PEER REVIEW SUMMARY REPORT

External Peer Review Meeting on the *Toxicological Review for Cerium Oxide and Cerium Compounds*

Prepared for:
Martin W. Gehlhaus, III
U.S. Environmental Protection Agency
National Center for Environmental Assessment (8601P)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Prepared by:
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Peer Reviewers:

Mitchell D. Cohen, Ph.D.
Joe L. Mauderly, D.V.M.
Günter Oberdörster, Ph.D., D.V.M.
Katherine S. Squibb, Ph.D.
John M. Veranth, Ph.D.

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I. INTRODUCTION

IRIS is an EPA database containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for non-cancer health effects resulting from oral exposure, a reference concentration (RfC) for non-cancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program, within EPA's National Center for Environmental Assessment (NCEA), developed a Toxicological Review of Cerium Oxide and Cerium Compounds, an assessment of which is not currently available on the IRIS database. Cerium was nominated in 2004 for IRIS assessment by EPA's Office of Air and Radiation. The draft document for external peer review contains a chronic Reference Concentration.

Peer Reviewers:

Mitchell D. Cohen, Ph.D.
New York University School of Medicine
Tuxedo, NY 10987

Joe L. Mauderly, D.V.M.
Lovelace Respiratory Research Institute
Albuquerque, New Mexico 87108

Günter Oberdörster, Ph.D., D.V.M.
University of Rochester School of Medicine and Dentistry
Rochester, NY 14642

Katherine S. Squibb, Ph.D.
University of Maryland School of Medicine
Baltimore, MD 21201

John M. Veranth, Ph.D.
University of Utah
Department of Pharmacology and Toxicology
Salt Lake City, Utah 84112

II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of cerium oxide and cerium compounds that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). There is currently no assessment on the IRIS database for the health effects associated with cerium oxide and cerium compounds.

The draft health assessment document includes a chronic Reference Concentration (RfC). Below is a set of charge questions that address scientific issues in the assessment of cerium oxide and cerium compounds. Please provide detailed explanations for responses to the charge questions.

(A) General Charge Questions:

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?

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.

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

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?

Chemical-Specific Charge Questions:

(B) Oral reference dose (RfD) for cerium

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.

(C) Inhalation reference concentration (RfC) for cerium

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.

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.

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?

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.

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?

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.

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.

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?"

(D) Carcinogenicity of cerium

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?

III. GENERAL IMPRESSIONS

Mitchell D. Cohen

The *Toxicological Review of Cerium Oxide and Cerium Compounds* is logical, clear, and for the most part, concise. The document provides Readers with an up-to-date overview of what is known about: chemical and physical characteristics of commonly-utilized/-encountered cerium agents; the toxicokinetics of how the metal is handled (i.e., absorption, distribution, metabolism, and elimination) following exposure of human subjects (occupational exposure scenarios) and animal models; critical hazard identification information based upon case reports, epidemiology, and clinical studies in humans as well as acute, subchronic, and chronic exposure(s) of animal models; both cancer and non-cancer effects of the cerium agents; dose-response assessments; and, potential mechanisms of action as they pertain to specific portal-of-entry effects. Overall, the EPA has clearly and objectively represented and synthesized the limited scientific evidence for the non-cancer and cancer hazards from exposure to CeO_2 and, at a lesser extent, to some other Ce (soluble) agents. For the most part, the majority of conclusions reported in the document are sound. However, there are some conclusions (for example, selection of the particular "critical effect" used in initial establishment of the point-of-departure [POD] value) that need to be discussed further. There are also a few questions as to the appropriateness of the level of uncertainty assigned to some factors used to help determine the inhalation reference concentration (RfC) for chronic cerium (specifically, cerium oxide) exposures. Once these various points have been resolved by the 2008 Peer Review Panel and any recommended changes made to the document, it is certain that a stronger document will ultimately be available for release.

Joe L. Mauderly

The Review is reasonably structured and the process was pursued appropriately EPA's framework. The report could have been improved somewhat by more intense review and revision; however, that would not have altered the outcome (i.e., EPA's choice to not estimate an RfD, magnitude of the RfC, and the decision not to characterize cancer hazard).

As is true for many review documents, readability is impaired somewhat because multiple terms are used for the same thing and some abbreviations are not conventional, simply because the report repeats various terms used in the original papers. Some judgment could have been applied in the writing of this document to use more uniform terminology. These factors, however, would not have affected the outcome.

There are places in the Review where the information could have been assimilated and interpreted more logically (e.g., the relevance of the Ce-fused clay particles to the issues at hand), or other readily-available information could have been accessed to address issues (e.g., the control incidence of lung tumors in F344 rats). Again, however, these improvements would not have affected the outcome.

Fundamentally, an RfC was derived in a manner that is logical within the constraints of EPA's process and mathematically correct, but it is based on weak information of questionable relevance. The problem is the information available, not necessarily EPA's process. The RfC is driven by a huge uncertainty factor that is based on standard EPA factors that may or may not have much bearing on the actual toxicity of Ce compounds in humans under realistic exposure scenarios. Although the methods by which the RfC was derived are clear, the choice of the point of departure is arguable. Serious consideration should be given to using alveolar hyperplasia, rather than lymph node hyperplasia, as the point of departure. The alveolar response is much more defendable as relevant to progressive adverse effects.

The biggest problem is that there has not been a study of inhaled Ce that is "adequate" for the purpose of setting an RfC for environmental exposures. EPA's judgment (and those of their previous external reviewers) that the BRL study was "adequate" must have been based on the fact that they believed that the results were accurately portrayed, rather than that the experimental design was adequate. This is the main fundamental flaw I find in the Review. The BRL study was not designed for this purpose. In my view, the BRL study was not "adequate" for the purpose of deriving an RfC for environmental exposures. It may have been adequate in regard to the accuracy of reported results (although never published in the peer reviewed literature) and it was the only study available, but it was certainly not adequate in terms of experimental design. First, the study did not address the material of primary concern for environmental exposures (CE in a form likely to be emitted from use as a fuel additive). Second, there was no exposure level even close to approximating upper bound environmental exposure levels. Third, the study included a very limited range of health outcome measures and did not address many types of outcomes that are of current concern for environmental exposures. Fourth, the study was of relatively short duration, which makes the applicability of its findings to lifetime exposures questionable at best.

EPA's review failed to offer any perspective on either the target form of Ce or the target exposure scenario. The BRL study used as a platform for cantilevering an RfC out of scanty data suffered by using only very high exposure concentrations (as well as other experimental design limitations). Even the lowest concentration would be predicted on the basis of experience with other materials to be an "overloading" concentration (5,000 $\mu g/m^3$). This makes the RfC for environmental exposures very uncertain. There are published estimates, based in part on actual measurements, that environmental exposures would likely range from less than one ng/m^3 to no more than 80 ng/m^3 at the extreme (Park et al., *Inhal. Toxicol*. 20:547-566, 2008). The proposed RfC is 200 ng/m^3, which suggests that Ce from engine emissions is not likely to be a significant environmental public health concern. However, the lowest concentration in the BRL study was 5,000,000 ng/m^3! Of course, the 2 μm diameter Ce_2 used in the BRL study does not represent the "nanoparticulate" form of Ce that would be emitted from engines. Although it is understood that it is not the purpose of this document to describe exposure parameters, the document is deficient for not having described these issues as part of giving the data proper context.

There is no way to determine whether the point of departure, lymphoid hyperplasia, would occur under upper-bound long-term environmental exposure scenarios, let alone whether it would be progressive. The alveolar epithelial hyperplasia is more readily envisioned as an "adverse" effect. The choice of EPA to use the lymphoid hyperplasia from the BRL study as the point of departure is the key debatable issue. I would not recommend setting an RfC at all, based on the BRL study, but perhaps EPA felt it had no other choice.

