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Report on the Peer Review of the U.S. Environmental Protection Agency's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

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Report on the Peer Review of the U.S. Environmental Protection Agency's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

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NOTICE

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This report was prepared by Eastern Research Group, Inc. (ERG), an EPA contractor (Contract No. 68-C-99-237, Task Order No. 58), as a general record of discussion for the peer review meeting. This report captures the main points of scheduled presentations, highlights discussions among the reviewers, and documents the public comments provided at the peer review meeting. This report does not contain a verbatim transcript of all issues discussed during the peer review, and it does not embellish, interpret, or enlarge upon matters that were incomplete or unclear. Except as specifically noted, no statements in this report represent analyses by or positions of EPA or ERG.

Table of Contents

Table of Contents (Continued)

Table of Contents (Continued)

Appendices

List of Abbreviations

AFRL	Air Force Research Laboratory
ANOVA	analysis of variance
AUCB	area-under-the-curve in blood
AUCT	area-under-the-curve in thyroid
BCF	bioconcentration factor
EPA	U.S. Environmental Protection Agency
Revised ERD	revised external review draft (i.e., the review document)
ERG	Eastern Research Group, Inc.
HPT axis	hypothalamic-pituitary-thyroid axis
K_m	Michaelis-Menten affinity constant
IRIS	Integrated Risk Information System
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
NCEA	National Center for Environmental Assessment
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIS	sodium (Na^+)–iodide (I^-) symporter
NOAEL	no-observed-adverse-effect level
PBPK	physiologically based pharmacokinetic
PWG	Pathology Working Group
RAIU	radioactive iodide uptake
RIA	radioimmunoassay
RfD	reference dose
STP	Society of Toxicologic Pathologists
T3	triiodothyronine
T4	thyroxine or tetraiodothyronine
TBG	thyroxine-binding globulin
TPO	thyroid peroxidase
TSH	thyroid-stimulating hormone
USAF	U.S. Air Force
Vmaxc	Michaelis-Menten maximum velocity capacity
WPAFB	Wright Patterson Air Force Base

Executive Summary

This report summarizes a peer review of the U.S. Environmental Protection Agency's external review draft document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization" (the Revised External Review Draft, or Revised ERD), and of associated studies published since 1999 that have not been published in externally-reviewed scientific literature. During the 2-day peer review meeting, 17 independent experts from a broad range of relevant scientific backgrounds and affiliations thoroughly discussed and evaluated the scientific analyses presented in the Revised ERD. The reviewers had favorable feedback on many issues, such as the proposed harmonized approach for evaluating cancer and noncancer endpoints, and constructive feedback on others. Reviewers expressed a diversity of opinions on several critical issues, including the role of human health data in the Revised ERD, the use of reported changes in rat brain morphometry as a point of departure, and the application of uncertainty factors.

The peer reviewers' main findings on the Revised ERD are summarized below, organized into topic areas covered during the peer review meeting. The remainder of this report documents the extensive discussions that led up to these main findings presented below, as well as deliberations on additional topics not noted in this Executive Summary.

- **Topic Area A: Hazard Characterization and Mode of Action (see Section 2).** The peer reviewers generally supported the proposed key event, mode of action, harmonized approach for characterizing cancer and noncancer toxicity, and approach for low-dose extrapolations. Some reviewers, however, questioned assumptions EPA made regarding perchlorate not being metabolized and being actively translocated into thyroid cells.

- **Topic Area B: Human Health Effects Data (see Section 3).** Several reviewers recommended that EPA consider deriving a reference dose using data from the human health effects studies, particularly those from a recent clinical study (the "Greer study"). On the other hand, some reviewers cautioned against using these studies, given their lack of control for confounding factors, limited exposure duration, consideration of only healthy adults, and focus on a narrow set of toxicologic endpoints.

- **Topic Area C: Laboratory Animal Studies (see Section 4).** The peer reviewers' comments on laboratory animal studies were made for the individual toxic endpoints:

  – The reviewers concluded that the two *developmental toxicity* studies completed since the 1999 peer review were scientifically sound and that both studies indicate that developmental toxicity appears to occur at doses orders of magnitude higher than those causing effects at other endpoints.

  – The reviewer found the two-generation *reproductive toxicity* study in rats conducted since the 1999 peer review to be thorough and well conducted and EPA's interpretations of these studies generally adequate. They recommended that EPA further investigate apparent dose-dependent decreases in sperm density and daily sperm production levels.

  – The reviewers with expertise in *endocrinology* noted that most laboratory animal studies of thyroid hormone levels have detected effects, although not consistently within and across studies—an outcome attributed primarily to limitations of the measurement techniques (i.e., decrements in certain hormone levels cannot be quantified reliably when baseline hormone levels are near the lowest range of the diagnostic kits' standard curves). The reviewers had different opinions on the most appropriate statistical approach for analyzing these data: one reviewer supported EPA's use of analysis of variance, while another advocated another advocated testing hypotheses with a complete pharmacokinetic and pharmacodynamic model.

  – The reviewer assigned to evaluate *thyroid histopathology* indicated that administrating low doses of perchlorate to rats and rabbits produced adaptive changes in thyroid histopathology: colloid depletion and epithelial hypertrophy. Higher doses (at least 1.0 mg/kg/day) are needed to produce hyperplasia, which presumably resulted from upregulation of thyroid-stimulating hormone (TSH). While dosage at considerably higher levels (30 mg/kg/day) produced follicular cell neoplasms in rats, several reviewers doubted humans would develop this cancer from environmental exposure to perchlorate at the currently reported levels.

  – The reviewers commented on two different types of studies evaluating *neurotoxicity*. First, the reviewers indicated that the two studies of motor activity in rats were conducted using rigorous methodologies. Moreover, they concluded that EPA's interpretations of these studies were appropriate and defensible, including EPA's identification of dose-related motor activity effects in the most recent study (Bekkedal et al. 2000).

Second, the reviewers had different opinions on the studies examining brain morphometry changes in rats. Most reviewers agreed that use of linear measurements to characterize brain dimensions is subject to artifacts. They had different perspectives, however, on how EPA should interpret the data given the limitations. Some reviewers argued that linear measurements of brain dimensions in rat pups are not reliable indications of brain morphometry changes. Other reviewers, however, believed that errors introduced by using linear measurements would be randomly distributed across dosage groups; this would most likely make it impossible to detect statistically significant effects, not to detect effects that do not exist. Overall, given the weaknesses in the study methodology and other concerns described later in this report, some reviewers felt that EPA should consider the brain morphometry data inconclusive. Other reviewers, on the other hand, did not support disregarding these data, especially considering that two studies and several re-analyses of them have all identified brain morphometric changes in consistent regions of the brain.

– The reviewer who addressed *immunotoxicity* focused primarily on the two studies that were performed since the 1999 peer review, including a study of contact hypersensitivity conducted in response to a recommendation of that peer review. This reviewer noted that both studies followed standard protocols and used validated assays to evaluate both the innate and acquired immune responses, considering most compartments of the immune system. Though this reviewer generally supported EPA's interpretations of these studies, he questioned the relevance to humans of the contact hypersensitivity findings, which exhibited no clear dose-response. This reviewer concluded (and several other reviewers agreed) that the immunotoxicity studies should not be used as a point of departure for the reference dose determination and do not provide an adequate basis for applying an uncertainty factor of 3 to account for database insufficiencies.

– No new *genotoxicity* studies have been published since the 1999 peer review. The reviewers supported the findings of the previous peer review panel (that perchlorate is not genotoxic) and EPA's relevant dose-response interpretations (that cancer endpoints can be evaluated using a nonlinear dose-response model).

• **Topic Area D: Ecological Risk Assessment and Evidence for Indirect Exposure (see Section 5).** The peer reviewers generally supported EPA's assimilation and interpretation of exposure and effects data that were available at the time the Revised ERD was prepared. The reviewers were concerned, however, by a study published after the Revised ERD was released that suggests amphibians may be experiencing toxic effects at perchlorate exposures considerably lower than those EPA previously predicted (Goleman et al. 2002). Though they identified potential limitations of the recent study, they concluded that its implications suggest that the current screening-level ecological risk assessment is not

adequate. The reviewers identified several issues that must be evaluated further if environmental exposure and ecological risk are to be assessed more thoroughly.

- **Topic Area E: Use of Physiologically Based Pharmacokinetic (PBPK) Modeling (see Section 6).** The peer reviewers found the structure, basic equations, and physiological parameters in the PBPK models to be generally adequate, though sometimes not documented in sufficient detail in the Revised ERD. They recommended that the PBPK models should include more refined descriptions of passive iodide uptake and active perchlorate uptake and the kinetic representation of these processes. The reviewers had different opinions on the proposed dose metric. Some concluded that use of area-under-the-curve perchlorate in serum is the most defensible dose metric and is suitable for purposes of interspecies extrapolation. The other reviewer, however, advocated the development of a biologically-based dose response model that would link pharmacodynamic changes in the thyroid hormones with internal perchlorate dose and iodide uptake inhibition.

- **Topic Area F: Human Health Dose-Response Assessment (see Section 7).** The reviewers comments on the human health dose-response assessment primarily addressed point of departure and the use of uncertainty factors. Consistent with their differing reviews of the brain morphometry study (summarized above), the peer reviewers had differing opinions on whether EPA should use the brain morphometry changes as a point of departure: some reviewers supported using the brain morphometry data in the reference dose derivation, while roughly an equal number of reviewers did not. Several reviewers, however, indicated that EPA may be able to justify using the brain morphometry data as a point of departure if they can be re-scored blindly and the effects still observed.

  The reviewers discussed other options for selecting a point of departure. Some suggested using data from human clinical studies, but others expressed concern about the limitations of these data sets. Though they acknowledged that EPA could derive a point of departure based on changes in thyroid hormone levels and iodide uptake inhibition in laboratory animals, several reviewers questioned whether such effects are adaptive or truly adverse. One reviewer noted that thyroid histopathology can be defended as a point of departure, but, regarding neoplastic sequelae, he recommended that EPA only consider hyperplasia as an adverse effect, with colloid depletion and hypertrophy being adaptive effects.

  Regarding uncertainty factors, most reviewers accepted the factors of 10 applied for intraspecies variability and extrapolating a lowest-observed-adverse-effect level to a no-observed-adverse-effect level. Nearly every reviewer, however, was against applying an uncertainty factor of 3 to account for database insufficiencies in immunotoxicity. Many reviewers supported the use of an uncertainty factor of 3 to account for the limited exposure duration of the laboratory animal studies, but some found this factor unnecessary. During this discussion, several reviewers proposed alternate statistical and modeling approaches to replace EPA's general practice of using discrete uncertainty factors.

- **Topic Area G: Risk Characterization (see Section 8).** The reviewers recommended that EPA revise the risk characterization to reflect any changes made when addressing the issues mentioned above. Moreover, they recommended that the risk characterization give greater context for the proposed reference dose and potential health risks, perhaps by describing public health consequences of exposure and by acknowledging the uncertainties associated with the reference dose derivation.

1.0 Introduction

This report summarizes a peer review by 17 experts of documents that the U.S. Environmental Protection Agency (EPA) prepared or evaluated when assessing human health and ecological risks associated with exposure to perchlorate. These documents are:

- The January 2002 external review draft of "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization" (EPA 2002). (Throughout this report, this document is referred to as the Revised External Review Draft, or Revised ERD.)

- Relevant studies performed since the 1999 peer review of EPA's perchlorate assessment, but provided as contractor reports or as preliminary findings (e.g., as an abstract or letter to the editor) and not yet vetted in the peer-reviewed literature at the time the current peer review was scheduled.

Eastern Research Group, Inc. (ERG), organized and implemented the peer review under a contract to EPA. The peer review took place in a meeting open to the public on March 5 and 6, 2002, in Sacramento, California. This introductory section provides background information on EPA's perchlorate assessment (Section 1.1), the scope of this peer review (Section 1.2), and the organization of this report (Section 1.3).

1.1 Background

Perchlorate (ClO_4^-) is an anion that contaminates groundwater and surface waters, where it originates from dissolution of ammonium, potassium, magnesium, or sodium salts. Perchlorate is exceedingly mobile in aqueous systems and can persist for many decades under typical groundwater and surface water conditions. A major source of perchlorate contamination is the manufacture of ammonium perchlorate for use as the oxidizer component and primary ingredient in solid propellant for rockets, missiles, and fireworks.

EPA issued a provisional toxicity assessment for perchlorate in 1992 and a revised provisional assessment in 1995, based on the effects of potassium perchlorate in patients with

Graves' disease (an autoimmune disease that results in hyperthyroidism). In March 1997, an independent non-EPA external peer review panel determined that the existing toxicologic database on perchlorate was inadequate for quantitative human health risk assessment. In May 1997, a perchlorate testing strategy was developed. This strategy initiated an accelerated research program to inform future human health and ecological risk assessment studies.

In December 1998, EPA developed a draft external peer review version (EPA 1998) of a document that assessed the most current information at the time on perchlorate toxicity. This document included a human health risk assessment, which incorporated results from health effects studies available as of November 1998, and a screening-level ecological assessment. The human health risk assessment presented a model (motivated by the mode of action) that harmonized noncancer and cancer evaluations to derive a single oral risk benchmark. This benchmark was based on precursor effects for both altered neurodevelopment and thyroid neoplasia.

In February 1999, an EPA contractor held an external peer review meeting to evaluate EPA's 1998 draft perchlorate assessment. The review panel endorsed the conceptual approach presented in the draft assessment, but recommended that new analyses be conducted and that new studies be planned and performed. After the 1999 external peer review, EPA prepared the Revised ERD of perchlorate toxicity (EPA 2002), which incorporates data from the studies that the previous peer review panel recommended. Both the supporting data from these studies and the Revised ERD are the subject of the current external peer review.

To evaluate whether the assumptions, methods, and conclusions of the Revised ERD are based on sound scientific principles, EPA decided, as per policy, to obtain an independent, expert peer review not only of the Revised ERD but also of the relevant studies performed since the 1999 peer review that are not documented in the peer-reviewed literature. Appendix A lists the studies that the reviewers evaluated during the current peer review. EPA hired ERG to implement the current peer review.

1.2 Scope of the Peer Review

ERG managed every aspect of the peer review, including selecting reviewers (see Section 1.2.1); coordinating selected activities prior to, during, and after the peer review meeting (see Sections 1.2.2, 1.2.3, and 1.2.4, respectively); and preparing this summary report, which describes the scope and findings of the peer review. The following subsections describe what each of these tasks entailed.

