burden of 95 and 60%, respectively, 4 days after exposure. Differences in clearance rates may have been dependent on particle size differences, with the smaller particles taking more time for elimination; however, the authors also stated that the difference may have resulted from a leak in the inhalation chamber used for the first dose group. Boecker and Cuddihy (1974) observed an early clearance of initial body burden from 35–80% for individual dogs 4 days after exposure. Thomas et al. (1972) exposed Holtzman rats to two concentrations (unreported) of aerosolized [^{144}Ce]-ceric hydroxide for 10 minutes and observed approximately 75–95% clearance of initial body burden within 2 weeks of exposure (Thomas et al, 1972). Sturbaum et al. (1970) reported clearance of 80% of initial cerium body burden by 7 days in Chinese hamsters exposed to [^{144}Ce]-cerous chloride aerosol for 20 minutes. After the initial clearance of cerium particles from the upper respiratory tract, pulmonary clearance is slower, with reported slow-phase clearance half-times ranging from 100 to 190 days in rodents (Lundgren et al., 1974; Thomas et al., 1972; Morgan et al., 1970; Sturbaum et al., 1970). The slow-phase clearance was slightly faster in beagles, with an estimated half-time of 63 days (Boecker and Cuddihy, 1974). Slow-

phase clearance from the lung is a combination of cerium dissolution and absorption (Morgan et al., 1970) and mechanical clearance from the respiratory tract (Sturbaum et al., 1970).

Elimination of orally administered soluble cerium has been shown to be age dependent in animals, with suckling animals absorbing cerium into the GI tissues (Inaba and Lengemann, 1972). This cerium may remain in the intestinal cells, may not be available systemically, and may eventually be eliminated in the feces.

Although quantitative estimates of cerium elimination are rare, it appears that the primary route of elimination for cerium, whether inhaled, ingested, or injected, is through the feces, with small (generally <10%) amounts eliminated in the urine (Lustgarten et al., 1976; Durbin et al., 1956). It has been suggested that the fecal excretion of systemically absorbed cerium is due to elimination in the bile (Lustgarten et al., 1976), since hepatic clearance was due primarily to biliary function.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

No physiologically based toxicokinetic (PBTK) models for cerium oxide or other cerium compounds were located in the evaluated literature.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Oral Exposure

An association between exposure to rare earth elements, cerium in particular, in food and the development of endomyocardial fibrosis has been suggested (Eapen, 1998; Kutty et al., 1996; Valiathan et al., 1989). Cerium levels were elevated in the endomyocardial tissue samples from patients who died of endomyocardial fibrosis compared with the tissues of controls who died of accidents or congenital heart disease (Valiathan et al., 1989). Although causality has not been conclusively demonstrated, a higher incidence of endomyocardial fibrosis has been reported among a population consuming tubers grown in a region of India with high soil cerium concentrations, compared to subjects consuming tubers grown in a soil with low cerium concentrations. Analysis of the geographic distribution of endemic endomyocardial fibrosis in India suggested a link to high cerium soil concentrations and possibly to magnesium deficiency during childhood (Kutty et al., 1996).

A case-control study was conducted by Gómez-Aracena et al. (2006) to investigate the role of chronic cerium exposure in coronary heart disease. Chronic cerium exposure was represented by toenail cerium concentrations, and the occurrence of first myocardial infarction was the characterization used for coronary heart disease. Odds ratios were calculated by comparing the four exposure groups of 111, 142, 171, and 257 μg/kg toenail cerium concentration to the control group. Gómez-Aracena et al. (2006) found an association between increased toenail cerium concentrations and the risk of first myocardial infarction, when controlling for confounding factors, such as smoking, body mass index history of hypertension, diabetes, family history of coronary heart disease, β-carotene, lycopene, α-tocopherol, selenium, mercury, and scandium. The odds ratio of first myocardial infarction in smokers with a toenail cerium concentration of 257 μg/kg and without adjusting for additional risk factors of myocardial infarction was 1.18 (95% confidence interval [CI]: 0.83–1.66), with a p value for trend of 0.020. In nonsmokers, the odds ratio of first myocardial infarction with toenail cerium concentrations of 169 and 227 μg/kg were 2.09 (95% CI: 1.05–4.16) and 2.81 (95% CI: 1.21–6.52), respectively, with a p value for trend of 0.011, when controlling for the confounding factors listed above. The results of Gómez-Aracena et al. (2006) suggest a relationship between chronic cerium exposure and increased risk of acute myocardial infarction, with the strongest association observed in nonsmokers.

4.1.2. Inhalation Exposure

Reports have been published describing numerous cases of workers who developed adverse lung effects, such as interstitial lung disease or pneumoconiosis, associated with accumulation of cerium in the lungs after prolonged occupational exposure to cerium fumes or dust (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). The workers in the above reports had been exposed to cerium for periods of 10–46 years, with exposures most commonly due to fumes from carbon arc lamps. These lamps, widely used in the past in the fields of cinematography and photoengraving, have a central core consisting of approximately 46% cerium oxide and smaller amounts of other rare earth oxides, including lanthanum, neodymium, praseodymium, and samarium (Waring and Watling, 1990). As the core burns, it emits oxide, and, to a lesser extent, fluoride dusts of cerium and the other rare earth elements. Cases of cerium pneumoconiosis not associated with carbon arc lamps all involved exposure to cerium oxide, either during processing or peripheral to its use as an abrasive to grind and polish lenses (McDonald et al., 1995). Exposure concentrations of cerium were not quantified in any of these studies.

Dendriform pulmonary ossification (DPO), a rare condition characterized by branching, bony spicules often containing marrow, which are found in the lung parenchyma and are associatied with pulmonary fibrosis, was observed in a 38-year-old man who worked as a polisher in a crystal factory for 3 years (Yoon et al., 2005). Diffuse reticulonodular infiltrates in the lung were observed in a chest radiograph, and a computer tomography (CT) scan showed diffuse, tiny, circular, or beak-like densities with branching structures in the interlobular septum. A CT scan with bone-setting showed branching, twig-like ossified masses in the right lower lobe and a few dot-like ossifications in both lower lobes. The lung surface appeared irregular, emphysematous, and mottled with anthracotic pigmentation during an open lung biopsy, with several thorn-like hard materials in the lung parenchyma. Microscopic examination revealed interstitial fibrosis, peripheral emphysema, multiple particles, and pneumonia. The particles (0.1–0.3 μm) were determined to be cerium oxide and phosphates of cerium and lanthanum by energy dispersive X-ray analysis, with particles of quartz, feldspar, mica, kaolinite, halloysite, talc, and TiO_2 infrequently detected. Yoon et al. (2005) characterized this case study as the first to present a case of DPO associated with pneumoconiosis caused by the inhalation of rare earth metals.

A 60-year-old male who worked as a movie projectionist and was exposed to rare earth dusts for 12 years presented with diffuse interstitial lung fibrosis, emphysema, and severe

obstructive impairment (Porru et al., 2001). An increase in the lung concentration of rare earth elements was evident in the subject with the highest concentration for cerium, compared with the five unexposed controls. Interstitial fibrosis accompanied by vascular thickening, reactive alveolar macrophages, abundant macrophages in the air space, and moderate chronic interstitial inflammation, along with small interstitial clumps of macrophages bearing scant deposits of grayish-black pigment, was observed in a 68-year-old man who was employed as an optical lens grinder for 35 years and smoked for 20 years (McDonald et al., 1995). Pairon et al. (1995) identified particles containing cerium, lanthanum, and phosphorus in the alveolar macrophages from a 58-year-old smoker with dyspnea who had been exposed to asbestos and rare earth dusts as a crystal manufacturer, polisher, and movie projectionist from 1951–1967. Diffuse fibrosis of interalveolar septa and perivascular hyalinized fibrosis was observed from the histologic analysis (Pairon et al., 1995). Mild interstitial fibrosis, peribronchiolar fibrosis, and diffuse interstitial fibrosis with emphysema were seen in a photoengraver or glass polisher, nonsmoking foundry worker, and a movie projectionist and glass polisher, respectively (Pairon et al., 1994).

Deposits of carbon arc lamp fumes were evident in the marcrophages in the tracheobronchial lymph nodes of a 66-year-old smoking movie projectionist of 25 years, although pneumoconiosis was not considered because respiratory symptoms and radiographic and histologic changes were not apparent (Waring and Watling, 1990). Dark particles, identified as cerium by energy dispersive X-ray analysis, were observed in lung tissue from a 48-year-old smoker employed as a photoengraver for 13 years (Sulotto et al., 1986). A chest X-ray showed a micronodular pattern extending to all lung fields although lung examinations were normal and the patient did not experience respiratory impairment. Vogt et al. (1986) observed five reproduction photographers, exposed to carbon arc fumes for more than a decade, with slowly progressive respiratory function restriction, as well as interstitial lung fibrosis and the accumulation of fine, granular dusts, characterized as rare earth minerals and primarily cerium, in the lung tissue.

Pulmonary hypertension with increased vascular resistance was observed in a 58-year-old man who worked in the photoengraving industry for 46 years (Vocaturo et al., 1983; Sabbioni et al., 1982). Rare earth elements, primarily cerium, were observed by neutron activation analysis in the lung and lymph node biopsies, and the concentrations of the elements in these tissues were greater than those in control subjects (Pietra et al., 1985).

Profuse discrete nodular shadowing was present in the chest X-ray of a 34-year-old employee at a glass rubbing polish plant, although pulmonary function appeared normal (Husain et al., 1980). The subject refused histologic examination, and an analysis of the occupational dust concentrations revealed high levels of cerium oxide (50%) and other rare earth oxides.

A 65-year-old man working in the photographic department of a printing plant demonstrated inactive right apical infiltrates in a chest roentgenogram in 1948; in 1951 diffuse spotty infiltrates were noted; in 1953 and 1959 the infiltrates were more pronounced in the middle and lower fields; and in 1965 slight fibrosis of the surrounding tissue was evident, along with a perifocal emphysema (Heuck and Hoschek, 1968). Heuck and Hoschek (1968) also documented fibrosis and small infiltrates in the lung of a 53-year-old male exposed to carbon arc lamp smoke in printing industries and infiltrates in a 67-year-old man, with chemotherapy-treated tuberculosis in both upper lobes of the lung, who worked with carbon arc lamps for 26 years.

Collectively, the available studies show that the defining characteristic of cerium pneumoconiosis is accumulation of cerium particles, as well as other rare earth particles, in the lungs and lymphoreticular system. In most cases, the initial indication of disease was the presence of diffuse interstitial or reticulonodular opacities in chest X-rays. Pulmonary function in the affected workers varied from normal to severe restrictive impairment. In several cases, thorium, a common impurity in rare earth minerals, and the naturally occurring radioisotopes of the rareearth elements were quantified, but, in each case, they were found to be present in quantities too small to produce any effect due to radiation. Exposure to silica, which is fibrogenic, may have contributed to the effects observed in the cerium oxide and glass workers but is not a factor for the workers exposed to carbon arc lamp dust, which includes most of the workers found to have fibrosis. Two reviews of the available case studies concluded that there is convincing evidence that accumulation of cerium and other rare earth metals in the lungs is causally related to the development of pulmonary interstitial fibrosis in workers (McDonald et al., 1995; Waring and Watling, 1990). The human data were inadequate to identify potentially sensitive subgroups.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

Kawagoe et al. (2005) studied the possible association between cerium exposure and oxidative stress in the mouse liver. Groups of male ICR mice (four per dose group) were administered a diet containing 0, 20, or 200 ppm cerium chloride ($CeCl_3$) for 6 or 12 weeks. This corresponds to doses of approximately 2.6 or 26 mg Ce/kg-day, assuming a reference food consumption of 0.005 kg/day and reference body weight of 0.022 kg (U.S. EPA, 1988). Evaluations conducted at termination included cerium levels in organs (liver, spleen, kidney, and lung), levels of glutathione (GSH) and metallothionein (MT) in liver, kidney, and lung, levels of

lipoperoxide in liver and plasma, superoxide dismutase (SOD) activity in blood and liver, as well as cholesterol levels, triglyceride levels, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in serum. Treatment with cerium did not affect food consumption or body weight but statistically significantly decreased lipoperoxide levels in hepatic tissues (33% at 20 and 38% at 200 ppm, 6 weeks; 29% at 20 ppm, 12 weeks), increased liver GSH levels (200 ppm, 12 weeks) and liver MT activity (20 ppm, 12 weeks; 200 ppm, 6 weeks), and decreased plasma SOD activity (20 and 200 ppm, 6 weeks). Cerium in the kidney, liver, lung, and spleen was statistically significantly elevated relative to controls in the organs of mice in the 200 ppm group at 6 and 12 weeks, with the lung and spleen containing higher cerium concentrations. Pathological alterations were not detected in the kidney, liver, lung, or spleen by microscopic observation. According to Kawagoe et al. (2005), the increases in hepatic GSH and MT activity represent a response to cerium-induced oxidative stress. It is unknown whether the decrease in hepatic lipoperoxide is a consequence of the increase in GSH and MT. The study authors suggest that the endpoints showing changes as a result of cerium exposure in this study are indicators of reactive oxygen species generation in the liver.

A study (Cheng et al., 2000) in male Wistar rats (six per dose group) dosed orally (further details not reported) with 0, 0.2, 2.0, or 20 mg cerium chloride/kg-day (0.1, 1.1, or 11.4 mg Ce/kg-day) for up to 105 days investigated the effects of $CeCl_3$ on the structure and oxygen affinity of hemoglobin in vivo. The highest dose, 20 mg/kg-day, produced a slight increase of hemoglobin content in the erythrocytes after 40 days of treatment, with an even greater increase in hemoglobin content after 80 days. The effect on the oxygen affinity of the hemoglobin was demonstrated by oxygen saturation curves for the dosed rats and control rats. Hemoglobin in cerium-treated rats also exhibited altered oxygen affinity up to 80 days of exposure, demonstrated by increased affinity up to 10 mm Hg and a double sigmoidal curve for rats treated for 40 days and increased affinity above 20 mm Hg for rats treated for 80 days. A significant change was not observed at 0.2 mg/kg-day for 105 days of exposure. These results suggest that the oxygen affinity of hemoglobin increases following long-term oral exposure to $CeCl_3$. When the 80-day cerium feeding period was followed by 15 days of cerium-free exposure, the oxygen affinity did not recover to normal levels. The investigators attributed the altered oxygen affinity up to 80 days of exposure to conformational changes of hemoglobin, hydrolysis of hemoglobin-bound diphosphoglyceric acid, and the partial oxidation of heme-Fe(II) to heme-Fe(III).

Kartha et al. (1998) examined the effects of cerium chloride, administered in the drinking water with other rare earth chlorides, on the heart of New Zealand white rabbits (10/group) fed diets with normal or restricted magnesium for 6 months. The rabbits were randomly distributed into 4 groups with a male to female ratio of 1:1 within each group. Group 1 was exposed to a

magnesium-sufficient diet only, group 2 was exposed to a magnesium-sufficient diet and cerium, group 3 was exposed to a magnesium-restricted diet, and group 4 was exposed to a magnesium-restricted diet and cerium. The drinking water for the rare earth chloride-exposed rabbits was adulterated with 1 g/L of rare earth chloride of which 56.6% was cerium (lanthanum 11.5%; praseodymium and neodymium 14.5%; samarium 2.6%). Histologic evaluation of the heart at the end of the study showed no cardiac lesions in the groups fed the normal magnesium diet, regardless of whether they consumed water with or without cerium. Cardiac lesions were evident in 6/10 rabbits from group 3, the magnesium-restricted diet, and in 9/10 rabbits from group 4, the magnesium-restricted diet and cerium chloride-exposed group. Rabbits fed the magnesium-restricted diets, treated with or without cerium, showed endocardial, subendocardial, interstitial, and perivascular fibrosis. The lesions were more severe in those with cerium added to the drinking water (group 4). The results suggest that cerium may intensify the effect of magnesium deficiency on heart tissue. A no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for cerium cannot be established for cerium chloride in this study because the cardiac lesions were observed in rabbits that were fed a magnesium-restricted diet.

The only chronic oral exposure study to stable cerium was reported by Kumar et al. (1996), who exposed mixed-sex (male to female ratio of 1:1) groups (n = 5–9) of Sprague-Dawley rats to cerium chloride and a magnesium-sufficient or magnesium-deficient diet for 13 months. The rats were randomly distributed into four groups, with groups 1 and 2 fed a magnesium-sufficient diet and groups 3 and 4 fed a magnesium-deficient diet. The rats in group 1 and 3 were exposed to 0 ppm cerium chloride, while the rats in groups 2 and 4 were exposed to 35 ppm cerium chloride in the drinking water. This cerium chloride exposure corresponds to a concentration of 19 mg/L, which in turn, assuming a reference water consumption of 0.046 and 0.038 L/day and reference body weight of 0.523 and 0.338 kg (U.S. EPA, 1988) in males and females, respectively, corresponds to an average daily dose in males of 1.7 mg/kg-day and in females of 2.1 mg/kg-day. At 13 months, the animals were sacrificed and cardiac tissue was collected for elemental analysis and histology. No statistically significant changes in serum or cardiac levels of magnesium or calcium were reported. Cerium levels in cardiac tissue were statistically significantly elevated in group 4. Cerium-treated animals had a significantly greater level of collagen in cardiac tissue, relative to group 1, with an enhanced effect in animals fed a magnesium-deficient diet (groups 3 and 4). No other endpoints were evaluated. This study was not used to identify a NOAEL or LOAEL due to the limited number of evaluated endpoints and the investigation of a single dose group.

4.2.2. Inhalation Exposure

No chronic inhalation studies on cerium toxicity are available. However, the National Toxicology Program (NTP) is considering an evaluation of the chronic inhalation toxicity of cerium oxide.

A subchronic inhalation study using cerium oxide (CeO_2; ceric oxide) was conducted in 7-week-old Sprague-Dawley rats (BRL, 1994). Cerium oxide is the form of cerium typically encountered in industrial exposures (Reinhardt and Winkler, 1986). This study is an unpublished study; accordingly, it was externally peer reviewed by EPA in August 2006 (external peer review report available at www.epa.gov/iris).

Groups of 15 male and 15 female Sprague-Dawley CD rats were given nose-only exposure to a dry powder aerosol (mass median aerodynamic diameter [MMAD] = 1.8–2.2 μm, geometric standard deviation [GSD] = 1.8–1.9) of cerium oxide at concentrations of 0, 0.005, 0.0505, or 0.5075 mg/L (0, 5, 50.5, or 507.5 mg/m^3) 6 hours/day, 5 days/week for 13 weeks. The cerium oxide test material was 99% rare earth oxide with a maximum of 75 ppm Fe_2O_3. Of the 99% rare earth oxide, 99.95% was cerium oxide with a maximum of 25 ppm of both Pr_6O_{11} and Nd_2O_3. Praseodymium and neodymium are also rare earth metals.

A functional observational battery was performed on all rats, as well as activity level testing, hematology, clinical biochemistry, urinalysis, ophthalmological examination, and a gross pathological examination of selected tissues weighed and retained for histopathologic examination. No deaths or clinical signs related to cerium oxide were noted. Food consumption and body weight gain were marginally, but statistically not significantly reduced in males at 507.5 mg/m^3 and were considered the result of cerium oxide exposure.

A functional observational battery detected a statistically significant ($p < 0.05$) 17% decrease in forelimb grip strength at week 13 in females exposed to 507.5 mg/m^3. No other changes were found in the functional observational battery. Motor activity, measured by photocells in a figure-8 enclosure, was unaffected by cerium oxide exposure. The ophthalmology examination was normal.

Hematological analysis revealed a statistically significant ($p < 0.05$) increase in absolute neutrophil counts of 105% in 6-week males at 507.5 mg/m^3, 130% in 6-week females at 50.5 mg/m^3, 85% in 13-week males at 50.5 mg/m^3, 75% in 13-week males at 507.5 mg/m^3, 210% in 13-week females at 5 mg/m^3, 177% in 13-week females at 50.5 mg/m^3, and 233% in 13-week females at 507.5 mg/m^3. Differential white blood cell counts were largely restricted to altered neutrophil counts (as shown in Table 4-1), with the exception being increased absolute lymphocyte and eosinophil counts in 13-week males at 50.5 mg/m^3 by 36% and 187%, respectively. Differential white blood cell counts revealed changes in relative percentages of

neutrophils and lymphocytes. The relative percentage of neutrophils and lymphocytes were significantly increased by 102 and 80% in 6-week and 13-week males, respectively, at 507.5 mg/m^3. In 13-week males, there was a corresponding 19% decrease in the relative percentage of lymphocytes. The 118% relative increase in neutrophils was accompanied by a 12% decrease in lymphocytes in 6-week females at 50.5 mg/m^3. In 13-week females, the 5, 50.5, and 507.5 mg/m^3 doses were associated with, respectively, 130, 130, and 164% relative increase in the percentage of neutrophils and 12, 10, and 14% decreases in lymphocytes. Clinical chemistry and urinalysis were normal.

Table 4-1. Hematological changes in male and female Sprague-Dawley rats following inhalation of cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks

Dose (mg/m^3)	Absolute neutrophils		Absolute lymphocytes		Absolute eosinophils		Relative neutrophils		Relative lymphocytes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Males, week 6										
0	1,509	1,697	10,057	2,455	122	299	11.3	7.5	85.4	8.3
5	1,440	720	9,891	2,602	102	136	12.5	5.9	84.6	6.5
50.5	2,560	882	11,478	1,944	83	125	17.7	5.6	79	5.7
507.5	3,088[a]	1,162	10,296	3,670	110	175	22.8[a]	6.8	73.7[a]	6.9
Females, week 6										
0	724	494	8,791	2,355	75	119	7.8	5.7	90.8	6.0
5	1,230	765	7,537	2,068	67	57	13.0	6.3	84.5	6.7
50.5	1,680[b]	651	8,083	1,737	92	88	17[a]	6.4	80.1[b]	7.5
507.5	1,328	562	8,916	3,678	87	104	13.3	4.3	84.9	4.5
Males, week 13										
0	1,541	909	6,243	1,575	62	94	18.4	6.4	77.5	8.7
5	1,638	622	6,254	1,521	85	83	20.1	6.9	76.1	7.9
50.5	2,844[a]	1,038	8,481[b]	3,009	175[b]	173	25.4	10.9	70.7	11.0
507.5	2,698[a]	1,101	5,238	1,910	73	98	33.2[a]	9.5	62.7[a]	11.0
Females, week 13										
0	325	162	3,624	1,365	47	58	8.3	4.2	85.7	5.5
5	1,006[c]	797	3,794	1,200	61	55	19.1[a]	10.0	75.3[a]	10.7
50.5	899[c]	414	3,802	1,228	36	44	19.1[a]	7.8	76.9[b]	8.5
507.5	1,081[c]	399	3,722	1,137	32	42	21.9[a]	6.3	73.9[a]	7.1

[a]Statistically significantly different from control (Dunnett's test; $p < 0.01$).
[b]Statistically significantly different from control (Dunnett's test; $p < 0.05$).
[c]Statistically significantly different from control (Dunn's test; $p < 0.01$).
Source: BRL (1994).