EPA rightly characterizes confidence in the RfC as low. If Ce compounds are considered an important environmental health issue, a more relevant study should be conducted. EPA characterizes confidence in the seminal (BRL) study as "medium". The reason for this characterization is not explained.

There is a small amount of more recent relevant literature that should be discussed in the document. The Park et al. (2008) paper is perhaps the best example, but not the only one.

Günter Oberdörster

Please see answers to the following charge questions.

Katherine S. Squibb

This review of the toxicity of cerium (Ce) oxide and cerium compounds is well done. It provides a comprehensive summary of available literature pertinent to a hazard risk assessment of insoluble Ce oxide and other soluble versus insoluble Ce compounds. The decision to develop an RfC for the inhalation of insoluble Ce oxide based on the Bio-Research Laboratories 90-day inhalation study is well supported within the document, with a clear explanation that the doses used were very high and the mechanism of action of the observed effects may have involved, at least in part, effects due to particle overload. These same mechanisms may not be involved in adverse effects of Ce oxide at lower, more environmentally relevant doses and data are currently inadequate to assess effects of chronic exposures to lower exposure doses of any of the soluble or insoluble Ce compounds. EPA needs to be sure that these qualifying factors are clearly described in the IRIS summary document that accompanies this review, in addition to emphasizing the importance of particle size and solubility of Ce compounds in Ce toxicity assessments. The decision not to develop an RfD for oral exposure to Ce compounds or a cancer assessment due to a lack of available, adequately designed studies is well justified. Overall, this IRIS assessment serves as a good review of our knowledge and lack of knowledge of Ce toxicity in humans.

John M. Veranth

The subject document systematically reviews the limited available human, animal, and cell-based literature on the toxicology of Ce metal, $Ce0_2$, and soluble Ce compounds. It was prepared in accordance with current agency policy guidance documents and follows the format of other EPA toxicology reviews. Methodology follows current practices and

the EPA guidance documents are generally consistent with recommendations in the scientific literature.

There is clear evidence that chronic exposure to unquantified, but presumably high, levels of cerium have adverse human health effects. Inhalation of cerium-derived particulate from carbon arc lamps is associated with observable pathology in the lung. Ingestion of cerium is associated with endomyocardial fibrosis and odds ratio of first myocardial infarction. Thus, there is justification for establishing human exposure limits.

Appropriate RfD and RfC values for cerium compounds are uncertain due to the limitations of available studies. From the standpoint of setting exposure guidelines the study limitations include inadequate characterization of the environmental exposure in human studies and inadequate study design in laboratory animal exposures.

A single 13-week study in rats provides the data that were used to establish an inhalation RfC for CeO_2. The process of selecting this study, selecting the critical effect and point-of-departure, and the uncertainty factors are presented in an objective and transparent manner. The exposure concentrations used, 5, 50.5 and 507 mg/m3, are exceptionally high compared to most inhalation exposure studies, especially with 2 micron particles. The higher concentrations are in the range of the short-term PM10 observed in a severe dust desert dust storm. The high exposures could have caused lung overloading, a non-specific response to any low solubility particle, and the observed effects may not be due to the chemical nature of cerium oxide.

A concern that increases uncertainty in the RfC is that the observed human health effects were associated with arc lamps which likely produced submicron or ultrafine particulate that was inhaled without significant atmospheric transformation. In contrast the BRL (1994) study used a dry powder with 1.8-2.2 MMAD. There is increasing literature suggesting that small particle size can increase particle uptake by cells, retention in tissues, and toxicity. The animal study may have underestimated the mass concentration of ultrafine CeO_2 necessary to cause lung damage.

The overall uncertainty factor used in setting the RfC was 3000 due to specific data deficiencies addressed below in the charge questions. This is the maximum combined uncertainty factor recommended in USEPA (2002). An oral RfD was not set due to database limitations and there was insufficient data to evaluate cancer risk. The data limitations encountered in this toxicological review are due in part to the high cost of conducting animal studies the include multiple species, both sexes, acute, subchronic and lifetime exposures, assessment of developmental as well as cancer endpoints, and sufficient statistical power. This problem emphasizes the need for scientific research and policy evaluation that can lead to the incorporation of validated alternative methods into regulatory risk assessments.

IV. RESPONSE TO CHARGE

(A) General Charge Questions

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?

Mitchell D. Cohen

The Review is logical, clear and, for the most part, concise. The latter at times comes into question, as there are many cases of repetition of sentences/paragraphs/points in differing subsections of the document. This Reviewer is certain that whether this is a (legal) requirement of this type of document or an editorial oversight will be made clear at the Review Panel session.

Overall, the EPA has clearly and objectively represented and synthesized the scientific evidence for the non-cancer and cancer hazards from exposure to (primarily) CeO_2 and, at a lesser extent, to some other Ce (soluble) agents. The degree of accuracy will be left to the Review Panel to finalize based upon the comments of each of the individual Reviewers.

Joe L. Mauderly

The review is adequately logical, clear, and concise – with exceptions as noted. The logic of the selected point of departure for the RfC is argued above. There are repetitive sections. Some sections give more verbiage to issues than they deserve in light of their irrelevance to the issue at hand.

Günter Oberdörster

The review is logical, clear but with some redundancies. 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.

Katherine S. Squibb

This toxicological review is an excellent summary of the scientific literature available for assessing the toxicity of Ce oxide and Ce compounds. It has adequately summarized each study, providing the critical information needed for the reader to make an independent judgment of the data available for a hazard assessment.

John M. Veranth

The toxicological review is logical, clear and concise and objectively presents the scientific evidence. It was prepared in accordance with current agency policy guidance documents and follows the format of other EPA toxicology reviews.

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.

Mitchell D. Cohen

As the charge here indicates, the following studies should be performed as they are either critical endpoints that were lacking in the Principal Study or they represent regimens that might bring about clearer POD values and a more precise understanding of the toxicity of CeO_2 and, potentially, other Ce agents:

- A study for a longer timeframe (i.e., 18 and 26 wk at 5 mg CeO_2/m^3) might provide clearer support for the current choice of the "critical effect" as a precursor of other adverse outcomes in an exposed host.

- Studies using exposure levels in the range of 0-5 mg CeO_2/m^3 in place of the current 5, 50, and 500 mg CeO_2/m^3 targets would (when certain more sensitive endpoints are examined in the exposed hosts) permit a narrower POD value to be generated.

- In any new investigation, analysis of pulmonary immunologic endpoints (including cellularity, cytokine/chemokine levels, growth factor expression) as immunomodulation in the lungs is a very sensitive early indicator of toxicologic effects and, more importantly, a predictor of adverse health outcomes in a host inhaling an agent of unknown/uncertain toxic potential.

- In any studies using exposures for increasing lengths of time, analyses of Ce burdens in the various lymph nodes, would help to clearly resolve how/ if particle clearance were truly being affected.

Joe L. Mauderly

There are no additional published studies to examine. One could certainly propose additional studies designed to support an RfC with greater confidence.

Günter Oberdörster

The important cerium-related and other relevant ancillary studies appear to have been included in the report; there are new data coming out in Europe and here about nano-Ceria studies, but these are not helpful for RfC calculations.

Katherine S. Squibb

Two studies demonstrating the hepatic toxicity of soluble $CeCl_2$ injected intravenously were not included in this review. Although these studies are not useful for establishing an RfD, they provide information on effects observed in the liver from systemic Ce and should be mentioned in Section 4.4.2.

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.

Two other studies (Stineman, et.al. 1978 and Magnusson,1963) that were already cited in other sections also addresses hepatic effects following acute exposure.