1.2.1 Selecting the Reviewers

ERG followed its long-standing procedures for conducting expert peer reviews to select 17 highly qualified and independent reviewers. The initial step was to establish reviewer selection criteria. The specific criteria for this peer review follow:

- Reviewers must be senior scientists or researchers with broad experience and expertise (as demonstrated by peer-reviewed publications, awards, and service to relevant professional societies) in the following fields: pharmacokinetics, endocrinology, neurotoxicology, epidemiology, statistics, immunotoxicology, thyroid pathology, developmental toxicology, reproductive toxicology, genetic toxicology, ecotoxicology, and environmental transport and biotransformation.
- Some reviewers should have working knowledge of EPA's risk assessment guidelines and methodologies, as well as familiarity with the content, format, and objectives of recent health assessments included in the Integrated Risk Information System (IRIS) database.
- Reviewers must be available to critique the review materials and present their comments at the peer review meeting.
- Reviewers must have no conflicts of interest in performing the peer review.

To implement the fourth selection criterion, ERG distributed a conflict-of-interest screening form to all candidate reviewers. ERG used the self-reported responses on the form to eliminate from consideration any candidates who have real or perceived conflicts of interest. For instance, ERG did not consider any candidates who have a vested interest, financial or otherwise,

in the outcome of the peer review or those who have conflicts of interest with EPA on pending scientific issues pertaining to this review. Further, ERG did not consider candidates who prepared or edited any section of the Revised ERD or other federal documents related to perchlorate. Finally, ERG did not consider candidates who have worked on Superfund sites at which perchlorate is a contaminant of concern, who have worked for potentially responsible parties for such sites, or who have worked for companies that are members of the Perchlorate Study Group.[1]

After establishing the selection criteria, ERG began to identify a large pool of highly experienced candidates. To identify qualified candidates, ERG conducted literature reviews to identify widely published researchers, contacted reviewers from the 1999 external peer review, and performed various other searches for experts in relevant disciplines. Overall, ERG contacted more than 250 candidate peer reviewers. ERG carefully reviewed the expertise and credentials of these candidates and selected the 17 most qualified individuals.

Appendix B lists the names and affiliations of the 17 peer reviewers, and Appendix C includes brief biographies that summarize the reviewers' areas of expertise. Recognizing that few individuals truly specialize in every technical area specified by the first reviewer selection criterion, ERG ensured that the collective expertise of the selected peer reviewers covers the required technical areas (i.e., at least one reviewer has expertise in immunotoxicity, at least one reviewer has expertise in neurotoxicity, at least one reviewer has expertise in reproductive toxicology, and so on). Moreover, ERG selected peer reviewers with a broad range of affiliations (e.g., academia, consulting, industry, other federal agencies), in hope that the expert panel would offer a balanced perspective on the scheduled discussion topics. ERG instructed the reviewers to remain independent throughout the peer review process, and therefore refrain from discussing the scientific merit of the Revised ERD with any of the identified stakeholders. ERG had copies of

[1] During the opening conflict-of-interest disclosures, one reviewer (Dr. Gary Williams) indicated that he had worked for Kerr-McGee (a member of the Perchlorate Study Group), but that he had learned that Kerr McGee has an interest in the Revised ERD only upon seeing that the company submitted public comments on EPA's document.

the peer reviewers' resumes and conflict-of-interest disclosure forms on display at the peer review meeting.

1.2.2 Activities Prior to the External Peer Review Meeting

ERG took several steps to ensure that the peer reviewers had the information necessary to conduct thorough, informed, and unbiased reviews of the Revised ERD. The specific activities that ERG conducted prior to the peer review meeting follow:

- *Prepare a charge to the reviewers.* ERG first worked with EPA to prepare written guidelines (commonly called a "charge") for the technical review. Specifically, EPA identified technical issues that the charge should address, and ERG incorporated these issues into 30 charge questions, organized into 8 topic areas. The charge included a question that asked the peer reviewers to comment on any topics not explicitly addressed by the other questions. Copies of the charge were available prior to the meeting, upon request, and at the peer review meeting; a copy is included in this report as part of Appendix C.

  In the charge, ERG assigned different responsibilities to the individual reviewers. Every reviewer was asked to read the entire Revised ERD, focusing on specific sections relevant to their areas of expertise. In the charge, ERG required almost every peer reviewer to evaluate some of the studies that were conducted since the 1999 peer review meeting and that were not published in the peer-reviewed literature at the time the March 2002 meeting was planned. ERG ensured that at least one expert peer reviewer critically evaluated every study listed in Appendix A. Finally, the charge identified the peer reviewers who would lead discussions on the eight topic areas during the peer review meeting.

- *Distribute review documents and communicate reviewer assignments.* On January 23, 2002, ERG sent every peer reviewer a package of review materials. These packages included the charge, the Revised ERD, copies of the studies completed since the 1999 peer review (see Appendix A), and logistical information regarding the peer review. Copies of these documents were made available to observers at the peer review meeting. ERG held several conference calls with the reviewers to confirm the shipment of the review materials and to answer any questions about the peer reviewers' assignments. ERG facilitated a conference call with the peer reviewers prior to the workshop to ensure that the reviewers understood their assignments. During this call, ERG informed the reviewers of the procedures they should follow to ask EPA or the various study authors questions of clarification prior to the external peer review meeting (see next bulleted item).

- *Facilitate questions of clarification.* When conducting their reviews, some peer reviewers informed ERG that they had questions of clarification for the authors of the recent studies and of the Revised ERD. ERG forwarded these questions to the appropriate individuals and then forwarded the responses to the peer reviewers. To maintain the independence of the peer review, ERG asked that the peer reviewers refrain from contacting the study authors or representatives from EPA directly with any such questions.

- *Obtain and compile the reviewers' premeeting comments.* In the weeks after the peer reviewers received the charge, ERG asked the reviewers to prepare their initial evaluations of the Revised ERD and the studies listed in Appendix A. ERG compiled these written premeeting comments, distributed them to the reviewers, and made copies available to observers during the peer review meeting. These initial comments are included in this report, without modification, as Appendix C. It should be noted that the premeeting comments are preliminary in nature. Some reviewers' technical findings changed after the premeeting comments were submitted. Therefore, the comments in Appendix C should not be considered the reviewers' final opinions.

- *Distribute public comments for the reviewers' consideration.* After receiving the reviewers' premeeting comments, ERG sent the peer reviewers two sets of copies of public comments. First, before the peer reviewers departed for the meeting, ERG sent them packages with copies of all comments that were received by February 19, 2002. Second, at the peer review meeting, ERG handed the peer reviewers copies of all public comments that were received between February 20, 2002, and March 5, 2002. ERG distributed the entire set of public comments prior to the meeting, so that the peer reviewers could factor any issues raised into the meeting deliberations. Appendix D includes an index of the public comments that ERG sent to the reviewers prior to the peer review meeting. Copies of these comments were made available to observers at the meeting.

1.2.3 Activities at the External Peer Review Meeting

The 17 peer reviewers[2] and approximately 150 observers attended the peer review meeting, which was held at the Holiday Inn hotel in Sacramento, California, on March 5 and 6, 2002. The peer review meeting was open to the public, and the meeting dates and times were announced in the Federal Register. Appendix F lists the observers who confirmed their attendance at the

[2] Dr. Michael Kohn could not attend the peer review meeting in person, but participated in the discussions relevant to his area of expertise via conference call. Dr. Michael Aschner attended the first day of the peer review meeting, but could not attend the second.

meeting registration desk. The schedule of the peer review meeting generally followed the agenda, presented here as Appendix G.

The meeting began with introductory comments by the meeting's facilitator (Jan Connery of ERG) and three representatives from EPA. All of these comments are summarized below. Before beginning their deliberations, the peer reviewers were asked to introduce themselves by stating their names, affiliations, areas of expertise, and any potential conflicts of interest they had. (Table 1, at the end of this section, summarizes the reviewers' specific remarks regarding conflicts of interest.) For the remainder of the meeting, the peer reviewers provided many comments, observations, and recommendations when answering the charge questions. ERG ensured that peer reviewers presented their own opinions on technical topics; no efforts were made to reach consensus on any issue. The meeting included three designated observer comment periods, when observers were allowed to offer verbal comments. Appendix H documents all observer comments presented at the peer review meeting and includes copies of written handouts that were distributed by those who spoke.

The remainder of this section summarizes the opening remarks provided by ERG and the three EPA representatives. Copies of Dr. Herman Gibb's and Ms. Annie Jarabek's presentation materials are included in Appendix I of this report.

- *Jan Connery, ERG, meeting facilitator.* In her opening remarks, Ms. Connery welcomed the reviewers and observers to the meeting, stated the purpose of the peer review, and identified the document under review. Later in the meeting, Ms. Connery explained the procedure observers should follow to make comments, both orally at the meeting and in writing to EPA. Ms. Connery noted that EPA had extended the public comment period through April 5, 2002, and that ERG would mail the peer reviewers copies of all public comments submitted by that date. She also summarized several key aspects of the peer review, including some activities that took place prior to the peer review meeting (see Section 1.2.2) and planned future activities (see Section 1.2.4).

- *Jane Diamond, Acting Director of Superfund in EPA Region IX.* Ms. Diamond's opening remarks addressed the challenges that EPA faces when examining sites with perchlorate contamination. Ms. Diamond said roughly 10% of Superfund sites in Region IX have perchlorate contamination, as do several other sites not on the National Priorities List. For these sites, she noted that the emerging science on perchlorate toxicity has affected how the Region determines action levels and makes remedial decisions. Given that the states within Region IX are developing their own "action levels" for perchlorate, and that none of the action levels are consistent, Ms. Diamond said she looks forward to EPA establishing an official reference dose (RfD). Finally, Ms. Diamond acknowledged the efforts of numerous parties (e.g., EPA's Office of Research and Development, the Department of Defense, other researchers, consultants, and community activists) for their ongoing work on evaluating perchlorate toxicity.

- *Dr. Herman Gibb, Acting Associate Director for Health at EPA's National Center for Environmental Assessment (NCEA).* Dr. Gibb's presentation provided context on EPA's ongoing activities addressing levels of perchlorate environmental contamination. Dr. Gibb acknowledged that recent advances in analytical methods have allowed scientists and regulators to characterize perchlorate environmental contamination at much lower concentrations than could be achieved with other methods. These advances helped reveal that perchlorate contamination is more widespread than previously thought—an observation that has heightened concern for potential toxicity of perchlorate exposures. Dr. Gibb noted that EPA has since sought more detailed information on the occurrence of this contamination, fate and transport of perchlorate in various media, the potential for both direct and indirect exposures, adverse effects on human health and ecosystems, and effective treatment technologies. Dr. Gibb indicated that many advances have been made in these fields, due largely to contributions from multiple parties, including the Department of Defense, the Perchlorate Study Group (a consortium of defense contractors), multiple EPA Offices and Regions, private researchers, and numerous local, state, federal, and tribal agencies.

  Focusing on the current peer review, Dr. Gibb explained that the Revised ERD not only accounts for comments raised and recommendations made during the 1999 peer review, but also incorporates data from studies conducted since that time. He noted that the goal of the current peer review is to critique the scientific studies and approaches in the Revised ERD and the supporting documents, not to address potential regulatory actions. Dr. Gibb added that the external peer review is one of many steps that EPA takes in reviewing and finalizing documents for IRIS. Dr. Gibb mentioned other steps in this process (e.g., disposition of comments, internal EPA consensus review) and presented a proposed schedule for these and other future activities in the perchlorate assessment.

- *Ms. Annie Jarabek, Chemical Manager for the Revised ERD, EPA NCEA.* Ms. Jarabek's presentation reviewed EPA's scientific analyses of perchlorate toxicity and highlighted notable milestones from the 1999 peer review through release of the Revised ERD. Ms.

Jarabek first summarized the content of the previous external review draft (EPA 1998). She presented the proposed mode of action, which linked exposure to the key event of iodide uptake inhibition with early biological effects (e.g., decrements in thyroid hormones) that ultimately lead to clinical disease. In the previous draft, EPA's dose-response analyses were based largely on thyroid histopathology findings observed in rat pups, which EPA viewed as biomarkers for adverse hormonal changes believed to occur *in utero*. Ms. Jarabek also indicated that the previous draft included a screening-level ecological risk assessment.

Summarizing the findings of the 1999 peer review, Ms. Jarabek noted that the panelists endorsed EPA's proposed mode of action and conceptual model, shared the Agency's concern about potential neurodevelopmental effects, and made several recommendations to conduct new toxicity studies and to perform additional statistical analyses of the existing data. Ms. Jarabek identified several of the peer reviewers' recommendations. For instance, the 1999 peer review panel recommended that EPA convene a Pathology Working Group (PWG) to review thyroid tissue slides and histopathology diagnoses, that additional laboratory animal studies be performed or repeated, and that EPA and other researchers conduct additional studies to characterize the environmental fate and transport of perchlorate. Ms. Jarabek then reviewed the many research projects completed since 1999, many of which were done specifically in response to the peer reviewers' recommendations.

Focusing on the Revised ERD, Ms. Jarabek briefly summarized EPA's analyses of human health effects data, laboratory animal studies, ecotoxicological studies, and human health dose-response. Specifically, when addressing human studies, Ms. Jarabek identified the new studies conducted since the 1999 peer review, clarified EPA's policy on considering third-party human dosing data in toxicity assessments, and emphasized that the Revised ERD considers all third-party human data that were available to the agency as of December 14, 2001. Similarly, Ms. Jarabek identified the various laboratory animal studies and ecotoxicological studies conducted since the 1999 peer review meeting and presented selected EPA interpretations of the findings. She emphasized that the Revised ERD presents a screening-level ecological risk assessment.

Ms. Jarabek then reviewed how EPA derived its proposed RfD for perchlorate from the available studies. After reviewing the effect levels EPA and study authors calculated for various endpoints, Ms. Jarabek summarized the Agency's weight-of-evidence approach used to identify a point of departure. The proposed value was a lowest-observed-adverse-effect level (LOAEL) of 0.01 mg/kg/day, based on effects (e.g., perturbation in thyroid and pituitary hormones, thyroid histopathology, and brain morphometry effects) observed in multiple laboratory animal studies at various life stages. Ms. Jarabek highlighted key features of the four physiologically based pharmacokinetic (PBPK) models that EPA used to extrapolate the dose-response data observed in the animal studies to humans. Finally, she identified the composite uncertainty factor (300) that EPA proposed for developing an

RfD and described the individual factors that constitute this value. Briefly, she noted that EPA proposes the following:

- An uncertainty factor of 3 for intrahuman variability.
- No uncertainty factor for interspecies extrapolations.
- An uncertainty factor of 10 to extrapolate the LOAEL to a no-observed-adverse-effect level (NOAEL).
- An uncertainty factor of 3 to account for subchronic data extrapolation and concern for *in utero* programming.
- An uncertainty factor of 3 to account for database insufficiencies, particularly those associated with immunotoxic effects.