At necropsy, there were treatment-related increases in the weight of the lungs and spleen that correlated with gross and microscopic findings. Absolute and relative lung weights were statistically significantly ($p \leq 0.001$) increased in both males and females at 50.5 and 507.5 mg/m^3 (Tables 4-2 and 4-3). Lung weights, relative to brain weights, were also statistically significantly increased in male and female rats at 50.5 and 507.5 mg/m^3. Relative spleen weight was statistically significantly ($p \leq 0.05$) increased in males at 507.5 mg/m^3 (Table 4-4). A statistically significant increase in absolute (29%) and relative (28%) thymus weight in male mice at 50.5 mg/m^3 was not considered by the study authors to be related to cerium oxide treatment. The increase in absolute and relative thymus weight was observed in only the mid-dose male rats and a dose-response relationship was not observed in male or female rats. Thus, the increase in thymus weight in male rats was not determined to be a biologically significant effect of cerium oxide exposure.

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Table 4-2. Absolute lung weight in rats exposed to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks

Dose (mg/m^3)	Males			Females		
	Mean weight (g)	SD	% Change	Mean weight (g)	SD	% Change
0	1.611	0.1088	–	1.145	0.0871	–
5	1.760	0.1398	9	1.311	0.1490	14
50.5	2.574[a]	0.3499	60	1.651[a]	0.2900	44
507.5	4.662[a]	0.5161	189	3.173[a]	0.3235	177

[a]Statistically significantly different from control (Dunn's test; $p < 0.01$).
Source: BRL (1994).

Table 4-3. Relative lung weight in rats exposed to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks

Dose (mg/m^3)	Males			Females		
	Mean weight (g%)	SD	% Change	Mean weight (g%)	SD	% Change
0	0.334	0.0256	–	0.477	0.0443	–
5	0.371	0.0270	11	0.544	0.0557	14
50.5	0.528[a]	0.0775	58	0.697[a]	0.1124	46
507.5	1.024[a]	0.1621	207	1.358[a]	0.1432	185

[a]Statistically significantly different from control (Dunn's test; $p < 0.01$).
Source: BRL (1994).

Table 4-4. Relative spleen weight in rats exposed to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks

Dose (mg/m^3)	Males			Females		
	Mean weight (g%)	SD	% Change	Mean weight (g%)	SD	% Change
0	0.162	0.0190	-	0.216	0.0281	-
5	0.169	0.0286	4	0.242	0.0259	12
50.5	0.178	0.0266	10	0.226	0.0445	5
507.5	0.188[a]	0.0298	16	0.222	0.0252	3

[a]Statistically significantly different from control (Dunnett's test; $p < 0.05$)
Source: BRL (1994).

Gross examination found discoloration or pale areas, pale foci, and uncollapsed parenchyma in the lungs of male and female rats (Table 4-5). Pale areas and discoloration in the lung were evident at 50.5 and 507.5 mg/m^3 in male and female rats, respectively, with uncollapsed parenchyma evident at 50.5 and 507.5 mg/m^3 in male rats and 507.5 mg/m^3 in female rats. Pale foci in the lungs were only seen in female rats exposed to 5 mg/m^3. The incidence of enlargement or pale discoloration of the mandibular, bronchial, mediastinal, and

pancreatic lymph nodes is shown in Table 4-6. Enlargement or pale discoloration of both lymph nodes that drain the lungs (the bronchial and mediastinal lymph nodes) was evident in both males and females at ≥5 mg/m^3 cerium oxide. The mandibular and pancreatic lymph nodes did not display a dose-response trend of enlargement or discoloration. The study authors (BRL, 1994) judged the mandibular lymph node enlargement, which was observed in control and cerium-exposed rats, not to be an effect of the cerium oxide exposure, while the pancreatic, bronchial, and mediastinal lymph node enlargement and/or discoloration were considered to be related to the cerium oxide treatment.

Table 4-5. Results of gross pathological examination of lungs of rats exposed to cerium oxide 6 hours/day, 5 days/week for 13 weeks

	Dose (mg/m^3)							
	Male				Female			
Lung	0	5	50.5	507.5	0	5	50.5	507.5
Pale foci	0/15	0/15	0/15	0/15	0/15	4/15	0/15	0/15
Pale areas or discoloration	0/15	0/15	15/15	15/15	0/15	0/15	15/15	15/15
Uncollapsed parenchyma	0/15	0/15	2/15	15/15	0/15	0/15	0/15	15/15

Source: BRL (1994).

Table 4-6. Results of gross pathological examination of bronchial, mediastinal, and pancreatic lymph nodes of rats exposed to cerium oxide 6 hours/day, 5 days/week for 13 weeks

	Dose (mg/m^3)							
	Male				Female			
Lymph nodes	0	5	50.5	507.5	0	5	50.5	507.5
Mandibular								
enlargement	4/15	2/15	5/15	4/15	2/15	4/15	3/15	1/15
discoloration	–	–	–	–	–	–	–	–
Bronchial								
enlargement	0/15	4/15	15/15	15/15	0/15	1/15	14/15	15/15
discoloration	0/15	13/15	15/15	15/15	0/15	15/15	15/15	15/15
Mediastinal								
enlargement	0/15	2/15	9/15	9/15	0/15	1/15	8/15	8/15
discoloration	0/15	2/15	9/15	10/15	1/15	10/15	9/15	10/15
Pancreatic								
enlargement	1/15	0/15	0/15	0/15	2/15	0/15	1/15	0/15
discoloration	–	–	–	–	0/15	0/15	0/15	1/15

Source: BRL (1994).

Histologic examination revealed dose-related alveolar epithelial and lymphoid hyperplasia and pigment accumulation in the lungs, lymph nodes, and larynx of male and female rats at ≥5 mg/m^3. The incidence data are presented in Table 4-7. The pigment, which was also

found in other parts of the respiratory tract, including the nasal cavities and the trachea, and the liver and spleen, was considered by the study authors to be the test compound or a product thereof. The lymphoid hyperplasia in the lymph nodes following cerium oxide exposure was characterized by the study pathologist as an increase in the number of lymphocytes, with lymph node paracortices and cortices expansion. The authors reported that the severity of the hyperplasia in a given tissue, lung or lymph node, was correlated with the amount of pigment accumulated in the tissue but did not present any supporting data. BRL (1994) considered these findings to be consistent with antigenic stimulation by cerium oxide; however, they did not discuss the possibility of non-antigenic stimulation. The metaplasia evident in the larynx was interpreted by the study pathologist as adaptive and reversible. Lesions were not observed in the testes or ovaries of the high-dose group.

The NOAEL and LOAEL values for the toxicological effects observed are included in Table 4-8. This study identified a LOAEL of 5 mg/m^3 in rats, based on the increased incidence of lymphoid hyperplasia in the bronchial lymph nodes of male and female rats. A NOAEL was not identified.

Table 4-7. Incidences of histopathologic effects in rats exposed to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks

Exposure (mg/m^3)	Control	5	50.5	507.5
Males				
Larynx				
metaplasia	0/15	3/15	9/15[a]	13/15[a]
pigment accumulation	0/15	6/15[a]	9/15[a]	12/15[a]
Lung				
lymphoid hyperplasia	0/15	0/15	0/15	12/15[a]
alveolar epithelial hyperplasia	0/15	1/15	11/15[a]	14/15[a]
pigment accumulation	0/15	15/15[a]	15/15[a]	15/15[a]
Bronchial lymph node				
lymphoid hyperplasia	0/15	11/13[a]	15/15[a]	15/15[a]
pigment accumulation	0/15	13/13[a]	15/15[a]	15/15[a]
Mediastinal lymph node				
lymphoid hyperplasia	0/0	2/2	9/10	9/9
pigment accumulation	0/0	2/2	8/10	9/9
Mandibular lymph node				
lymphoid hyperplasia	0/15	0/3	0/5	2/15
pigment accumulation	0/15	0/3	0/5	6/15[a]
Pancreatic lymph node				
lymphoid hyperplasia	–	–	–	–
pigment accumulation	–	–	–	–
Spleen				
pigment accumulation	0/15	0/15	0/15	6/15[a]
Females				
Larynx				
metaplasia	0/15	3/15	6/15[a]	9/15[a]
pigment accumulation	0/15	0/15	7/15[a]	9/15[a]
Lung				
lymphoid hyperplasia	0/15	0/15	1/15	7/15[a]
alveolar epithelial hyperplasia	0/15	0/15	5/15[a]	15/15[a]
pigment accumulation	0/15	15/15[a]	15/15[a]	15/15[a]
Bronchial lymph node				
lymphoid hyperplasia	0/15	13/15[a]	15/15[a]	15/15[a]
pigment accumulation	0/15	14/15[a]	15/15[a]	15/15[a]
Mediastinal lymph node				
lymphoid hyperplasia	0/1	10/10	9/9	9/10
pigment accumulation	0/1	10/10	9/9	9/10
Mandibular lymph node				
lymphoid hyperplasia	0/15	0/5	0/3	0/15
pigment accumulation	0/15	0/5	0/3	6/15[a]
Pancreatic lymph node				
lymphoid hyperplasia	0/2	0/0	1/1	0/1
pigment accumulation	0/2	0/0	1/1	1/1
Spleen				
pigment accumulation	0/15	0/0	0/0	3/15

[a]Significantly different from vehicle control group ($p < 0.05$ by Fisher's exact test)
Source: BRL, 1994.

Table 4-8. NOAEL and LOAEL values for effects observed in BRL (1994).

Toxicological effect	Sex	NOAEL (mg/m^3)	LOAEL (mg/m^3)
Organ weight changes			
Absolute lung weight	M, F	5	50.5
Relative lung weight	M, F	5	50.5
Absolute spleen weight	M, F	507.5	–
Relative spleen weight	M	50.5	507.5
	F	507.5	–
Gross pathological lesions in lung			
Pale areas	M, F	5	50.5
Discoloration	M, F	50.5	507.5
Uncollapsed parenchyma	M	5	50.5
	F	50.5	507.5
Gross pathological lesions in the lymph nodes			
Mandibular enlargement	M, F	–	5
Bronchial enlargement	M, F	–	5
Bronchial discoloration	M, F	–	5
Mediastinal enlargement	M, F	–	5
Mediastinal discoloration	M, F	–	5
Histopathologic lesions			
Metaplasia, larynx	M, F	–	5
Pigment accumulation, larynx	M	–	5
	F	5	50.5
Lymphoid hyperplasia, lung	M	50.5	507.5
	F	5	50.5
Alveolar epithelial hyperplasia, lung	M, F	5	50.5
Pigment accumulation, lung	M, F	–	5
Lymphoid hyperplasia, bronchial lymph node	M, F	–	5
Pigment accumulation, bronchial lymph node	M, F	–	5
Pigment accumulation, spleen	M	50.5	507.5
Pigment accumulation, mandibular lymph node	M	50.5	507.5
Lymphoid hyperplasia, mediastinal lymph node	M, F	_	5
Pigment accumulation, mediastinal lymph node	M, F	–	5

Intratracheal instillation of 50 mg of a dust suspension of cerium oxide produced mild changes in the lung and spleen of white rats examined 8 months later (Mogilevskaya and Raikhlin, 1967). Conglomerates of dust particles deposited intracellularly and small particles were observed in the cytoplasm of alveolar macrophages. There was a moderate proliferative response in the lungs and bronchi, with occasional increased lymphocyte numbers and slight development of connective tissue and collagen fibers, but diffuse or nodular fibrotic changes

were lacking. In the spleen, macrophages and large multinucleated cells accumulated. No histologic changes were found in any of the other major organs.

Lundgren et al. (1996) conducted an investigation into the pulmonary carcinogenicity of beta-particle radiation from inhaled $^{144}CeO_2$ in F344/N rats, with stable cerium oxide serving as the control group (n = 1,049). The radiation doses ranged from 3.6 to 37 Gy. The control rats received stable cerium oxide in a single inhalation dose of comparable mass concentration to the dose groups, although the concentration was not specified by the study authors. The control rats were held for life-span observation and were evaluated histologically. The histologic evaluation showed nonneoplastic lesions, such as inflammation (5.1%), fibrosis (5.6%), alveolar-epithelial hyperplasia (4.5%), and alveolar macrophage hyperplasia (7.1%), in the lungs of control rats. Seven primary lung neoplasms were also apparent in the control rats and included four alveolar or papillary adenomas; one alveolar, papillary, or tubular adenocarcinoma; one squamous cell carcinoma; and one fibro- or osteosarcoma. Control rats, unexposed to cerium oxide, in a separate study of inhaled $^{144}CeO_2$ in F344/N rats demonstrated an incidence of lung tumors of 6/110 (Lundgren et al., 1992). The observed tumors included two papillary adenomas, two papillary adenocarcinomas, one adenosquamous carcinoma, and one mesothelioma.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Kawagoe et al. (2008) administered 200 mg/kg cerium chloride by gavage to pregnant ICR mice on day 11.5 of gestation and the embryos were examined on gestation day 14.5. Histopathology was performed on the liver, spleen, and lungs of the embryos. Pulmonary and hepatic vascular congestion were observed in the fetal mice. However, evidence of altered tissue morphology was not observed. In addition, Kawagoe et al. (2008) administered a single dose of 200 mg/kg cerium chloride by gavage to dams immediately after parturition to observe the effects of cerium chloride on neonatal mice through lactation. Neonatal mice were smaller compared to controls through lactation. There were no differences in survival of neonatal mice and controls. As early as 1 day post-administration, pulmonary hemorrhage in the peripheral alveoli, vascular congestion, and neutrophil infiltration were observed in the lungs of adult mice. In neonatal mice exposed lactationally, pulmonary hemorrhage, hepatic vascular congestion, and trachea hemorrhage were also observed.

Yu et al. (2001, English translation of abstract) administered 0, 200, or 800 mg/kg-day cerium to male mice (five to eight per group) in the diet for 30 or 45 days. This exposure increased the rate of unspecified sperm abnormalities but did not statistically significantly affect testes weight or alter serum levels of testosterone. The increased rate of the apparent sperm abnormalities was dose and time dependent, with a 17 and 22% increase in abnormalities 30

days postexposure for 200 and 800 mg/kg-day, respectively, and a 28 and 32% increase in abnormalities 45 days postexposure at the same dose levels, respectively.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

As indicated in Section 3.1.1, cerium compounds are poorly absorbed from the GI tract of animals; thus, parenteral administration of cerium compounds has been the preferred route of exposure to study the systemic effects of these compounds.

4.4.1. Acute Toxicity Studies (Oral and Inhalation)

Single dose and/or acute oral data are limited but include a report of splenic lesions, including hypertrophy, reticuloendothelial hyperplasia, and hyperactive lymphoid follicles, and GI irritation, characterized by gastritis and enteritis with focal hemorrhage and necrosis of the mucosa in the stomach and duodenum, in mice given single gavage doses of 1,000 or 1,163 mg/kg (1:3 cerium chloride-sodium citrate complex) (Stineman et al., 1978). Open field behavior was not affected and the 7-day LD_{50} was 1,291 mg/kg cerium (95% CI: 1,198–1,440). Bruce et al. (1963) identified an oral LD_{50} of 4,200 mg/kg for cerium nitrate in female Sprague-Dawley rats (n=30).

There is a report of piloerection, hunched posture, unsteady gait, and dark coloring of eyes in male and female rats, along with lethargy, abnormal respiration, and prostration in females, given single gavage doses of 5,000 mg/kg cerium as cerium sulfide (Rhodia Inc., 1998). Ji and Cui (1988) list an oral LD_{50} in female mice of 1,178 mg/kg (95% CI: 1,043–1,331) for cerium nitrate ($Ce[NO_3]_3$) and 622 mg/kg (95% CI: 550–702) for cerium oxides.

Cerium chloride was administered by gavage to male and female adult ICR mice as a single dose of 200 or 500 mg/kg (Kawagoe et al., 2008). The mice were sacrificed and necropsied 1, 3, or 7 days post-administration. Mortality in the mice that received 500 mg/kg cerium chloride was 60% within 48 hours of gavage, while the mice in the 200 mg/kg group survived at least 8 weeks after dosing. The mice receiving cerium chloride demonstrated pulmonary hemorrhage, vascular congestion, and thickened alveolar septae in the lungs; focal hepatocellular necrosis and neutrophil infiltrations in the midzone of periportal areas in the liver; congestion of hepatic central and portal veins and sinusoids; kidney vascular congestion, particularly of the renal tubules; and pancreatic vascular congestion (Kawagoe et al., 2008).

Data on the toxicity of single exposures to airborne cerium in animals are limited. A review by the HEI (2001) reported that the LC_{50} for cerium oxide in rats was >50 mg/m^3 in a study by Rhone-Poulenc (1983), but the study was unavailable for examination and no further details were reported.

4.4.2. Acute Studies (Injection)

4.4.2.1. *Neurobehavioral and Neurodevelopmental Effects*

D'Agostino et al. (1982, 1978a, b) studied the neurodevelopmental effects of cerium following subcutaneous injection in mice. The three separate publications present the same experimental design. Pregnant mice were administered a single dose of 80 mg/kg cerium (sodium/citrate complex) or citrate (control) on gestation day (GD) 7 or 12 or postnatal day (PND) 2. In order to differentiate the gestational effects from lactational effects or changes in maternal behavior, a full cross-foster study design was employed. A cross-fostering design distributed the offspring (3 males and 3 females per dam) of mothers receiving cerium or citrate during gestation to lactating dams exposed to either cerium or citrate. Body weight and gross activity of the neonates were assessed on PNDs 7 or 12 (D'Agostino et al., 1978a, b) or PNDs 8 or 13 (D'Agostino et al., 1982), whereas open-field behavior, accelerating rotarod performance, and passive avoidance learning were assessed on PNDs 60–65. Maternal offspring retrieval latency was measured on PND 3. The growth and behavioral data were analyzed via a multivariate analysis.

Pups exposed to cerium in utero on GDs 7 and 12 demonstrated a statistically significant decrease in body weight on PNDs 7 and 12 (D'Agostino et al., 1978a, b), whereas the body weight of offspring of cerium-exposed dams on GD 7 did not differ significantly from that of control offspring (D'Agostino et al., 1982). Offspring of dams exposed to cerium on GD 12 showed significantly decreased body weights on PNDs 8 and 13 (D'Agostino et al., 1982). Pups exposed to cerium in utero were retrieved by dams and replaced in the nest significantly faster than controls. When the dams were injected on PND 2, neonatal weights on PNDs 7 and 12 and 8 and 13 were statistically significantly reduced (D'Agostino et al., 1982, 1978a, b). The study authors suggested that these effects may be due to altered maternal behavior (e.g., ineffective suckling or lack of grooming) rather than cerium being transmitted to offspring in the milk. In addition, offspring of rats exposed to cerium on GD 7 showed a higher frequency of rearings than controls when evaluated on PNDs 60–70 (D'Agostino et al., 1982, 1978a, b). Rats exposed to cerium on GD 7 demonstrated a decrease in activity, measured by the circular runway, on PND 12 (D'Agostino et al., 1978a, b). Other behavioral differences were not reported. No possible mechanism of action was discussed to explain the neurodevelopmental effects of cerium administration. The lack of information on maternal health status following administration of cerium limits the usefulness of these studies.

4.4.2.2. *Neurological Effects*

Morganti et al. (1978) examined the open-field and exploratory behavior of 6- to 8-week-old male Swiss ICR mice following subcutaneous administration of cerium. Three days after receiving a dose of 20 mg/kg cerium (from a 1:3 cerium chloride-cerium citrate complex), 10 mice were observed in the open-field and exploratory apparatuses and then sacrificed. This procedure, including injection, was repeated every 3 days until the last group of mice had received a total of 200 mg/kg cerium. Control mice were injected with sodium citrate. The results showed that cerium exposure statistically significantly ($p \leq 0.05$) depressed ambulations, marginally depressed explorations, but did not affect rearings. Behavioral measures were not correlated with cerium levels in tissues. According to Morganti et al. (1978), the lack of correlation between the behavioral measures and the cerium levels in tissue indicated that the effect of cerium is not a one-step process depending directly on the level of cerium in a single tissue but instead involves a biological chain of events.

In a different study by the same group of investigators (Stineman et al., 1978) in which male Swiss mice received single, subcutaneous injections of cerium (136 or 173 mg/kg), there was an inverse relationship between open field behavior (ambulations and rearings) and levels of cerium in blood, brain, lung, stomach, intestine, and kidney. The levels of cerium in the brain had the strongest association with decreased open field behavior, followed by the lung. However, splenic levels of cerium were positively correlated with behavior, as mice with higher levels of cerium in the spleen exhibited less altered behavior. Stineman et al. (1978) suggested that the spleen may protect against the cerium-induced effects by sequestering cerium from the circulation after the removal of damaged erythrocytes and leukocytes.

In a subsequent study (Morganti et al., 1980), 6- to 8-week-old adult male Swiss ICR mice administered single, subcutaneous injections of 136 or 173 mg/kg cerium showed depression of general activity, as measured in an activity wheel study and a passive avoidance study. However, the subcutaneous injection of cerium did not significantly affect two-way active avoidance learning or social behavior, although gross activity was depressed in the social behavior study (Morganti et al., 1980). The study authors' interpretation of the study data was that cerium exposure did not affect simple and complex learning, as measured in the above tests.

4.4.2.3. *Hepatic Effects*

Numerous studies have examined the effects of cerium on liver parameters following parenteral dosing. The i.v. administration of cerium oxide (0.5, 1.0, 2.0, 3.5, or 4.5 mg/kg to females and 0, 3.5, 7.0, or 14.0 mg/kg to males) to Carworth Farms Nelson rats resulted in an increased level of liver lipids compared to controls, but a dose response trend among the dose groups was not apparent (Snyder et al., 1959). In a second part of the study, i.v. administration

of 3.5 mg/kg cerium oxide to male and female Sprague-Dawley, Charles River, Carworth Farms Nelson and Wistar rats resulted in an increase in liver lipids, with a more pronounced increase in liver lipids in female rats (Snyder et al., 1959).