John M. Veranth

The cutoff for material included in this review was June 2007. One study since then is relevant to in vitro responses to nano-sized CeO_2 , but this paper would not affect the conclusions of the toxicological review. 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. There has also been a new study on ingestion of CeCl3 by mice by Kawagoe et al. J Trace Elem Med Bio (2008). This was an acute exposure with small animal groups and inclusion would not affect the conclusions of the toxicological review. After the meeting the panel members received one public comment letter from a manufacturer of cerium-based fuel additives which cited four papers. I reviewed the abstracts of these papers. The papers are consistent with the material included in the EPA review and provide no information that would affect the conclusions on RfD and RfC.

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

Mitchell D. Cohen

See bulleted items in response A2 above.

Joe L. Mauderly

There is little opportunity for improved epidemiological studies. Improved animal studies would focus on inhaled material of the type thought most likely to present environmental exposures, at concentrations beginning at the lower end of the range of the BRL study (5,000 $\mu g/m^3$) as a benchmark to existing literature, and extending downward for at least two lower concentrations in a plausible environmental range (e.g., 100 & 10 $\mu g/m^3$, which would still be 20+-fold higher than expected exposures to Ce compounds). In addition, a thorough attempt would include additional health outcomes of current concern, such as exacerbation of respiratory allergic responses, impairment of lung defenses, and cardiovascular effects.

Günter Oberdörster

Additional studies needed are related to the biokinetics of cerium compounds, in particular with specific and different particle sizes; also effects determined on alveolar macrophage clearance function to objectively determine as to whether this is impaired due to CeO_2 overload or due to a direct cytotoxic effect. Studies on the dissolution rate of cerium-oxide at different pHs including different particle sizes would also be very useful to be able to predict the behavior of inhaled cerium-oxide particles in the respiratory tract, mechanical clearance rates *vs*. clearance due to dissolution at the acidic pH of phagolysosomes or at extracellular more neutral pH. This is in particular valuable for nano-Ceria particles. Also, better characterization of the BRL Ceria particles is needed: What is the geometric diameter of the individual particles, do they consist of agglomerates? Same information for airborne particles available?

Katherine S. Squibb

It would be helpful to have inhalation studies that extend the work reported in the BRL (1994) study using lower doses that would not cause effects due to particle overload, to determine whether the same or different types of effects would be present at lower exposure doses. It would also be helpful to include a group of animals exposed to a soluble form of Ce (and/or partially soluble) to determine systemic absorption and target organ effects other than the lung at extended time periods after exposure. Cardiotoxicity should be examined by histologic evaluation of the heart, as well as hepatic and renal toxicity by standard assays that identify changes in organ function. These studies should also be designed to determine whether effects in the lungs are reversible following cessation of exposure. Chronic inhalation exposure studies should also be conducted.

Chronic oral exposure studies to soluble Ce compounds should also be conducted using a range of doses to establish an RfD for Ce. This study should include an assessment of cardiotoxicity, as well as hepatic and renal effects. If possible, the impact of magnesium deficiency should also be assessed.

John M. Veranth

There is a need for a more comprehensive animal exposure study that: 1) uses ultrafine cerium oxide, not micron-sized powder; 2) incorporates current recommendations for aerosol characterization (see Warheit comments on BRL study (external letter peer review p4); and includes more sensitive toxicological endpoints (see Mark Noble comments on BRL study (external letter peer review p4). Since cerium-based fuel additives are a potential source of environmental exposure the particle size should be consistent with expected emissions. I have seen conference presentations suggesting that cerium fuel additives produce submicron to ultrafine particulate, and this result is consistent with current understanding of metals in combustion systems. Testing the effect of ultrafine CeO_2 in animals might best be addressed in the context of an NTP study.

Occupational exposure studies that include retrospective evaluation of likely exposure in terms of particle size distribution and concentration would be especially useful in reducing the uncertainty regarding human response to ultrafine cerium. Also, follow-up studies on the association between areas with high soil Ce and endomyocardial fibrosis are needed to retrospectively estimate the ingested dose. International collaboration on this issue could provide the essential data needed to establish an oral RfD for cerium.

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?

Mitchell D. Cohen

There is no issue with the selection of the types/sources of uncertainty applied to the POD for determination of the RfC. Each appears sufficiently justified and objectively described. However, there are some issues (as outlined in later Section C responses) that pertain to some of the individual UF component values. These include:

- A potential "missing factor" is the lack of accounting for potential mouth breathing of Ce particles by laborers during heavy work. It is not clear how, or if, this "factor" could be incorporated into the UF_A of if it needs to be an additional UF component.

- The underlying premise for the choice of the POD is debatable. As such, it might be more prudent to use a greater (or the default) UF_L value for generating the final RfC.

- Even with the special consideration of the information pertaining to the deposition and absorption of CeO_2, the effects observed in humans following prolonged exposure, the mode of action data, and the similar effects observed in animals in the principal study, in the absence of a criteria table/chart to explain the basis for assignation of this (or any other) UF value, it is not clear how the UF_D value was selected. However, this opinion does not negate the fact that the document clearly lays out the deficiencies in the scientific database regarding CeO_2 and other Ce compounds.

Joe L. Mauderly

The choices and their impacts are clear. The source of uncertainty that I would argue is not dealt with adequately is the relevance of the BRL study to environmental exposures. There is substantial evidence from studies of other materials that the lowest exposure level in that study most likely caused proliferative effects that would not have been caused at upper-bound environmental exposure levels.

Günter Oberdörster

Key sources of uncertainty have been discussed, although there should be more emphasis on the importance of particle size-related differences in the biokinetics (esp. nano-Ceria) and potential effects. There should also be more discussion on the differences between effects elicited in the alveolar region of the lung *vs*. the conducting airways – tracheobronchial region – which may well explain the lymph node hyperplasia observed in this study. Consider the much greater deposition per unit surface area of the

conducting airways. Although the choices and assumptions made with respect to uncertainty have been well-described, there is a question as to whether the selected endpoint, lymphoid hyperplasia, is most adequate or whether alveolar hyperplasia should be considered as well. There could also be more of a discussion as to why the BMC approach was not considered to be appropriate.

Katherine S. Squibb

Sources of uncertainty are well described in Sections 5 and 6, and the choice of UFs is carefully justified. It is, as described, primarily a lack of available data that creates the low confidence in the derived RfC, and the lack of ability to derive an RfD. Again, it should be emphasized that the derived RfC is based on a mechanism of action of limited to insoluble Ce oxide (within a specific particle size range) at doses that were high enough to cause particle overload in the lung. Thus, uncertainty for the derived RfC increases when it is applied generally to Ce compounds.

John M. Veranth

In general, the document appropriately identifies and characterizes the sources of uncertainty. More specific comments appear under items C5, C6, and C7. The product of uncertainty factors used equals 3000 which is the maximum combined uncertainty factor recommended in USEPA (2002).

The high uncertainty reflects the serious limitations of the available studies and these limitations are objectively presented in the toxicological review. This high uncertainty factor is partially justified by the issue of supermicron particles being used in the BRL study versus the likely exposure to ultrafine particles in the human exposures where pathology symptoms were observed. The issue of particle size effects is not adequately discussed in the toxicological review, but is acknowledged on p56 lines 32-35. The issue of nanoparticles should be more completely discussed in the final document. Example: the use of a carbon arc to generate laboratory ultrafine particles, e. g. the PALAS generator, strongly suggests that the occupational exposures from arc lamps were ultrafine.

(B) Oral reference dose (RfD) for cerium

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.