Based on these factors and the selected point of departure, the Revised ERD proposed an RfD of 0.00003 mg/kg/day. Ms. Jarabek presented two comparative risk calculations, one based on the use of the human clinical data from the Greer study and the other based on thyroid tumors observed in the two-generation study. Both of these alternative derivations resulted in RfD estimates within the range of the proposed value. Ms. Jarabek also presented hypothetical calculations of Drinking Water Equivalent Levels (DWELs) that might result from the proposed RfD, though she emphasized that the proposed RfD is based entirely on scientific analyses of the available toxicity studies and is not a regulatory standard.

Following these opening presentations, Dr. Ron Wyzga, a peer reviewer and the designated chair of the meeting, opened the technical discussions among the peer reviewers. Dr. Wyzga explained that he would ask the designated discussion leaders to facilitate the reviewers' discussions for the individual topic areas. The discussion leaders drew from the reviewers' premeeting comments to initiate discussions. ERG notes that the discussions at the meeting (and not the premeeting comments) should be viewed as the reviewers' final opinions on the Revised ERD. The technical discussions among the peer reviewers focused almost entirely on answering the 30 charge questions. The only instances in which individuals other than ERG or the peer reviewers spoke were when the reviewers asked EPA questions of clarification, which were facilitated by either Ms. Connery or Dr. Wyzga, and when the observers gave comments during the designated observer comment period, as documented in Appendix H.

1.2.4 Activities Following the External Peer Review Meeting

Following the peer review meeting, ERG's involvement in the peer review was limited to two activities. First, ERG distributed written public comments received after the peer review meeting. Specifically, ERG mailed to the peer reviewers copies of all public comments that EPA received between March 6, 2002, and the close of the extended public comment period (April 5, 2002). Appendix E includes an index of the comments that ERG distributed during that time. The peer reviewers were given the option to submit post-meeting comments based on the information presented in these supplemental public comments. Appendix J presents all the post-meeting comments that the peer reviewers submitted to ERG.

Second, an ERG technical writer who attended the peer review meeting prepared this summary report. ERG distributed a draft of this report to the 17 peer reviewers and asked them to verify that it accurately reflected the tone and content of the discussions at the peer review meeting. After every peer reviewer submitted suggested revisions to the summary report or indicated that the report was a faithful account of the peer review meeting, ERG submitted the final peer review report (i.e., this report) to EPA.

1.3 Report Organization

The structure of this report follows the order of the reviewers' discussions during the peer review meeting. For instance, Section 2 summarizes the reviewers' responses to the charge questions in topic area A (hazard characterization and mode of action), Section 3 summarizes the discussions on topic area B (human health effects data), and so on. Finally, Section 10 provides references for all documents cited in the text. Throughout the main body of the report, the reviewers' initials are used to attribute technical comments, suggestions, and recommendations to the peer reviewers who made them. The following key lists the initials used:

WA = Dr. William Adams, Ph.D.
MA = Dr. Michael Aschner, Ph.D.
NC = Dr. Nancy Carrasco, M.D.
MC = Dr. Michael Collins, Ph.D.
TC = Dr. Thomas Collins, Ph.D.
AC = Dr. Anthony Cox, Ph.D.
TF = Dr. Teresa Fan, Ph.D.
DH = Dr. David Hoel, Ph.D.
DJ = Dr. David Jacobson-Kram, Ph.D.
MK = Dr. Michael Kohn, Ph.D.
LK = Dr. Loren Koller, Ph.D.
KK = Dr. Kannan Krishnan, Ph.D.
MP = Dr. Merle Paule, Ph.D.
MR = Dr. Mehdi Razzaghi, Ph.D.
GW = Dr. Gary Williams, M.D.
RW = Dr. Ronald Wyzga, Ph.D.
TZ = Dr. Thomas Zoeller, Ph.D.

The appendices to this report include extensive background information on the peer review meeting. This information includes items made available to all meeting attendees, as well as items generated since the peer review meeting (e.g., a final list of attendees). Specifically, the following information is included as appendices:

- A list of studies performed since the 1999 peer review, but not documented in the peer-reviewed literature at the time when the March 2002 peer review was organized (Appendix A).
- A list of the peer reviewers (Appendix B).
- Premeeting comments and the charge to the reviewers (Appendix C).
- An index of written public comments submitted before the peer review meeting (Appendix D).
- An index of written public comments submitted after the peer review meeting (Appendix E).
- A list of registered observers of the peer review meeting (Appendix F).
- The agenda for the peer review meeting (Appendix G).
- Public comments given during the peer review meeting (Appendix H).
- Copies of EPA's opening presentation materials (Appendix I).
- Copies of post-meeting comments submitted by the peer reviewers (Appendix J).

- An index of written materials that observers handed to peer reviewers during the observer comment period (Appendix K).

Table 1
Conflict-of-Interest Disclosures Made at the Peer Review Meeting

Peer Reviewer	Conflict-of-Interest Disclosure
Dr. William Adams	Dr. Adams noted that a perchlorate groundwater contamination plume is in the vicinity of, but not associated with releases from, his employer.
Dr. Michael Aschner	Dr. Aschner indicated that he has recently applied for a research grant with the Department of Defense (a stakeholder in EPA's perchlorate work), but for a research topic not relevant to perchlorate.
Dr. David Hoel	Dr. Hoel mentioned that he is a member of the EPA Science Advisory Board's Environmental Health Committee. He added that he advises the Department of Defense (a stakeholder in EPA's perchlorate work) on issues pertaining to depleted uranium and nerve agents.
Dr. David Jacobson-Kram	Dr. Jacobson-Kram noted that the laboratory where he works (BioReliance Corporation) conducted one of the mutagenicity studies cited in the Revised ERD.
Dr. Michael Kohn	Dr. Kohn mentioned that he has conducted past projects in which he modeled changes in thyroid hormone levels. He noted that this past work was relevant to perchlorate, but he did not specify whether it explicitly considered perchlorate.
Dr. Merle Paule	Dr. Paule indicated that he has recently been pursuing employment opportunities with EPA.
Gary Williams, M.D.	Dr. Williams indicated that he currently serves as a medical expert in a lawsuit in which Kerr McGee (a stakeholder in EPA's perchlorate work) is a defendant. He noted that this work does not involve perchlorate and that he has not actually met with Kerr McGee employees. Dr. Williams added that he learned that Kerr McGee has an interest in the Revised ERD only upon seeing that the company submitted public comments on EPA's document.
Dr. Thomas Zoeller	Dr. Zoeller noted that he serves on the Screening and Testing Workgroup of EPA's Endocrine Disruptor Screening and Testing Advisory Committee. He mentioned that he was a panelist for the 1999 peer review of EPA's perchlorate risk assessment, after which multiple news organizations, other parties, and various sectors contacted him for comment.

Notes: The ten peer reviewers not listed in this table all stated that they have no known conflicts of interest in performing their review.
This table summarizes the peer reviewers' disclosures at the meeting, which may or may not present true conflicts of interest. However, most of the reviewers listed above explicitly stated at the meeting that they did not perceive their past or ongoing activities as conflicts of interest.

2.0 Responses to Questions in Topic Area A: Hazard Characterization and Mode of Action

This section summarizes the reviewers' comments relevant to hazard characterization and mode of action for perchlorate toxicity. Dr. Thomas Zoeller, the designated discussion leader for this topic area, facilitated the reviewers' responses to charge questions A.1 through A.4. The peer reviewers answered these questions by summarizing their premeeting comments and discussing additional topics not raised in those comments. A general record of the peer reviewers' discussions on these questions follows. Readers interested in the peer reviewers' major findings on hazard characterization and mode of action should refer to the Executive Summary of this report.

2.1 Charge Question A.1—*Have all the relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized? Have the similarities and differences in the toxicity profile across species been adequately characterized?*

The peer reviewers offered many comments when responding to this charge question. Most discussion focused on the nature of the interaction between perchlorate and the sodium (Na^+)–iodide (I^-) symporter (NIS) and whether perchlorate is translocated into thyroid cells. Most of the peer reviewers' comments suggested that the Revised ERD present more detailed information on toxicokinetics and toxicodynamics. A summary of the specific comments, organized by topic, follows:

- *Is perchlorate translocated into thyroid cells?* One reviewer (NC) stated that perchlorate is not translocated into thyroid cells, as the Revised ERD currently states. This reviewer first critiqued a study cited in the Revised ERD that suggests such translocation occurs (Anbar et al. 1959). In the study, rats and rabbits were dosed with radioactive perchlorate, and researchers quantified perchlorate accumulation in the thyroid by measuring the radioactivity released when incinerating the glands. Using this study design, the reviewer argued, one cannot discern whether perchlorate translocated into thyroid cells or simply bound to them.

This reviewer then noted that the recent cloning of NIS has enabled laboratories to conduct more rigorous research on iodide uptake inhibition. Specifically, this reviewer indicated that her research group and another research group in Japan have completed studies showing that perchlorate interacts with (and thus inhibits iodide uptake at) the NIS by creating a conformational change, but does not translocate into the thyroid cells. She recommended that EPA incorporate the findings from these studies into its discussion of perchlorate toxicokinetics.

Given that perchlorate does not translocate into thyroid cells, the reviewers briefly discussed what terminology most accurately describes perchlorate interaction with NIS. One reviewer (KK), for instance, noted that a recent study (Eskandari et al. 1997) and other studies cited in the premeeting comments (see the comments in Appendix C submitted by Nancy Carrasco, M.D.) suggest that perchlorate is a "blocker" rather than a "competitive inhibitor," the term EPA uses throughout the Revised ERD. Another reviewer (NC) noted that both terms characterize perchlorate interaction with NIS. The reviewers revisited this topic when discussing assumptions and kinetic constants considered in the PBPK models (see Section 6). Regardless of the terminology EPA eventually adopts, both reviewers recommended, the Agency should update the Revised ERD to reflect current findings on whether perchlorate is translocated into thyroid cells.

- *Is NIS inhibition reversible? Is perchlorate metabolized?* When discussing the implications of iodide inhibition at NIS, one reviewer (GW) asked the others if this inhibition is reversible. One reviewer (NC) responded, noting that preliminary, unpublished research in her laboratory has shown that some NIS inhibition may be irreversible. This reviewer added that the potential irreversibility of NIS inhibition raises questions about the extent to which perchlorate is metabolized. She indicated that EPA's assertion that perchlorate is "excreted virtually unchanged after absorption" is based in part on a study (Anbar et al. 1959) in which four humans were dosed with radioactive double-labeled potassium perchlorate ($K^{36}Cl^{18}O_4$). Noting that the subjects' urine contained $^{36}ClO_4^-$ and $^{36}Cl^-$, not only $^{36}Cl^{18}O_4^-$, this reviewer suggested the study implies that some ingested perchlorate may, in fact, be metabolized. This reviewer noted that her research group has hypothesized how perchlorate may be oxidized by molecular residues on the NIS molecule, but she added that these hypotheses are the subject of ongoing studies.

- *NIS in other tissues.* One reviewer (NC) noted that the Revised ERD provides limited information on other tissues known to contain NIS (e.g., lactating mammary gland, placenta, stomach, salivary glands, choroid plexus), and whether perchlorate exposure can lead to adverse effects through iodide uptake inhibition in these tissues. She was particularly concerned that maternal exposure to perchlorate may inhibit iodide transport both across the placenta and into breast milk, which is the primary source of iodide for fetuses and nursing neonates, respectively. Because iodide deficits in infants may decrease thyroid hormone production, which, in turn, may affect the development of the nervous

system, this reviewer thought the Revised ERD should provide much more information on the potential for perchlorate to inhibit NIS in other tissues.

- *General comments on NIS inhibition and upregulation.* The peer reviewers identified additional areas where more detailed information on toxicokinetics is warranted. One reviewer (NC), for example, recommended that the Revised ERD more prominently acknowledge that NIS's affinity for perchlorate is roughly an order of magnitude greater than its affinity for iodide, as she demonstrated by comparing published values for the Michaelis-Menten affinity constant (K_m) for iodide transport via the NIS to published values for the inhibition constant for perchlorate. Further, this peer reviewer recommended that the Revised ERD note that the NIS upregulation mechanism triggered by the hypothalamic-pituitary-thyroid axis (HPT axis) regulates expression of NIS only in the thyroid, not in the other tissues mentioned in the previous bulleted item.

- *Comments on toxicodynamics.* Regarding toxicodynamics, the peer reviewers offered several comments. Most of these identified specific topics in the Revised ERD that should include more detailed information. Summarizing the peer reviewers' premeeting comments, one reviewer (TZ) noted that the Revised ERD lacks detail both on exactly how increases in thyroid-stimulating hormone (TSH) lead to cancer and on the similarity between the specific sequence of events in rodents and humans. Elaborating on this topic, another reviewer (AC) suggested that the Revised ERD could improve its discussion of carcinogenesis by describing more steps in the sequence of events, such as how perchlorate exposure leads to compensating proliferation or excess mitoses per unit time. This reviewer acknowledged that the background discussions in the Revised ERD present some detailed information on toxicodynamics, but he added that those details are not quantitatively incorporated into the PBPK models.

  When discussing carcinogenesis, one peer reviewer (GW) noted that none of the laboratory studies being critiqued at the current meeting were designed to evaluate cancer as an endpoint. He reminded the peer reviewers that much of the information in the Revised ERD on carcinogenesis is based on observations of precursor lesions, which do not necessarily result in cancer. The peer reviewers discussed this issue further when addressing charge questions relevant to thyroid pathology (see Section 4.4). On a similar note, another reviewer (AC) suggested that EPA apply the term "precursor" carefully: it conventionally refers to an event that lies on the causal pathway of an adverse effect. This reviewer recommended that EPA list, possibly in a table, all events that are considered precursors and the specific adverse effects that ensue.

- *Other comments.* When summarizing the reviewers' pre-meeting comments, the discussion leader (TZ) identified other sections of the Revised ERD that EPA could clarify, but these comments were not discussed in detail. First, he suggested that EPA provide additional detail on the re-analysis of the radioimmunoassay (RIA) data. The Revised ERD, he noted, should document how analyses were conducted, what standard curves were used, and

whether data points were interpolated from the standard curves or extrapolated to levels below the lowest available standard. Second, he noted that the Revised ERD lacks detail on the levels of iodide in maternal serum, how perchlorate may affect these levels, and the potential consequences to fetuses and neonates. Finally, he briefly mentioned the potential for re-programming of the HPT axis following perchlorate exposure and suggested that some current research on the hypothalamic-pituitary-adrenal axis might offer perspective on the issue. Another reviewer (NC) indicated that iodide uptake is an electrogenic process, not one that is driven by adenosine triphosphate (ATP), as the Revised ERD states.