The i.v administration of a dose of 3 mg/kg cerium (specific form not specified) to rats resulted in increased serum levels of ornithine-carbamyl transferase (OCT), which reached a peak 1–2 days after dosing (Magnusson, 1962). Blood glucose levels were reduced markedly in females 2–3 days after dosing but recovered by day 4. No macroscopic changes were seen in the liver of males, but fatty liver and fatty degeneration were evident in females 1 day after treatment. Males showed hydropic changes and isolated necrotic cells 1–4 days after dosing. In females, changes to the ultrastructure of the liver cells were seen 12 hours after administration of cerium and appeared to reach a maximum 2–3 days later, with only slight ultrastructural changes observed in males.

Several additional reports have confirmed and expanded on the Magnusson (1962) findings. Marciniak and Baltrukiewitcz (1981, 1977) showed that the increase in serum OCT activity after cerium injection was linear in the range 1.5–4.5 mg/kg cerium. Lombardi and Recknagel (1962) observed that i.v. administration of 5 mg/kg cerium (form not specified) to female Sprague-Dawley rats produced a fourfold increase in liver triglyceride levels 24 hours after treatment. Salas et al. (1976) showed that a single i.v. dose of 10 mg/kg cerium chloride administered to female Sprague-Dawley rats decreased the total hepatic adenosine triphosphate level by 12 hours postexposure and depleted liver glycogen levels and increased liver triglyceride levels within 48 hours. Ultrastructurally, the rough endoplasmic reticulum was the main target of cerium toxicity, with marked dilation and degranulation, as well as the appearance of free ribosomes in the cytoplasm, 24 hours postexposure. The hepatic changes returned to normal between the 5^{th} and 8^{th} day postexposure.

Arvela and Karki (1971) observed fatty degeneration on the first day following i.v. administration of 2 mg/kg cerium chloride in Sprague-Dawley rats. On the third day after i.v. administration, there was a 53% reduction in the level of CYP450 in the liver of male Sprague-Dawley rats. Arvela et al. (1991) showed that C57BL/6N male mice were more resistant to the hepatotoxic effects, including necrosis, cell membrane disintegration, and microsteatosis, of cerium than were DBA/2 mice. However, the concentration of cerium in the livers of C57BL/6N mice was about 50% higher at 72 hours than in the livers of DBA/2 mice. The authors also showed that in C57BL/6N mice the slight to moderate liver injury 72 hours post administration was associated with an increase in coumarin 7-hydroxylase (COH) activity; however, at doses that caused severe damage in DBA/2 mice, COH activity 72 hours post administration was drastically reduced. It was also reported that the total amount of CYP450 in the liver was

significantly increased in both strains after a dose of 1 mg/kg cerium, but higher doses tended to decrease CYP450 content, producing a biphasic relationship. A follow-up study found that cerium increased the amount of hepatic CYP2A5 mRNA only in DBA/2 mice (Salonpää et al., 1992), which have been shown to be more sensitive to cerium exposure than the C57BL/6N strain. Since the CYP2A5 gene encodes P450 isozymes catalyzing COH activity in mice, the study authors suggested that some association exists between the development of liver damage and COH induction.

Strubelt et al. (1980) reported dose-dependent increases in AST (reported using the older name of glutamic-oxaloacetic transaminase) and ALT (reported using the older name of glutamic-pyruvic transaminase) and sorbitol dehydrogenase (SDH) in male Wistar rats after i.v. administration of 3, 5, 7, and 10 mg/kg cerium nitrate.

Sex differences in sensitivity were also reported by Wiener-Schmuck et al. (1990). Intravenous injection of 1.3 mg/kg of cerium chloride to rats caused lipid deposition, damage to mitochondria, and invaginations of the nuclear membrane in hepatocytes 48 hours after dosing. These changes, which were accompanied by increased activities of serum transaminases, were reversible and occurred only in females. However, when isolated hepatocytes of female rats were incubated in medium with cerium chloride for 20 hours, there was no sign of cell damage. The lack of toxicity of cerium to isolated hepatocytes in vitro suggests that hepatocellular lesions and related changes observed following injection of cerium result from an indirect cause. Wiener-Schmuck et al. (1990) suggested that deposition of injected rare earths in Kupffer cells leads to blockage of the reticuloendothelial system in the liver, inducing damage to macrophages, which release mediators that in turn damage the hepatocytes.

A more recent paper showed that administration of the metal chelator ethylene glycol bis(2-aminoethylether)tetraacetic acid (EGTA) to mice after dosing with cerous sulfate for 7 days decreased the severity of the histologic effects of cerium on the liver (Shrivastava and Mathur, 2004). The histologic effects that resulted from this 7-day subcutaneous exposure of 0.5 mL cerous sulfate included lymphocytic infiltration, hepatocyte hypertrophy, cytoplasmic vacuolation, and stumpy Kupffer cells. In addition to the histologic effects, the activity of ALT and AST were statistically significantly increased, with maximum increases of 350 and 98%, respectively, and hepatic acid, alkaline phosphatases, and succinic dehydrogenase were statistically significantly decreased, 42, 69, and 46%, respectively. The decrease in severity of the histologic effects would indicate that the presence of circulating cerium is necessary for hepatotoxicity but does not provide information on a possible mechanism of hepatotoxicity.

In summary, administration of cerium to rodents caused lipid deposition, mitochondrial damage, and invaginations of the nuclear membrane in hepatocytes, as well as morphological

changes in the liver, characterized by fatty liver, fatty degeneration, and necrosis. In addition, histologic effects included lymphocytic infiltration, hepatocyte hypertrophy, cytoplasmic vacuolation, and stumpy Kupffer cells. Arvela and Karki (1971) suggested that liver toxicity may result indirectly from induction of CYP2A5 and COH.

4.4.2.4. *Cardiovascular Effects*

Information regarding effects of cerium on the human heart derives mainly from a series of studies (see Section 4.1.1) in which naturally high levels of cerium in the soil in certain geographical regions appear to be correlated with higher levels of cerium in serum and cardiac tissue of individuals with endomyocardial fibrosis (Eapen, 1998; Kutty et al., 1996; Valiathan et al., 1989).

The i.v. administration of a single dose of 1.3 mg/kg cerium as cerium chloride to female Sprague-Dawley rats resulted in a statistically significant twofold increase in protein synthesis ($p \leq 0.001$) and transcription ($p \leq 0.01$) in cardiac muscle relative to controls 24 hours after injection (Kumar et al., 1995). This was consistent with findings by the same group of investigators who reported that incubation of cardiac fibroblasts in vitro with 100 nM cerium increased RNA synthesis approximately 64%, but the rate of DNA synthesis was unaffected (Shivakumar et al., 1992). However, it should be noted that higher concentrations of cerium in the medium were inhibitory. This was taken as evidence suggesting that cerium at low levels may act at the level of transcription to stimulate collagen and non-collagen protein synthesis. This, in turn, may contribute to the accumulation of collagen in endocardial fibrosis. In a follow-up study, Kumar and Shivakumar (1998) reported that a single i.v. dose of 1.3 mg/kg cerium increased lipid peroxidation by 30% in cardiac tissue of Sprague-Dawley rats and increased proliferation of cardiac fibroblasts by 23%. Treatment with cerium also statistically significantly decreased collagen degradation by 7% and increased the rate of deposition of newly synthesized collagen by 27% in cardiac tissue 48 hours after cerium administration (Kumar and Shivakumar, 1998).

In summary, the limited data from oral exposure experiments in rats and rabbits (see Section 4.2.1) and i.v. administration studies in rats suggest that cerium increases collagen accumulation in cardiac muscle by increasing synthesis and decreasing degradation by unknown mechanisms.

4.4.2.5. *Hematological Effects*

The i.v. administration of a single dose of 10 mg/kg cerium as the chloride, citrate complex, or ethylenediamine tetraacetic acid (EDTA) complex to anesthetized dogs every

10 minutes for 10 total doses, significantly increased prothrombin levels and coagulation time within minutes of the injection (Graca et al., 1964). The magnitude of the increased prothrombin levels and coagulation time was greatest for cerium chloride, followed by the citrate complex, and finally the EDTA complex. No other hematological endpoint was significantly affected by cerium exposure. Talbot et al. (1965) implanted a pellet of cerium metal under the skin of C57BL mice and collected blood samples from five male and five female mice every 6 months for hematological determinations. They stated that there were no significant differences between coagulation times of cerium-implanted mice and controls; however, a table shows that after 6 months, coagulation time in implanted male mice was approximately twice that in controls. In this study, the only statistically significant difference ($p < 0.05$) between cerium implanted and nonimplanted mice was a decrease in total leukocyte counts in males and females at 6, 12, and 18 months relative to controls. However, analysis of differential counts showed no significant differences between treated and control mice. In another study, Shrivastava and Mathur (2004) injected subcutaneous doses of cerous sulfate ($Ce_2[SO_4]_3$) daily for 7 days into male mice and reported 18 and 37% decreases in hemoglobin and red blood cell counts, respectively, and 93 and 39% increases in sedimentation rate and hematocrit, respectively, all of which appeared to reach a maximum on day 14 (7 days after the last injection) and appeared to return to control levels approximately 60 days posttreatment.

The information available from these few studies is insufficient to determine a possible mechanism of action by which cerium might be causing hematological alterations. Alterations in prothrombin and coagulation times could be secondary to liver dysfunction, but more information is necessary to confirm this hypothesis.

4.4.2.6. *Renal Effects*

The i.v. administration of a single dose of 2 mg/kg of cerium chloride to adult male DBA/2 and C57BL/6 mice resulted in a more than fourfold increase in COH activity in the kidneys of DBA/2 mice, but less than a twofold increase in C57BL/6 mice (Salonpää et al., 1992). The effect was maximized in DBA/2 mice 4 hours after dosing, and the enzyme activity returned to predosing levels 6 hours after treatment. Cerium also increased CYP2A5 mRNA in the kidneys of DBA/2 mice sevenfold 6 hours after dosing and sixfold 1 day after the injection, but no such increase occurred in C57BL/6 mice.

Injection of subcutaneous doses of 0.5 ml (1 mM) cerous sulfate to male mice for 7 days produced statistically significant ($p < 0.05$) decreases up to 62 and 42% in the activities of renal acid and alkaline phosphatases, respectively, and up to 31% in succinic dehydrogenase activity (Shrivastava and Mathur, 2004). Cerium also induced histologic damage to the kidneys,

consisting of hypertrophy in the epithelial cells, deformed Bowman's capsules, exfoliated nuclei in tubular lumen, and leukocyte infiltration. Maximum injury was observed on day 21 (14 days after the last injection). As with the findings regarding liver toxicity, mice administered EGTA after cerium exhibited less severe necrotic effects in the kidney than mice not receiving EGTA. The mechanism of kidney toxicity of cerium is unknown.

In summary, cerium exposure initiated an increase in COH activity and CYP2A5 expression and decreased acid and alkaline phosphatases and succinic dehydrogenase activities, as well as kidney epithelial hypertrophy, exfoliated nuclei in the tubular lumen, and leukocyte infiltration.

4.4.3. Genotoxicity

No information was located regarding genotoxic effects of cerium or cerium compounds in humans and only three studies were identified with pertinent information in mammals and in lower organisms (Sharma and Taluker, 1987; Shimizu et al., 1985; Nishioka, 1975). The available information is insufficient to ascertain the genotoxicity of cerium.

Shimizu et al. (1985) examined the potential mutagenicity of cerium oxide in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) and in *Escherichia coli* WP2uvrA. Tests were conducted with and without metabolic activation (S9 fraction from male Sprague-Dawley rats). Eight different concentrations were tested, ranging from 1 to 5,000 μg/plate. No increase in the number of revertant colonies per plate was observed at any dose level; in some cases the highest concentration produced growth inhibition. In a short communication, Nishioka (1975) reported that cerium chloride did not induce DNA damage in two strains of *Bacillus subtilis* using the rec-assay. Cerium nitrate was reported to induce chromosomal breaks and reduce the mitotic index in rat bone marrow in vivo (Sharma and Talukder, 1987).

4.5. MECHANISTIC DATA AND OTHER STUDIES

4.5.1. In Vitro Studies

The cytotoxicity of soluble cerium chloride and insoluble cerium oxide was assayed in Sprague-Dawley rat pulmonary alveolar macrophages (PAMs) and compared to the cytotoxic and fibrogenic cadmium chloride and oxide (Palmer et al., 1987). Cell viability, effect on lysosomal enzyme release, and cell morphology were investigated. Cerium chloride was cytotoxic to rat PAMs with an LC_{50} (concentration inducing 50% cell death) of 29 μM, while cerium oxide was less toxic, with an LC_{50} of approximately 4,700 μM; the LC_{50} values in this assay for cadmium chloride and oxide were 28 and 15 μM, respectively. Cerium chloride and

oxide did not affect lysosomal enzyme release, although the sensitivity of this assay was questioned by the study authors. Cerium oxide induced an increase in cells with a featureless surface and a decrease in cells consistent with the control population. Cells typified by blebs on the cell surface and/or surface structure were absent. The induction of cells with a featureless surface was considered by the study authors to be minimal. Cerium chloride was not evaluated for effect on cell morphology due to an experimental error during cell culture preparation. Cerium chloride was more cytotoxic than cerium oxide and of similar cytotoxicity to cadmium chloride, which had an LC_{50} of 28 μM.

Shivakumar and Nair (1991) conducted an in vitro study to examine the effect of cerium on protein synthesis in cultured rat heart cells and human lung fibroblasts exposed to normal and reduced levels of magnesium in the growth medium. Cerium exposure resulted in the inhibition of protein synthesis in rat heart cells and human lung fibroblasts, evident by the decreased amount of [^{3}H]-tyrosine incorporated into heart cell and fibroblast proteins, 73 and 76%, respectively; the cell cultures grown on low-magnesium medium displayed a more pronounced decrease in protein synthesis, with [^{3}H]-tyrosine incorporation decreased 51 and 40% in heart cell and fibroblast proteins, respectively. However, the mechanism of protein synthesis inhibition is unknown.

Another in vitro study was conducted to investigate the toxicity of lanthanides, which includes cerium, towards cultured rat alveolar macrophages (Lizon and Fritsch, 1999). Rat alveolar macrophages were acquired by pulmonary lavage of 2-month-old male Sprague-Dawley rats and were cultured for 1 day on culture medium and then for 3 days on medium containing soluble cerium concentrations of 1×10^{-4} and 5×10^{-6} M. The fraction of apoptotic cells increased with concentration and as a function of time, with observations at 24, 48, and 78 hours. As cerium concentration increased from 10^{-6} to 5×10^{-5} M, the fraction of normal cells decreased and the fraction of post-apototic and unstained cells increased. The LC_{50} for cerium was approximately 5×10^{-5} M. Cerium exposure to cultured rat alveolar macrophages resulted in significant alveolar macrophage death.

Studies with cardiac fibroblasts from neonatal rats in vitro showed that the stimulation of fibroblast proliferation was accompanied by an increase in the generation of free radicals. Preeta and Nair (1999) isolated cells from the hearts of 3–4-day-old neonatal Wistar rats. Fibroblasts were selected from the cultured isolated heart cells via selective adhesion and reincubated in fresh medium. To assess fibroblast growth, the selected fibroblasts were exposed to cerium concentrations of 0.1, 0.5, 1, 10, and 100 μM and were harvested 96 hours postexposure. Growth dynamics were evaluated at 24, 48, and 96 hours postexposure. Immunohistochemical labeling for proliferating cell nuclear antigen (PCNA) was also conducted to determine if an

increase in cell number was due to cell proliferation. Intracellular free-radical generation was determined by spectrophotometric assay with reduced nitroblue tetrazolium, with the cardiac fibroblasts exposed to the same cerium concentrations as in the growth assessment. In addition, the role of free radicals in cell proliferation was investigated using cardiac fibroblasts exposed to 0.5 μM cerium, SOD (100 U/mL), and catalase (120 U/mL) with cell counts measured after 96 hours.

The results of this investigation showed that cardiac fibroblast proliferation followed a concentration-dependent response to cerium exposure, with increased proliferation at low concentrations and decreased proliferation at high concentrations (Preeta and Nair, 1999). Increased proliferation was evident from 0.1 to 1 μM, with a statistically significant ($p < 0.01$) peak at 0.5 μM, while decreased proliferation was evident from 10–100 μM. A statistically significant increase in the proliferation of PCNA immunoreactive cells was evident at low cerium concentrations (0.1–1.0 μM), while a decrease was evident at higher concentrations. The addition of SOD inhibited the increased PCNA expression. The reduction of nitroblue tetrazolium to formazan, which peaked at 0.5 μM, showed the increase in free radicals from the fibroblasts exposed to cerium. An increase in free radical production resulted from the fibroblast exposure to cerium at 0.5 μM, and the cerium-induced stimulatory response was inhibited by the addition of SOD. This in vitro study demonstrates that low concentrations of cerium stimulate cardiac fibroblast proliferation in association with an increase in intracellular generation of free radicals.

A comparative study of cardiac and pulmonary fibroblasts in vitro by Nair et al. (2003) was conducted to investigate the mitogenic effect of cerium. Fibroblasts were isolated from heart and lung tissue of 3- to 4-day-old neonatal Wistar rats through selective adhesion. The cardiac and pulmonary fibroblasts were exposed to fetal bovine serum, a known nonspecific mitogen, which resulted in cell proliferation in association with intracellular free radical generation in both types of fibroblasts. Exposure to SOD resulted in significant reductions in intracellular superoxide anion content and cell density for both types of fibroblasts. The cardiac fibroblast proliferation was stimulated by exposure to cerium at 0.5 μM for 96 hours, whereas lung fibroblast proliferation was not stimulated by 0.5 μM cerium exposure for 96 hours. These data indicate that cardiac fibroblasts may be more sensitive to cerium exposure than lung fibroblasts.

Du et al. (2001) exposed packed human erythrocytes to cerium chloride at concentrations ranging from 4.9×10^{-5} to 3.9×10^{-3} M Ce^{3+} to investigate the aggregation of membrane proteins after exposure to cerium. At the highest concentration tested, 3.9×10^{-3} M, aggregation of membrane proteins was clearly evident, with aggregation increasing gradually with increasing

concentration from 4.9×10^{-5} to 3.9×10^{-3} M. Further analysis, using SDS-PAGE of the membrane proteins and light scattering measurements, showed that the membrane protein aggregation was mainly due to non-covalent cross-linking and to a lesser extent oxidative cross-linking through disulfide bond formation.

4.5.2. Ex Vivo Studies

Manju et al. (2003) utilized isolated papillary muscle in an effort to assess the mechanical response of the myocardium to varying levels of cerium. Cerium chloride concentrations of 0, 0.1, 0.5, 1, 5, 10, 20, 50, and 100 μM were applied to papillary muscle isolated from Sprague-Dawley rats and the force of contraction was recorded using a force transducer. The role of SOD was also investigated by superfusing the muscle with SOD prior to cerium chloride exposure and recording of contractile force. A statistically significant ($p \leq 0.01$) reduction in contractile force was evident at cerium chloride exposures as low as 0.1 μM, with the lowest dose reducing the force of contraction approximately 15% (Manju et al., 2003). Complete recovery of contractile force was apparent at or below 0.5 μM when cerium exposure was removed. The addition of SOD completely inhibited the effect of cerium exposure, up to 5 μM, on the contractile force of the isolated papillary muscle. These results provide further evidence for the involvement of reactive oxygen species in the effects of cerium on the heart.

4.5.3. Nanoparticle Studies

In addition to the in vitro studies, data on nano-sized cerium particles provide information on absorption and cytotoxicity of cerium. Limbach et al. (2005) measured the uptake of cerium oxide nanoparticles at four different sizes, 20–50, 40–80, 80–150, and 250–500 nm, into cultured human lung fibroblasts. The cultured fibroblasts absorbed the nanoparticle cerium linearly with exposure time, with absorption occurring at concentrations as low as 100 ng/g. Particles were not found outside of the vesicles or flowing freely in the cytoplasm and were present exclusively in the form of agglomerates. The size of the cerium nanoparticle greatly affected the amount of cerium incorporated into the cell, with better absorption of larger nanoparticles. Particle size was a more important factor in absorption than particle number and total surface area (Limbach et al., 2005).

Brunner et al. (2006) evaluated the cytotoxicity of nanoparticle CeO_2 to human mesothelioma and rodent fibroblast cell lines by measuring metabolic activity and cell proliferation. Cerium oxide was tested at exposures of 0, 3.75, 7.5, and 15 ppm for 6 days and 0, 7.5, 15, and 30 ppm for 3 days. The cerium oxide particles had a specific surface area derived particle size of 6 nm and a hydrodynamic particle size of 19 nm, a specific surface area of

124 ± 3% m^2/g, and a GSD of 1.49. Metabolic activity was spectroscopically measured as the total mitochondrial activity through the conversion of the leuko form of a formazan-type dye to the active dye, and cell proliferation was measured by the total DNA content of the cells measured spectroscopically after converting DNA with an intercalating dye into a highly fluorescent complex (Brunner et al., 2006). The human mesothelioma cells were more sensitive to CeO_2 than the rodent fibroblast cells, with cell activity and DNA content decreased approximately 50% after 3 days. The mesothelioma and fibroblasts were, however, not completely killed at 30 ppm. After 6 days, the cell activity was not significantly altered and DNA content was increased slightly in the mesothelioma cells; in the rat fibroblasts, the DNA content increased slightly and the cell activity was not significantly affected.

The impact of a model water dispersion of 7 nm CeO_2 nanoparticles (specific surface area of 400 m^2/g) on *E. coli* and cytotoxicity, assessed by counting colony-forming units (CFUs), was investigated at concentrations ranging from 0 to 730 mg/L by Thill et al. (2006). Cerium was almost completely adsorbed to the bacteria cell surface at a concentration of approximately 30 mg/L, and above this concentration an increasing amount of cerium was found in the supernatant with a maximum adsorbed concentration of approximately 48 mg/L. The adsorption appeared to be due to electrostatic attraction between the cerium oxide nanoparticles and the cell membrane. The percentage of CFUs was strongly affected as the CeO_2 nanoparticle concentration increased, with a 50% survival rate at around 5 mg/L and no survival above 230 mg/L. Thill et al. (2006) also showed that the speciation of the adsorbed cerium was modified and the nanoparticles reduced. The study authors concluded that direct contact between the *E. coli* and the CeO_2 nanoparticles needs to be established for CeO_2 cytotoxicity to occur and that the reduction of the nanoparticles occurs at or close to the surface of the bacteria and may be associated with cytotoxicity.