Mitchell D. Cohen

The document clearly describes the poor absorption of Ce (soluble) agents from the GI tract in numerous adult and newborn/young animal models (cf. p. 8-11, p. 29) provided the test agents in the diet, drinking water, or solutions administered intragastrically. The results of some studies describing specific toxicologic outcomes are also provided (cf. p. 19-21, p. 50-51). However, experimental deficiencies associated with many of these studies (i.e., small sample size, lack of multiple doses, relevant exposure routes) that are objectively described provide the needed justification for not selecting any of these as a Principal Study for use in defining an RfD.

Based on the documented poor absorption of soluble Ce agents from the gut, it is not clear which endpoints should be considered for the selection of a critical effect in any future study. If it is critical to define this effect, one should focus attention upon changes to and/or within the tissues comprising the GI itself, as this would be the most likely site of any "portal-of-entry" effect(s). Should there be evidence that some of the Ce leaves the GI and enters the systemic circulation, attention should focus on the liver and its associated (hepatic) functions for any potential early changes induced by the metal agent(s).

Joe L. Mauderly

The Review handles this issue appropriately.

Günter Oberdörster

Not to do an oral RfD for cerium-oxide was the right choice given the paucity of data.

Katherine S. Squibb

None of the existing oral exposure studies are adequate for derivation of an RfD. They were not designed for this purpose, and fall short primarily because they did not include multiple exposure doses. They do serve as a good basis for identifying the principle target organ(s) and the critical effects, however. The existing animals and in vitro studies, as well as the human data, suggest that the cardiotoxicity, as well as hepatic toxicity, should be examined carefully.

John M. Veranth

It appears from the studies cited in the toxicological review that effects of soluble cerium are observed in animals at 20-30 mg/kg/day. However the review adequately presents the rationale for not using any of these studies to establish a point-of-departure for a RfD.

(C) Inhalation reference concentration (RfC) for cerium

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.

Mitchell D. Cohen

Selection of the BRL (1994) study (cf. p. 51) appears to be solely justified by (a) the lack of other relevant studies and (b) experimental deficiencies associated with the available human studies. However, the report indicates that the BRL study was externally peer-reviewed (2006) and deemed adequate (cf. p. 68). While the BRL study is transparently and objectively described in the document, the criteria and rationale for its selection are vague (apart from the above-cited reasons). To date, no other studies are available in the literature that might adequately address many of the points that were raised by the 2006 External Review Panel members or likely to be raised by the present panel. As such, no other studies can be recommended at this time as candidates for "Principal Study."

Joe L. Mauderly

The BRL study was the only option, if EPA felt compelled to select a study regardless of its relevance. My view is that the selection was <u>not</u> adequately justified on the basis of existing knowledge. My view is that existing knowledge overwhelmingly argues that the lowest exposure concentration in the BRL study was not only absurdly higher than the highest expected environmental concentration, but was also a concentration that would predictably cause nonspecific effects of the type seen due to particle "overloading" (a jargon term, but one defined repeatedly in the literature and understood well by those having expertise in the field). Existing knowledge would not support the notion that 2 μm diameter CeO_2 was relevant to the most likely target form of Ce.

Günter Oberdörster

The choice of the BRL, 1994, study was appropriate and is scientifically justifiable given that there is no other study available. This selection has been clearly justified and well-described.

Katherine S. Squibb

The decision to develop an RfC for Ce oxide based on the BRL (1994) study is well supported in the document. Based on the summaries of other available studies, it is clear that they were not adequately designed for deriving an NOAEL or LOAEL. Details of the BRL study are well described in the document, and the review of the study by the peer-review panel is very helpful in substantiating the strengths and weaknesses of the study.

John M. Veranth

The BRL 1994 report is the best available study. The comments from the External Letter Peer Review were helpful the BRL study.

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.

Mitchell D. Cohen

The EPA considers the increased incidence of lymphoid hyperplasia in the bronchial lymph nodes to be a precursor to an adverse effect – and so, the critical toxicological effect here. A good portion of the document is devoted to justifying this selection. Whether this effect is a true "precursor" is somewhat challenged by the facts that neither increases in blood levels of PMN nor in the incidence of alveolar epithelial hyperplasia occurred at 5 mg CeO_2/m^3 even though bronchial lymph node lymphoid hyperplasia was already strongly evident (cf. Tables 4-1 and 4-7). This suggests that some "threshold" of Ce burden had to have been crossed to begin to bring about the hematological/epithelial outcomes – irrespective of if these changes were associated with/dependent upon the onset of lymphoid hyperplasia or not. Optimally, a study for a longer timeframe (i.e., 18 and 26 wk at 5 mg CeO_2/m^3) might have more strongly supported the EPA viewpoint. Specifically, those types of studies might have shown that these blood/epithelial changes developed *subsequent* to the initial appearance of the lymph node hyperplasia. In the absence of these types of studies, it is only weakly sufficient to claim this effect as the "critical" effect. It is more semantics than not, i.e., this is an adverse effect as opposed to 'no effect' or a 'beneficial effect.' Thus, to claim that it is a "precursor" is not sufficiently justified.

It is unclear from the charge whether the identification and rationalization for any other endpoints to be selected as the critical toxicologic effect are to be limited to the Principal Study. If so, none of the other endpoints evaluated would appear to suffice to replace the choice of the lymphoid hyperplasia in the bronchial lymph nodes (i.e., several are too non-specific like changes in blood hematological parameters). If not, and if other endpoints could be suggested for study in a new investigation, an emphasis on analysis of pulmonary immunologic endpoints (including cellularity, cytokine/chemokine levels, growth factor expression) would be warranted. Many studies have shown that immunomodulation in the lungs is a very sensitive early indicator of toxicologic effects and, more importantly, a predictor of adverse health outcomes in a host inhaling an agent of unknown/uncertain toxic potential. Such studies might also help to address several of the points raised by the 2006 Reviewers and provide further evidence to support/refute some of the statements in the current document (i.e., cf. p. 56 and p. 65).

Joe L. Mauderly

The point of departure was an effect that often occurs before more clearly adverse effects are observed with continued exposure of rats to poorly-soluble particles of relatively low toxicity. However, the lymphoid hyperplasia is not known to be on the pathogenetic pathway for those effects, or to represent an adverse effect in itself. Thus, current knowledge would argue that the chosen point of departure was most likely not an environmentally relevant adverse outcome. Of course, this cannot be proven. If EPA is determined to use the BRL study, it should consider using the alveolar hyperplasia as the point of departure, rather than the lymphoid hyperplasia. This issue (the key issue of the entire document) was not dealt with adequately in the Review.

It is not clear that the increased cellularity of lung-associated lymph nodes *per se* would have evolved into a detrimental effect. Studies of such effects in rats exposed under other overloading conditions have shown that enlarged lymph nodes can occur without impairment of the antibody-forming function of the lymphocytes (for example, Bice et al. *Fund. Appl. Toxicol*. 5: 1075-1086, 1985). Continued exposure would most likely have resulted in other outcomes (e.g., lung fibrosis and epithelial hyperplasia, metaplasia, and neoplasia) that would have been considered more clearly adverse. Diesel exhaust presents a case study, in which the lymphoid hyperplasia occurred (Bice citation above), but non-cancer lung epithelial effects were selected for derivation of an RfC.

In summary, I do not agree that the lymphoid hyperplasia observed in the BRL study is an appropriate point of departure for an RfC intended to be focused on environmental exposures. If EPA felt compelled to derive an RfC from the BRL study regardless of its relevance to environmental exposures, lung epithelial hyperplasia was a more logical choice.