2.2 Charge Question A.2—*The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at NIS. Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?*

Summarizing premeeting comments submitted by several peer reviewers, the discussion leader (TZ) indicated that the Revised ERD, while it clearly articulates the mode of action and identifies the subsequent neurodevelopmental and neoplastic sequelae, does not characterize the mechanisms by which inhibition of iodide uptake (and subsequent decrements in thyroid hormones and increases in TSH) eventually alter neurodevelopmental and neoplastic processes. As a suggested improvement, this reviewer recommended that the Revised ERD discuss more thoroughly the role of different thyroid hormones (e.g., T3 and T4) on other biological processes. More detailed information on the various mechanisms, he noted, might enable EPA to relate adverse effects observed in laboratory animals to those anticipated to occur in humans more effectively.

The peer reviewers also discussed differential sensitivity to perchlorate exposure across species, particularly between rodents and humans. Noting that rodents are much more susceptible to thyroid peroxidase (TPO) inhibitors than humans are, one reviewer (GW) asked if similar data

have been collected on differential susceptibility to NIS inhibitors. In response, another reviewer (NC) indicated that transporters, like NIS, are among the most conserved proteins across species. Specifically, citing data collected in her laboratory, she indicated that rat NIS and human NIS are extremely similar (>93% homologous). This reviewer also noted that the K_m for iodide translocation at the NIS and the inhibition constant for perchlorate at the NIS are nearly identical in rats and in humans. Based on these observations, this reviewer concluded that rat NIS is essentially as susceptible to inhibitors as human NIS. She and another reviewer (GW) recommended that the Revised ERD highlight the similarities in the NIS protein between rodents and humans.

During these comments, reviewers raised additional topics that were not discussed extensively: one reviewer (KK) reiterated that the discussion of mode of action in the Revised ERD should clarify that NIS does not translocate perchlorate, though inhibition of iodide uptake clearly occurs; another reviewer (NC) summarized studies of how perchlorate inhibition of iodide uptake affects iodide discharge from the thyroid; and another reviewer (TZ) noted that a submission in the premeeting comments questioned the relevance of "cold exposure" (i.e., not radiolabeled) to the Revised ERD (see page 39 of the premeeting comments in Appendix C).

2.3 Charge Question A.3—*The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?*

When responding, the peer reviewers focused on two general topics—the use of thresholds in low-dose extrapolations (even for cancer endpoints) and the choice of dose metric—as the following paragraphs indicate:

- *Use of nonlinear low-dose extrapolations.* Three peer reviewers (KK,LK,GW) said EPA's use of nonlinear low-dose extrapolations is appropriate and adequately defended. Two of these reviewers (KK,LK) noted that the Revised ERD provides compelling evidence that

perchlorate is not genotoxic. They supported the idea that cancer endpoints may result from non-genotoxic mechanisms having anti-thyroid effects. Another reviewer (GW) acknowledged that proving a chemical has a toxicity threshold is difficult given that studies test a limited number of subjects at a limited number of dosage levels. In the case of perchlorate, however, this reviewer indicated that EPA presents clear and convincing mechanistic arguments supporting that the toxicity resulted from a nonlinear process. Given the large number of NIS molecules in the thyroid, he argued, iodide deficiency in the thyroid would not result from a single, low perchlorate exposure, but would rather likely require sustained, elevated exposures.

One reviewer (AC) suggested that EPA use toxicodynamic arguments or modeling to support the approach of nonlinear low-dose extrapolations. As one example of defending the low-dose extrapolation approach, this reviewer indicated that EPA could use PBPK modeling to quantify the extent of NIS inhibition needed to observe downstream adverse effects. He added that such models can elaborate the relative importance of various sequelae. Ms. Annie Jarabek (EPA) clarified that EPA used the models that were available from the Air Force Research Laboratory (AFRL). She added that it was originally planned to develop a biologically-based dose response model, but this modeling effort did not succeed to that extent.

- *Selection of area-under-the-curve in blood (AUCB) as a dose metric.* Several peer reviewers commented on the dose metric selection, and most offered differing insights. One reviewer (MK) indicated that the selected dose metric appeared to be simply a convenient choice, rather than a selection that EPA justified mechanistically. This reviewer expressed particular concern about using area-under-the-curve of perchlorate in the thyroid (AUCT) as a dose metric, given that perchlorate is not translocated into the thyroid cells. Another reviewer (KK) clarified, however, that EPA's proposed dose metric is not the AUCT, but rather the AUC of perchlorate in the blood (AUCB). These reviewers discussed the dose metric selection in greater detail when responding to charge question F.2 (see Section 7.2).

  Another reviewer (AC) questioned whether the selected dose metric was the best predictor of toxic effects. He suggested that measurements of intermediate responses on the causal pathway (e.g., compensating hyperplasia, excess mitoses per unit time) may be better indicators of adverse effects, particularly for neoplasia. As evidence of his concern, this reviewer noted that the studies presented in the Revised ERD imply that iodide uptake inhibition (a dose metric that EPA considered) appears to be a poor indicator of adverse effects. Another reviewer (NC) disagreed, cautioning about what inferences can be drawn from human dosing studies of short duration (e.g., 2 weeks or less). Because humans have relatively vast reservoirs of iodinated thyroglobulin in the colloid of thyroid follicles, she noted, humans can produce thyroid hormones for up to a few weeks, even in the absence of iodide uptake. As a result, this reviewer emphasized that perchlorate exposure clearly may cause changes in thyroid hormones and TSH levels in humans, even if these effects are

not observed within the first 2 weeks of exposure. The peer reviewers revisited this argument when critiquing the human studies (see Section 3).

2.4 Charge Question A.4—*A harmonized approach to characterize the potential risk of both noncancer and cancer toxicity has been proposed based on the key event of iodide uptake inhibition. Comment on whether the approach is protective of both.*

Before asking for responses, the discussion leader summarized the proposed harmonized approach: by focusing on a key event (iodide uptake inhibition) that precedes neoplasia, neurodevelopmental effects, and other noncancer effects, EPA's toxicity model protects against both cancer and noncancer outcomes. Summarizing the premeeting comments, the discussion leader noted that the peer reviewers generally supported EPA's proposed harmonized approach. No peer reviewers offered conflicting opinions.

3.0 Responses to Questions in Topic Area B: Human Health Effects Data

This section summarizes the peer reviewers' comments on human health effects data, as summarized primarily in Chapter 4 of the Revised ERD. Dr. David Hoel was the designated discussion leader for this topic area, and he initiated and moderated the peer reviewers' responses to charge questions B.1 through B.5. The peer reviewers offered comments on many of the human studies presented in the Revised ERD, including both ecological epidemiological studies (e.g., Crump et al. 2001) and clinical studies (Greer et al. 2000; Greer et al. 2002 - In Press; Lawrence et al. 2000, 2001). The reviewers focused largely on studies that were completed since the 1999 peer review, but were not yet published in the peer-reviewed literature. This section summarizes the peer reviewers' comments on human health effects in detail. Readers interested in the peer reviewers' major findings on human health effects data should refer to the Executive Summary of this report.

Note: Before the peer reviewers discussed the human health effects studies in detail, Ms. Annie Jarabek (EPA) clarified that all human clinical data presented in the Revised ERD were considered in the Agency's toxicity assessment and none were excluded due to the policy issued on December 14, 2001, on the use of third-party human data. Ms. Jarabek specifically noted that EPA evaluated the Greer study, including information published in an abstract (Greer et al. 2000) and the raw data presented in a quality assurance/quality control report (Merrill 2001a). Further, ERG distributed to the peer reviewers the manuscript of the Greer study (Greer et al. 2002 - In Press), which has been accepted for publication in a scientific journal.

3.1 Charge Questions B.1 and B.2—*Review of the Human Clinical Data Published Since 1999 That Have Not Undergone Peer Review*

The charge to the peer reviewers identifies relevant publications (Greer et al. 2000; Lawrence et al. 2001; Merrill 2001a) that were completed since the 1999 peer review but were not published in the peer-reviewed literature when the current meeting was planned. As a general comment, one reviewer (DH) recommended that EPA update Table 4-5 in future releases of the

Revised ERD to include findings from the most recent human health effects studies. Specific comments on the publications follow:

- *Comments on the Greer study (Greer et al. 2000; Greer et al. 2002 - In Press; Merrill 2001a).* The "Greer study" is a clinical study that involved administering perchlorate in drinking water to 24 euthyroid adults over 14 days and testing for radioactive iodide uptake (RAIU) and thyroid hormone levels at selected days during and after exposure. Three doses were considered, with 8 adults in each dosage group. When ERG originally distributed the peer review materials, the study was only available as an abstract (Greer et al. 2000) with much of the raw data from this study (plus data from 7 additional adults in a lower dosage group, 0.007 mg/kg/day) documented in a separate report (Merrill 2001a). Prior to the meeting, the Greer study was accepted for publication in the peer-reviewed literature, and ERG distributed copies of that manuscript to the peer reviewers (Greer et al. 2002 - In Press).

  Summarizing the peer reviewers' premeeting comments, the discussion leader (DH) indicated that the Greer study was generally well conducted and informative. It suffers from several limitations, though: lack of control for potential confounders (most notably dietary iodine intake), small sample size, consideration of only healthy adults, and use of 2-week exposure duration when human thyroid reservoirs can generate thyroid hormones for weeks after iodide uptake is inhibited. The other peer reviewers expanded on these concerns. One reviewer (MR), for example, expressed concern about the lack of information provided on the subjects, other than their age (18 to 57 years old). He noted that one must consider more detailed information to evaluate the study's findings—perhaps information on weight, smoking habits, health status, and other potential confounders. This reviewer also questioned the study's use of a simplistic three-point loglinear regression dose-response model; he recommended more sophisticated statistical analyses for modeling the dose-response behavior.

  Another reviewer (TZ) had additional concerns, primarily associated with a key conclusion in the abstract that reads ". . . water supplies containing less than [250 μg/L perchlorate] should not affect human thyroid function" (Greer et al. 2000). Noting that the study considered dosing for only 2 weeks and only among euthyroid adults, this reviewer did not think the Greer study's data supported such a general finding. He questioned whether the study would have reached similar findings had more subjects been considered, had the subjects included pregnant women, neonates, and other potentially susceptible populations, and had the dosing period been longer than 2 weeks. Later in the discussion, another peer reviewer (NC) indicated that she also strongly disagreed with this conclusion presented in the Greer abstract.

  Regarding the issue of susceptible populations, one reviewer (TZ) asked if researchers have observed any differences in iodide uptake inhibition between neonates and adults.

Another reviewer (NC) responded that iodide uptake kinetics have not been studied in neonates. This reviewer (NC) was concerned, however, that a study of eight euthyroid adults does not reflect the variability in thyroid function in humans, as evidenced, she argued, by the considerable variability observed in the baseline 8-hour RAIU levels in the Greer study (i.e., 6% to 25% of administered dose). Given this variability, this reviewer questioned whether the Greer study truly supports a no-effect level.

Finally, one peer reviewer (NC) noted that some findings of the Greer study are generally consistent with expectations. Specifically, those individuals who experienced iodide uptake inhibition had perchlorate serum concentrations greater than the inhibition constant for iodide uptake at the NIS.

- *Comments on the Lawrence publications (Lawrence et al. 2000; 2001).* The "Lawrence study" is a clinical study with resulted documented in two separate publications: an article with data presented for some doses (Lawrence et al. 2000), and a letter to the editor that describes another dose tested in the study (Lawrence et al. 2001). The Lawrence study is a clinical study that, like the Greer study, administered perchlorate in drinking water for 14 days to euthyroid adults. The study considered nine subjects and reported RAIU, circulating thyroid hormone levels, urine and serum perchlorate levels, and other parameters at selected days during and after exposure. In his opening remarks, the discussion leader (DH) indicated that the results of this study are generally consistent with those of the Greer study. Like the Greer study, the Lawrence study suffers from several limitations (e.g., limited sample size, lack of control for dietary intake and other confounders, short exposure duration). The peer reviewers did not offer any additional specific comments on the Lawrence study during their deliberations.

3.2 Charge Question B.3—*Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?*

Summarizing the peer reviewers' discussions, the discussion leader (DH) indicated that the reviewers generally thought that EPA adequately summarized the human health effects studies. Some reviewers commented that the findings from these studies should receive greater attention throughout the Revised ERD, but others felt that a more cautious interpretation of the studies is appropriate. The peer reviewers' specific comments follow:

- *Suggestions that EPA more prominently acknowledge findings from human health effects studies.* When discussing this charge question, two peer reviewers (AC,GW) suggested that EPA place greater emphasis on the findings from the human health effects studies—a

suggestion that other peer reviewers (DH,LK) echoed later in the peer review meeting. As an example of the concerns expressed, one reviewer (AC) indicated that the Revised ERD adequately outlines the limitations of the studies but does not prominently acknowledge notable gaps in the dose-response of perchlorate exposure in the rat compared to the dose-response observed in humans. Another reviewer (GW) agreed, noting that the Crump study (Crump et al. 2000, see below) suggests that humans can experience perchlorate exposures much higher than the proposed point of departure without having impaired thyroid function.

When discussing the relevance of the human health effects studies, a reviewer (DH) cautioned about inferring insights on causality from ecological studies (see the next bulleted item). Agreeing that such studies have potential limitations, another reviewer (AC) suggested that EPA present a balanced overview of these limitations for all ecological studies, including those with positive findings. He specifically referred to the Schwartz study (Schwartz 2001, see below), which EPA refers to as "by far the most convincing of the neonatal studies" (page 4-13, line 11).

Two reviewers (AC,GW) made two recommendations for revising the Revised ERD to emphasize the findings of the human health effects studies. First, noting that the human studies appear to show the absence of excess health risks (particularly for thyroid tumors) among highly exposed populations, one reviewer (AC) suggested that the Revised ERD highlight the apparent differences in toxic responses observed in laboratory animals and in humans, the limitations of the human studies notwithstanding. Second, this reviewer (AC) recommended that EPA use results from the human studies to conduct a sensitivity analysis on the point of departure the Agency used in the Revised ERD. This reviewer specifically encouraged comparing the point of departure EPA derived from the laboratory studies to that which would be based on human studies, rather than comparing RfDs calculated from those points of departure using multiple uncertainty factors. The other reviewer (GW) supported these recommendations, indicating that a greater emphasis on the human health effects data could provide some perspective on toxicity thresholds derived from animal studies.