The toxicity of cerium nanoparticles was also investigated using the human bronchioalveolar carcinoma-derived cell line A549 (Lin et al., 2006). The A549 cell line was exposed to 20 ± 3 nm cerium oxide nanoparticles at 0, 3.5, 10.5, or 23.3 μg/mL for 24, 48, or 72 hours. The cells were evaluated for cytotoxicity, as well as for intracellular reactive oxygen species generation, lactate dehydrogenase (LDH) activity, dichlorodihydrofluorescein diacetate (DCF) fluorescence, GSH levels, α-tocopherol levels, and malondialdehyde (MDA) levels. Cell viability was decreased at all three dose levels and exposure durations and followed a dose- and time-dependent decrease, with viability at 3.5, 10.5, and 23.3 μg/mL at 72 hours decreased 12, 22, and 46%, respectively. LDH levels, indicative of cell membrane damage, increased 15, 32, and 71% at 24, 48, and 72 hours, respectively, and were greatest at 23.3 μg/mL. DCF fluorescence, indicative of oxidative stress, increased 70, 139, and 181% after exposure to 3.5,

10.5, and 23.3 μg/mL cerium oxide, respectively. Antioxidant levels were decreased, with cellular GSH levels reaching a maximal decrease of approximately 70% at 48 hours, with a possible recovery of GSH levels at 72 hours, and α-tocopherol levels decreased 38, 76, and 88% at 3.5, 10.5, and 23.3 μg/mL, respectively. MDA levels, indicative of lipid peroxidation, were significantly increased in a dose- and time-dependent manner in the 3.5, 10.5, and 23.3 μg/mL dose groups at 48 and 72 hours. Lin et al. (2006) demonstrated the induction of significant oxidative stress at levels of 3.5, 10.5, and 23.3 μg/mL of 20 nm cerium oxide particles. The elevated reactive oxygen species levels, increased lipid peroxidation, increased membrane damage, and reduced antioxidant levels are evidence of the increased oxidative stress from cerium oxide nanoparticle exposure.

An investigation by Park et al. (2007) described an in vitro screening strategy to examine the potential for human health effects following exposure to cerium oxide nanoparticles (9 nm mean particle size). The in vitro assays applied in this strategy were used to investigate local toxicological effects (skin irritation), cytological effects (structural, functional, or pathological effects on cells), and genotoxic effects (Ames test). An in vivo immobilization study was also conducted in Daphnia magna. In the Epiderm™ Skin Irritation Test, nano-cerium oxide and non-nano-cerium oxide (320 nm mean particle size) particles were not cytotoxic in the MTT assay (Park et al., 2007). The MTT cytotoxicity assay measures the amount of MTT converted by the mitochondria of the cell and a reduction of activity is indicative of cell death (Fletcher et al., 2001). Cytotoxicity was not demonstrated in the BS EN ISO 10993-5 cytotoxicity assay for both nano-cerium oxide and non-nano-cerium oxide. A cytotoxicity grade of 0 for both nano- and non-nano-cerium oxide was the same as the cytotoxicity grade for the negative control (polypropylene pipette tips) and less than the cytotoxicity grade of 3 for the positive control (tin-impregnated PVC strips) (Park et al., 2007). An increase in the frequency of revertant colonies in the OECD 471 Ames assay were not observed for any of the tested *Salmonella* strains (TA100, TA1535, TA102, TA98, TA1537) at any dose level of nano- or non-nano-cerium oxide with or without metabolic activation (Park et al., 2007). There were no toxic effects observed at any time period in the immobilization study in Daphnia magna. The study authors concluded that the results support a lack of any additional toxicological effects for nano-cerium oxide when compared to non-nano-cerium oxide (Park et al., 2007).

Fall et al. (2007) exposed lung slices from female Wistar rats to a continuous flow of nano-sized cerium oxide particles for 3 hours at 14, 76, or 157 mg/m^3 to evaluate the potential biological impact of cerium oxide on lung slices. The cerium oxide particles were approximately 10 nm in diameter, with a mean mobility diameter of approximately 140 nm. The following biological endpoints were investigated: cell viability (ATP levels and total glutathione), tumor

necrosis factor (TNF)-α levels, and analysis of a range of antioxidant enzyme levels including selenium-dependent glutathione peroxidase, catalase, and superoxide dismutase. The viability of the lung tissue slices, glutathione-dependent metabolism, superoxide dismutase activity, TNF-α, and proinflammatory factors were not affected by the 3-hour exposure to nanoparticle cerium oxide. Catalase levels were statistically significantly increased over basal levels at all three exposure levels, but did not lead to a reduction in cell viability.

A study by Park et al. (2008a) was conducted to examine a range of in vitro cell and cell-free endponts to assess the toxicity of cerium oxide nanoparticles. The assays included in this assessment included; general toxicity assays, studies using organotypic cultures of lung slices, studies on oxidative potential and cytotoxicity, and interleukin (IL)-8 release by A549 cells. The general toxicity assays conducted by Park et al. (2007) were reported again by Park et al. (2008a). The studies using organotypic cultures of lung slices used a continuous flow of cerium oxide aerosol over a 3-hour period were conducted and reported by Fall et al. (2007). The studies on oxidative potential and cytotoxicity included three experiments: 1) ascorbate depletion by metal oxide nanoparticles in simple, single antioxidant model, 2) respiratory tract lining fluid (RTFL) model studies with metal oxide nanoparticles, and 3) cytotoxicity – in vitro cell viability using A549 cells.

Nano-cerium oxide did not significantly deplete ascorbate in the single antioxidant model, but did decrease ascorbate in a dose-dependent manner in the RTLF model (Park et al., 2008a). Nano-cerium oxide at concentrations up to 1000 μM had no effect on A549 cells viability, while copper oxide, zinc oxide, and titanium dioxide decreased the viability of A549 cells with LD_{50} of 300, 375, and 1600 μM, respectively (Park et al., 2008a). Nano-cerium oxide, at concentrations of 6.25-100 μg/ml, did not cause any detectable cytotoxicity to epithelial cells based on LDH leakage and did not have a significant stimulating effect on IL-8 (Park et al., 2008a).

Park et al. (2008b) evaluated the cytotoxic effect of cerium oxide nanoparticles using BEAS-2B cells and compared the results to the cytotoxicity in the T89G and H_9C_2 cell lines. The BEAS-2B cell line is derived from human bronchial epithelial cells, where as the T98G cell line is from human brain fibroblasts and the H_9C_2 cell line is from rat cardiomyocytes. BEAS-2B, T89G, and H_9C_2 cell viability was tested following treatment with 10, 20, and 40 μg/ml of 30 nm-sized particles for 24, 48, 72, and 96 hours. BEAS-2B, T89G, and H_9C_2 cells were also treated with 15, 25, 30, or 45 nm-particles to compare cytotoxicities of different size nanoparticles. To measure reactive oxygen species (ROS) generation, BEAS-2B cells were pretreated with 5, 10, 20, 40 μg/ml of 30 nm-sized particles for 24 hours and then incubated with 40 μM 2,7-dichlorofluoroscein diacetate for 30 minutes. The relationship between the increased

ROS and the level of antioxidant materials in the cells was investigated by treating cells with 30 nm-particles at 5, 10, 20, or 40 μg/ml for 24 hours. Caspase-3 activity was determined by incubating cells with 5, 10, 20, or 40 μg/ml 30 nm-particles and lysing the cells and testing for enzyme activity. BEAS-2B cells were also treated with 40 μg/ml of 30 nm-particles for 0.5, 1, 2, 4, and 8 hours for inclusion in gene expression analysis.

BEAS-2B cells viability was decreased by treatment with nanoparticles (15 – 45 nm) in a time- and dose-dependent manner, and differences in cerium oxide nanoparticle size had no effect on cell viability (Park et al., 2008b). Cell viability was not reduced in T98G or H_9C_2 cells (Park et al., 2008b). ROS were generated in a dose-dependent manner by 5, 10, 20, or 40 μg/ml 30 nm cerium oxide particles in BEAS-2B cells, and the levels of GSH were decreased in the nanoparticle-treated groups (Park et al., 2008b). Caspase-3, which plays a key role in the apoptotic pathway, was increased following treatment with 5, 10, 20 or 40 μg/ml 30 nm cerium oxide particles (Park et al., 2008b). In addition, oxidative stress-related genes, including catalase, glutathione-S-transferase, heme oxygenase-1, and thioredoxin reductase, were induced by 40 μg/ml of 30 nm-particles in the BEAS-2B cells (Park et al., 2008b). The cytotoxicity in BEAS-2B cells appears to be caused by oxidative stress, as cerium oxide nanoparticles increased the level of ROS with the induction of ROS-related gene expression and decreased the level of intracellular GSH (Park et al., 2008b). The cerium oxide nanoparticles were also absorbed by the cells and were observed around the peri-region of the nuclear membrane using a phase-contrast microscope (Park et al., 2008b).

In addition to the evidence of cytotoxicity, there is evidence of neuroprotective and cardioprotective effects of nanoparticle cerium exposure. Schubert et al. (2006) exposed the HT22 hippocampal nerve cell line, derived from the rodent nervous system, to CeO_2 nanoparticles and monitored the intracellular generation of reactive oxygen species with a nonfluorescent compound that fluoresces when in contact with reactive oxygen species. The cerium oxide nanoparticles were characterized as single, monodisperse crystals. The CeO_2 nanoparticles were, for the most part, nontoxic to the HT22 cell line, with the exception of the 1 μm CeO_2 nanoparticles at concentrations ≥20 μg/mL. The 6 and 12 nm, as well as 1 μm, cerium particles were protective of oxidative stress to HT22 cells, and a difference in level of protection offered by the 6 nm, 12 nm, and 1 μm sized particles could not be produced. It was also shown that 12 nm CeO_2 particles at 20 and 200 μg/mL were able to rapidly reduce pools of reactive oxygen species formed by the cells after 8 hours of exposure to glutamate. Cerium nitrate and cerium chloride, when tested at the same concentrations as cerium oxide, displayed no protective effects. Schubert et al. (2006) were able to provide evidence that cerium oxide

nanoparticles have antioxidant properties that promote nerve cell survival under oxidative stress conditions.

The cardioprotective effects of nano-sized CeO_2 were evident in cultured cells from MCP mice administered 15 nmol of 7 nm CeO_2 intravenously twice a week for two weeks (Niu et al., 2007). The cardiac-specific expression of monocyte chemoattractant protein (MCP-1) in mice causes ischemic cardiomyopathy. The treatment of MCP mice with nanoparticle CeO_2 inhibited monocyte/macrophage infiltration into the myocardial interstitial space, suppressed proinflammatory cytokine production in the myocardium, and limited myocardial oxidative stress.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

No studies evaluating the oral toxicity of cerium in humans were located, but an association between exposure to cerium in food and the development of endomyocardial fibrosis has been suggested (Eapen et al., 1998; Kutty et al., 1996; Valiathan et al., 1989). Long-term studies in animals are limited to a 13-month drinking water study in rats, which investigated the effects of a single dose group (Kumar et al., 1996), a 6-month drinking water study in rabbits in which the administered dose consisted of a mixture of rare earth chlorides (Kartha et al., 1998), a 12-week dietary study in mice (Kawagoe et al., 2005), and a 105-day gavage study in rats (Cheng et al., 2000). The study of Kumar et al. (1998) suggested that cerium may increase the levels of collagen in the heart, while the findings of Kartha et al. (1998) suggested that cerium may intensify the cardiac effects of magnesium deficiency. Kawagoe et al. (2005) suggested that cerium may increase oxidative stress in tissues of mice, and Cheng et al. (2000) reported that cerium increased the oxygen affinity of hemoglobin in rats.

A single dose study in mice observed splenic lesions and GI irritation in treated mice (Stineman et al., 1978). Other acute oral data consist of a report of piloerection, hunched posture, unsteady gait, and dark coloring of eyes in male and female rats (along with lethargy, abnormal respiration, and prostration in females only) given single gavage doses of 5,000 mg/kg cerium as cerium sulfide (Rhodia Inc., 1998); a 7-day LD_{50} in mice of 1,291 mg/kg cerium as the chloride/citrate complex (Stineman et al., 1978); and an LD_{50} of 1,178 mg/kg for cerium nitrate and 622 mg/kg for cerium oxide, also in mice (Ji and Cui, 1988).

The few long-term studies available in animals identify cardiac tissue and hemoglobin oxygen affinity as possible health effects, but the animal studies are of limited scope and insufficient duration and experimental design. Toxicokinetics data have shown that orally

administered cerium compounds are poorly absorbed, and this may be reflected in the results of the studies conducted with these compounds by the oral route of exposure.

4.6.2. Inhalation

Inhalation data in humans consist of reports describing numerous cases of workers who developed pneumoconiosis associated with accumulation of cerium in the lungs after prolonged occupational exposure to cerium fumes or dust (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). In these cases, the exposure was to cerium oxide, and cerium-induced pneumoconiosis was characterized by accumulation of cerium particles (and other rare earth particles) in the lungs and lymphoreticular system. Exposure was not quantified in any of these cases. Potentially sensitive subgroups were not identified in the available human studies.

Information regarding long-term inhalation exposure in animals is derived from a single subchronic study in rats (BRL, 1994). Sprague-Dawley rats were exposed nose only to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks. Endpoints evaluated included a functional observational battery, hematology and clinical chemistry, urinalysis, and gross and microscopic morphology of tissues. The results revealed statistically significant increases in absolute and differential neutrophil counts in the blood, treatment-related increases in the absolute and relative weight of the lungs in both males and females dosed at 50.5 and 507.5 mg/m^3 and in the relative spleen weight of male rats at 507.5 mg/m^3, discoloration or pale areas and uncollapsed parenchyma in the lungs of male and female rats at $\geq$50 mg/m^3 and pale foci in female rats at 5 mg/m^3, and dose-related alveolar epithelial and lymphoid hyperplasia and pigment accumulation in the lungs, lymph nodes, and larynx of males and females at $\geq$5 mg/m^3. The lowest exposure level, 5 mg/m^3, was a LOAEL for lymphoid hyperplasia of the lymph nodes. A NOAEL was not identified.

Acute inhalation data are limited to the determination of an LC_{50} greater than 50 mg/m^3 for cerium oxide in rats (Rhone-Poulenc, 1983) and a report of mild histologic alterations, including slight connective tissue and collagen fiber development and increased lymphocyte numbers, in the lung, as well as the accumulation of macrophages and large, multinucleated cells in the spleen, of rats that received an intratracheal instillation of cerium oxide 8 months prior to examination (Mogilevskaya and Raikhlin, 1967).

4.6.3. Mode-of-Action Information

4.6.3.1. *Respiratory Tissues*

The mode of action for pulmonary toxicity in humans following chronic inhalation of cerium oxide has not been demonstrated. However, pathological data from human case reports demonstrate accumulation of cerium in the lungs following occupational exposure to cerium fumes or dust. Animal data provide evidence that supports a hypothesized mode of action for effects observed in the lungs. The occurrence of alveolar epithelial and lymphoid hyperplasia in the lungs of test animals accompanied by pigmentation in the lungs due to the accumulation of cerium oxide suggests that the mode of action for cerium oxide inhalation toxicity may be mediated by cytokine and fibrogenic effects resulting from pulmonary macrophage activation and immobilization. Taken together, the accumulation of insoluble cerium particles in the respiratory tract of humans and animals following chronic and subchronic inhalation exposures, respectively, suggests that at high exposure concentrations impaired clearance may influence pulmonary toxicity for both species. Pathology observed in the lung may be due to an immune response to inhaled cerium dust in which clearance by pulmonary macrophages becomes overloaded.

One question related to the impairment of clearance in the lung is whether the toxicity results from the chemical characteristics of cerium oxide versus the physical presence of the particle. The concept of dust overloading of the lungs refers to inhalation exposures that are sufficiently intense as to overwhelm the pulmonary clearance mechanisms, specifically, macrophagic phagocytosis in the alveolar spaces with mucociliary escalation in the bronchial airways (Morrow, 1988). The concept of particle overload applies to particles of relatively low cytotoxicity that secondarily induce pulmonary toxicity via chronic activation of immune-responsive cells (Oberdorster, 1995). In addition to immune cellular activation, the uncleared particles may traverse the pulmonary epithelial boundary to the interstitial spaces, where fibrogenesis may occur (Oberdorster, 1995).

Haley (1991) proposed that rare earth metals, including cerium, may exert toxicity both from innate chemical characteristics as well as pulmonary dust burden. A suggested mechanism explaining both inflammatory and fibrogenic responses involves the activation of PAMs following particle overload. Tissue-destructive release products of activated PAMs include acid hydrolases, elastases, collagenases, and several reactive oxidant species (Haley, 1991). Additionally, the release of fibroblast growth factor and fibronectin may stimulate the proliferation of fibroblasts, and PAMs may release neutrophil attractant factors (Haley, 1991). The subsequently enlisted neutrophils can release several oxidants and proteases that are known to result in connective tissue damage, possibly stimulating fibrogenesis (Hunninghake et al., 1984). Although BRL (1994) did not collect data on macrophage activation or the release of chemokines, the significant increase in mature neutrophils in rats exposed to high levels of

cerium oxide observed in this study is consistent with stimulation of neutrophils by PAMs. The exposure-related increase in pigment accumulation and lymphoid hyperplasia in lymph nodes draining the lungs (i.e., bronchial and mediastinal lymph nodes) observed in the BRL (1994) study and the absence of significant effects in pancreatic and mandibular lymph nodes further supports the role of pulmonary macrophages.

The immobilization of PAMs by excessive cerium dust loads, as hypothesized by Morrow (1992, 1988), results in macrophages carrying a heavy cerium oxide load to lose the ability to move either toward the mucociliary escalator system, for subsequent clearance to the gut, or to the lymphoid vasculature. A dense tissue population of activated, yet immobile, macrophages may serve to induce significant cell damage by effectively increasing the concentration of inflammatory cytokines and fibrogenic growth factors within the pulmonary epithelium. Reduction in the ability to clear insoluble cerium oxide from the lung spaces due to macrophage immobilization is consistent with reports of extracellular presence of pigment in mid- and high-dose rats relative to low-dose animals (BRL, 1994). The presence of inflammatory cytokines and growth factors was not examined.

The ability of immobilized macrophages to induce toxicity via concentration of immune signaling requires minimal cytotoxicity of the macrophages themselves. In vitro experiments have shown that cytotoxicity of rat pulmonary macrophages is high for soluble cerium (cerium chloride) (Lizon and Fritsch, 1999; Palmer et al., 1987) but quite low for the insoluble salt (cerium oxide) (Palmer et al., 1987). This suggests that the pulmonary responses to inhaled cerium reported in human cases and animal bioassays (which predominantly involved insoluble cerium oxide) may not have caused significant macrophage cell death. This finding is consistent with pathological findings in human case reports, animal studies, and in vivo assays of PAMs in which cerium-related granule pigmentation was visible in the lung and lymphoid tissues, suggesting that the cerium sequestration and concentration occurred in viable PAMs.

The precipitation and concentration of both soluble cerium chloride and insoluble cerium oxide within cytosolic lysosomes has been demonstrated (Berry et al., 1997; Berry, 1996). The high phosphate concentration and extensive enzymatic hydrolysis activity (acid phosphatase) within lysosomes was shown to effectively precipitate cerium as cerium phosphate. Rats exposed to 5 μg insoluble cerium oxide via intratracheal dosage for 30 days displayed rat alveolar macrophages that contained very fine needles or granules in the lysosomes (Berry et al., 1997). The needle or granule inclusions contained both phosphorus and cerium. After 2 days of intratracheal dosage, the rat alveolar macrophage lysosomes contained cerium particles approximately 1 μm in length and displayed a crystalline structure. Rats exposed to soluble cerous chloride displayed fine granules and needles in the lysosomes (Berry et al., 1997). Berry

(1996) showed that cerium precipitated with phosphorus in the lysosomes of hepatocytes and of bone marrow and splenic macrophages of rats after intraperitoneal injections of cerium. Granular or needle-like precipitate deposits were observed in the lysosomes of alveolar macrophages of rats exposed to an aerosol solution of cerium chloride (Galle et al., 1992). The authors (Berry et al., 1997; Galle et al., 1992) suggest that the precipitation and concentration of cerium within the lysosomes may serve to inhibit diffusion of the metal to other tissues.

The presence of insoluble cerium oxide particles in the alveolar macrophages of rats exposed via the intratracheal route (Mogilevskaya and Raikhlin, 1967) is consistent with the appearance of cerium-related particles within the alveolar macrophages of a worker in an occupation associated with cerium oxide exposure (McDonald et al., 1995). The particles in the human alveolar macrophages ranged from small, pinpoint particles to oblong or needle-shaped. Pairon et al. (1995) revealed particles of phosphorus, calcium, lanthanum, and cerium in human alveolar macrophages following occupational exposure and identified interstitial macrophages that contained both phosphorus and rare earth elements. Cerium was essentially always associated with phosphorus in the biological samples studied by Pairon et al (1995).

The presence of the insoluble cerium oxide particles in the rat alveolar macrophages (Berry et al., 1997) and cerium in the lysosomes of hepatocytes and splenic macrophages (Berry, 1996) is also consistent with the involvement of macrophages in the appearance of cerium-related pigmentation in the rat lung, liver, and spleen following inhalation exposures (BRL, 1994).

Shivakumar and Nair (1991) have shown that cerium exposure resulted in the inhibition of protein synthesis in human lung fibroblasts, while lung fibroblast proliferation was not stimulated by 0.5 μM cerium exposure (Nair et al., 2003). However, cell types other than fibroblasts may be involved in the inflammatory response associated with cytokine and growth factor release that leads to a fibrotic response (Nair et al., 2003).