Günter Oberdörster

The choice of lymphoid hyperplasia in the bronchial lymph nodes can be questioned, since the major critical effect addressed in the document relates to alveolar hyperplasia in the lung periphery. It is not clear as to whether the lymphoid hyperplasia is a precursor effect of the alveolar hyperplasia. There are other scenarios, possibly even more likely ones, where the lymphoid hyperplasia reflects an immune response due to the cerium-oxide particles delivered by dendritic cells from the tracheobronchial region to the bronchial lymph nodes. This would make sense, also given that the response in the mediastinal lymph nodes are less than those in the bronchial lymph nodes. Thus, I strongly suggest to use of alveolar hyperplasia as the critical effect. In addition to making more sense from a mechanistic point of view, there is the advantage that a NOAEL would be available in this study, showing a very nice dose-response relationship which could be used for a BMC approach. Of course, a limitation with regard to environmental exposures is still the large particle size. This could be further discussed at the face-to-face meeting of the review committee.

Katherine S. Squibb

Selection of the increased incidence of lymphoid hyperplasia in the bronchial lymph nodes as the critical effect for cerium oxide is justified based on the fact that it is the most sensitive effect following the Ce oxide exposure in the BRL study and can be a precursor to alveolar hyperplasia. The fact that this mode of action, which involves particle overload, would not be important at most environmental exposures and would increase the uncertainty of this RfC when applied to environmental exposure scenarios is something to be considered however.

John M. Veranth

The discussion on P52 establishes the link between lymphoid hyperplasia, impaired particle clearance, and chronic inflammation. In risk assessment there is always the question of whether a response is an "adverse effect" or a normal biochemical defensive response. The toxicological review calls lymphoid hyperplasia a "precursor of adverse effect" but then associates it with inflammation resulting from impaired clearance. Review of a comprehensive review text on human lung pathology (Travis 2002) indicates that bronchial lymphoid hyperplasia is not considered clinically to be a diagnostic marker of specific diseases. Rather, it appears to be a secondary response to other conditions, such as autoimmune disease. While the conclusions regarding using lymphoid hyperplasia as the critical effect are justified in the review, the panel discussion made a persuasive case for using alveolar epithelial hyperplasia instead as the critical toxicological effect. The alveolar hyperplasia is more clearly recognized as an "adverse effect" in the lung and the BRL study data provide both LOAEL and NOAEL levels for alveolar epithelial hyperplasia. Also, this indicator provides well differentiated dose-response (strictly, concentration-response) data.

William D Travis. T Colby, M. Koss, M. Rosado-de-Christenson, N. Muller, T. King "Non-neoplastic Disorders of the Lower Respiratory Tract" American Registry of Pathology & Armed Forces Institute of Pathology, Washington DC. (2002) Ref Pages 277-281

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?

Mitchell D. Cohen

There is no challenge to the statement 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." In addition, there is no problem with the statement that "delayed clearance leads to increased accumulation of cerium oxide particles in the respiratory tract, an inflammatory response, and subsequent cell proliferation". However, the claim 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" requires discussion. This particular statement unfortunately could be interpreted to imply that there is an "all-or-none" phenomenon at work with the inhaled CeO_2. Specifically, it does not delineate if the hyperplasia occurred even while there was ongoing deposition (albeit increasingly impaired) of Ce-bearing PAM into the lymph nodes over the entire 13-wk period or if the translocation stopped at some point during the 13-wk timeframe. The observation of significant increases in bronchial/mediastinal lymph node enlargement as the exposure dose increased from 5 to 50 mg CeO_2/m^3 suggests either that local PAM dose-dependently ingested greater amounts of the CeO_2 particles that were, in turn, translocated (which runs counter to the "overladen" phenomenon [cf. p. 44]) or that movement of PAM out of the lung continued apace during the 13-wk period (which should not have been the case had the system been "overwhelmed" under the *in situ* conditions that would be associated with the 5 CeO_2/m^3 dose). As noted above, optimally, studies using 5 CeO_2/m^3 exposures for various lengths of time - in conjunction with analyses of Ce burdens in the various lymph nodes - would help to resolve how/ if particle clearance was being affected.

The MOA for CeO_2-induced bronchial lymph node lymphoid hyperplasia is questionable. If anything, that hyperplasia is occurring there is suggestive of problems with clearance of the particles (or more appropriately, the Ce-bearing PAM) out of the nodes and not necessarily the airways themselves. As noted earlier, the suggestion that the lymph node hyperplasia is an "immunological response to the CeO_2 particles" (cf. p. 56) has been previously challenged by the 2006 External Review Panel and remains suspect. Thus, this MOA overall is not justified based on the sole evidence provided in the 1994 BRL study.

Joe L. Mauderly

It is not certain that hyperplasia in lung-associated lymph nodes is a direct result of "overloading" exposures. It typically appears early in the sequence of findings from studies in which such exposures continue, but that does not necessarily mean that the finding itself is a result of overload. Particles are well-known to be translocated to the lymph nodes under exposure scenarios that do not subsequently overwhelm particle clearance from the lung. Again, I do not agree that the Review adequately characterized the likelihood that the effect would occur at environmental exposure levels.

Günter Oberdörster

There is a heavy and sole-focus in this document on lung particle overload as the only mechanism driving the observed effects. However, the BRL study does not give any evidence of impaired clearance (it was simply not measured) nor does it give evidence of other key findings related to dust overload, such as type II cell proliferation, altered alveolar epithelial integrity, chronic acute inflammation with a heavy influx of PMNs, despite the extremely high exposure concentrations of 500 mg/m^3 in the highest exposure group. Alternative MOAs ought to be discussed as well in this document. See above, immune response *via* DCs. Lymph node hyperplasia may be part of overload, but overload starts before, *i.e.,* with impaired clearance.

Katherine S. Squibb

The MOA action data presented do support the proposed MOA for the Ce oxide-induced bronchial lymphoid hyperplasia observed in the BRL study. This is well described in the document. It's important to remember, however, that this mode of action occurs only at the high doses of Ce oxide that overwhelm the particle clearance mechanisms in the lung. It is possible that chronic exposure to lower doses could cause effects via a different MOA. Thus, a chronic inhalation study needs to be conducted.

John M. Veranth

Lymphoid hyperplasia at similar exposure concentrations has been reported in the toxicological reviews for nickel sulfate (TR-454), cobalt sulfate (NIH 91-3124), and vanadium pentoxide (TR-507). There is some concern that the lymphoid hyperplasia might be a very non-specific response to particles and not strictly an effect of cerium oxide. Also, it has been hypothesized that particle overload effects in rats may result in unique defense responses, especially cellular proliferation leading to tumors, that are not seen in other species. See Warheit, D. B. (2006). Effects of Engineered Nanoscale Particulates on the Lung, in Toxicology of the Lung, Fourth Ed. Ed. D. E. Gardner. Boca Raton, Taylor & Francis. 537-557.

As an alternative critical toxicological effect, increased neutrophils (both absolute and relative) was statistically significant in females at 5 mg/m3. This provides another marker of inflammation with the same LOAEL. Neutrophil counts appear on P 23 but were not included in Figure 5-1. Blood count endpoints have the limitation of being a non-specific

response that many not constitute an "adverse effect." Thus the alveolar lymphoid hyperplasia may be the best critical toxicological effect.

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.

Mitchell D. Cohen

Assuming that the threshold for LOAEL is "any" effect in the CeO_2-exposed hosts, and using the criteria accepted by the EPA during their review of the BRL Principal Study, this appears to be the best approach to determining the point of departure (POD). However, as noted in response C2, use of other endpoints and some intermediary CeO_2 concentrations could result in a dramatically different chronic RfC value being generated.

Joe L. Mauderly

Once the decision was made to use lymphoid hyperplasia in the BRL study as an acceptable point of departure, the approach used thereafter was reasonable within EPA's framework.

Günter Oberdörster

Again, the BMC approach should be considered for alveolar hyperplasia, although there may be some scientific objection (which mathematical model does best fit the data?) to do this which should be discussed at the review committee meeting.