- *Concerns about relying too heavily on the human health effects data.* Three reviewers supported the way in which the Revised ERD currently presents the human health effects studies and cautioned EPA against basing key conclusions on these studies, given their limitations. One reviewer (DH), for instance, cautioned EPA against using the results of the ecological epidemiological studies to draw firm conclusions about perchlorate toxicity. To illustrate his concern, this reviewer referred to an extensive ecological epidemiological study that found a negative association between exposure to low levels of radon gas and incidence of lung cancer—a result that contradicts the findings of many case-control epidemiologic studies. EPA should remember the potential limitations of ecological epidemiological studies, this reviewer noted, when interpreting results of such studies, regardless of whether they have positive or negative findings.

Two reviewers (NC,TZ) questioned the utility of the human health effects data in the Revised ERD, given at least three specific limitations in the available studies. First, one reviewer (TZ) emphasized that the available studies may not have considered the most sensitive health endpoints. Referring to an earlier comment about thyroid tumors not being observed in highly exposed human populations, this reviewer noted that the absence of these tumors does not imply that other toxic effects are not occurring. He added that the available studies considered only certain endpoints, perhaps not including the most sensitive ones (e.g., neurodevelopmental effects). Second, noting that most humans can continue producing thyroid hormones for weeks following iodide uptake inhibition in the thyroid (see Section 2.3), another reviewer (NC) explained that the human studies with exposure durations of 2 weeks or less (e.g., Greer et al. 2000; Greer et al. 2002 - In Press; Lawrence et al. 2000, 2001) likely do not identify toxic effects that may occur for long exposure durations. Third, the same reviewer indicated that no single metric of human thyroid function identifies potentially significant thyroid impairment and toxic effects. She cited a study in which researchers found associations between intellectual deficits in children and their mothers' having decreased T4 levels during pregnancy, but not increased TSH levels. (Another reviewer [TZ] later noted that his laboratory has observed changes in gene expression in the brains of laboratory animals related to decrements in circulating thyroid hormone levels—the magnitudes of such decrements not resulting in upregulation by TSH.) These findings, said the reviewer (NC), show that adverse effects may result from impaired thyroid function, even if evidence of upregulation is not observed.

Synthesizing these comments and providing his own insights, another reviewer (KK) noted that EPA carefully and systematically reviewed the human health effects studies and eventually concluded that the studies' limitations preclude derivation of a LOAEL or NOAEL that can be used as a point of departure. This reviewer supported presenting comparative risk analyses in Chapter 7, in which EPA calculates an RfD that it might have derived from human data, assuming a defensible calculation can be made.

- *Comments on the Schwartz study (Schwartz 2001).* The peer reviewers offered various insights on the findings in, and EPA's interpretation of, the Schwartz study. First, one reviewer (RW) recommended that EPA eventually consider any publication that may result from the Schwartz thesis. Ms. Annie Jarabek (EPA) responded, noting that EPA considers graduate dissertations and theses to be peer-reviewed publications. Second, reiterating his concern about the inferences that can be drawn from ecological studies, one reviewer (DH) cautioned EPA against the conclusions that can be drawn from the Schwartz study. Finally, a third reviewer (MR) recommended that EPA interpret the significance of the transient changes in T4 levels observed in the newborns (see lines 18 through 20 on page 4-12 of the Revised ERD).

- *Comments on the Crump study (Crump et al. 2000).* The peer reviewers had various insights on EPA's interpretation of the Crump study. First, based on comments submitted by an author of the study, one reviewer (GW) recommended that EPA rewrite its review of the Crump study (pages 4-7 to 4-8 of the Revised ERD), but this reviewer did not specify the nature of the necessary revisions. Second, the same reviewer (GW) questioned why EPA dismisses the negative findings in the Crump study for exposure to perchlorate, which were observed in a population of school children in Chile, while results of other epidemiological studies of cohorts in Chile have been widely used to develop dose-response data for exposure to nitrates and nitrites in drinking water and its associated effects. Third, another reviewer (NC) noted that the Crump study has paradoxical findings, namely lower TSH levels observed among more highly exposed individuals. Finally, yet another reviewer (TZ) found the incidence of goiter among the study population (>20% for some subsets of school children) to be surprisingly high. No other reviewers commented further on these topics.

- *Comments on balanced presentation of human health effects studies.* Citing statements made in the reviewers' premeeting comments, the discussion leader (DH) indicated that one reviewer (AC) suggested that the findings reported in two publications (Crump et al. 2000; Soldin 2001) deserve more discussion in the Revised ERD. Citing his own premeeting comments, he further noted that the Revised ERD gives disproportionately great attention to the Lawrence study, as compared to the Greer study.

- *General comments.* Two peer reviewers offered general insights on iodide uptake inhibition and dietary iodine deficiency during this discussion. First, when interpreting data on circulating TSH levels, one reviewer (NC) noted that iodide uptake does not have to be completely inhibited for thyroid upregulation to occur. Upregulation, she argued, may likely begin to occur when thyroid uptake decreases by a factor of 2, with far greater upregulation resulting from any further inhibition. Second, one reviewer (NC) indicated that iodide uptake inhibition may have serious consequences, especially considering that iodine deficiency among pregnant mothers is one of the most preventable causes of mental retardation in the world. Another reviewer (GW) agreed and suggested that humans with iodine deficiency may be a susceptible population, but he added that most U.S. residents' dietary intake of iodine is currently far higher than the recommended levels. Though she agreed that iodine deficiency is not a widespread problem in the United States, another reviewer (NC) indicated that some sub-populations in the United States do not meet their iodine dietary intake requirements, particularly during pregnancy and lactation.[3]

[3] When reviewing the draft of this report, one reviewer (TF) recommended that EPA consult with expert clinical endocrinologists when characterizing the incidence of iodine deficiency among the population.

3.3 Charge Question B.4—*Are the exposure measures constructed from data in the epidemiological studies sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies?*

The peer reviewers discussed several issues when responding to this charge question. First, the discussion leader (DH) summarized the reviewers' premeeting comments: despite limitations of epidemiological studies and clinical trials, several reviewers recommended that EPA, at a minimum, explicitly compare effect levels observed in laboratory animal studies to those observed in humans. Expanding on this subject, one reviewer (AC) suggested that the Revised ERD include much more specific summaries and analyses of the human health effects studies (e.g., at what levels have perchlorate-related effects been observed in humans? at what levels would one predict health effects to occur?). Ms. Jarabek (EPA) asked if the peer reviewers could provide specific suggestions on how such predictions can be made, given that the available epidemiological studies examined changes in circulating thyroid hormone levels, but did not consider neurodevelopmental and neoplastic sequelae. Another peer reviewer (KK) noted that the Revised ERD uses two approaches to construct exposure measures from the epidemiological studies: calculating actual exposure doses (e.g., as EPA reported on pages 4-15 and 4-18 for selected human studies) and using PBPK models to calculate internal doses (e.g., as EPA did to compare doses between selected human and laboratory animal studies). This reviewer supported these approaches, and indicated that he saw no better alternative for constructing doses to relate the human and laboratory animal studies.

Second, two reviewers discussed the differential sensitivity of rats and humans to perchlorate exposure. On the one hand, one reviewer (GW) indicated that rats and humans have dramatically different thyroid physiology, noting that perturbations in the thyroid economy lead to far greater effects (in terms of circulating thyroid hormone levels) in rats than in humans. On the other hand, another reviewer (TZ) cautioned against inferring that rats are more sensitive than humans to perchlorate exposure. This reviewer explained that no researchers have established the exact amount of thyroid hormone decrements that result in adverse neurodevelopmental effects in

rats and humans, and therefore no conclusions should be drawn on whether rats are more sensitive to perchlorate exposure than humans, at least in terms of neurodevelopmental and adult neurological sequelae.

Three peer reviewers raised additional issues during this discussion or later in the peer review meeting. First, one peer reviewer (DH) indicated that additional comparisons can be made across the occupational and clinical studies regarding the perchlorate doses needed to elicit changes in thyroid hormone levels. Second, a peer reviewer (GW) recommended that the Revised ERD include some text on the prevalence of goiter among populations exposed to perchlorate, considering that goiter is widely observed among individuals with iodine deficiencies. Third, another peer reviewer (NC) expressed concern about serum perchlorate levels observed in the human health effects studies, based largely on analyses presented in a review of these studies (Soldin 2001). The review, she explained, reported that humans exposed to 10 mg of perchlorate a day had serum concentrations of 0.6 µg/ml, while perchlorate was not detected in the serum of humans exposed to 3 mg of perchlorate a day. This reviewer could not understand the non-detects in this latter group, because she expected that those receiving the 3 mg/day dose would have serum concentrations of approximately 0.2 µg/ml—a level about 50 times higher than detection limits commonly reported for ion chromatography. The other reviewers could not explain this apparent discrepancy.

3.4 Charge Question B.5—*Are the associations observed in the epidemiological data consistent with the proposed mode of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?*

Summarizing the reviewers' premeeting comments, the discussion leader (DH) indicated that the findings of the human health effects studies are generally consistent with the proposed mode of action. He added that EPA identified limitations in these studies, such as limited power for detecting certain effects and lack of control for potential confounding factors. The discussion

leader noted that the effect of potential confounders on the selected outcomes has not been estimated. The peer reviewers did not comment further on this response.

4.0 Responses to Questions in Topic Area C: Laboratory Animal Studies

This section summarizes the peer reviewers' comments on laboratory animal studies of perchlorate toxicity, as documented in Chapter 5 of the Revised ERD. The peer reviewers initially focused on specific toxicologic endpoints (e.g., developmental, reproductive, neuroendocrine) and then offered general comments on EPA's synthesis of the available data. During these discussions, the peer reviewers critiqued the protocols, performance, and results of those laboratory animal studies completed since the 1999 peer review. They also commented on EPA's interpretations of the studies. This section includes detailed summaries of the peer reviewers' comments; readers interested in the main findings regarding laboratory animal studies should refer to the Executive Summary of this report.

4.1 Comments on Developmental Toxicity

The discussion leader (MC) presented his comments on developmental toxicity and facilitated subsequent discussions among the reviewers on this topic. A summary of the reviewers' discussions follows:

- *Review of developmental studies completed prior to the 1999 peer review.* The discussion leader (MC) briefly summarized findings from four relevant developmental toxicity studies published prior to the 1999 peer review (Postel 1957; Brown-Grant 1966; Lampé et al. 1967; Brown-Grant and Sherwood 1971). For each study, he indicated the exposure dose, the gestational days over which doses were administered, the endpoints that were evaluated, and whether or not effects occurred. He emphasized three key points to consider when interpreting these studies: all four studies considered relatively large doses, ranging from 100 mg/kg/day in one study (Lampé et al. 1967) to 2,660 mg/kg/day in another (Brown-Grant and Sherwood 1971); fetuses in the studies were not examined for a wide range of developmental effects; and the window of exposures in some studies did not include the most critical time frames for organogenesis. The peer reviewers did not comment further on these studies.

- *Detailed comments on the two most recent developmental studies.* The discussion leader (MC) provided various insights on the two most recent developmental toxicity studies. This included one study (Argus 1998) that was peer reviewed by the panel in 1999 and has

since been published as a journal article and another study (Argus 2000) that was completed since the 1999 peer review, but had not been peer-reviewed when this meeting was organized. In general, the discussion leader indicated that the two studies were apparently well conducted and considered an appropriate number of animals to detect effects. But, he questioned the need to evaluate teratological outcomes so thoroughly, given that adverse neurotoxic outcomes reportedly occur at considerably lower exposure doses (see Section 4.5). This specific comments on the individual studies follow:

– Regarding the 1998 study of developmental toxicity in New Zealand White rabbits (Argus 1998a), the discussion leader (MC) identified the dose ranges (0 to 100 mg/kg/day), the time frame over which doses were administered (gestational days 6 to 28), and some study conclusions (e.g., decreases in thyroid weight in dams that were not statistically significant, decreases in T4 levels, no significant changes in T3 or TSH levels). He indicated that the study administered doses at the appropriate gestational time for organogenesis. The study reported a NOAEL greater than 100 mg/kg/day for fetal developmental toxicity, other than for potential thyroid effects.

– Regarding the "Segment II Developmental Study" in rats (Argus 2000), the discussion leader (MC) indicated the range of doses considered (0.01 to 30 mg/kg/day), when they were administered (starting 15 days prior to cohabitation and ending at sacrifice), and some study conclusions (increases in localized alopecia in dams in two dose groups, "questionable changes" in pre-implantation loss, decreases in ossification sites at sternal centers and forelimb phalanges in the highest dose group). The discussion leader found two elements of the study design unusual: the decision to begin dosing animals 15 days prior to cohabitation and the notable gap between the highest dose (30 mg/kg/day) and the second highest dose (1 mg/kg/day). He noted that EPA and the study authors interpret the observed effects at the highest dose group differently. While Argus reports 30 mg/kg/day as a NOAEL for developmental toxicity based on the endpoints considered, EPA considers this value a LOAEL. The reviewers briefly discussed the different interpretations. One reviewer (KK) indicated that 30 mg/kg/day is an appropriate LOAEL. The discussion leader (MC) acknowledged that the distinction is debatable, but he eventually agreed that 30 mg/kg/day could be viewed as a LOAEL.

• *Additional discussions.* Following the review of the two most recent studies, one reviewer (GW) asked the discussion leader to comment on the teratogenicity of perchlorate. The discussion leader (MC) indicated that teratologists typically consider frank anatomic malformations as teratogenic endpoints, and not the various other endpoints examined in the two studies (e.g., changes in thyroid hormone levels). From this perspective, the discussion leader (MC) said, perchlorate does not appear to be teratogenic, except perhaps at the high exposures considered in the historical studies (see the first bulleted item in this

list). Another reviewer (GW) concurred, adding that the malformations observed in the various developmental studies appear to result from hypothyroidism, and not directly from perchlorate exposure.

4.2 Comments on Reproductive Toxicity

The discussion leader (TC) presented the majority of comments on the reproductive laboratory animal studies, and EPA's interpretations of the studies' results. First, he summarized the design of the main reproductive toxicity study that was conducted since the 1999 peer review meeting but had not been peer-reviewed when the current peer review was planned. This study (Argus 1999) examined reproductive effects in Sprague-Dawley rats across two generations: the P, F1, and F2 generations were all exposed to perchlorate, with doses ranging from 0 to 30 mg/kg/day, and a variety of endpoints were considered.[4] The discussion leader offered generally favorable comments on the study design and methods. The only general methodological weakness he identified was waiting until day 1 of lactation to weigh pups; he did not consider this to be a critical shortcoming. Specific comments on the reproductive toxicity study follow:

- *Comments on reproductive endpoints.* The discussion leader (TC) first summarized the study's findings regarding reproductive endpoints: nearly every endpoint revealed little evidence of reproductive effects, even in the high dose group (30 mg/kg/day). Though some changes were observed in pregnancy rates and the number of stillborn pups, these and other findings were not statistically significant. Based on these observations, the discussion leader and another reviewer (KK) agreed with the study's finding that 30 mg/kg/day is the appropriate NOAEL for most reproductive effects considered, with the possible exception of selected male reproductive endpoints.