Inhalation data on other poorly soluble metal oxides provides support for the hypothesized mode of action for cerium oxide-induced lung effects. Cullen et al. (2000) demonstrated overwhelmed pulmonary clearance in male Wistar rats following inhalation exposure to the poorly soluble metal oxide, titanium dioxide (TiO_2), in which the particles were of similar size and concentration to that used for cerium oxide in the BRL (1994) study. Cullen et al. (2000) investigated pulmonary effects in male Wistar rats following the 4- and 7-month inhalation exposure of TiO_2 particles with a MMAD of 2.1 μm administered at 25 mg/m^3 for 209 calendar days and 50 mg/m^3 for 118 calendar days. To compare the pulmonary clearance and effects of TiO_2 to another poorly soluble particle, barium sulfate particles, with a MMAD of 4.3 μm, were administered to male Wistar rats at 37.5 mg/m^3 for 203 days and 75 mg/m^3 for 119

days. The lymph node burdens, measured in mg/day, for TiO_2 demonstrated the initiation of overload retardation after approximately 50 days at 50 mg/m^3 and 150 days at 25 mg/m^3. In addition, inhalation of TiO_2 led to an accumulation of pulmonary macrophages around dust deposition sites, and most of the macrophages contained phagocytized dust particles. Cullen et al. (2000) did not include exposure concentrations as low as the BRL cerium oxide study (i.e., 5 mg/m^3); however at the lowest concentration of TiO_2 examined, overload was also observed, although time to overload was longer at the lower concentration (140 days at 25 mg/m^3). Overwhelmed pulmonary clearance, as marked by increased translocation of dust to lymph nodes, was not observed for barium sulfate ($BaSO_4$) (Cullen et al., 2000). The demonstration of pulmonary overload by Cullen et al. (2000) in an exposure time frame and at a concentration of TiO_2 particles with a similar MMAD to the cerium oxide particles in the BRL (1994) study supports the proposed mode of action of overwhelmed pulmonary clearance for the relatively insoluble cerium oxide. Although pulmonary overload is a mode of action that may apply to other relatively insoluble particles of low cytotoxicity, it is clear from the Cullen et al. (2000) study that there are strong differences between exposures to the dust of different chemicals, such that exposure to TiO_2 leads to overloading of the lung, whereas overloading was not observed for $BaSO_4$. Tran et al. (2000) hypothesizes that the differences between the TiO_2 and $BaSO_4$ in the level of inflammation and translocation to the lymph nodes may be explained when the lung burden is expressed as total particle surface area. Barium sulfate, with a low specific surface area, may be cleared effectively from the lung, as the volumetric capacity of the alveolar macrophage was not exceeded and overload was not initiated (Tran et al., 2000).

An alternative mode of action may involve the presentation or delivery of cerium oxide particles to the bronchial lymph nodes from the tracheobronchial region of the lung by dendritic cells. An important requirement in the induction of adaptive immunity is the transport of antigen to the T-cell area of the draining lymph nodes from the initial exposure region (Lambrecht et al., 2001). The lymphoid hyperplasia in the bronchial lymph nodes may represent the presentation of cerium oxide particles to the lung-draining lymph nodes by dendritic cells. While this mode of action provides a biologically plausible basis for cerium oxide-induced pulmonary toxicity, data are lacking that directly evaluate adaptive immunity and cerium oxide exposure.

The proposed mode of action for the pulmonary effects observed following cerium oxide exposure is the overloading of the PAMs by cerium oxide particles at high doses, leading to immobility of the PAMs in the alveoli, resulting in the sustained release of inflammatory cytokines and fibrogenic growth factors and subsequent cell damage. However, additional data demonstrating pulmonary overload, such as type II cell proliferation, altered alveolar epithelial

integrity, and/or chronic acute inflammation with an influx of PMNs are unavailable following cerium oxide exposure.

4.6.3.2. *Other Tissues*

No mode of action for cerium compounds in non-respiratory tissues was identified from the available studies. The studies utilized primarily parenteral routes of exposure and suggest that the production of free radicals may be involved in cerium-associated toxicity, as well as actions at the RNA transcription level. However, details of potential mode(s) of action in non-respiratory tissues are lacking.

Cerium, as a cerium chloride-cerium citrate complex, administered subcutaneously was found to have minor effects on some tests of spontaneous motor behavior in young adult mice (Morganti et al., 1980, 1978; Stineman et al., 1978) and in mice exposed in utero (D'Agostino et al., 1982, 1978a, b); however, a mode of action was not discussed or apparent in these studies. Interestingly, behavioral measures were not correlated with cerium levels in tissues, which suggested an unknown, indirect mode of action involving a biological chain of events (Morganti et al., 1978).

Liver effects have been reported in several studies in rats and mice following i.v. administration of cerium compounds. The effect is characterized by fatty liver, fatty degeneration, and necrosis (Magnusson, 1962; Lombardi and Recknagel, 1962). Female rats appeared more sensitive than male rats (Wiener-Schmuck et al., 1990; Magnusson, 1962). Incubation of hepatocytes from female rats with cerium did not result in damage to the cells (Wiener-Schmuck et al., 1990), suggesting that the damage is not a result of direct contact of cerium with the hepatocytes but that cerium exposure may trigger a series of events that ultimately result in liver damage. This seems consistent with the results of a study in two strains of mice having different sensitivities to cerium toxicity that showed that cerium levels in the liver of the more resistant strain were 50% higher than those in the more sensitive strain (Arvela et al., 1991). The latter study and an additional study by Salonpää et al. (1992) presented evidence suggesting that some association exists between the development of liver damage and COH induction. Enzyme induction was accompanied by increased expression of CYP2A5 mRNA, but the mechanism by which cerium increases CYP2A5 expression is unknown.

Cerium chloride exposure has been found to increase the levels of collagen in the heart of animals in an oral study (Kumar et al., 1996) and following i.v. dosing (Kumar and Shivakumar, 1998). Cerium chloride exposure decreased collagen degradation and increased the rate of deposition of newly synthesized collagen in cardiac tissue of rats (Kumar and Shivakumar, 1998). The increase in collagen synthesis is consistent with a marked increase in mRNA in

cardiac fibroblasts incubated with cerium chloride (Shivakumar et al., 1992), pointing to an action at the level of transcription. Cerium-induced production of free radicals was also associated with stimulation of fibroblast proliferation in studies in vitro (Nair et al., 2003; Preeta and Nair, 1999). While cerium-induced collagen proliferation can explain the occurrence of cardiac fibrosis, the mechanism by which cerium triggers this event at the molecular level remains unknown.

In vitro studies demonstrated that low cardiac fibroblast proliferation is stimulated by exposure to cerium (0.5 μM) (Nair et al., 2003), that low concentrations of cerium stimulate cardiac fibroblast proliferation in association with an increase in intracellular generation of free radicals (Preeta and Nair, 1999), and that cerium exposure results in the inhibition of protein synthesis in rat heart cells (Shivakumar and Nair, 1991). Kuruvilla and Kartha (2006) demonstrated that cerium reduced the incorporation of [H3]-thymidine into DNA of cardiac fibroblasts grown in endocardial endothelial cells conditioned medium. These results suggest that the cardiac lesions in endomyocardial fibrosis may result from the direct stimulation, and not through toxic effects on the endocardial endothelium, of subendomyocardial fibroblasts by cerium (Kuruvilla and Kartha, 2006).

Cerium inhibited contraction of isolated rat ventricular papillary muscle, an effect that could be partially prevented by the free radical scavenger SOD (Manju et al., 2003), which adds support for the involvement of free radicals in the cerium-induced cardiac effects.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Data were unavailable regarding the carcinogenicity of cerium compounds in humans or experimental animals. In accordance with U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, there is "inadequate information to assess the carcinogenic potential" of cerium in humans.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

No relevant human or animal data are available. In addition, the available information is insufficient to ascertain the mutagenicity of cerium compounds. The NTP has recently begun an evaluation of the chronic inhalation toxicity of cerium oxide, including a cancer bioassay. The date of completion and public availability of the results is unknown but may be expected in 2009.

A study in various strains of *S. typhimurium* demonstrated negative evidence of mutagenicity under the conditions of the assay (Shimizu et al., 1985). Cerium chloride did not

induce DNA damage in two strains of *B. subtilis* by using the rec-assay (Nishioka, 1975), but cerium nitrate was reported to induce chromosomal breaks and reduce the mitotic index in rat bone marrow in vivo, and cerium sulfate was reported to cause differential destaining of chromosomal segments in plants (Sharma and Talukder, 1987).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No studies were located regarding possible childhood susceptibility to cerium compounds. Kutty et al. (1996) stated that high cerium soil concentrations and magnesium deficiency during childhood may lead to endomyocardial fibrosis, although causation has not been established. Increased GI uptake of cerium in preweaning, suckling animals compared with adults is apparent, due primarily to pinocytosis in intestinal cells, but it is unclear if this has any toxic consequences, since the cerium remains in the intestinal cells, may be minimally available systemically, and is eventually eliminated in the feces as the intestinal cells die and are replaced (Kargacin and Landeka, 1990; Kostial et al., 1989a, b; Inaba and Lengemann, 1972).

4.8.2. Possible Gender Differences

No information was located regarding gender differences in humans in response to exposure to cerium compounds. Information addressing gender differences in animals is from a study by Magnusson (1962) in which i.v. administration of cerium to rats resulted in noticeably more severe liver effects in females than in males. This was confirmed in another study in rats also treated intravenously with cerium (Wiener-Schmuck et al., 1990). There is no explanation for this gender-related difference in susceptibility, and it is unknown whether it also applies to endpoints other than the liver. Additionally, it appears that female rats may be more susceptible to the hematological changes (Table 4-1) observed as the result of cerium oxide inhalation (BRL, 1994).

4.8.3. Possible Susceptible Populations

An association between exposure to rare earth elements, cerium in particular, in food and the development of endomyocardial fibrosis has been suggested (Eapen, 1998; Kutty et al., 1996; Valiathan et al., 1989). Cerium levels were elevated in the endomyocardial tissue samples from patients who died of endomyocardial fibrosis compared with the tissues of controls who died in accidents or from congenital heart disease (Valiathan et al., 1989). A higher incidence of endomyocardial fibrosis has been reported among a population consuming tubers grown in a region of India with high cerium soil concentrations, compared to subjects consuming tubers

grown in a soil with low cerium concentrations. Analysis of the geographic distribution of endemic endomyocardial fibrosis in India suggested a link to high cerium soil concentrations and possibly to magnesium deficiency during childhood (Kutty et al., 1996). Populations exposed to both cerium in food and a diet deficient in magnesium may be susceptible to cardiac effects, although causality has not been conclusively demonstrated.

A variety of health effects could result from the accumulation of a sufficient amount of persistently retained particles in the lung (Morrow, 1988). Smoking has suppressive effects on pulmonary clearance in humans (Morrow et al., 1992), and the co-exposure of cerium with cigarette smoke, or particulates that accumulate in the lungs, may potentially lead to more severe pulmonary effects in exposed populations. Chen et al. (2006) found that pulmonary inflammation, in this case from lipopolysaccharide treatment, may play an integral role in enhancing the extrapulmonary translocation of particles.

Particle size is another factor that influences overload and toxicity that may be particularly relevant for human exposure if human exposure is associated with fumes from carbon arc lamps, as fumes include smaller particles from the low nm range to <1 μm aggregates. Impaired lung clearance and lung effects in rats exposed to ultrafine particles (<100 nm) occur at lower mass concentrations than in rats exposed to fine particles (<10 μm) (Baan et al., 2006). Translocation of particles to the insterstitium is a function of number of particles, and appears to be dependent on the dose and particle size (Ferin et al., 1992). Excessive translocation into the insterstitium may cause damage to epithelial cells, pulmonary edema, and eventual fibrosis (Ferin 1994, Oberdorster et al., 1990). Populations exposed to ultra-fine cerium oxide may have a higher than expected toxicity when compared to cerium oxide particles of a larger size, as ultrafine particles have higher than expected toxicity when compared to similar particles of a larger size (Ferin, 1994).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

The available human and animal studies demonstrate that cerium may have an effect on cardiac tissue and hemoglobin oxygen affinity. However, an RfD for cerium was not derived because the available studies were not suitable for quantitation of effects for various reasons, including unknown exposure concentrations in the available human studies, lack of a dose-response, uncertain toxicological significance (e.g., changes in measures of oxidative stress), and study design (e.g., effects noted only under conditions of a restricted diet).

An association between exposure to cerium in food and the development of endomyocardial fibrosis has been suggested (Eapen et al., 1998; Kutty et al., 1996; Valiathan et al., 1989). In addition, Gómez-Aracena et al. (2006) suggest a relationship between chronic cerium exposures, characterized by cerium toenail concentrations, and increased risk of acute myocardial infarction.

The data set for long-term animal studies consists of a 13-month drinking water study in rats (Kumar et al., 1996), a 6-month drinking water study in rabbits (Kartha et al., 1998), a 12-week dietary study in mice (Kawagoe et al., 2005), and a 105-day gavage study in rats (Cheng et al., 2000).

Kumar et al. (1996) demonstrated increased, highly variable cerium levels in cardiac tissue. Cerium chloride-treated rats, relative to untreated controls, had an increased level of collagen in the cardiac tissue, with an enhanced effect in rats fed a magnesium-deficient diet. This study suggested that cerium might increase the levels of collagen in the heart. This study was conducted on a small number of rats at one dose level (control and dosed rats) and evaluated few endpoints, and the observed effects were highly variable and not statistically significant.

Kartha et al. (1998) suggested that cerium chloride may intensify the cardiac effects of magnesium deficiency. Cardiac lesions were apparent in 6/10 rabbits fed a magnesium-deficient diet with no cerium exposure and 9/10 rabbits fed a magnesium-deficient diet with cerium exposure. Rabbits fed magnesium-restricted diets, treated with or without cerium, showed endocardial, subendocardial, interstitial, and perivascular fibrosis and the lesions were more severe in those with cerium added to the drinking water. Cardiac lesions were absent from the groups fed the normal magnesium diet regardless of whether they consumed water with or without cerium. However, this study used only one dose group. The authors reported that cerium may intensify the cardiotoxicity associated with a magnesium-deficient or restricted diet but did not elicit a cardiac effect when tested under conditions of a normal magnesium diet.

Cheng et al. (2000) reported that cerium chloride exposure in rats produced a slight increase of hemoglobin content in erythrocytes after 40 days of treatment with an even greater increase in hemoglobin content after 80 days of exposure. The effect on the oxygen affinity of hemoglobin was demonstrated by altered oxygen saturation curves for the dosed rats compared to control rats. Hemoglobin in cerium-treated rats exhibited altered oxygen affinity up to 80 days of exposure, demonstrated by increased affinity up to 10 mm Hg and a double sigmoidal curve for 40-day rats and increased affinity above 20 mm Hg for 80-day rats.

Kawagoe et al. (2005) demonstrated that cerium chloride statistically significantly decreased liver lipoperoxide levels, increased liver GSH levels and liver MT activity, and decreased plasma SOD activity in mice. Cerium concentrations in the kidney, liver, lung, and spleen were statistically significantly elevated relative to controls, with the lung and spleen containing the highest levels. The study authors suggested that the endpoints showing changes as a result of cerium exposure in this study are indicators of reactive oxygen species generation and resultant oxidative stress but indicated that their toxicological significance was uncertain. The authors did not report any other effects in the liver.

5.1.2. Previous RfD Assessment

This is the first IRIS assessment for cerium oxide and cerium compounds; thus, no oral RfD was previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Exposure to cerium compounds in the environment is most likely through cerium (ceric) oxide. There are numerous case reports of workers who developed pneumoconiosis or interstitial lung disease associated with the accumulation of cerium particles in the lungs after prolonged occupational exposure to cerium fumes or dust (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). In these cases, the inhalation exposure was primarily to cerium oxide, and cerium-induced pneumoconiosis was characterized by accumulation of cerium particles (and other rare earth particles) in the lungs and lymphoreticular system. Cerium exposure in humans was associated with interstitial, peribronchial, and perivascular fibrosis, a restriction of respiratory function and/or pulmonary hypertension (Porru et al., 2001; Pairon et al., 1995, 1994; Vogt et al., 1986; Vocaturo et al., 1983; Sabbioni et al., 1982). The available human studies were not selected for the derivation of an inhalation RfC

because the cerium exposures were not available in any of the case reports.

Information regarding long-term inhalation exposure in animals is derived from a single subchronic study in rats (BRL, 1994). The BRL (1994) study is an unpublished study; accordingly, it was externally peer reviewed by EPA in August 2006 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. Sprague-Dawley rats (n = 30) were exposed nose only to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks. Endpoints evaluated included a functional observational battery, hematology and clinical chemistry, urinalysis, and gross and microscopic morphology of tissues, as discussed in Section 4.2.2. As in humans, BRL (1994) demonstrated that the lung and lymphoreticular system appeared to be the targets of toxicity in rats following inhalation exposure to cerium oxide. Low-dose effects observed included a statistically significant increase in absolute and relative lung weight with discoloration or pale areas and uncollapsed parenchyma in the lungs in both male and female rats at $\geq$ 50.5 mg/m^3. Female rats also showed pale foci in the lungs at $\geq$ 5 mg/m^3. In addition, the study authors reported an increased incidence of pigment accumulation and lymphoid hyperplasia in the bronchial lymph nodes at $\geq$ 5 mg/m^3 in both males and females. In the lungs, an increased incidence of alveolar epithelial hyperplasia and pigment accumulation was observed at $\geq$ 50 and 5 mg/m^3, respectively, in both males and females. The authors reported that the severity of the hyperplasia in a given tissue, lung or lymph nodes, was correlated with the amount of pigment accumulated in the tissue (data not reported). BRL (1994) considered these findings to be consistent with antigenic stimulation by cerium oxide; however, they did not discuss the possibility of non-antigenic stimulation. Other effects observed included an increased incidence of metaplasia and pigment accumulation in the larynx in males at $\geq$ 50.5 mg/m^3 and at 50.5 and 5 mg/m^3, respectively, in females. The metaplasia observed in the larynx was interpreted by the study pathologist to be adaptive and reversible. Hematological changes reported were statistically significant increased numbers of absolute neutrophils in males at $\geq$ 50 mg/m^3, absolute and relative neutrophils in females at $\geq$ 5 mg/m^3, and relative lymphocytes in females at $\geq$ 5 mg/m^3 at 13 weeks. The biological significance of the observed hematological changes is not known.

The mode of action for cerium oxide-induced lung and lymphoreticular toxicity is not known. However, the available MOA information from humans and animals (Section 4.6.3.1) suggests that cerium toxicity may be the result of nonspecific stimulation of PAMs that are activated and immobilized by the accumulation of insoluble cerium particles. The subchronic BRL (1994) study in rats displayed increased lung weight; discoloration or pale areas, pale foci, and uncollapsed parenchyma in the lungs; enlargement or pale discoloration of the bronchial, mediastinal, and pancreatic lymph nodes; and dose-related alveolar epithelial and lymphoid

hyperplasia and pigment accumulation in the lungs and lymph nodes. These effects are similar to the pneumoconiosis described in the human case reports, which were characterized by the accumulation of cerium particles in the lungs and lymphoreticular system and histologic effects throughout the lung. In addition, Hahn et al. (2001, 1999) demonstrated the retention of cerium-alumniosilicate particles in the tracheobronchial lymph nodes of dogs for several years following a single inhalation exposure.

Clearance of foreign substances from the lung by macrophages involves removal to the stomach and GI tract, the lymph nodes, and the pulmonary vasculature (Witschi and Last, 2001). Thus, it would be expected that following inhalation exposure to cerium oxide, macrophages that have phagocytized cerium particles and have retained their mobility, would be removed from the lung and may accumulate in the lymph nodes. However, if this process is overwhelmed, PAMs that have been activated and immobilized by the absorption of cerium oxide particles may be observed. Therefore, the effects observed in the lymph nodes and lungs of male and female rats following inhalation exposure to cerium oxide in the BRL (1994) study were considered to be indicative of overwhelmed clearance and potential key events in the proposed MOA.

The only available study reporting low-dose effects of subchronic inhalation exposure to cerium oxide in animals, BRL (1994), was chosen as the principal study. This study was well designed with three dose groups of 30 animals per group per sex. Numerous tissues and endpoints were assessed, and methods and observed effects were thoroughly reported. This study identified statistically significant dose-dependent effects on the lungs and lymphoreticular system in both male and female rats. The observed effects included increased lung weight; discoloration or pale areas, pale foci, and uncollapsed parenchyma in the lungs; enlargement or pale discoloration of the bronchial, mediastinal, and pancreatic lymph nodes; and dose-related alveolar epithelial and lymphoid hyperplasia and pigment accumulation in the lungs and lymph nodes. The lung and lymphoreticular system effects observed by BRL (1994) are comparable to the effects observed in humans, that were characterized by the accumulation of cerium particles in the lungs and lymphoreticular system and histologic effects throughout the lung. EPA has selected lymphoid hyperplasia in the bronchial lymph nodes as the critical effect because it was determined that this effect represents the most sensitive endpoint occurring at the lowest dose that was indicative of lung and lymphoreticular system toxicity.

5.2.2. Methods of Analysis—Including Models (PBTK, BMD, etc.)

A NOAEL/LOAEL approach was used to derive the RfC for cerium oxide. Benchmark dose (BMD) modeling was not utilized because the incidences of lymphoid hyperplasia in the

bronchial lymph nodes of male and female rats approached 100% at the lowest dose tested (5 mg/m^3) and were not amenable to modeling. Thus, the RfC is based on the LOAEL of 5 mg/m^3 as the point of departure. Additionally, there was an increase in the incidence of lymphoid and alveolar epithelial hyperplasia in the lung at 50.5 mg/m^3. The selected point of departure is considered to be protective of the pulmonary effects.

The human equivalent concentration (HEC) was calculated from the point of departure by adjusting to a continuous exposure (24 hours a day, 7 days a week) and multiplying by a dosimetric adjustment factor (DAF), which, in this case, was the regional deposited dose ratio (RDDR) for the pulmonary region of the lung. Adjustment to a continuous exposure was calculated as follows:

$$LOAEL_{ADJ} = LOAEL \times (6 \text{ hours})/(24 \text{ hours}) \times (5 \text{ days})/(7 \text{ days})$$
$$= 5 \text{ mg/m}^3 \times 0.25 \times 0.71$$
$$= 0.89 \text{ mg/m}^3$$

The RDDR was calculated using the RDDR v.2.3 program (Table 5-1), as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). The pulmonary region of the lung was selected as the deposition site because the critical effect and the mode-of-action data indicate that the pulmonary region of the lung is the initial location of cerium oxide toxicity.