Katherine S. Squibb

The NOAEL/LOAEL approach is the only one that could be taken if the lymphoid hyperplasia is the chosen critical toxicological effect, due to the nature of the results of the BRL study that showed statistically significant effects at the lowest dose examined.

John M. Veranth

This is adequately justified on P 53. The data documented for lymphoid hyperplasia in Table 4-7 are not suitable for benchmark curve fitting. However, the data for alveolar lymphoid hyperplasia are suitable for benchmark dose calculations.

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?

Mitchell D. Cohen

There is no issue with the selection of the uncertainty factors applied to the POD for the determination of the RfC. Each appears to be sufficiently justified and objectively described (cf. p. 54-56). The only potential "missing factor" that might be considered is that there is no accounting for potential mouth breathing of the Ce particles by laborers during heavy work. This Reviewer is not clear how, or if, this "factor" could be incorporated into the UF_A of if it needs to be an additional UF.

Joe L. Mauderly

The use of uncertainty factors was described in a transparent manner. The types of factors are reasonable. The magnitudes of the factors are largely arbitrary, and may or may not be the most appropriate. Because they portray ranges of potential uncertainty, one can neither verify them nor argue strongly against them. In aggregate, I view them as resulting in a very conservative adjustment factor. However, the more important point is that an RfC attended by such high uncertainty is of questionable value regardless of how it is derived. In this case, the most relevant point is that the uncertainties are all simply adjustment factors for an outcome that is probably not relevant to environmental exposures in the first place.

Günter Oberdörster

The uncertainty factors seem to be well chosen, although the last one, related to the potential of systemic effects should be considered in more depth and particularly address the issue of nano-sized cerium-oxide which, indeed, may give rise to systemic effects that could not be observed with the larger particle size used in the BRL study.

Katherine S. Squibb

The UFs selected are fairly standard given the nature of the BRL study (the lack of a NOAEL) and the lack of a chronic study. In the discussion of the factor of 10 selected to account for variation in susceptibility among members of the human population (page 54 lines 28-30), there is no mention of the studies suggesting magnesium deficiency might increase a person's susceptibility to cardiotoxic effects of Ce. Since the results of a number of studies have been consistent with this hypothesis, it would strengthen this document to refer to these studies as a potential mechanism causing increased risk within human subpopulations.

John M. Veranth

The UFs are appropriately discussed on P 54-56. In the present case the calculation is 3 x 10 x 10 x 3 x 3 = 3000 where 3 is the rounded value for $10^{0.5}$. The values used are consistent with both EPA guidance and the findings of statistical studies of toxicological uncertainty in the peer reviewed literature.

The product of uncertainty factors used equals 3000 which is the maximum combined uncertainty factor recommended in USEPA (2002). The cited guidance document discourages the use of a combined uncertainty of 10^4 when four factors are at maximum uncertainty. The guidance suggests that when uncertainty reaches 4 factors at the maximum value there is a question of whether the information is sufficient to set a limit. In the present case the calculation is 3 x 10 x 10 x 3 x 3 where 3 is the rounded value for $10^{0.5}$; that is two factors at maximum uncertainty and 3 factors at lesser uncertainty.

A consequence is that the toxicological review has taken a minor 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. All steps in the process are clearly stated, transparent, and consistent with agency guidance.

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.

Mitchell D. Cohen

The underlying premise for the choice of the POD, and so a UF_L of 3 (cf. p. 55) has already been deemed insufficiently justified (see responses C2 and C3). As such, it might be more prudent to use a greater (or the default) UF value for generating the final RfC.

Joe L. Mauderly

There is nothing wrong with the uncertainty factor of 3 in this case. The fact that it is admittedly being applied to a potentially progressive result of overloading exposures is the problem.

Günter Oberdörster

The uncertainly factor of 3 has been used 3 times. One was used for extrapolation from animals to humans, which is justifiable based on using dosimetric interspecies adjustment. The second refers to the LOAEL-to-NOAEL extrapolation. I am not sure that the lymphoid hyperplasia represents the point at which normal clearance of particles from the lung by macrophages has become overwhelmed; rather it signifies events that happen after macrophage clearance has been overwhelmed. The first symptom would be accumulation of particles in the lymph nodes which, unfortunately, has not been measured in this study. Again, a different explanation may be that we are dealing here with an immune response initiated by cerium-oxide particles translocated to the lymph node by dendritic cells as antigen presenting cells. This can be discussed further as an alternative hypothesis and explanation. The LOAEL-to-NOAEL adjustment can be avoided by using alveolar hyperplasia because that has NOAEL. A third uncertainty factor of 3 is addressed already in the previous point.

Katherine S. Squibb

The fact that lymphoid hyperplasia is a sensitive precursor of an adverse effect is a reasonable explanation for the UF of 3, however the fact that this endpoint does not show a dose response (i.e. the response in the lowest dose group is as high as that in the highest group) in the BRL study, selecting a UF of 10 should also be considered.

John M. Veranth

The flat response for hyperplasia shown in 4-7 (Females 13/15 and 15/15 at 5 and 50 mg/m^3 respectively) makes extrapolation below the lowest tested dose very uncertain. Alexeeff et. Al. compared the data base for LOAEL to NOAEL ratios for acute inhalation effects and found the ratios were 2.0, 5.0, 6.3, and 10.0 for the 50th, 90th, 95th, and 99th percentiles respectively, and recommended a value of 6 for extrapolating to from a LOAEL to a NOAEL. Note that reported LOAEL and NOAEL points are limited by the finite steps in applied dose, a limitation not shared by the benchmark dose approach. The factor of 3, rather than 10, is justified by the comment that lymphoid hyperplasia is a sensitive effect occurring early in the events leading to lung damage. The role of lymphoid hyperplasia as a sensitive biomarker could be more clearly stated on P 52. If alveolar hyperplasia is used as the POD then the UF for LOAEL to NOAEL extrapolation is eliminated, reducing overall RfC uncertainty.

GV Alexeef, R Broadwin, J Laiw, SV Dawson, "Characterization of the LOAEL-to-NOAEL Uncertainty Factor for Mild Adverse Effects from Acute Inhalation Exposure" Regulatory Pharmacology and Toxicology 36;1 p96-105 2002.

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.

Mitchell D. Cohen

The scientific rationale and justification for the selection of the UF_D are clearly delineated (cf. p. 55-56). The document clearly indicates that there is a paucity of data regarding the toxicology of CeO_2 (apart from the numerous generic case reports), a lack of exposure and recovery studies, and the need to rely upon inhalation studies (such as the 1994 BRL study) as "toxicity via the inhalation route is expected to be a portal-of-entry effect." Though the document briefly addresses what is known about potential reproductive and/or developmental toxicity of CeO_2/Ce agents (cf. p. 29), it also clearly states that the database is lacking and not sufficient to rely upon for the determination of potentially lower POD values than that derived from the 1994 Principal Study. As noted in response C2, studies that would assess immunologic endpoints in the lung would present a great opportunity for a potential lower POD to be generated. Of course, this is predicated on these types of studies utilizing exposure levels in the range of 0-5 mg CeO_2/m^3 in place of the current 5, 50, and 500 mg CeO_2/m^3 targets.

Even with the 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, in the absence of a criteria table/chart to explain the basis for assignation of this (or any other) UF value, it is not clear as to how the value of 3 was selected for the UF_D. However, this opinion does not negate the fact that the document clearly lays out the deficiencies in the scientific database regarding CeO_2 and other Ce compounds.