  The discussion leader (TC) was not convinced, however, that the two-generation reproductive toxicity study fully evaluated the potential for male reproductive endpoints. He listed several reasons for this concern. First, a dose-related decrease (not statistically significant) in sperm density and spermatid density was apparent in the F1 generation, but not in the P generation. Further, the discussion leader presented his own calculations of daily sperm production, which also showed a (not statistically significant) dose-related decrease in the F1 generation, but not in the P generation. Moreover, the daily sperm

[4] Preliminary data on the F1 generation were reviewed during the 1999 peer review meeting, but the entire set of data for all generations were compiled after that meeting was conducted.

production levels observed in the two highest dose groups in the F1 generation were notably lower than the levels he routinely measures in rats in his laboratory, using the same measurement device. Finally, the discussion leader said the number of animals with low sperm counts increased with dose in the F1 generation. He expressed concern about the possibility of sperm effects manifesting in later generations, where accumulation of perchlorate exposure effects might occur.

Based on these observations, the discussion leader recommended further analysis of the existing sperm data in the Argus study. This reviewer acknowledged that an earlier publication (Springborn Laboratories 1998) examined selected parameters as a satellite to another 90-day study. This publication reported no evidence of perchlorate affecting sperm counts and motility, but he indicated that more detailed review of the Argus study is warranted. For example, this reviewer suggested that the study authors re-evaluate the histological testes slides to evaluate appropriate male reproductive endpoints more thoroughly; he asked that the authors clarify the unexpected, and considerable, difference in sperm density between the P and F1 generation control groups; and he recommended that the authors evaluate the reliability of the computer-assisted semen analysis measurement device used to generate much of the sperm data. These follow-up activities are needed to satisfy this reviewer that 30 mg/kg/day truly is an appropriate NOAEL for reproductive toxicity.

Note: After the peer review meeting, the reviewer (TC) who expressed concern about the sperm data reported in the Argus study had follow-up questions for the study's authors. ERG forwarded these questions to the study's authors and returned the responses to the peer reviewer. The peer reviewer submitted a post-meeting comment (see Appendix J) with additional insights on the sperm data.

- *Comments on all other endpoints.* When evaluating the two-generation reproductive study, the discussion leader (TC) summarized findings for other endpoints, primarily the thyroid histology and thyroid and pituitary hormone levels. This reviewer indicated that the findings for the decreased thyroid hormone levels, particularly in the adult rats, are generally consistent with EPA's proposed mode of action. The other peer reviewers discussed the histology and thyroid hormone endpoints in greater detail later in the meeting (see Sections 4.3 and 4.4). Ms. Annie Jarabek (EPA) asked the peer reviewers if they had any comments to make on effects observed in the F2 generation. None of the peer reviewers offered detailed insights on this matter.

4.3 Comments on Endocrine and Neuroendocrine Toxicity

Dr. Tom Zoeller led the discussions on endocrine and neuroendocrine toxicity, which focused primarily on how perchlorate exposure affected circulating thyroid hormone levels in rabbits and rats, as reported for two laboratory animal studies (Argus 1998a; Argus 2001). A

summary of the comments made during this segment of the peer review meeting follows, but the peer reviewers revisited issues of endocrine toxicity when discussing thyroid pathology and neurotoxicity (see Sections 4.4 and 4.5, respectively).

- *Comments on concordance among thyroid endpoints.* Because of the mode of action for perchlorate, the discussion leader (TZ) said, concordance is expected among multiple thyroid endpoints, such as decreases in T4, increases in TSH, and changes in thyroid histopathology. The reviewers discussed various findings regarding thyroid hormone levels, saving their comments on thyroid histopathology for the following presentation (see Section 4.4). The discussion leader found a high degree of concordance among the thyroid hormone endpoints in a recent laboratory animal study (Argus 2001) (e.g., dose-dependent increases in TSH and decreases in T4 and T3), but acknowledged that earlier studies (e.g., Argus 1998a) did not observe similar results across all three hormones. The discussion leader suspected that the lack of concordance across endpoints in the earlier study may have resulted from poor measurement techniques (see the next bulleted item).

- *Comments on sources of inconsistencies across studies.* The reviewers addressed certain inconsistencies between the thyroid hormone findings of two laboratory animal studies (Argus 1998a; Argus 2001). Two reviewers (MA,MP) found these inconsistencies somewhat troublesome, but the discussion leader (TZ) suspected that the inconsistencies likely resulted from how the researchers used RIA kits to measure thyroid hormone levels. He explained that many measurements documented in the laboratory animal studies (e.g., Argus 1998a; Argus 2001), particularly for T4, appear to be at levels near or below the range of the standard curves. Measurements of such trace amounts, he argued, are known to be highly variable. Furthermore, because the studies did not document inter-assay and intra-assay variability, the precision of the RIA measurements is unknown, complicating efforts to interpret results. Because of these concerns, the discussion leader suspected that the poor measurement techniques caused the lack of concordance among thyroid endpoints within studies and lack of consistency in outcomes across studies. The discussion leader added that he can confidently dismiss certain inconsistent results, given the measurement techniques used and the extensive mechanistic knowledge of how perchlorate exposure inhibits iodide uptake at the thyroid.

- *Comments on the shape of the observed dose-response.* Three peer reviewers commented on the dose-response relationship observed for changes in thyroid hormone levels, namely that monotonic dose-response behavior was not identified. One reviewer (MP) indicated that many studies over the years have identified non-monotonic dose-response behavior, such as U-shaped or inverted dose-response curves. This reviewer himself observed such dose-response patterns when investigating nicotine-related behavioral effects and when others at his institution conducted studies on the chronic administration of endocrine disruptors. He added that the animal studies for perchlorate are all based on relatively

short dosage periods, and the shape of the observed dose-response curves may reflect the nature of an acute response. As a result, this reviewer cautioned against disregarding any study's findings only because the observed dose-response is non-monotonic. Another reviewer (MA) agreed with these observations, but added that the lack of consistency across studies is more troubling than the reported shape of the dose-response curve.

Though not disagreeing with these comments, the discussion leader (TZ) offered different insights on the observed dose-response behavior. Based on the proposed mode of action, he indicated, a monotonic dose-response relationship for changes in thyroid hormone levels[5] is expected. The absence of monotonic dose-response in certain dosage groups and generations, he reiterated, may simply result from failed application of the RIA kits and measurement of thyroid hormones (particularly T4) at levels at or below the range of the standard curves.

- *Comments on statistical analyses of thyroid hormone levels.* Ms. Annie Jarabek (EPA) asked the panelists to comment on the statistical methods used to evaluate the thyroid hormone levels, asking specifically if they support a recommendation from the 1999 peer review that EPA use analysis of variance (ANOVA) for these evaluations, rather than t-tests that do not take into account the litter of the individual animals. One reviewer (RW) indicated that he supported the recommendations made at the previous meeting by Dr. Joseph Haseman. The discussion leader (TZ) agreed, noting that multiple t-tests are clearly inadequate. He supported use of ANOVA, since repeated measures of thyroid hormone levels in many studies are not available. Moreover, due to concerns about inconsistent uses of the RIA kits, he cautioned EPA against pooling measurements from multiple studies into a single statistical analysis.

  Another reviewer (AC) recommended approaches other than ANOVA, particularly if EPA is most interested in evaluating the nature of the dynamic response to iodide uptake inhibition. This reviewer stressed that aggregate statistics (e.g., correlations, regression models) will not adequately capture such a dynamic response. His recommended approach is for EPA to first develop pharmacodynamic hypotheses (e.g., increases of TSH should follow decrements in thyroid hormones) and then use non-parametric statistical methods to test them. Though not disagreeing with this alternate approach, the discussion leader (TZ) noted that the available data on thyroid hormone levels probably will not support extensive dynamic response modeling, particularly for hormones released in a pulsed manner (i.e., TSH).

[5] This reviewer (TZ) emphasized that the monotonic dose-response behavior is anticipated for changes in thyroid hormone levels, but the nature of the dose-response for the downstream effects of these changing hormone levels cannot be predicted given that the mechanisms by which such effects occur have not been fully established. The reviewers revisited this issue when discussing neurotoxicity (see Section 4.5).

4.4 Comments on Thyroid Pathology (Including Cancer Effects)

Dr. Gary Williams moderated the discussions on thyroid pathology, which considered the findings EPA reported for colloid depletion, hypertrophy, hyperplasia, and cancer. Considering all studies together, this reviewer noted that the thyroid pathology analyses focus strictly on histopathology. He added that perchlorate does not appear to exhibit cellular toxicity, as would be characterized by cell death, cell degeneration, and other outcomes. The emphasis of the Revised ERD, therefore, is appropriately on thyroid histopathological changes resulting from impaired thyroid function (itself caused by perchlorate inhibition of iodide uptake). A summary of the reviewers' specific comments on thyroid histopathology follow:

- *Comments on the diets used in a study reporting thyroid pathology (Argus 2001).* The discussion leader (GW) expressed concern that rats in a key laboratory animal study (Argus 2001) were fed "certified rodent diet 5002"—a diet he said he had never seen used in toxicity studies. He noted that this particular feed is soy-based, and therefore likely contains goitrogens. As a result, he wondered if the soy-based diet might have exaggerated the observed effects of perchlorate exposure, though he acknowledged that no adverse thyroid pathologies were observed in the control group. Another reviewer (TC) had a different opinion: he thought this particular feed is cereal-based and widely used in laboratory animal studies, in accordance with Good Laboratory Practices. This issue was not resolved at the peer review meeting, but two reviewers (LK,GW) recommended that EPA investigate this issue further.

- *Are colloid depletion, hypertrophy, and hyperplasia adverse effects?* The discussion leader (GW) questioned whether the observed thyroid histopathologies (primarily colloid depletion, hyptertrophy, and hyperplasia) should be considered adverse effects, especially when some of the outcomes are apparently reversible and are not associated with compromised thyroid function. As an example of his concern, this reviewer indicated that colloid depletion is basically an adaptive effect. This reviewer acknowledged that onset of hyperplasia suggests that the thyroid has lost its ability to compensate adequately, but he emphasized that the observed hyperplasia appears to be reversible, based largely on findings from a laboratory animal study that considered a 90-day dosing period followed by a 30-day recovery period (Springborn Laboratories 1998). He quoted EPA's interpretation of this study: "recovery of the thyroid histopathological changes was essentially complete by 30 days post-exposure . . ." (Page 5-26, lines 21–22). Noting that rats in a control group from another study (Argus 2001) were diagnosed with hyperplasia, he expressed further concerns about the biological significance of these diagnoses.

Because of these observations, the discussion leader (GW) was not convinced that the observations of thyroid lesions are truly adverse effects. He noted that sustained hyperplasia in the thyroid gland would likely proceed to thyroid follicular neoplasms, but he did not think the studies provided evidence of such advanced effects (see the following bulleted items).

One other peer reviewer (DJ) addressed these comments, noting that reversible hyperplasia does not necessarily suggest that adverse effects will not occur. He explained that some non-genotoxic carcinogens (e.g., phenobarbital) may cause transient hyperplasia, with tumors occurring later. The discussion leader (GW) agreed, but emphasized that these "delayed" tumors following transient hyperplasia would only occur in the presence of continued exposure to the non-genotoxic carcinogen.

- *Comments on the diagnoses of thyroid adenomas.* The discussion leader (GW) expressed concern about whether the thyroid adenomas identified in the two-generation reproductive toxicity study (Argus 1999) were truly neoplasms, and he asked EPA to identify the criteria that the Pathology Working Group used to make these diagnoses.[6] This reviewer noted that the STP criteria, which are based strictly on histopathology, may lead to false positive diagnoses—a concern he based on a previous experience in which he noted ovarian histopathology in rats that met the STP criteria for a granulosis cell tumor, but the "tumors" later vanished after the treatment ceased. Questioning whether the thyroid adenomas identified by the Pathology Working Group may instead be advanced, but reversible, stages of hyperplasia, this reviewer indicated that the tumor diagnoses for these rats are not compelling.

  Another reviewer (KK) had a different opinion. He indicated that the Revised ERD presents strong evidence that the highest perchlorate exposure doses (30 mg/kg/day) produced cancer in the rat. He added that the presence of the tumors only in the highest dosage group supports EPA's inference that perchlorate exposure leads to neoplastic outcomes. The discussion leader (GW) later agreed, adding that the presence of tumors in only the highest dosage group suggests that rats exposed at this level (30 mg/kg/day) for a lifetime would get thyroid tumors.

- *Are rodents a good model for neoplastic outcomes in humans?* The discussion leader (GW) questioned whether the thyroid tumors reported for rats are relevant to perchlorate toxicity in humans. Rodent neoplasia, he indicated, is generally a much simpler process than human neoplasia. As evidence of this, he noted that *in vitro* studies of rodent cells

[6] Dr. Doug Wolf (EPA) clarified during this discussion that the Pathology Working Group used the standard National Toxicology Program criteria to diagnose tumors. These criteria, he explained, use Society of Toxicologic Pathology's (STP's) Standardized System of Nomenclature and Diagnostic Criteria (SNNDC). He added that the Pathology Working Group included both external experts and pathologists from the National Toxicology Program. Finally, Dr. Wolf noted that the diagnoses of adenomas are based on the morphology of the lesions on the slides, not on suspected biology, which cannot be determined from the morphology alone.

have identified neoplastic transformations following as few as two gene mutations (typically in an oncogene and a tumor suppressor gene), while similar studies of human cells have required between four and seven gene mutations to achieve similar neoplastic transformations. Focusing specifically on thyroid neoplasia, he commented that thyroid tumors are relatively easy to induce in rats, while no evidence of perchlorate-related tumors has been observed in humans. Based on these and other arguments, he concluded that rats are not useful models for thyroid neoplasia in humans. The other peer reviewers did not comment on this issue.

- *Comments on the use of Bayesian statistics for the cancer effects.* Several peer reviewers commented on EPA's Bayesian analysis of tumor incidence. One reviewer (KK) found the analyses elegant. Another reviewer (MR) commended EPA on its use of the Bayesian hierarchical model, but commented that the software (BUGS) used for this analysis is not based on the most sophisticated approach for drawing numbers for the numerical simulations (i.e., it uses correlated draws, rather than independent ones).[7] Two other reviewers, however, had concerns about the Bayesian analyses of tumor incidence.