The human parameters used were body weight, 70 kg; minute volume, 13.8 L; extrathoracic surface area, 200 cm^2; tracheobronchial surface area, 3,200 cm^2; and pulmonary surface area, 54.0 m^2. The parameters used for the rat were body weight, 345 g (calculated by averaging the mean body weights for each dose group for the course of the experiment); minute volume, 234.18 mL; extrathoracic surface area, 15 cm^2; tracheobronchial surface area, 22.50 cm^2; and pulmonary surface area, 0.34 m^2. The GSD for the cerium oxide particles in the BRL (1994) study ranged from 1.8 to 1.9 and the MMAD for the particles was approximately 2.0 μm. The RDDRs calculated from the above model parameters vary depending upon the deposition site in the lung (Table 5-1). The RDDR for pulmonary depositon is 0.536. The calculation is as follows:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RDDR$$
$$LOAEL_{HEC} = 0.89 \text{ mg/m}^3 \times 0.536$$
$$LOAEL_{HEC} = 0.48 \text{ mg/m}^3$$

Table 5-1. Output from RDDR v.2.3 used in the analysis in Section 5.2.2

Species		Body weight (g)	VE (ml)	Extrathoracic		Tracheobronchial		Pulmonary	
				SA (cm^2)	dep	SA (cm^2)	dep	SA (m^2)	dep
Rat		345	234.2	15.000	0.542	22.500	0.042	0.340	0.049
Human		70000	13800.0	200.000	0.352	3200.000	0.085	54.000	0.245
	Ratio	0.005	0.017	0.075	1.540	0.007	0.490	0.006	0.199
	RDDR			0.348		1.183		0.536	
				Thoracic		Total RT		Extrarespiratory	
				SA (cm^2)	dep	SA (cm^2)	dep	SA (m^2)	dep
Rat				0.342	0.091	0.344	0.632	345	0.632
Human				54.320	0.125	54.340	0.682	70000	0.682
	Ratio			0.006	0.724	0.006	0.927	0.005	0.927
	RDDR			0.738		2.487		3.191	

MMAD = 2.00; Sigma g = 1.85.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The $LOAEL_{HEC}$ value of 0.48 mg/m^3 for lymphoid hyperplasia in the bronchial lymph nodes of male and female CD rats (BRL, 1994) was used to derive the RfC for cerium oxide. A total UF of 3,000 was applied to this point of departure: 3 for extrapolation from animals to humans (UF_A: animal to human), 10 for consideration of interindividual variability (UF_H: human variability), 10 for extrapolation from a subchronic study (UF_S), 3 for LOAEL-to-NOAEL extrapolation (UF_L), and 3 for database deficiencies (UF_D). The rationale for the application of the UFs is described below.

A factor of 3 was selected to account for uncertainties in extrapolating from rats to humans (UF_A). This value is adopted by convention where an adjustment from an animal-specific $LOAEL_{ADJ}$ to a $LOAEL_{HEC}$ has been incorporated. Application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a HEC as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method.

A factor of 10 was used to account for variation in susceptibility among members of the human population (UF_H). Insufficient information is available to predict potential variability in susceptibility among the population to inhaled cerium oxide and cerium compounds.

A factor of 10 was used to account for uncertainty in extrapolating from a subchronic to chronic (UF_S) exposure duration, since the BRL (1994) study, which was selected as the principal study, is a subchronic study. The critical effect, increased incidence of lymphoid hyperplasia in the bronchial lymph nodes, may be more pronounced at longer exposure durations.

A factor of 3 was used to account for uncertainty in extrapolating from a LOAEL to a NOAEL (UF_L) because the point of departure was a LOAEL. The critical effect selected to

determine the point of departure for this analysis, lymphoid hyperplasia in the bronchial lymph nodes, represents a minimally biologically significant effect; therefore, an uncertainty factor of 3 was applied.

A UF of 3 was used to account for deficiencies in the cerium oxide database. The database includes multiple case reports of inhalation exposure to workers and a single 13-week subchronic inhalation study in rats. The effects from the subchronic rat inhalation study that are used for the derivation for the RfC (i.e., bronchiolar lymph node hyperplasia) may be early indicators of the more overt toxicity that is found in humans (i.e., interstitial lung disease) exposed to cerium oxide in the workplace. The database does not include an exposure and recovery study that could demonstrate the persistence or, conversely, the adaptive nature of the lymphoid hyperplasia in the bronchial lymph nodes.

Toxicity via the inhalation route is expected to be a portal-of-entry effect. Cerium oxide is a relatively insoluble metal oxide and absorption or translocation from the lung to the circulation is expected to be minimal at low doses. The pulmonary effects observed in the human case reports and in the BRL (1994) study are likely due to the physical deposition of cerium oxide particles in the lung and the immunological reaction to the particles, and are not due to a chemical reaction of cerium oxide with lung tissues. The lymphoid hyperplasia in the bronchial lymph nodes is an immunological response to the cerium oxide particles and is not due to cytotoxicity with regenerative cell growth. The observed immunological response is a portal-of-entry effect and systemic circulation and effects are not expected because of the insoluble nature of the cerium oxide particles.

In considering the impact of database deficiencies on the derivation of the RfC, substantial weight was given to the available data demonstrating similarities between the effects observed in humans following prolonged exposure to cerium oxide and the effects observed in rats in the subchronic principal study, along with the data on deposition and absorption of cerium oxide in the lung. Thus, these data support the assumption that the respiratory system may be the most sensitive target of toxicity following inhalation exposure to cerium and that inhalation exposure to cerium may primarily involve portal-of-entry effects.

The database for cerium oxide lacks both a two-generation reproductive toxicity bioassay and a developmental toxicity bioassay. Systemic effects following the inhalation of cerium oxide, with an MMAD of approximately 2.0 μm and a GSD from 1.8 to 1.9, are not likely to be observed. While it is recognized that the investigation of systemic effects following cerium oxide exposure has not been the focus of existing studies, there is no reason to expect that reproductive, developmental, or other systemic effects would occur, and a UF of 3 is sufficient for the absence of data on these effects.

The chronic RfC for cerium oxide was calculated as follows:

$$RfC = LOAEL_{HEC} \div UF$$
$$= 0.48 \text{ mg/m}^3 \div 3000$$
$$= 2 \times 10^{-4} \text{ mg/m}^3 \text{ (rounded to one significant figure)}$$

Note that the RfC was quantified for cerium oxide particles with an MMAD of approximately 2.0 μm and a GSD from 1.8 to 1.9, and may not characterize the potential toxicity from exposures to cerium oxide particles with smaller MMADs and GSDs, including nano-sized cerium particles. The use of the RfC for cerium compounds other than cerium oxide is not recommended as the similarity between this form of cerium and other cerium compounds is unknown.

5.2.4. RfC Comparison Information

Figure 5-1 is an exposure-response array, which presents NOAELs, LOAELs, and the dose range tested corresponding to selected health effects from the BRL (1994) study, some of which were considered candidates for chronic RfC derivation. Figure 5-2 presents the point of departure, calculated as a human equivalent dose, applied uncertainty factors, and derived sample chronic inhalation reference values for selected endpoints from Figure 5-1. This comparison is intended to provide information for additional endpoints associated with cerium oxide inhalation.

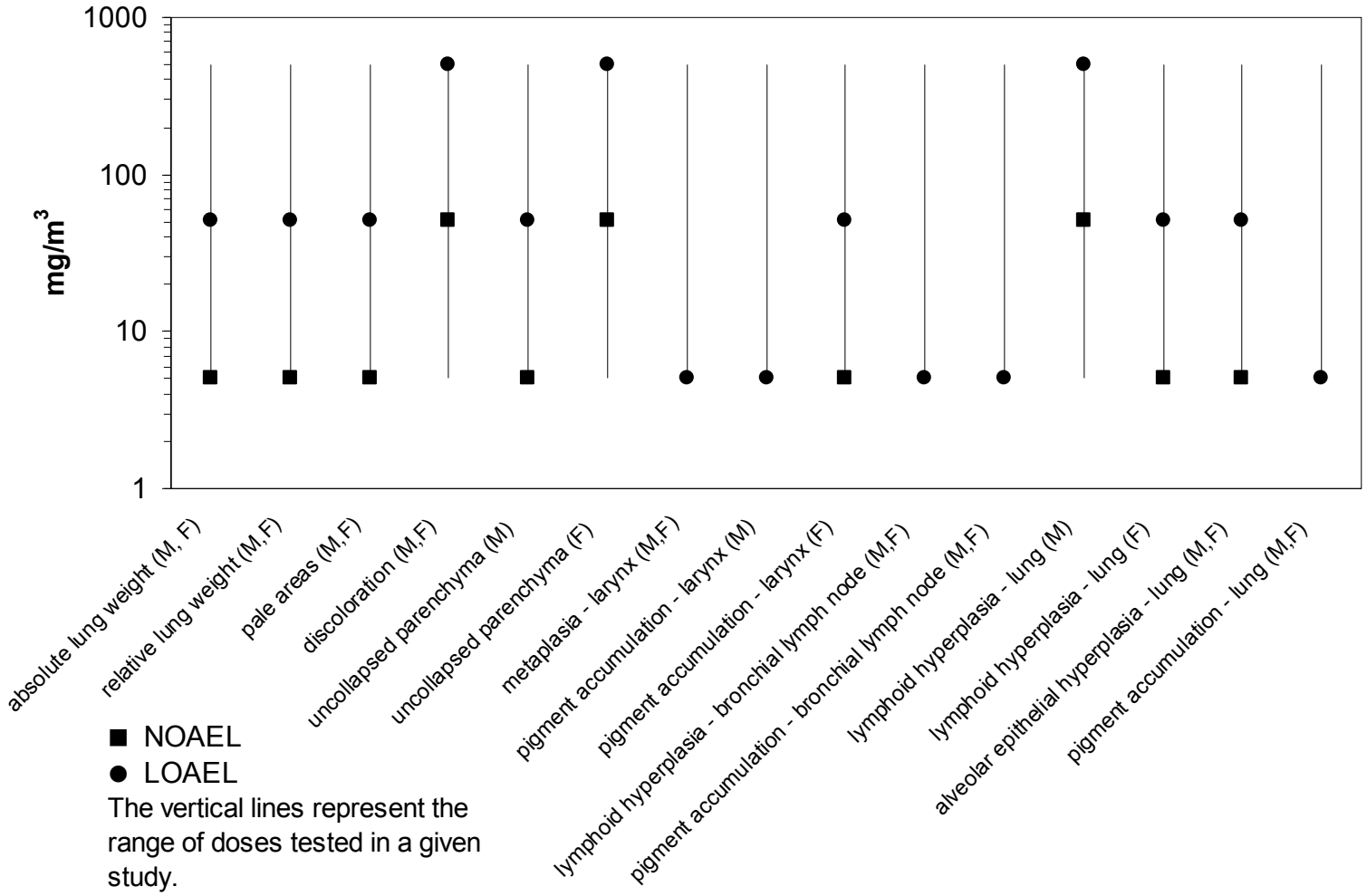
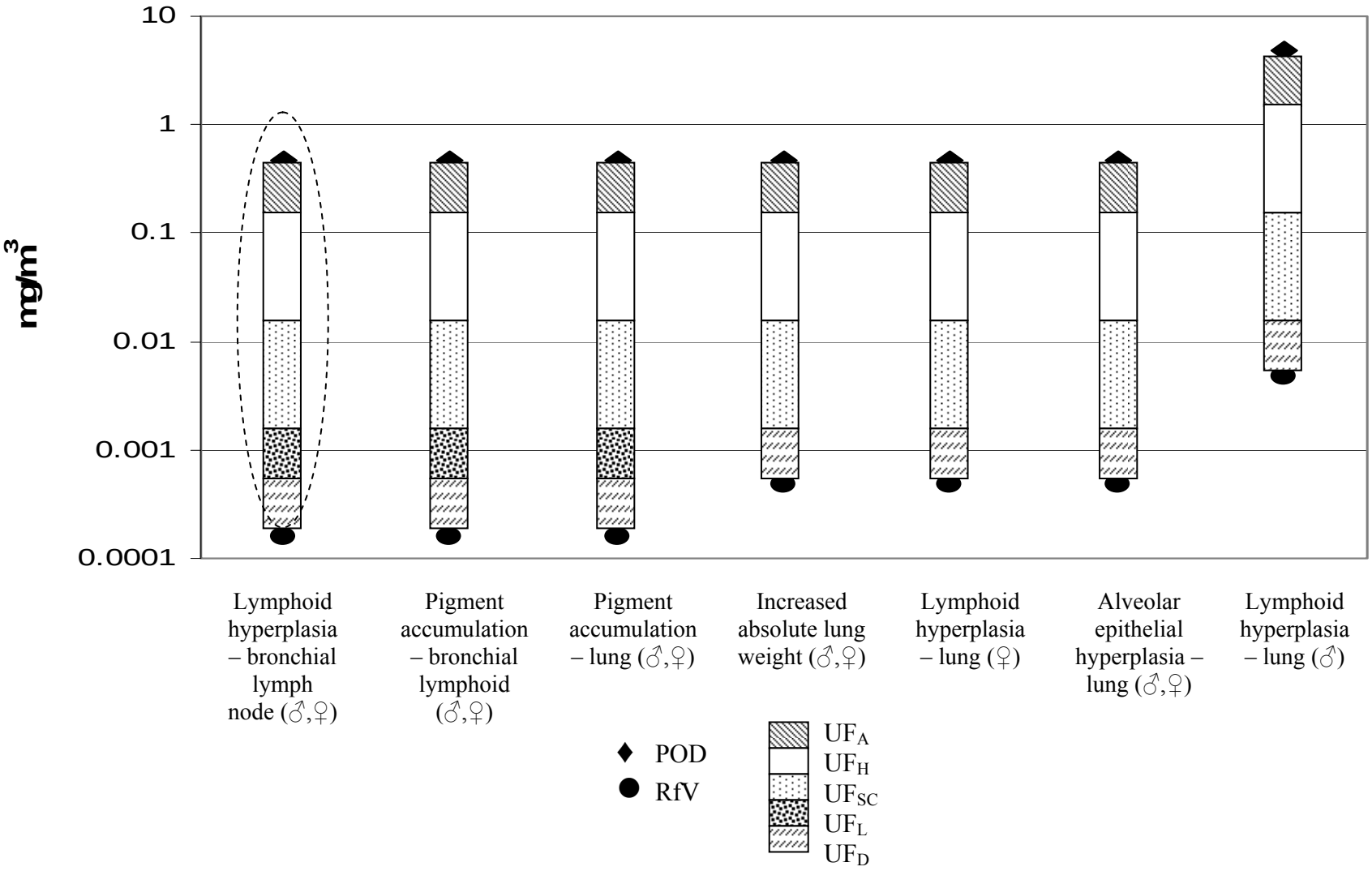


Figure 5-1. Exposure-response array of selected toxicity effects from the BRL (1994) study

Source: BRL, 1994.

Figure 5-2. Points of Departure for selected endpoints (with critical effect circled) from Figure 5-1 with corresponding applied uncertainty factors and derived chronic inhalation RfVs.



5.2.5. Previous RfC Assessment

This is the first IRIS assessment for cerium oxide and cerium compounds; thus, no inhalation RfC was previously available on IRIS.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE (RfD) AND INHALATION REFERENCE CONCENTRATION (RfC)

Risk assessments need to portray associated uncertainty. The following discussion identifies uncertainties associated with the RfC for cerium oxide. As presented earlier in this chapter (Sections 5.2.2 and 5.2.3), uncertainty factors were applied to the point of departure for the RfC. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure, a diverse population of varying susceptibilities, extrapolating from a subchronic to chronic exposure duration, extrapolating from a LOAEL to a NOAEL, and database deficiencies.

A limited range of animal toxicology data is available for the hazard assessment of cerium oxide, as described throughout the previous sections (see Sections 4 and 5). For the oral route, human studies showing an association between exposure to cerium in food and the development of endomyocardial fibrosis are available. Long-term oral studies in animals are limited to a 13-month drinking water study in rats, which investigated the effects of a single dose group; a 6-month drinking water study in rabbits in which the administered dose consisted of a mixture of rare earth chlorides; a 12-week dietary study in mice; and a 105-day gavage study in rats. An RfD for cerium oxide and cerium compounds was not derived since the available studies were not suitable for quantitation of effects for various reasons, including unknown exposure concentrations in the available human studies, lack of a dose response, uncertain biological significance (e.g., changes in measures of oxidative stress), and study design (e.g., effects noted only under conditions of a restricted diet).

The inhalation database includes numerous case reports of workers who developed pneumoconiosis associated with accumulation of cerium particles in the lungs after prolonged occupational exposure to cerium fumes or dust and a single, 13-week subchronic inhalation bioassay of cerium oxide exposure in rats (BRL, 1994). The exposure concentrations utilized in the BRL (1994) study were higher than expected human environmental exposures to cerium oxide. Thus, the selection of the BRL (1994) as the principal study may overestimate the risk to cerium oxide particles. In addition, due to significant uncertainty related to toxicokinetic and toxicodynamic differences between soluble and insoluble cerium compounds, the RfC for cerium oxide should not be applied to other cerium compounds.

Consideration of the available dose-response data to determine an estimate of inhalation exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 13-week subchronic inhalation bioassay in Sprague-Dawley rats (BRL,

1994) and increased incidence of lymphoid hyperplasia in the bronchial lymph nodes of male and female rats as the principal study and critical effect for deriving the RfC for cerium oxide.

The mode of action for these lymphoreticular system effects, along with the observed pulmonary effects, is not known. The hypothesized mode of action for the pulmonary effects observed following cerium oxide exposure is the overloading of the PAMs by cerium oxide particles, leading to immobility of the PAMs in the alveoli, resulting in the sustained release of inflammatory cytokines and fibrogenic growth factors and subsequent cell damage. This proposed mode of action is based on the results from mechanistic studies, toxicity reviews of cerium oxide or similar compounds, in vitro cerium oxide and soluble cerium studies, intratracheal cerium oxide instillation studies, and human case reports. Additional data demonstrating particle overload following cerium oxide exposure, such as type II cell proliferation, altered alveolar epithelial integrity, and/or chronic acute inflammation with an influx of PMNs, are unavailable. The data demonstrating particle overload following exposure to cerium oxide were at relatively high doses. Thus, the mode of action for toxicity induced by chronic exposure to lower doses of cerium oxide may not be related to overloaded pulmonary clearance mechanisms, and the effects observed following low dose chronic exposures may also be different. An alternative mode of action may involve the presentation or delivery of cerium oxide particles to the bronchial lymph nodes from the tracheobronchial region of the lung by dendritic cells and subsequent induction of immune responses.

Based on the available evidence the effects observed in the lymph nodes and lungs of male and female rats following inhalation exposure to cerium oxide in the BRL (1994) study were considered to be indicative of overwhelmed pulmonary clearance and potential key events in the proposed MOA. However, as described in Section 4.6.3.1, evidence for overwhelmed pulmonary clearance as a mode of action for pulmonary and lymphoreticular system effects observed with inhalation exposure to cerium oxide relies on data from other relatively insoluble particles (such as titanium dioxide) due to the limited mechanistic data on cerium oxide. In the absence of evidence indicating otherwise, these effects are assumed to represent cerium oxide-induced toxicity.

The derived RfC was quantified using a LOAEL for the point of departure. A point of departure based on a LOAEL or NOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a point of departure defined as an effect level concentration (i.e., benchmark concentration [BMC]) obtained from benchmark dose-response modeling. In this assessment, the exposure-related increase in pigment accumulation and lymphoid hyperplasia in lymph nodes draining lungs, the bronchial and mediastinal lymph nodes, in combination with the hypothesized mode of action, the overloading of the PAMs by cerium oxide particles, further supports the role of pulmonary macrophages in

the nonspecific response. Thus, the lymphoid hyperplasia in the bronchial lymph nodes, which was observed in 80% of the exposed rats, may be an early manifestation of the nonspecific response to cerium oxide.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and its magnitude associated with the concentration at the point of departure in rodents are extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the effects observed following inhalation exposure to cerium oxide. For the RfC, a HEC was calculated from the point of departure by multiplying the $LOAEL_{ADJ}$ by a DAF. The calculated DAF in this assessment is an RDDR. The RDDR was calculated using the RDDR v.2.3 program, as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b).

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration, also, in extrapolating doses from a subset or smaller sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population. Human variation may be larger or smaller; however, cerium-specific data to examine the potential magnitude of over- or underestimation is unavailable.

Critical data gaps have been identified with uncertainties associated with regards to chronic toxicity, especially evidence demonstrating a persistence of lymphoid hyperplasia, and reproductive and developmental toxicity associated with inhalation exposure to cerium oxide. The available oral cerium exposure information in both humans and rats identifies cardiac tissue and hemoglobin oxygen affinity as possible health effects, but the animal studies are of insufficient duration and experimental design and the human studies do not provide exposure characterization. The lack of a study to derive an RfD given the possible effects demonstrated represents a critical data gap in the oral database. In addition, it is unclear whether the cerium oxide particles in the lung or lymph nodes can disassociate and release cerium systemically, possibly causing systemic effects, such as cardiotoxicity.

A chronic, cerium oxide inhalation exposure study in animals is unavailable; however, the workers in the human case reports were exposed to cerium oxide for periods of 10 to 46 years, and, while the case reports do not contain the necessary exposure analysis for dose response assessment, they do provide evidence of respiratory effects following long-term exposure to cerium oxide. There are limited data available addressing possible reproductive or neurodevelopmental toxicity following exposure to cerium. The accumulation of insoluble cerium particles in the respiratory tract of humans and animals following chronic and subchronic inhalation exposures, respectively, suggests that impaired clearance may influence pulmonary

toxicity in both rats and humans and limit systemic availability; therefore, possible reproductive or developmental toxicity may be expected to occur, if at all, at doses higher than those at which portal-of-entry effects occurred.

The RfC derived in this assessment is for cerium oxide particles with an MMAD of approximately 2.0 μm and a GSD of 1.8 to 1.9, and the application of the RfC to cerium oxide particles with smaller MMADs and GSDs, including nano-sized cerium particles is not recommended. The lack of data to support the derivation of an RfC for nano-cerium oxide is a significant area of uncertainty.

5.4. CANCER ASSESSMENT

Studies addressing the carcinogenic effects of cerium or cerium compounds upon which to base a cancer assessment are unavailable. Lundgren et al. (1996) observed lung neoplasms (7/1,049) in cerium oxide exposed control F344/N rats in an investigation of the carcinogenicity of the beta-particle emitter, ^{144}Ce. The incidence of lung neoplasms in the stable cerium oxide exposed rats was not compared to a treatment-free group or to historical background levels in F344/N rats.