Joe L. Mauderly

There is nothing wrong with the magnitude of the uncertainty factor in this case. Again, it is the point of departure that is the problem. It is not appropriate to speculate on the counterfactual. It cannot be known whether studies of other designs would result in a lower point of departure for Ce toxicity. Existing knowledge would argue that the outcome selected as the point of departure in this case would not occur at all at upper-bound environmental exposure levels, but that cannot be proven. It is pure speculation to opine on whether other adverse outcomes would be seen at environmental exposure levels of Ce. Certainly, there is much literature reporting statistically significant

biological effects from exposures to other particles at lower concentrations, but none of those studies have addressed Ce compounds. If EPA were to elect to set a lower concentration for Ce because of effects from other types of particles, then there is no reason to set composition-specific RfCs at all (just set an RfC for all particles regardless of type). That has not been the strategy to date.

Günter Oberdörster

Again, my suggestion is to consider different particle sizes of cerium-oxide rather than just focusing on what size happened to be used in the BRL, 1994 study. In particular, with the increasing use of nanotechnology and the associated potential for human exposure at the workplace as well as by consumers, this should be part of the consideration for a different uncertainty factor. In fact, what could be done is to perform some modeling exercises of deposition of inhaled cerium-oxide particles at different sizes, such as the one used in the BRL study, using the MPPD deposition model and assuming normal clearance in rats and in humans, and contrasting the result with a particle size of 20 or 50 nm cerium-oxide particles to obtain some information about differences in lung deposition and CeO_2 accumulation.

Katherine S. Squibb

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 Ce systemically, possibly causing cardiotoxicity over time. Also, on page 56 (lines 6-8), in the section discussing the pulmonary effects observed in the BRL (1994) study, the statement is made that the lymphoid hyperplasia in the bronchial lymph nodes is not due to cytotoxicity. There was no direct evidence of this in the BRL study, however. It is possible that overtime, there could be slow release of Ce ions from the Ce oxide particles in the lymph nodes, which could have local cytotoxic effects.

John M. Veranth

This factor is appropriate given the limitations of available studies. Specifically there are no exposure studies covering the neonatal period where rapid lung development is taking place nor exposure studies with ultrafines.

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?"

Mitchell D. Cohen

The statement to not use the RfC for CeO_2 for other Ce compounds is justified. The title "Toxicologic Review of Cerium Oxide and Cerium Compounds" is appropriate in that the chemistries and toxicologic effects of several non-CeO_2 agents are reviewed and discussed in various parts of the document.

Joe L. Mauderly

Yes, that position is justified. No scientific justification is presented in the Review for declaring that the RfC applies to all Ce compounds, and the literature doesn't support it. The fact is that there are only substantive inhalation data for CeO_2.

Günter Oberdörster

The use of the RfC for cerium-oxide and cerium compounds is probably justifiable; however, it needs some more discussion at the end of the document that given the greater solubility of cerium salts, the case of cerium-oxide may represent the most critical case because of the long retention as particle, provided very low inhaled concentrations are present. For concentrations that are very high, even approaching the mg/m^3 range (which is really unlikely), acute effects of soluble salts may indeed be much more severe.

Katherine S. Squibb

Yes, it is important to not use the RfC for cerium compounds other than cerium oxide. In fact, the written document should emphasize more than it does now that the RfD established is for Ce oxide – an insoluble form of Ce. Since occupational exposures are most likely to be to Ce oxide, this is an appropriate reference concentration to establish. However, statements in the text such as "the respiratory system may be the most sensitive target of toxicity following inhalation exposure to cerium" (page 56, lines 15-16) should specifically state "cerium oxide" or at least "insoluble forms of cerium." The IRIS Summary written from this Toxicological Review needs to be very specific about the applicability of the derived RfD for Ce compounds other than Ce oxide at the particle size used in the BRL study.

There is value in not re-naming the document so the title includes only cerium oxide. The current document does a good job of describing the differences between the soluble and insoluble Ce compounds with respect to their toxicokinetics and toxicity, which is important for people to know. In the uncertainty sections, it would be useful to include a discussion of the importance to clearly distinguish between the effects of insoluble and soluble forms of Ce. It may well be that the most sensitive target of inhalation exposure

to a soluble form of Ce (such as $CeCl_3$) may not be the respiratory system. Since soluble Ce is systemically absorbed from the lung after inhalation exposure (Morgan et al, 1970 reference from draft document), other organs, such as the liver, kidney, or heart, may be affected as demonstrated by single dose injection studies using soluble Ce compounds. There are insufficient data to evaluate this at this time, since the focus of inhalation studies has primarily been the lung following exposure to insoluble Ce compounds.

John M. Veranth

The data on soluble forms are inadequate to set a RfC. However, the literature on clearance of soluble cerium is well reviewed and this toxicological review provides a valuable reference on "cerium compounds" and the title should be retained.

(D) Carcinogenicity of cerium

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?

Mitchell D. Cohen

The statement that there is "*inadequate information to assess the carcinogenic potential"* of cerium compounds is justified. The cancer weight of evidence characterization (cf. p. 47) appears to be justified and reinforced by the statements about lack of cancer-inducing effects using non-radioactive (stable) forms of the metal (cf. p. 62). Appropriately, the document: (a) reports the results of the studies examining potential carcinogenic outcomes from host exposure to ^{144}Ce (as inhaled $^{144}CeO_2$; cf. p. 28-29) and the lack of significant effects therein; and, (b) that there is an ongoing inhalation study that will include a cancer bioassay (with date of completion/availability of results in 2009).

Whether sufficient scientific justification for not deriving a quantitative risk estimate has been fully provided is uncertain. In several sections, the document defaults to the phrase "In accordance with US EPA (2005a) *Guidelines for Carcinogen Risk Assessment*" to cover the conclusions presented. Providing potential readers some short synopsis/bullet points about these guidelines (i.e., what precisely allowed for the declaration about "inadequate information to assess….") would be helpful.

Joe L. Mauderly

Yes, I agree with the position taken in the Review. Under the circumstances, it is the only viable position.

Günter Oberdörster

There is, indeed, no information in the scientific literature on cancer-inducing potential of cerium-oxide, and this needs to be identified as another research need, although I would not give it a very high priority.

Katherine S. Squibb

With so little data available on the carcinogenicity of Ce oxide and other Ce compounds, the conclusion that a quantitative risk assessment cannot be conducted is well justified. Mutagenicity and chromosomal damage data suggest potential mechanisms by which Ce compounds could be carcinogenic, however, available studies provide no dose/effect information from which to derive a quantitative assessment.

John M. Veranth

There are no data in the review or available in the publically available literature on which to assess carcinogenic potential of any cerium chemical form, therefore the statement that the database is inadequate in Section 6.2.3 is fully supported by the review document.

V. SPECIFIC COMMENTS

Mitchell D. Cohen

Specific items dealing with scientific content have been cited in the responses above.

Joe L. Mauderly

P 11, L 32: The "controls" were obviously exposed to some extent, or Ce would not have been measurable. They are not "unexposed."

P 12-13, Section 3.2: This section is nearly devoid of dose information. Giving the administered concentration of Ce compounds is meaningless without also giving the amount of solution, or preferably just the dose itself. Doses of inhaled aerosols can be at least estimated.

P 13, L 7 and 22: The "fused aluminosilicate" and "fused clay" is the same thing. Both were generated as aerosols of solubilized montmorillonite clay fused to a cewramic-like or glass-like material by passing through a heating column. This is important because the Ce incorporated in such particles is explicitly intended to be insoluble, or at most very poorly soluble.

P 14, L 23-24: The abbreviations "D2" and "B6" for mice are neither adequate nor common.

P 15, L 9: The fact that there might have been a leak in the inhalation chamber would only be significant if it affected the calculation of dose. It is the actual dose that is important, not the intended target dose.

P 16, L 28: What was the odds ratio for smokers <u>with</u> adjustment? That would seem to be the more relevant metric.

P 17, L 16-17: Of course the occupational exposures were not quantified. However, can any reasonable bounds be put on the probably exposure levels? Has anyone tried?