  First, though he acknowledged that Bayesian analysis is a powerful statistical tool, one reviewer (AC) cautioned about using Bayesian analyses to detect certain outcomes when the expected ones are not initially observed. He noted that the Revised ERD does not explain exactly why Bayesian analyses were conducted and what possible outcomes were examined. He thought EPA could instead have used Bayesian analyses to test multiple hypotheses, which would avoid the perception that the Agency was seeking a particular effect. This reviewer also questioned the utility of control groups in laboratory animal studies, since EPA's statistical analyses instead considered outcomes observed in historical control groups.

  Second, another reviewer (DH) expressed concern about assumptions EPA made to compare the tumor incidence observed in rats after 19 weeks in the laboratory animal study (Argus 1999) to that observed among rats aged 2 years in historical laboratory controls—assumptions that were needed to compare the cancer incidences over the same time frame. Citing his experiences extrapolating cancer incidences in laboratory animals from one age to another, this reviewer emphasized that the reliability of these extrapolations decreases with increased time frames. Though he indicated that some researchers have performed reasonable extrapolations of cancer incidence data over relatively short time frames (e.g., using data observed at 18 months to predict incidence at 24 months), he noted that some efforts to extrapolate incidence over longer time frames have generated "bogus" results. Based on these concerns, this reviewer viewed the Bayesian analysis as a modeling exercise and questioned whether EPA can state the

[7] Dr. David Dunson, from the National Institute of Environmental Health Sciences (NIEHS), clarified that the BUGS software was used to analyze the motor activity data because multi-dimension integration was required. However, S-Plus with independent draws was used to conduct the Bayesian analyses of the tumor data.

probability of cancers occurring at 19 weeks with as much confidence ($p = 0.005$) as reported in the Revised ERD.[8]

4.5 Comments on Neurotoxicity

Dr. Michael Aschner facilitated the discussions on neurotoxicity, during which the reviewers commented on relevant laboratory animal studies and EPA's interpretations of these studies. Specifically, the discussions focused on the studies of brain morphometry (Section 4.5.1) and motor activity (Section 4.5.2) in rats.

4.5.1 Comments on Studies of Brain Morphometry

The peer reviewers discussed the studies of brain morphometry at length, considering both the most recent study of this endpoint (Argus 2001) and an earlier study that followed a similar methodology (Argus 1998b). The discussion leader (MA) initiated the comments by reviewing the scope of the most recent study. Then he said the study has many potential flaws, though he acknowledged that it was very extensive, used an adequate number of animals, and was conducted using thorough quality control procedures. The peer reviewers had differing opinions on these flaws and the extent to which they may have affected the study's findings, as the following summary indicates:

- *Methodological concerns.* The discussion leader (MA) identified several aspects of the most recent study (Argus 2001) that could have biased the measured dimensions of brain sections. For instance, linear measurements of brain dimensions are subject to artifacts from fixation, sectioning, and positioning of the grid for viewing sections. The discussion

[8] Dr. David Dunson (NIEHS) provided several clarifications when the reviewers discussed interpretations of thyroid tumor incidence. First, he clarified that the motivation for conducting the Bayesian analysis was the strong weight of evidence from previous studies that thyroid tumors are very rare in young animals. Given that the thyroid is the hypothesized target for the mode of action for perchlorate, Dr. Dunson noted, data reviewers could not simply discard the diagnoses, especially because they were unanticipated. Second, regarding the Bayesian analyses, Dr. Dunson noted that evaluating trends among historical controls is a well-established technique for enhancing the sensitivity of statistical analyses, and relevant controls (i.e., same strain of rats) were appropriately considered.

leader indicated that volumetric measurements of brain dimensions are preferred. (Note: Ms. Annie Jarabek, from EPA, clarified that Agency guidelines for brain morphometric studies currently require the use of linear measurements. She also noted that the purpose of the most recent study was to repeat the same measures used in the earlier study.) Moreover, the discussion leader questioned both whether inconsistent sectioning of the brains may have biased results and how EPA decided which samples to include and exclude from its statistical analyses.[9] Given the overall dimensions of brains in rat pups, the discussion leader was particularly concerned that small deviations in sectioning practices could lead to substantial errors in fine-scale measurements. Finally, he pointed out that the analyses of brain sections were not blinded.

Though not disagreeing with these potential methodological weaknesses, another reviewer (TZ) was not convinced that they invalidate the data. This reviewer explained that the sectioning practices and other aspects of the study methodology surely introduce variability into the observations, but he found no evidence that these factors introduce any systematic bias. This alleviated some of his concerns about the study's methods.

- *Is hypothyroidism expected to increase the size of selected brain regions?* The peer reviewers debated the biological plausibility of hypothyroidism causing increased dimensions in specific brain sections. The discussion leader (MA), for example, expected hypothyroidism to result in decreased sizes of brain sections, based on studies published in the literature. Another reviewer (TZ) did not share this expectation. He cautioned that the published studies linking hypothyroidism to decreased sizes of brain sections are based largely on subjects with severe hypothyroidism or on subjects given thyroid hormone replacement to treat severe hypothyroidism—not on subjects experiencing the impaired thyroid function believed to result from perchlorate exposure. This reviewer noted that, because researchers have yet to quantify the dose-response behavior for how changes in thyroid hormone levels affect linear measurements of brain sections, he has no clear expectation for what changes in brain morphometry might result from small decrements in thyroid hormones. In summary, several reviewers commented that a more complete mechanistic understanding of how hypothyroidism alters central nervous system development is desired, but one reviewer (TZ) noted that such an understanding currently does not exist.

  Nonetheless, for greater confidence that the observed brain morphometry changes are indeed related to impaired thyroid function, the discussion leader (MA) suggested that EPA examine the existing data, or possibly control data from the literature, to determine if

[9] Dr. Andrew Geller (EPA) indicated that the Agency used all brain sections for its primary statistical analyses. He noted that EPA's profile analysis was run using all of the data from all brain sections and using only the data from the two brain levels that were not purported to show bias (i.e., omitting data from the posterior corpus callosum and hippocampus structures). Both of these analyses showed dose-related alterations in the pattern of brain development when compared to controls.

the observed brain morphometry changes are truly associated with other thyroid endpoints, such as changes in thyroid hormone levels or thyroid histopathological effects (i.e., colloid depletion, hypertrophy, hyperplasia). Based on his review of the data, he noted that the dosage groups that exhibited brain morphometric changes did not consistently exhibit significant effects in terms of decreased thyroid hormone levels, which made him question whether the brain morphometric changes can be mechanistically linked to hypothyroidism, as the proposed mode of action suggests. To address these and other concerns, the discussion leader (MA) recommended several future actions, such as making specific toxicological hypotheses for studying specific brain regions and integrating observations from brain morphometry, thyroid hormone levels, and neurobehavioral endpoints into a single evaluation.

- *Variability in measurements.* The discussion leader (MA) expressed concern about the variability in the brain section measurements, particularly among pups in the same litter. He cited some observations in which the Argus study reports a linear dimension of the corpus callosum for a pup in one litter to be more than twice as large as that for another pup from the same litter, sacrificed on the same day. He suspected that this considerable variability results from the weaknesses in the study methodology (see the first bullet in this list). Dr. Kevin Crofton (EPA) noted that the data cited by the discussion leader present the range of measurements and not the variability; Dr. Crofton explained that the coefficients of variation for most measurements were typically on the order of 15% for animals within a given dose group.

- *Consistencies and inconsistencies in observed results.* The reviewers had different opinions on the implications of consistencies, and lack thereof, between laboratory animal studies. On the one hand, the discussion leader (MA) was very concerned about inconsistent findings across the two studies of brain morphometry (Argus 1998b; Argus 2001). He acknowledged the two studies had some consistent results, but was troubled by many inconsistent findings, including the following: the recent study (Argus 2001) found no significant effect in the size of the corpus callosum in female rats, but the previous study (Argus 1998b) found a significant—and much larger—effect in the females; some results differed between the right and left hemispheres[10]; and the previous and recent studies had

[10] Dr. Andrew Geller (EPA) acknowledged that some differences were observed among measurements taken in the right and left brain hemispheres. He added, however, that no systematic biases were observed and that the differences between the two hemispheres were generally smaller than the brain size differences that appeared to result from perchlorate dosing. He also noted several consistencies across the brain morphometry studies: (1) In the 1998 study, an increase in the size of the corpus callosum was observed; in the 2001 study, an inverted U-shaped dose response in corpus callosum was observed (this study considered higher doses). (2) In the 1998 study, there was an inverted U-shaped dose response in cerebellar size (A-P dimensions); in the 2001 study, there was also an inverted U-shaped dose response in cerebellar measures. (3) In the 1998 study, there was a U-shaped dose response in caudate putamen; in the 2001 study, there was a U-shaped dose response in striatum (a large portion of which is caudate putamen). (4) In the 1998 study, there was a U-shaped dose response in hippocampal gyrus; in the 2001 study, there was a U-shaped dose-response in CA3.

inconsistent results in the different age groups and treatment groups considered. The discussion leader said that these inconsistencies, coupled with his concerns about the study methods, leave him little confidence in the observed effects.

On the other hand, other reviewers had different insights on the inconsistencies and offered reasons why they may have been observed. For example, one reviewer (MP) indicated that inconsistencies in rodent studies can result simply from studies being conducted in different seasons. As evidence of this, he noted that some laboratory animal studies have observed that lethal doses to half a subject population vary from one season to the next by as much as a factor of 2. This reviewer suggested that EPA consider the seasons when the two brain morphometry studies occurred when commenting on inconsistent findings.

- *Are changes in brain morphometry adverse?* One reviewer (LK) asked if the observed brain morphometry changes have been associated with any functional, cognitive, or other type of adverse effects. The discussion leader (MA) replied that the Revised ERD does not correlate the brain morphometry findings with observations from any other endpoint; he suggested that EPA evaluate whether such correlations exist. A third reviewer (GW) cited the following quote from the most recent study of brain morphometry (Argus 2001), indicating that it did consider other endpoints: "Detailed microscopic analysis . . . failed to indicate any evidence of treatment-related neuropathologic effects." Another reviewer (KK) indicated that EPA considers any alteration in brain structure as an adverse effect, regardless of whether its potential impacts, if any, have been identified.

- *General comments.* The peer reviewers made additional comments on the brain morphometry studies that do not fall under the categories listed above. First, the discussion leader (MA) was concerned that the Revised ERD relies too heavily on the more recent brain morphometry study (Argus 2001), without fully integrating the findings from the previous study (Argus 1998b). Second, another peer reviewer (AC) recommended that EPA's statistical analyses include some adjustment for multiple comparisons to determine if a dose-related signal exists across the two brain morphometry studies. Third, though not disagreeing that additional statistical analyses may be helpful, the discussion leader (MA) emphasized that no statistical analyses can correct for the methodological weaknesses he identified in the study. Finally, the discussion leader (MA) wondered if any mechanistic arguments could explain the U-shaped dose-response curve.[11]

[11] Dr. Andrew Geller (EPA) offered some clarifications on the issues of mechanisms. Specifically, he noted that profound hypothyroidism results in both reduced programmed cell death and reduced myelination in corpus callosum. He added that literature examples show that increased numbers of fibers absent of myelin result in smaller structures. He suspected that the dose responses to hypothyroidism for programmed cell death and myelination differ. However, Dr. Geller noted that it is not difficult to imagine that when multiple mechanisms contribute to a gross measurement (e.g., brain structure size), these mechanisms' competing or complementary effects may result in non-linear results.

- *Overall conclusions.* Two peer reviewers offered their individual conclusions on the brain morphometry studies and EPA's interpretation of them. The discussion leader (MA) acknowledged that both brain morphometry studies (Argus 1998b; Argus 2001) provide evidence suggesting an association between perchlorate exposure and changes in brain morphometry in rats. But, given limitations of the study methodology and inconsistencies in results between the two studies, the discussion leader said one cannot be certain that the observed effects are not the result of sampling error, selection bias, or some other artifact. As a result, he found the results of the brain morphometry studies to be inconclusive.

  Another peer reviewer (KK) offered a different conclusion. Noting that EPA considers any changes in brain structure as an adverse effect, he indicated that EPA has appropriately designated the observed brain morphometry changes as a LOAEL, even though some concerns remain about the study methodology and the shape of the dose-response curve. This reviewer, and several others, commented further on EPA's interpretations of the brain morphometry data when responding to charge question F.1 (see Section 7.1).

4.5.2 Comments on Studies of Motor Activity

The peer reviewers had fewer comments on studies of motor activity in rats, primarily as documented in one study completed since the 1999 peer review (Bekkedal et al. 2000). Summarizing the peer reviewers' premeeting comments, the discussion leader (MA) noted that the reviewers found the recent motor activity study to be rigorous and EPA's interpretations appropriate. One peer reviewer (MP) recommended that EPA consult with the study's authors about the timing with which behavioral observations were collected, suspecting that the considerable variability observed in certain parameters might result from observations for the different dosage groups being collected at different times of the day, rather than being consistently collected during a specified window of time.

This reviewer (MP) also addressed the consistency of findings across the two studies that examined motor activity. An earlier study (Argus 1998) discounted potential motor activity effects. EPA, however, believed effects were evident. Given the agency's concern regarding potential effects, another study (Bekkedal et al. 2000) was conducted. The authors of this study again also found no statistically significant differences in any motor activity measure. EPA's Bayesian analyses of the motor activity data, however, identified behavioral effects in both the

1998 and 2000 studies. The reviewer (MP) found EPA's statistical analyses compelling and agreed that they indicate that behavioral effects did occur in both studies, thus making the two studies consistent in a general sense (i.e., they both demonstrated behavioral effects). This reviewer added that perfect replication across two such behavioral studies is not expected, given his previous comments on seasonal differences observed in laboratory animal studies and variability in motor activity with time of day.

4.6 Comments on Immunotoxicity

Dr. Loren Koller, the designated discussion leader for immunotoxicity, provided the majority of comments on this topic. He had generally favorable comments both on the two immunotoxicity studies completed since the 1999 peer review and on EPA's interpretations of these studies, but he did not support EPA's proposed uncertainty factor to account for database insufficiencies regarding perchlorate immunotoxicity. A summary of the comments related to immunotoxicity follows:

- *Comments on study of B6C3F1 mice (Keil et al. 1999).* The discussion leader (LK) reviewed the design and key findings from this study, focusing primarily on endpoints relevant to immunotoxicity. He commented on several findings that EPA summarizes in Table 5-2 of the Revised ERD. For most endpoints considered, either no effects were observed or the observed effects were not consistent across dosage groups and exposure durations (i.e., 14 days and 90 days). Of particular note, the discussion leader indicated that no effects were observed in one of the most sensitive indicators for whether a chemical is immunosuppressive (i.e., spleen antibody responses to challenges with sheep red blood cell antigens). He then commented on the one parameter that exhibited some consistency in its effect—decreased macrophage phagocytosis was observed in all dosage groups, albeit not in a dose-dependent fashion, for the 90-day experiment conducted during this study, but the effect was not detected 30 days after perchlorate dosage ceased. Given that the decreased macrophage phagocytosis was not observed across all experiments and was not accompanied by any sign of compromised host resistance (e.g., response to challenges by *Listeria monocytogenes*), and that no consistent effects were observed for the many other parameters considered, the discussion leader concluded that this laboratory animal study (Keil et al. 1999) indicates that perchlorate exposure results in minimal immunotoxic effects.