As discussed in Section 4.7, data were unavailable regarding the carcinogenicity of stable cerium in humans or experimental animals. In accordance with U.S. EPA (2005a) guidelines for carcinogen risk assessment, there is "inadequate information to assess the carcinogenic potential" of cerium oxide and cerium compounds. Lack of carcinogenicity data precludes derivation of an oral slope factor or inhalation unit risk. Genotoxicity evidence was insufficient to assess the genotoxic potential. This overall lack of information represents a data gap and does not allow for a quantitative assessment of the carcinogenicity of cerium oxide and cerium compounds. A previous cancer assessment was not available for cerium oxide and cerium compounds.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Cerium oxide (CeO_2; ceric oxide) is used either in the pure form or in a concentrate as a polishing agent for glass mirrors, plate glass, television tubes, ophthalmic lenses, and precision optics (Kilbourn, 2003; Reinhardt and Winkler, 2002). Cerium oxide is used as a glass constituent to prevent solarization and discoloration (especially in the faceplates of television screens) (Reinhardt and Winkler, 2002). Cerium oxide is also used as a diesel fuel-born catalyst to reduce particulate matter emissions (HEI, 2001). Cerium is not expected to exist in elemental form in the environment since it is a reactive metal (Lewis, 2001). Cerium compounds are not expected to volatilize and will exist in the particulate form if released into the air.

Toxicokinetic studies in rodents have examined the absorption, distribution, metabolism, and elimination of cerium. In adult rats, cerium compounds are poorly absorbed following oral exposure, while suckling animals exhibit higher absorption and retention of cerium in the GI tissues (Kostial et al., 1989a, b; Inaba and Lengemann, 1972). Following inhalation exposure, cerium, a poorly-soluble particle, behaves like other airborne particles, depositing within the respiratory tract based on aerodynamic character (for review, see Schulz et al., 2000). Cerium has been detected in lung tissues and in alveolar macrophages of subjects believed to have been exposed occupationally (Vocaturo et al., 1983; Sabbioni et al., 1982), with cerium concentrations in the lung tissues 2,800–207,000 times higher than those found in the urine, blood, or nails (Pietra et al., 1985). The early clearance of the radioactive cerium administered dose suggests that the majority of cerium aerosol is deposited in the airways, where it is subject to removal via the mucociliary escalator, swallowing, and elimination in the feces.

Once absorbed into the body, cerium tends to accumulate primarily in the bone, liver, heart, and lung. Cerium has been observed to be localized in the cell, particularly in the lysosomes, where it is concentrated and precipitated in an insoluble form in association with phosphorus. As an element, cerium is neither created nor destroyed within the body. The particular cerium compound (e.g., cerium chloride, cerium oxide) may be altered as a result of various chemical reactions within the body, particularly dissolution, but data have not demonstrated a change in the oxidation state of the cerium molecule (Berry et al., 1989, 1988).

Following inhalation exposure, the initial rapid elimination of cerium from the body is due primarily to transport up the respiratory tract by the mucociliary escalator and eventual swallowing of the material, as with other poorly soluble particles (Boecker and Cuddihy, 1974). After the initial clearance of cerium particles from the upper respiratory tract, pulmonary clearance is slower, with reported slow-phase clearance half-times ranging from 100 to 190 days in rodents (Lundgren et al., 1974; Thomas et al., 1972; Morgan et al., 1970; Sturbaum et al.,

1970). Elimination of orally administered cerium has been shown to be age dependent in animals, with suckling animals absorbing cerium into the GI tissues (Inaba and Lengemann, 1972).

An epidemiological study reports a higher incidence of endomyocardial fibrosis among a population consuming tubers grown in a high cerium soil in India (Eapen, 1998; Kutty et al., 1996; Valiathan et al., 1989), and a case control study found an association between increased toenail cerium concentrations and the risk of first myocardial infarction (Gómez-Aracena et al., 2006). The few long-term animal studies available included dietary, gavage, and drinking water administrations, although clear indicators of health effects were not evident.

Numerous case reports have been published describing cases of workers who developed adverse lung effects, such as interstitial lung disease or pneumoconiosis, associated with accumulation of cerium in the lungs after prolonged occupational exposure to cerium fumes or dust (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). The human cases of cerium exposure demonstrate the accumulation of cerium particles in the lungs and lymphoreticular system, with pulmonary function varying from normal to severe restriction and interstitial fibrosis in one case and granulomas in another. Interstitial fibrosis accompanied by vascular thickening, reactive alveolar macrophages, abundant macrophages in the airspace, and moderate chronic interstitial inflammation, along with small interstitial clumps of macrophages bearing scant deposits of grayish-black pigment, was observed in a 68-year-old man who was employed as an optical lens grinder for 35 years (McDonald et al., 1995). Additionally, particles characterized as cerium were identified in alveolar macrophages, macrophages in the tracheobronchial lymph nodes, and lung and lymph node tissue (Porru et al., 2001; Pairon et al., 1995; Waring and Watling, 1990; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985). Cerium exposure was associated with interstitial, peribronchial, and perivascular fibrosis, a restriction of respiratory function, and/or pulmonary hypertension (Porru et al., 2001; Pairon et al., 1995, 1994; Vogt et al., 1986; Vocaturo et al., 1983; Sabbioni et al., 1982).

Data from a subchronic toxicity test in Sprague-Dawley rats (BRL, 1994) identified an increased incidence of lymphoid hyperplasia in the bronchial lymph nodes as the critical effect for noncancer effects. Histologic examination revealed dose-related alveolar epithelial and lymphoid hyperplasia and pigment accumulation in the lungs, lymph nodes, and larynx of male and female rats at $\geq$5 mg/m^3.

The mode of action for cerium-oxide induced lung and lymphoreticular system effects observed in rats is not known. However, evidence suggests that the overloading of PAMs by cerium oxide particles at high doses, leading to immobility of PAMs in the alveoli, resulting in

the sustained release of inflammatory cytokines and fibrogenic growth factors and subsequent cell damage may be a plausible mode of action for these effects. The accumulation of insoluble cerium oxide particles in the respiratory tract of humans and rodents following chronic and subchronic exposure, respectively, suggests that impaired clearance may influence pulmonary toxicity for rats and humans. A population of immobilized, activated macrophages may serve to induce significant cell damage by effectively increasing the concentration of inflammatory cytokines and fibrogenic growth factors within the pulmonary epithelium.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The database for oral exposure to cerium is limited to geographical distribution studies evaluating the possible association between exposure to cerium in food and the development of endomyocardial fibrosis (Eapen et al., 1998; Kutty et al., 1996; Valiathan et al., 1989), and long-term animal studies, including a 13-month drinking water study in rats (Kumar et al., 1996), a 6-month drinking water study in rabbits (Kartha et al., 1998), a 12-week dietary study in mice (Kawagoe et al., 2005), and a 105-day gavage study in rats (Cheng et al., 2000).

The geographical distribution studies suggest that there is an association between exposure to cerium in food and the development of endomyocardial fibrosis but are unsuitable for RfD derivation. The above animal studies are unsuitable for derivation of an RfD for various reasons. The 13-month study by Kumar et al. (1996) was conducted on a small number of rats at only one dose level (control and dosed rats) and evaluated few endpoints, and the health effects were highly variable and not statistically significant. Kartha et al. (1998) conducted a 6-month drinking water study in rabbits that utilized a rare earth chloride mixture in the drinking water, which suggests that cerium may enhance the effect of magnesium deficiency in heart tissue. The Cheng et al. (2000) study was limited in scope, since it investigated the effects of cerium chloride exposure on the structure and affinity of hemoglobin in rats. The evaluations conducted in Kawagoe et al. (2005), showing statistically significant changes as a result of cerium exposure (e.g., decreased lipoperoxide levels), increased GSH levels and MT activity, and decreased SOD activity, are of unknown biological significance and may represent oxidative stress in response to cerium exposure.

The available oral cerium exposure information in both humans and rats identifies cardiac abnormalities and changes in hemoglobin oxygen affinity as possible effects, but the animal studies are of insufficient duration and experimental design and the human studies do not provide adequate exposure characterization. The lack of a study to derive an RfD given the effects demonstrated represents a critical data gap in the oral database.

6.2.2. Noncancer/Inhalation

There are numerous case reports of workers who developed pneumoconiosis, associated with accumulation of cerium particles in the lungs, after prolonged occupational exposure to cerium fumes or dust (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). Information regarding long-term inhalation exposure in animals is derived from a single subchronic study in rats (BRL, 1994). Sprague-Dawley rats were exposed nose only to cerium oxide aerosol 6 hours/day, 5 days/week for 13 weeks. Endpoints evaluated included a functional observational battery, hematology and clinical chemistry, urinalysis, and gross and microscopic morphology of tissues.

Consideration of the available dose-response data to determine an estimate of inhalation exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 13-week subchronic inhalation bioassay in Sprague-Dawley rats (BRL, 1994) and increased incidence of lymphoid hyperplasia in the bronchial lymph nodes of male and female rats as the principal study and critical effect for deriving the RfC for cerium oxide. EPA selected lymphoid hyperplasia in the bronchial lymph nodes as the critical effect because it was determined that this effect represents the most sensitive endpoint occurring at the lowest dose that was indicative of lung and lymphoreticular system toxicity. Based on the available evidence the effects observed in the lymph nodes and lungs of male and female rats following inhalation exposure to cerium oxide in the BRL (1994) study were considered to be indicative of overwhelmed pulmonary clearance and potential key events in the proposed MOA. However, as described in Section 4.6.3.1, evidence for overwhelmed pulmonary clearance as a mode of action for pulmonary and lymphoreticular system effects observed with inhalation exposure to cerium oxide relies on data from other relatively insoluble particles (such as titanium dioxide) due to the limited mechanistic data on cerium oxide. In the absence of evidence indicating otherwise, these effects are assumed to represent cerium oxide-induced toxicity.

The RfC was derived using a LOAEL for the point of departure. A point of departure based on a LOAEL or NOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a point of departure defined as an effect level concentration (i.e., BMC) obtained from benchmark dose-response modeling. In this assessment, the exposure-related increase in pigment accumulation and lymphoid hyperplasia in lymph nodes draining lungs (the bronchial and mediastinal lymph nodes), in combination with the hypothesized mode of action (the overloading of the PAMs by cerium oxide particles), further supports the role of pulmonary macrophages in the nonspecific response. Thus, the lymphoid hyperplasia in the bronchial lymph nodes is an early manifestation of the nonspecific response to cerium oxide.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and its magnitude associated with the concentration at the point of departure in rodents are extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible for the toxicity observed following inhalation exposure to cerium oxide. For the RfC, an HEC was calculated from the point of departure by multiplying the $LOAEL_{ADJ}$ by a DAF. The calculated DAF in this assessment is an RDDR. The RDDR was calculated using the RDDR v.2.3 program, as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b).

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration, also, in extrapolating dose from a subset or smaller-sized population, say of one sex or a narrow range of life stages typical of occupational epidemiologic studies, to a larger, more diverse population. Human variation may be larger or smaller; however, cerium-specific data to examine the potential magnitude of over- or underestimation are unavailable.

The RfC of 2×10^{-4} mg/m^3 was calculated from a $LOAEL_{HEC}$ of 0.48 mg/m^3 for increased incidence of lymphoid hyperplasia in the bronchial lymph nodes in male and female rats following subchronic cerium oxide inhalation exposure (BRL, 1994). A total UF of 3,000 was used: 3 for interspecies extrapolation, 10 for intraspecies variability, 10 for subchronic to chronic extrapolation, 3 for extrapolating from a LOAEL to a NOAEL, and 3 for database deficiencies.

A factor of 3 was selected to account for uncertainties in extrapolating from rats to humans, which is adopted by convention where an adjustment from an animal-specific $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Insufficient information is available to predict potential variability in susceptibility among the population, thus a human variability UF of 10 was applied. A 10-fold UF was used to account for uncertainty in extrapolating from a subchronic to chronic exposure duration. A 3-fold UF was applied to account for the extrapolation from a LOAEL to NOAEL because the POD was a LOAEL. The critical effect for this analysis, lymphoid hyperplasia in the bronchial lymph nodes, represents a minimally biologically significant effect; therefore, an uncertainty factor of 3 was applied. Data gaps have been identified with uncertainties associated with database deficiencies with regards to reproductive and developmental toxicity associated with cerium inhalation exposure. A database UF of 3 was applied with special consideration of the information pertaining to the deposition and absorption of cerium oxide, the effects observed in humans following prolonged exposure, the mode-of-action data, and the effects observed in animals in the BRL (1994) study, in addition to the lack of reproductive and developmental studies.

The overall confidence in this RfC assessment is low. Confidence in the principal study (BRL, 1994) is medium. EPA conducted an external peer review to evaluate the accuracy of the experimental procedures, results, and interpretation and discussion of the results presented in this study. The peer reviewers considered the BRL (1994) study conclusions to be supported by the data. The peer reviewers were not specifically asked to comment on their confidence in the study. In addition, the results observed in the BRL (1994) study were consistent with the observed effects in the human case reports (Yoon et al., 2005; Porru et al., 2001; McDonald et al., 1995; Pairon et al., 1995, 1994; Sulotto et al., 1986; Vogt et al., 1986; Pietra et al., 1985; Vocaturo et al., 1983; Sabbioni et al., 1982; Husain et al., 1980; Kappenberger and Bühlmann, 1975; Heuck and Hoschek, 1968). Confidence in the database is low. The database lacks chronic exposure information on cerium via any route of exposure and multigenerational developmental and reproductive toxicity studies. However, there is evidence of cerium pneumoconiosis in humans exposed to cerium compounds, and the anticipated critical effects observed are point-of-entry effects that would be expected in humans. Reflecting medium confidence in the principal study and low confidence in the database, confidence in the RfC is low.

6.2.3. Cancer/Oral and Inhalation

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for cerium oxide and cerium compounds is inadequate to assess the human carcinogenic potential and to calculate quantitative cancer risk estimates.

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of cerium oxide and cerium compounds has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a). The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

On April 10, 2008, EPA introduced revisions to the IRIS process for developing chemical assessments. As part of the revised process, the disposition of peer reviewer and public comments, as found in this Appendix, and the revised IRIS Toxicological Review was provided to the external peer review panel members for their comment on October 15, 2008. Any additional peer review panel comments received as part of this second review and EPA's responses are included at the end of this Appendix. On March 20, 2009, the external peer review panel members were given an additional opportunity to comment on the revised IRIS Toxicological Review. The comments from this third review are also included at the end of this Appendix.

EXTERNAL PEER REVIEW PANEL COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

In addition, the external peer reviewers commented on decisions and analyses in the Toxicological Review under multiple charge questions, and these comments were organized under the most appropriate charge question.

A. General Comments

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Comment: The reviewers concluded that the Toxicological Review was logical, clear,

and concise, and that EPA has accurately, clearly, and objectively represented and synthesized the scientific information. The Toxicological review was characterized as 'repetitive' by some of the reviewers. One reviewer stated that the synthesis of the scientific evidence for noncancer hazard appears to be more subjectively represented and is in part more speculative than proven by the data.

Response: EPA has reviewed and modified the document to address the reviewer concerns about the repetitious nature of the document.
The evidence provided by the human case reports and the BRL (1994) study is sufficient to formulate a hazard identification. The only available study reporting low-dose effects of subchronic inhalation exposure to cerium oxide in animals, BRL (1994), was chosen as the principal study. This study was well designed with three dose groups of 30 animals per group per sex. Numerous tissues and endpoints were assessed, and methods and observed effects were thoroughly reported. This study identified statistically significant dose-dependent effects on the lungs and lymphoreticular system in both male and female rats. The observed effects included increased lung weight; discoloration or pale areas, pale foci, and uncollapsed parenchyma in the lungs; enlargement or pale discoloration of the bronchial, mediastinal, and pancreatic lymph nodes; and dose-related alveolar epithelial and lymphoid hyperplasia and pigment accumulation in the lungs and lymph nodes. The lung and lymphoreticular system effects observed by BRL (1994) are consistent with effects observed in humans, that were characterized by the accumulation of cerium particles in the lungs and lymphoreticular system and histologic effects throughout the lung. Thus, EPA selected this study as the principal study for the derivation of the RfC and chose lymphoid hyperplasia in the bronchial lymph nodes as the critical effect because it was determined that this effect represents the most sensitive endpoint occurring at the lowest dose that was indicative of lung and lymphoreticular system toxicity.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of cerium oxide and cerium compounds.

Comment: The following references demonstrating the hepatic toxicity of soluble $CeCl_2$ injected intravenously were recommended by a reviewer:

Hirano, S. and K.T. Suzuki. Exposure, metabolism, and toxicity of rare earths and related compounds. Env. Hlth. Perspect. 104 Suppl. 1: 85-95, 1996

Snyder, F. Cress, E.A., Kyker, G.C. Liver lipid response to intravenous rare earths in rats. J. Lipid Res. 1: 125 - 1131, 1959

Response: The references cited in Hirano and Suzuki (1996) that address cerium-induced liver toxicity are referenced in Section 4.4.2.3, Hepatic Effects, of the draft Toxicological Review. Hirano and Suzuki (1996) was not added to the document because it is a review and the references presented in the article are already included in the document. Snyder et al. (1959) was added to Section 4.4.2.3, *Hepatic Effects*.

Comment: The following references were recommended by a reviewer; the first reference provides in vitro data for nano-sized CeO_2 and the second reference is a new study of the ingestion of $CeCl_3$ by mice.

EJ Park, J Choi, YK Park, K Park, "Oxidative stress induced by cerium oxide nanoparticles in BEAS-2B cells." Toxicology 245 p 90-100, 2008

Kawagoe et al. J Trace Elem Med Bio (2008)

Response: Both references were added to the document. The Park et al. (2008) reference was added to Section 4.5.3, *In Vitro and Ex Vivo Nanotoxicology Studies*. The Kawagoe et al. (2008) reference was added to Section 4.3, *Reproductive/Developmental Studies – Oral and Inhalation* and Section 4.4.1, *Acute Toxicity Studies (Oral and Inhalation)*.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of cerium oxide.

Comment: Reviewers identified the following areas of research that would likely increase the confidence in the database:

- Study with a longer timeframe than 13-weeks to provide clearer support for the current choice for the "critical effect"
- Study using an exposure level in the range of 0-5 mg CeO_2/m^3 to permit a narrower point of departure to be generated
- A new investigation should include an analysis of pulmonary immunologic endpoints and of cerium burdens in the various lymph nodes
- Improved animal study with concentrations beginning at the lower end of the range of the BRL (1994) study and extend downward toward a plausible environmental range

- Studies related to the biokinetics of cerium compounds, in particular with specific and different particle sizes, and determine alveolar macrophage clearance function in an effort to determine the cause of particle overload
- Animal bioassay of soluble forms of cerium to determine systemic absorption and target organ effects, with evaluations of cardiotoxicity, hepatotoxicity, and renal toxicity
- More comprehensive animal exposure study that uses ultrafine cerium oxide and includes more sensitive toxicological endpoints

Response: EPA agrees that the above research recommendations would improve future hazard identifications of cerium compounds.

4. Please comment on the identification and characterization of sources of uncertainty in Sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Comment: The relevance of the BRL (1994) study to environmental exposures is an area of uncertainty that a reviewer highlighted as being inadequate.

Response: Text has been added to Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*, addressing uncertainty associated with the high exposure concentrations of the BRL study and the potential for overestimating risk.

Comment: Another area of uncertainty that was inadequately discussed was the importance of particle size-related differences in the biokinetics and potential effects, especially for nano-cerium oxide.

Response: Text has been added to Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*, discussing the uncertainty of the RfC and it's application to nano-sized cerium oxide. However, data are insufficient to derive an RfC for nano-cerium oxide, and the RfC derived in the draft document is for cerium oxide particles with an MMAD of approximately 2.0 μm and a GSD of 1.8 to 1.9.

Comment: More discussion is also needed highlighting the differences between effects elicited in the alveolar region of the lung and the conducting airways (tracheobronchial region).

Response: Effects were observed in the alveolar region of the lung and in the bronchial lymph nodes of rats exposed to cerium oxide aerosol for 13-weeks. The bronchial lymph nodes, in addition to the mediastinal lymph nodes, are part of the lymphatic system that collects particles from the lung as a whole. The exposure-related increase in pigment accumulation and lymphoid hyperplasia in the lymph nodes draining the lung supports the role of pulmonary macrophages in the clearance of particles deposited in the lung. The deposition of cerium oxide particles in the pulmonary region of the lung may lead to pulmonary alveolar macrophage (PAM) activation and migration of PAMs containing cerium oxide particles to the tracheobronchial lymph nodes.

Comment: One reviewer recommended that the discussion of the mode of action should be limited to situations where exposure to insoluble cerium oxide is at high doses. In addition, this reviewer suggested that the uncertainty for the derived RfC increases when it is applied generally to cerium compounds.

Response: Text has been added to Section 5.3, *Uncertainties in the Oral Reference Dose and Reference Inhalation Concentration*, stating that the proposed mode of action for toxicity induced by lower dose chronic exposures to cerium oxide may not be related to overloaded pulmonary clearance mechanisms, and the effects observed following low dose chronic exposures may also be different.

The derived RfC, 2×10^{-4} mg/m^3, is not recommended for cerium compounds other than cerium oxide. Text has been added to Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*, addressing uncertainty associated with the application of the derived RfC to cerium compounds other than cerium oxide.

B. Oral Reference Dose (RfD) for cerium oxide and cerium compounds

1. A chronic RfD for cerium compounds has not been derived. Has the scientific justification for not deriving an RfD been transparently and objectively described? Please identify and

provide the rationale for any studies that should be selected as the principal study. Please identify and provide the rationale for any endpoints that should be considered in the selection of the critical effect.

Comments: The reviewers did not identify any studies for consideration as the primary study or endpoints for selection as the critical effect. One reviewer commented that the available studies indicate potential target organs (cardiac and hepatic) for toxicity following oral exposure to soluble cerium compounds.

Response: While there may be potential for cardiac effects following oral exposure, the cardiac effects were observed in conjunction with a magnesium-restricted diet. An association between exposure to rare earth elements, cerium in particular, in food and the development of endomyocardial fibrosis in humans has been suggested (Eapen, 1998; Kutty et al., 1996; Valiathan et al., 1989); although, analysis of the geographic distribution of endemic endomyocardial fibrosis in India suggested a link to high cerium soil concentrations and possibly to magnesium deficiency during childhood (Kutty et al., 1996).