P 18, L 18, 30, & 35: "Chest X-ray," "X-ray," and "roentgenogram" are various terms commonly used for the same thing. The former two are most commonly used, but only the last is an accurate term.

P 19, L 11: Separate "rare" and "earth."

P 21, L 27-29: It is not worth noting that Ce is being considered for testing by NTP, unless this information can be extended to describe the study and when it would start. NTP always has many compounds on their list to "consider".

P 22, L 1: It should be made clear that the exposure concentrations in this study were absurdly high compared to any human exposure scenario under consideration.

The lowest concentration would be an upper bound for a meaningful bioassay for this purpose. The problem lies not in how EPA reports the study, but in the study itself. Frankly, one doesn't care what effects might result from an exposure at 500 mg/m^3, and no environmental exposure resulting from the use of Ce as a fuel additive would approach within orders of magnitude of even the lowest concentration.

P 23: Table 4-1: The term "relative neutrophils (and lymphocytes)" is neither meaningful nor conventional. One presumes that this means % neutrophils. If so, it should be labeled as such. Also, why are there two different footnote indicators for the same p value? One might guess that footnote c is actually $p<0.10$, but one should not have to guess.

P 24, Tables 4-3 and 4-4: Same comment as above. Presumably "relative lung weight" etc. means lung weight/body weight. If so, it should be labeled as such.

P 26, L 5: It is not at all clear what "antigenic stimulation" cerium oxide might cause. I have not encountered antigenic stimulation defined in this way (something caused by a non-antigen). Antigens are ordinarily proteins, or at least organic materials. Perhaps the thought is that cerium oxide might have enhanced a response to some other antigen. The authors might have been thinking about effects from beryllium (another metal), but there is no evidence that Ce would act in a manner similar to the action of beryllium. If the term "immune" is to be invoked, its meaning in this case ought to be explained.

P 29, L 4: The percentage values should be clearly defined. One might presume that these are the percentages of animals having the lesions, but there could be other definitions. One should not have to presume.

P 29, L 6-9: The numbers of lesions are not meaningful unless one knows the numbers of animals examined for the lesions.

P 29-32: None of the information in 4.4 is relevant to the issues at hand. The doses are absurdly high and injected Ce is not relevant to environmental exposures. The authors undoubtedly recognized this, but could have simply stated the case instead of going through the studies. Moreover, the final summary of section 4.4.2.4 mentions oral studies in rabbits, and I don't find those described.

P 35, L 23: Giving the concentration of administered material does not communicate the dose unless the volume of solution is also given. A concentration alone is meaningless.

P 39, L 11-12: At which doses did these effects occur?

P 42, L 16: Describing a 13 wk study as "long-term" is misleading. That's only about 10% of the subjects' life spans. 13 wk studies can be useful to be sure, but they are not "long-term".

P 43, L 3: Again, characterizing the response as an "immune" response is questionable. BRL's speculation that lymphoid hyperplasia resulted from "antigenic stimulation" was just that – pure speculation. Apparently, the speculation was not contested by EPA's external peer reviewers. Oberdörster 1995 is cited as a reference for this designation, yet that paper does not use the "immune" terminology at all. Increased cellularity of lung-associated lymph nodes is a common finding in studies in which poorly soluble particles accumulate there. There is little evidence that immune responses per se play significant roles. Here, and in several sentences to follow, the presumption seems to be that particle overload is an immune-based phenomenon. One can hypothesize immune mechanisms, but immune mechanisms are certainly not necessary to explain the syndrome. Inflammatory and proliferative responses to particle accumulation are not necessarily "immune" responses. Regardless, the designation makes no difference in interpretation or the final outcome regarding the RfC.

P 44, L 3-9: This paragraph misses the key point. There is a big difference in solubility between TiO_2 and $BaSO_4$. The latter has tremendously greater solubility than the former. The latter is a soluble salt and the former is a covalently-bound compound. That is why the latter does not cause classical "overload," which is associated with poorly-soluble particles. This is an "apples and oranges" issue.

P 46, L 8-10: Here we have an accurate characterization of our understanding of the overloading phenomenon. It says nothing about "immune" mechanisms.

P 48, L 4-6: At what dose does cerium nitrate cause clastogenic and mutagenic effects in this test system? What is its mutagenic potency relative to known mutagens?

P 48, Section 4.8.2: The paragraph cites gender differences in responses to i.v. administration, for which there is no current explanation. That is fine as far as it goes. However, the paragraph does not mention the gender differences observed in the BRL (1994) inhalation study. The weaknesses of that study aside, greater responses were observed in females than in males. Nothing is mentioned in the paragraph of the well-known fact that greater effects are typically found in females in studies of repeated inhalation exposures to toxicants, and especially particulate toxicants. The reason for this is not completely known, but one likely contributing factor is that females deposit greater amounts of respirable particles in the lung than males, no matter what the exposure. All studies including actual measures of lung burdens of particles in both genders have shown this. Whether or not the gender difference in response is proportional to the gender difference in accumulation of particles is less clear. Regardless, the paragraph should mention the inhalation work as well as the i.v. work. Inhalation is certainly the more relevant.

P 49, L 6: Current understanding suggests that translocation is certainly not just a function of the number of particles. It is also a function of the size and physical-

chemical characteristics of the particles. Although the sentence goes on to mention size, it is odd that it starts our stating that it is a function of number.

P 52, L 26-28: Again, the "cerium-fused aluminosilicate" particles were completely different types of particles than any of concern for environmental or occupational exposures. A small amount of Ce was incorporated into high temperature-fused "ceramic" or "glass" spheres as a means of getting radioactive Ce in the body in a highly insoluble form in several Lovelace studies (as was done with other radionuclides as well). Some control groups received non-radioactive Ce in the same delivery form. It is extremely unlikely that the body "perceived" any Ce exposure at all in this delivery mode. The approach was suitable for the questions being addressed by those studies, but has nothing to do with assessing the toxicity of stable Ce.

P 53, L 29-36: This paragraph cites none of the sources for factors used in estimating doses. For one, the pulmonary surface area of 0.34 for Sprague-Dawley rats seems low. For another, estimating minute volume to hundredths of a ml is completely absurd. No direct measurement could be that accurate, let alone a "normal value" for estimating dose! Because the results are entirely dependent on the factors used to estimate them, the source of factors should be made explicit.

P 62, L 21- 23: Although the Lundgren paper may not have compared carcinogenicity in controls to those in unexposed control rats, there is lots of information available to EPA for doing this. There have been many long-term carcinogenicity studies of F344 rats, and a review of the literature would indicate that the historical control incidence is approximately 1.0%. Indeed, this was one factor in the recent shift by NTP to Wistar rats, so the literature was recently reviewed. The tumor incidence in the CeO_2-exposed control rats in the Lundgren study is listed here as 1/1049, or 0.7%. It is simple to make the comparison to historical data, and the comparison would suggest that the CeO_2 was not carcinogenic in the Lundgren study.

Günter Oberdörster

A major omission in my view is that there is no section on airborne exposure concentrations of cerium-oxide, at workplaces, in the ambient environment. That should have been inserted at the beginning, following Chapter 2, Chemical and Physical Information. Also, there are several places in the text throughout the document that require changes to avoid misunderstandings/misconceptions; however, this does not affect the major objective of risk characterization.

Point out need for more realistic long-term study with respect to selected conc. and particle size (nano-Ceria).

Emphasize that RfC should not apply to nano-Ceria due to lack of data for inhaled nano-Ceria.

Katherine S. Squibb

The text on lines 27-30, page 36 that describes the morphological changes in the PAMs exposed to Ce oxide in PMS is not clear and should be reworded.

John M. Veranth

Report is well written and carefully edited. I found no typographic errors worth mentioning.