Kartha et al. (1998) suggested that cerium chloride may intensify the adverse cardiac effects of magnesium deficiency; however, the authors reported that cerium may intensify the cardiotoxicity associated with a magnesium-deficient or restricted diet but did not elicit a cardiac effect when tested under conditions of a normal magnesium diet.

Parenteral administration of cerium to rodents caused lipid deposition, mitochondrial damage, and invaginations of the nuclear membrane in hepatocytes, as well as morphological changes in the liver, characterized by fatty liver, fatty degeneration, and necrosis. Unfortunately, these effects were not observed following oral administration of cerium chloride and do not inform the oral hazard identification analysis.

C. Inhalation Reference Concentration (RfC) for cerium oxide and cerium compounds

1. A chronic RfC for cerium oxide has been derived from the 13 week inhalation study (BRL, 1994) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Are the criteria and rationale for the selection of this study transparently and objectively described in the document? Please identify and provide the rationale for any other

studies that should be selected as the principal study.

Comment: One reviewer commented that the study was not adequately justified because the lowest exposure concentration used in the study was not environmentally relevant, and is a concentration that would predictably cause nonspecific effects due to particle overload. In addition, the reviewer stated that existing knowledge would not support the notion that 2 μm diameter CeO_2 as relevant to the most likely target form of cerium oxide.

Response: Text was added to Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*, of the document addressing the utilization of high exposure concentrations in the BRL (1994) study and the possibility that the utilization of the BRL study may overestimate the risk to cerium oxide particles.

It should be noted that while the low dose in the BRL (1994) study was considered high in comparison to environmental concentrations, it should not be assumed to predictably cause nonspecific effects due to pulmonary overload. For example, $BaSO_4$, at higher exposure levels than those of the BRL (1994) study, did not elicit a nonspecific overload response, whereas, pulmonary overload was demonstrated by TiO_2 at similar exposures but higher particle surface area. It was hypothesized that the particle surface area of $BaSO_4$ ($BaSO_4$ has a low specific surface area) influences the level of inflammation (Tran et al., 2000). The data for TiO_2 and $BaSO_4$ suggests that a high particle exposure level does not necessarily lead to a pulmonary overload response. In the case of cerium oxide, the exposure was such that pulmonary clearance was overloaded.

The derived RfC is relevant to 2 μm diameter CeO_2 particles and this is specified in Section 5.2.3, *RfC Derivation-Including Application of Uncertainty Factors*. Data are currently unavailable to better evaluate the hazard identification and dose-response of nano-sized cerium oxide.

2. Increased incidence of lymphoid hyperplasia in the bronchial lymph nodes of male rats was selected as the critical toxicological effect. The selection of increased incidence of lymphoid hyperplasia in the bronchial lymph nodes as the critical effect for cerium oxide is because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed

explanation. Please comment on whether EPA's rationale about the adversity of the critical effect has been adequately and transparently described and is supported by the available data. Please identify and provide the rationale for any other endpoints that should be used instead of lymphoid hyperplasia to develop the RfC.

Comment: Several reviewers disagreed with the selection of lymphoid hyperplasia in the bronchial lymph nodes as the critical effect and recommended using the alveolar hyperplasia as the critical effect. One reviewer stated that it was only weakly sufficient to claim that the lymphoid hyperplasia in the bronchial lymph nodes as the "critical" effect, while another questioned whether the data supported the conclusion that lymphoid hyperplasia in bronchial lymph nodes was a precursor effect. Several reviewers questioned the proposed mode of action for the critical effect, stating that lymphoid hyperplasia is not known to be on the pathogenic pathway to more clearly adverse effects and may not be adverse itself. The reviewers commented that the alveolar epithelial hyperplasia in the lungs may be relevant to progressive adverse effects.

Response: BRL (1994) identified statistically significant dose-dependent effects on the lungs and lymphoreticular system in both male and female rats. The lung and lymphoreticular system effects observed by BRL (1994) are consistent with effects observed in humans, that were characterized by the accumulation of cerium particles in the lungs and lymphoreticular system and histologic effects throughout the lung. EPA has selected lymphoid hyperplasia in the bronchial lymph nodes as the critical effect because it was determined that this effect represents the most sensitive endpoint occurring at the lowest dose that was indicative of lung and lymphoreticular system toxicity.

The mode of action for pulmonary toxicity in humans following chronic inhalation of cerium oxide is unknown. The proposed mode of action is based on the results from, in general, mode of action studies, toxicity reviews of cerium oxide or similar compounds, in vitro cerium oxide and soluble cerium studies, intratracheal cerium oxide instillation studies, and human case reports; however, additional data demonstrating particle overload following cerium oxide exposure, such as type II cell proliferation, altered alveolar epithelial integrity, and/or chronic acute inflammation with an influx of PMNs, are unavailable. In addition, the evidence for overwhelmed pulmonary clearance as a mode of action for pulmonary effects observed with inhalation exposure to cerium oxide relies on data from other relatively insoluble particles (such as

titanium dioxide) due to the limited mechanistic data on cerium oxide. Nevertheless, the effects observed in the lymph nodes and lungs of male and female rats following inhalation exposure to cerium oxide in the BRL (1994) study were considered to be indicative of overwhelmed clearance and potential key events in the proposed MOA. In the absence of evidence indicating otherwise, these effects were considered to represent cerium-induced toxicity.

3. Some mode of action evidence exists suggesting that lymphoid hyperplasia in the bronchial lymph nodes represents a sensitive endpoint that occurs early in a series of critical events leading to more severe effects in the lung. Specifically, the data suggest that lymphoid hyperplasia in the bronchial lymph nodes may represent the point at which normal clearance of particles from the lung by alveolar macrophages becomes overwhelmed and particles are no longer cleared effectively. This delayed clearance leads to increased accumulation of cerium oxide particles in the respiratory tract, an inflammatory response, and subsequent cell proliferation. Please comment on whether the available mode of action data supports this proposed MOA for cerium oxide-induced bronchial lymphoid hyperplasia. Is this proposed MOA scientifically justified and transparently and objectively described?

Comment: One reviewer commented that the mode of action is not justified based on the sole evidence provided in the BRL (1994) study. Specifically, the hyperplasia may be suggestive of problems with the clearance of the particles out of the lymph nodes and not necessarily the airways. Another reviewer commented that the BRL study does not give any evidence of impaired clearance nor does it provide evidence of other key results related to dust overload, such as type II cell proliferation, altered alveolar epithelial integrity, and/or chronic acute inflammation with an influx of PMNs, and recommended the addition of an alternative mode of action to the discussion. The alternative mode of action proposed included an immune response due to the cerium oxide particles delivered by dendritic cells from the tracheobronchial region to the bronchial lymph nodes. A third reviewer stated that it is not certain that hyperplasia in lung-associated lymph nodes is a direct result of overloading exposures. A reviewer commented that chronic exposure to lower doses could cause effects via a different mode of action. Finally, a reviewer also expressed concern that the lymphoid hyperplasia may be a non-specific response to particles and not strictly an effect of cerium oxide.

Response: The proposed mode of action analysis, while including a discussion of the

results from the BRL (1994) study, also incorporated pulmonary overload and particle clearance studies, toxicity reviews, in vitro studies, intratracheal instillation studies, and the pulmonary effects observed in human studies to propose a potential mode of action for cerium oxide toxicity. The mode of action analysis included a discussion of the concept of dust overload in the lung following inhalation exposure, an example of overwhelmed pulmonary clearance following exposure to titanium dioxide, the comparison of symptoms of pulmonary overload with effects observed in the BRL (1994) study, and an analysis of cellular responses to insoluble particles and the relationship to pulmonary overload. EPA recognizes that the proposed mode of action is based, in part, on data for other insoluble particles (titanium dioxide for example), and that there is uncertainty for the mode of action due to the lack of comprehensive data from cerium oxide on some aspects of the mode of action. Text was added to Section 5.3, *Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration*, discussing the absence of key pulmonary overload data.

Chronic exposure to lower doses of cerium oxide may cause effects via a different mode of action; however, data and/or information on an alternative mode of action at lower doses are unavailable. Text was added to Section 5.3, *Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration*, stating that lower doses of cerium oxide may act through a different mode of action.

The delivery of cerium oxide particles to bronchial lymph nodes from the tracheobronchial region of the lung by dendritic cells is a proposed alternative mode of action. Text has been added to Section 4.6.3.1 *Respiratory Tissues* addressing this potential alternative mode of action.

In response to the External Peer Review comments, the critical effect has been reevaluated. However, EPA has selected lymphoid hyperplasia in the bronchial lymph nodes as the critical effect because it was determined that this effect represents the most sensitive endpoint indicative of lung and lymphoreticular system toxicity occurring at the lowest dose. Please see Section 5.2.1, *Choice of Principal Study and Critical Effect—with Rationale and Justification*, for a discussion on the selection of the critical effect.

The proposed mode of action for cerium oxide may, in fact, be a non-specific response to cerium oxide in the lung, i.e. the physical presence of cerium oxide in the lung leading to pulmonary toxicity. The available data support the proposed mode of action of overloading of PAMs by cerium oxide particles, leading to the release of inflammatory cytokines and fibrogenic growth factors and subsequent cell damage, observed as alveolar epithelial hyperplasia in the lung. However, the mode of action for

pulmonary toxicity in humans following chronic inhalation of cerium oxide is not known.

4. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to define the point of departure. Please provide comments with regards to whether this is the best approach for determining the point of departure. Please identify and provide rationale for any alternative approaches for the determination of the point of departure, and if such approaches are preferred to EPA's approach.

Comment: Four of the reviewers stated that the use of the NOAEL/LOAEL approach to define the point of departure was a reasonable selection and that this decision was adequately justified. Several reviewers stated that data for alveolar epithelial hyperplasia should be selected as the critical effect for determination of the point of departure using benchmark dose modeling.

Response: EPA has selected lymphoid hyperplasia in the bronchial lymph nodes as the critical effect. Please see response to comment under charge question C.2.

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfC. For instance, are they scientifically justified and transparently and objectively described in the document?

Comment: One reviewer commented that the database uncertainty factor (UF_D) should consider and address the issue of nano-cerium oxide, which may give rise to systemic effects that could not be observed with the larger particle size used in the BRL (1994) study.

Response: The database uncertainty factor is applied to the point of departure for cerium oxide particles with an MMAD of approximately 2.0 μm and a GSD of 1.8 to 1.9. It would not be appropriate to apply a database UF to this point of departure for lack of data for another particle size. Text was added to Section 5.3 *Uncertainties in the oral reference dose (RfD) and inhalation reference concentration (RfC)* was added to address the lack of nano-particle cerium oxide data as an uncertainty in the assessment.

Comment: One reviewer commented that the uncertainty factors are adjustments for an outcome that is probably not relevant to environmental exposures. Additionally, a

reviewer commented that the Toxicological Review has taken a response to a high exposure level, applied a large combined uncertainty factor, and ended up with a reference concentration comparable to the values for better studied materials with well-demonstrated toxicity.

Response: Please see first comment and response under A.4. for a discussion of the hazard identification and relevancy to environmental exposures.

In this draft document, uncertainty factors have been applied to a point of departure for the critical effect from the principal study to derive a reference concentration for cerium oxide.

Comment: A reviewer recommended including a discussion of magnesium deficiency and susceptibility to cardiotoxic effects of cerium. The document would be strengthened by referring to these studies as a potential mechanism causing increased risk within human subpopulations.

Response: The studies suggesting magnesium deficiency might increase susceptibility to cardiotoxic effects of cerium chloride were not included in the discussion of the intraspecies uncertainty factor for cerium oxide. The studies that demonstrated that magnesium deficiency might increase the susceptibility to cardiotoxic effects of soluble cerium chloride exposure are oral studies, and the qualitative extrapolation of these results to the derivation of the inhalation RfC is not supported. The cerium compound in the oral studies is cerium chloride, a soluble cerium compound, while the RfC was derived for cerium oxide, an insoluble cerium compound. The difference in solubility may have a significant effect on the toxicokinetics and toxicodynamics of the cerium compound, and qualitatively extrapolating the potential for cardiac effects from the oral studies of cerium chloride to the portal-of-entry effects following cerium oxide exposure via inhalation is not appropriate. Text has also been added to Section 4.8.3, *Possible Susceptible Populations*, highlighting magnesium deficiency and susceptibility to cardiac effects.

6. Please comment on the transparency, scientific rationale and justification for the LOAEL-to-NOAEL uncertainty factor of 3. Are the criteria and rationale for this selection transparently and objectively described in the document? The point of departure for this analysis was based on the critical effect of lymphoid hyperplasia in the bronchial lymph nodes. This effect is described as a

sensitive effect occurring early in the series of critical events leading to more severe effects in the lung, and hence a default 10-fold uncertainty factor was not applied. The mode of action for the critical effect is thought to be related to pulmonary clearance overload, in which normal clearance of particles from the lung by alveolar macrophages becomes overwhelmed and particles are no longer cleared effectively, leading to an increasing accumulation of particles in the lung and airways, an inflammatory response, and subsequent cell proliferation. Please comment on whether the justification for selection of the LOAEL-to-NOAEL uncertainty factor based on these data is scientifically justified and transparently described.

Comment: One reviewer commented that the underlying premise for the choice of the point of departure, and a UF_L of 3, was insufficiently justified based on the critical effect selected and mode of action proposed. One reviewer commented that because the lymphoid hyperplasia in the bronchial lymph nodes does not show a dose response in the BRL (1994) study, a UF_L of 10 should also be considered.

Response: The BRL (1994) study and human case reports support the conclusion that lymphoid hyperplasia in the bronchial lymph nodes represents the most sensitive effect that may occur early in a series of critical events leading to more severe effects in the lung. The critical effect along with other effects observed in the lungs and lymph nodes may be representive of disruption of the normal clearance of particles from the lung by alveolar macrophages. Specifically, alveolar macrophages become overwhelmed and particles are no longer cleared effectively. Delayed clearance could possibly lead to increased accumulation of cerium oxide particles in the respiratory tract, an inflammatory response, and subsequent cell proliferation. A factor of 3 was used to account for uncertainty in extrapolating from a LOAEL to a NOAEL (UF_L) because the point of departure was a LOAEL. The critical effect selected to determine the point of departure for this analysis, lymphoid hyperplasia in the bronchial lymph nodes was considered to be minimally biologically significant; therefore, an uncertainty factor of 3 was applied.

Comment: One reviewer was not sure that the lymphoid hyperplasia represents the point at which normal clearance of particles from the lung by macrophages has become overwhelmed. The reviewer proposed an alternative mode of action in which an immune response is initiated by cerium-oxide particles translocated to the lymph node by dendtritic cells. The LOAEL-to-NOAEL uncertainty factor can be avoided by using alveolar epithelial hyperplasia as the critical effect and benchmark dose modeling.

Response: Please see comment under charge question C.2. and the corresponding response. Text addressing an alternative mode of action was added to the mode of action discussion in Section 4.6.3.1, *Respiratory Tissues*.

7. Please comment on the transparency, scientific rationale and justification for the selection of the database uncertainty factor. Please comment on whether the application of the database uncertainty factor adequately addresses the lack toxicity data for cerium oxide. Specifically, please comment on whether studies addressing additional endpoints of concern (e.g. reproductive and developmental toxicity studies) would likely result in a lower point of departure. Are the criteria and rationale for this selection transparently and objectively described in the document? An uncertainty factor of 3 was applied with special consideration of the information pertaining to the deposition and absorption of cerium oxide, the effects observed in humans following prolonged exposure, the mode of action data, and the similar effects observed in animals in the principal study.

Comment: One reviewer commented that a lower point of departure might be developed if studies assessing immunologic endpoints in the lung were available.

Response: While it is possible that additional immunologic studies may lead to a lower point of departure, there are no data to inform this assumption. No changes have been made in response to the comment.

Comment: One reviewer suggested EPA consider different particle sizes of cerium oxide rather than just focusing on what size happened to be used in the BRL (1994) study. For example, the reviewer recommended modeling the deposition of inhaled cerium oxide particles at different sizes by using the MPPD deposition model and assuming normal clearance in rats and humans.

Response: While toxicity resulting from particle overload in the lungs may be expected with other micro-sized cerium oxide particles, the toxicity and mode of action of nano-sized particles is unknown. Extrapolating the toxicity resulting from exposure to micro-sized cerium oxide to nano-sized cerium oxide exposures would likely impart significant uncertainty. It was not undertaken as a part of the assessment.

Comment: One reviewer commented that the lack of a lifetime exposure/recovery study in a second animal species with a longer lifetime is a major shortcoming since 1) progressive effects (such as fibrosis and lung cancer) cannot be observed in from short term studies, and 2) it's unclear whether the particles in the lung and/or lymph nodes slowly release cerium systemically, possibly causing cardiotoxicity over time.

Response: The lack of a lifetime exposure/recovery study is a data gap in this assessment. In considering this data gap, an uncertainty factor of 10 was used to account for the extrapolation from a subchronic to chronic exposure duration. The lack of a chronic study is also addressed in Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*.

Cerium as an element is a strong oxidizing agent and is stabilized when associated with an oxygen ligand (Kilbourn, 2003). Text was added to Section 5.3, *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)* presenting the possibility for systemic effects, including cardiotoxicity, following inhalation absorption.

8. The RfC has been derived using data from inhalation exposure to cerium oxide (BRL, 1994). Is the statement to not use the RfC for cerium compounds other than cerium oxide scientifically justified? Is there enough information on and discussion of cerium compounds to warrant the title "cerium oxide and cerium compounds?"

Comment: One reviewer commented that the end of the document needs additional discussion of cerium oxide as the most critical compound of exposure (given the solubility of cerium salts) because of the long retention as a particle, provided low inhaled concentrations are present.

Response: Text was added in Section 6.1, *Human Hazard Potential*, to highlight cerium oxide exposures as the most relevant of the cerium compounds.

Comment: One reviewer commented that statements in the text drawing conclusions or making generalizations should specifically state the cerium compound being discussed or reference the solubility of the cerium.

Response: The document has been revised, where feasible, in order to specifically state the cerium compound being discussed by each reference article.

D. Carcinogenicity of cerium oxide and cerium compounds

1. Under the EPA's 2005 Guidelines for carcinogen risk assessment (www.epa.gov/iris/backgr-d.htm), there is "inadequate information to assess the carcinogenic potential" of cerium compounds. Please comment on the scientific justification for the cancer weight of the evidence characterization. Has the scientific justification for the weight of evidence characterization been sufficiently, transparently, and objectively described? Has the scientific justification for not deriving a quantitative risk estimate been transparently and objectively described?

Comment: The reviewers agreed with the conclusion that data were inadequate to assess carcinogenic potential of cerium oxide.

PUBLIC COMMENTS

A. Inhalation Reference Concentration (RfC) for cerium oxide and cerium compounds

Comment: A public submission provided three references addressing nanoparticle cerium oxide:

- Park et al., Particle and Fibre Toxicology 4:12, 1 (2007)
- Park et al., Inhalation Toxicology 20:6, 547 (2008)
- Fall et al., Nanotoxicology 1(3):227 (2007)

Response: The Park et al. (2007) and (2008) and Fall et al. (2007) references were added to Section 4.5.3, *Nanotoxicology Studies* of the document.

ADDITIONAL EXTERNAL PEER REVIEW PANEL COMMENTS – SECOND REVIEW IN RESPONSE TO REVISIONS AS INDICATED ABOVE

Comment: One commenter requested an explanation as to why alveolar epithelial hyperplasia

was selected as the critical effect in the derivation of the RfC, as opposed to another effect in the lung, such as lymphoid hyperplasia.

Response: The critical effect selected for the derivation of the chronic RfC was reevaluated in response to the external peer review panel's final comments. Please see comment under charge question C.2. and the corresponding response.

Comment: A commenter stated that the NOAEL of 5 mg/m^3 for the increased incidence of alveolar epithelial hyperplasia in male rats should be characterized as a LOAEL.

Response: The document has been modified to reflect the change from a NOAEL to a LOAEL of 5 mg/m^3 for the increased incidence of alveolar epithelial hyperplasia in male rats. The incidence of alveolar epithelial hyperplasia in male rats at 5 mg/m^3 is 1/15.

Comment: A reviewer commented that the absence of a rationale as to why the increased hyperplasia in the lymph nodes was not associated with the proposed mode of action is a limitation. The reviewer proposed reasons why the lympoid hyperplasia in the lymph nodes was not associated with the MOA; 1) because a larger number of particles are carried to the bronchial lymph nodes by dendritic cells from the tractheobronchial region; 2) the hyperplasia in the lymph nodes may be the result of a stimulation of a cell-mediated immune response.

Response: Text has been included in Section 5.3., *Uncertainties in the Oral Reference Dose (RfD) and the Inhalation Reference Concentration (RfC)*, addressing the association between the observed increased incidence of lymphoid hyperplasia in the bronchial lymph nodes and the proposed mode of action. While the increased lymphoid hyperplasia in the bronchial lymph nodes has been demonstrated in BRL (1994), there is uncertainty as to whether this effect is the result of the proposed mode of action, the overload of pulmonary clearance. In the absence of evidence indicating otherwise, this effect is considered to represent cerium-induced toxicity.

EPA believes the text in Section 5.2.1, *Choice of Principal Study and Critical Effect—with Rationale and Justification*, as well as in Sections 5.3 and 6.2.2 and in Appendix A, C.2., adequately presents the critical effect and the rationale for its selection.

ADDITIONAL EXTERNAL PEER REVIEW PANEL COMMENTS – THIRD REVIEW IN RESPONSE TO REVISIONS AS INDICATED ABOVE

Comment: Several of the external peer reviewers maintained support for alveolar epithelial hyperplasia in the lung as the critical effect in the derivation of the reference concentration.

Response: The comments provided by the panel addressing the mode of action for the lung and lymphoreticular effects are greatly appreciated. These comments contributed significantly to the mode of action analysis and led to a reorganization and revision of the toxicological review. However, an understanding of the mode of action, while informative, is not a condition for the selection of the critical effect.

Lymphoid hyperplasia in the bronchial lymph nodes has been retained as the critical effect for the determination of the POD in the quantitative dose-response assessment. The lung and lymphoreticular system effects observed by BRL (1994) are consistent with effects observed in humans that were characterized by the accumulation of cerium particles in the lungs and lymphoreticular system and histologic effects throughout the lung. In addition, this effect was chosen as the critical effect because it represents the most sensitive endpoint occurring at the lowest dose that was associated with cerium oxide exposure.