- *Comments on study of CBA/J Hsd mice (BRT-Burleson Research Technologies 2000a,b,c).* To review this study, the discussion leader (LK) first noted that mice had either no significant change in, or an enhanced, antibody response to sheep red blood cells using the plaque-forming cell assay—a finding that he considered consistent with the evaluation of humoral antibody response in the other study of immunotoxicity. He then addressed evaluations of dermal contact hypersensitivity to 2,4-dinitrochlorobenzene, as determined by the local lymph node assay (LLNA). Although exacerbated sensitivity was identified in some dosage groups after 14 days, he noted, no clear dose-response relationship emerged and consistent findings were not observed in the dosage groups following 90 days of exposure. Moreover, the study reported a lack of negative controls. As a result, the discussion leader questioned the relevance of the contact hypersensitivity findings, as described further in the next bulleted item.

- *Comments on EPA's overall interpretations of immunotoxicity.* The discussion leader (LK) indicated that the two immunotoxicity studies were comprehensive in evaluating both innate and acquired immune responses, followed standard protocols, and used validated assays. Perchlorate exposure showed no effects for most endpoints. Although negative effects were observed for some endpoints, no clear dose-response relationship was identified. Finally, the remaining detected effects could be viewed as protective or favorable (i.e., there are signs of an enhanced immune response).

  The discussion leader generally supported EPA's interpretations of the immunotoxicity studies, but he did not support the Agency's proposed uncertainty factor to account for database deficiencies regarding contact hypersensitivity. He listed several reasons why he found the uncertainty factor unnecessary: the doses at which perchlorate affects thyroid hormone levels are much lower than the doses where contact hypersensitivity was observed; the contact hypersensitivity findings have inconsistencies and were inappropriately controlled, thus leaving questions as to whether perchlorate causes the observed effect; the relevance of skin rashes and agranulocytosis in Graves' disease patients being treated with perchlorate is questionable, given that no such effects have been observed in rodents or in humans receiving lower doses of perchlorate; the only immunotoxic effect that exhibited some consistency (i.e., decreased phagocytosis) appears to be reversible; and the contact hypersensitivity effects that occur in rodents may not be a good model for such effects occurring in humans. Another reviewer (GW) did not agree with this final argument due to the widespread use of LLNA for assessing contact hypersensitivity for various beauty products. He suggested that EPA revise a sentence in Chapter 5 (lines 1–2 on page 5-109) that implies LLNA responses are not physiologically relevant.

During these discussions, Dr. Ralph Smialowicz (EPA) offered several clarifications. Regarding one study's (Keil et al. 1999) finding of decreased macrophage phagocytosis, he indicated that the 1999 peer review panelists did not think the *in vitro* assay was an appropriate test for phagocytic capacity of the macrophages. Though that panel recommended an *in vivo* clearance assay be used in future studies, Dr. Smialowicz noted that this recommendation was not heeded. Further, in response to a question asked by the discussion leader, Dr. Smialowicz explained that mice, and not rats, are typically used for these types of immunotoxicity studies.

5.0 Responses to Questions in Topic Area D: Ecological Risk Assessment and Evidence for Indirect Exposure

This section documents the peer reviewers' comments on ecological risk assessment and evidence for indirect exposure—issues that EPA covers primarily in Chapters 8 and 9 of the Revised ERD. Drs. William Adams and Teresa Fan, the two reviewers with demonstrated expertise in ecological risk assessment, provided the majority of comments on this topic area. These reviewers evaluated both the relevant studies conducted since the 1999 peer review and EPA's interpretations of those studies, where applicable. The following subsections present detailed accounts of the peer reviewers' discussions and recommendations; readers interested in the peer reviewers' major findings on this topic area should refer to the Executive Summary of this report.

5.1 Charge Questions D.1 and D.2—*Review of the Relevant Studies Published Since 1999 That Have Not Undergone Peer Review*

The discussion leader (WA) indicated that only two studies conducted since 1999 have been published (Goleman et al. 2002; Smith et al. 2001). The remaining studies (Condike 2001; EA Engineering 1999; EA Engineering 2000; Parsons Engineering Science 2001), he noted, are either memos, internal reports, draft reports, or laboratory reports that were not conducted in accordance with Good Laboratory Practices and should be viewed strictly as screening-level, informational studies. Further, this reviewer indicated that most of these studies did not include measured test concentrations, which he considered to be a major limitation.

The reviewers' other responses to this question primarily addressed the disparate findings between the various studies of perchlorate uptake by plants. Specifically, a reviewer (TF) noted that field studies suggest that perchlorate bioconcentration factors (BCFs) for terrestrial and aquatic plants appear to be less than one (see the graphs on page 31 in the premeeting comments in Appendix C), while laboratory studies suggest that the BCFs are much greater than 1, and as

high as 75, for several different terrestrial plant species (see data tabulated on pages 29–30 in the premeeting comments in Appendix C). This reviewer (TF) identified reasons why both the laboratory studies and the field studies may not be characterizing plant uptake accurately.

Regarding the laboratory studies, the reviewer noted that uptake was quantified only from measurements of the amount of perchlorate depleted from the water in which the plants grew, without consideration for perchlorate possibly absorbing to soils—an assumption that she questioned. A more rigorous study design, this reviewer noted, would consider additional measurements (e.g., concentrations of perchlorate in the plant at the end of the study) to verify the assumption that all perchlorate depleted from the water is indeed taken up by the plant.

Regarding the field studies of plant uptake, the reviewer was concerned that the sediment sampling results from a particular study (Parsons Engineering Science 2001) may not be representative of actual environmental contamination levels. Specifically, she noted that sediment samples were collected with excess water, not just interstitial water. Noting that the study evaluated sediment concentrations on a wet weight basis, this reviewer wondered if the field sampling methodology might have diluted the measured sediment concentrations.

The discussion leader (WA) offered several explanations for the apparent disparity between the studies regarding plant uptake. The widely ranging BCFs, he said, might reflect differences among the species considered, the differing matrices (water, soil, and sand) in which the plants grew, or an effect of the age of the plants considered. He noted, for instance, that the laboratory studies considered young plants, which were likely growing rapidly.

5.2 Charge Question D.3—*Comment on whether the assays selected for evaluation in the ecological screening and site-specific analyses can be reasonably expected to identify potential ecological effects of concern.*

Though the reviewers agreed that the existing data provide useful insights into potential ecological effects of concern, they indicated where data on additional species and specific life stages are needed. Regarding the existing studies, the discussion leader (WA) noted that some of the aquatic species considered (e.g., daphnids and fathead minnows) are known to be very sensitive to exposures to environmental contamination. Given the mechanisms of perchlorate toxicity, however, the discussion leader suggested that EPA consider broadening its evaluation of these species—for example, by conducting a longer-term study that evaluates both the reproductive success of fathead minnows and the ability of the juvenile fish to survive, grow, and mature. Furthermore, due to concerns raised by the most recent ecotoxicological study (Goleman et al. 2002), two reviewers (WA,TF) strongly supported further studies of amphibians.

The reviewers identified additional future research areas. The discussion leader (WA), for example, indicated that EPA should consider evaluating herbivorous avian species, given the fact that plant uptake of perchlorate has been reported. He emphasized, however, that this suggestion is not based on any perceived sensitivity of these species to perchlorate. Another reviewer (TF) noted that study of herbivorous terrestrial wildlife (e.g., voles, harvest mice) may be warranted, given the results of the laboratory animal studies and the evidence of plant uptake. Finally, the discussion leader (WA) recommended further screening with other algae species and with macrophytes.

5.3 Charge Question D.4—*Comment on whether the goals and objectives of this ecological screening analysis have been adequately described and to what extent these have been met.*

The two peer reviewers who commented on the ecological risk assessment offered different responses. The discussion leader (WA) indicated that he had thought the goals of the ecological screening analysis were met until he reviewed the results of the most recent ecotoxicological study (Goleman et al. 2002), which was published after EPA released the Revised ERD. Because that study suggests that effects may be occurring at water concentrations two orders of magnitude lower than the proposed no-effect level (0.6 ppm), this reviewer was no longer certain that the screening analysis truly achieves these goals (see lines 1–8 on page 8-2 of the Revised ERD).

On the other hand, the other reviewer (TF) listed two reasons why she was not convinced that the screening-level analysis had met its goals and objectives, even without considering the most recent data. First, because the laboratory toxicity tests do not include measured body burdens, and therefore cannot be directly compared to field studies, this reviewer questioned whether EPA can fully integrate the findings from these two types of studies. Second, she expressed concern that no studies have considered organisms whose anion transport mechanisms might be impacted by exposure to perchlorate—an issue she considered particularly important for organisms requiring higher intakes of silicate or nitrate than do *Selenastrum capricornutum*.

5.4 Charge Question D.5—*Do the analyses support the summary and conclusions presented? Are relevant and important aspects of uncertainty addressed sufficiently?*

The discussion leader (WA) provided insights on this charge question during his opening presentation for this topic area. He noted that he had originally had a favorable opinion of EPA's analyses, but, after reviewing a laboratory toxicity study (Goleman et al. 2002) published since EPA released the Revised ERD, his opinions changed. He now highly recommends that EPA

revise its analyses to incorporate this study's findings. His specific comments on EPA's analyses and the recent laboratory toxicity study follow:

- *Comments on EPA's analyses of the data available prior to the publication of Goleman's study*. The discussion leader (WA) noted that the Revised ERD integrates all data that were available on exposure and effects into an initial, screening-level ecological risk assessment. He added that EPA successfully analyzed the limited data to derive its conclusions, based largely on its derivation of "Tier II water quality values" (see pages 8-17 to 8-21 in the Revised ERD). This reviewer noted that EPA's proposed values—5 ppm for a secondary acute value and 0.6 ppm for a secondary chronic value—compare well with values he derived from the same set of data using species sensitivity distribution techniques (Aldenberg and Slob 1993). He concluded, therefore, that EPA's analyses of the existing effects data were sound.

- *Comments on the implications of more recent data (Goleman et al. 2002)*. Two reviewers (WA,TF) indicated that a recent laboratory toxicity study of developing *Xenopus laevis* (African frogs), if valid, completely changes their views of the findings reported in the Revised ERD. They were specifically concerned about the 70-day exposure experiment, in which multiple endpoints, including hind-limb length, fore-limb emergence, and tail resorption, all showed effects at water concentrations far lower than the secondary chronic value (0.6 ppm) that EPA reported in the Revised ERD. For instance, inhibited fore-limb emergence was observed at water concentrations as low as 0.005 ppm. The two reviewers emphasized that the endpoints considered (e.g., inability to produce limbs) have the potential to affect the growth of individuals in this species, which in turn can impact population levels. Based on these observations and the dramatically lower effect levels reported in the Goleman study, the two reviewers strongly recommended that EPA critically evaluate the study in subsequent releases of the Revised ERD.

  Though concerned about the implications of the Goleman study, the discussion leader (WA) noted that EPA should carefully evaluate three aspects of the study before making any interpretations. First, he noted that the 70-day exposure duration for the frog embryos is much longer than that which is conventionally evaluated, but he would not speculate on how this exposure duration might have influenced the study results. Second, he noted that effects were quite common in the controls (e.g., approximately 40% of the controls had inhibited fore-limb emergence), which made him question the significance of the effects observed at low-dosage levels. Finally, he had concerns about the use of a test solution composed chiefly of deionized water and perchlorate, with non-detectable levels of pesticides, metals, and organics. Given that some metals are essential for development, he wondered if the lack of essential elements in the test solution might have accounted for the effects observed in the controls.

5.5 Charge Question D.6—*Comment on the strengths and limitations of the available data to characterize transport and transformation of perchlorate in the environment, including soil, plants, and animals.*

The peer reviewers agreed that the available data characterizing the fate and transport of perchlorate are limited. The discussion leader (WA) summarized the results from the various studies by showing graphs comparing perchlorate concentrations in one environmental medium (e.g., water) to those in another (e.g., aquatic vegetation, sediment, fish). Copies of these graphs are shown on pages 31 through 33 of Appendix C. The peer reviewers' specific comments on these data, and assumptions regarding transport and transformation, follow:

- *Transport of perchlorate.* The discussion leader (WA) noted that no studies have extensively characterized the factors that affect perchlorate transport in soils and groundwater (e.g., soil partitioning, sorption of perchlorate to organic carbon or other surfaces). Another reviewer (TF) agreed, and added that the available studies provide conflicting information on perchlorate transport. For instance, she noted, some studies report that perchlorate does not sorb to sand, while others suggest that considerable sorption occurs. Further, she referred to a recent abstract according to which pH and organic content largely determine the extent to which soil sorption occurs. Based on the limited and conflicting findings, this reviewer concluded that no study has definitively documented the extent to which perchlorate absorbs to soils. She cautioned EPA against inferring that perchlorate does not sorb to soils, simply because the chemical is anionic and hydrophilic in nature. She explained that her own research has demonstrated that anionic chemicals sorb to local positively charged clusters that may be present in the organic or mineral matrices of soils.

- *Transformation of perchlorate.* The peer reviewers briefly discussed the extent to which perchlorate is transformed, both biologically and chemically, in the environment. Regarding biological transformation, the discussion leader (WA) noted that the available data demonstrate that plants and micro-organisms in anaerobic environments (e.g., sediments) reduce perchlorate. Another reviewer (TF) agreed, and added that a study suggests that perchlorate is transformed in humans (see the bulleted item "Is NIS inhibition reversible? Is perchlorate metabolized?" in Section 2.1). Regarding chemical transformations, one reviewer (TF) questioned statements suggesting that chemical reduction is limited, especially considering that similar chemicals (e.g., sulfates) are reduced in groundwater under certain conditions.

5.6 Charge Question D.7—*Comment on the strengths and limitations of the available data to suggest sources of perchlorate exposure other than drinking water.*

The reviewers provided few comments on this charge question. First, the discussion leader (WA) indicated that future research is needed to develop more sensitive analytical methods, not only for water but also for biotic tissues, soils, and sediment. Another reviewer (TF) agreed, indicating that ion chromatography analyses potentially suffer from matrix interference and interfering ions. She noted, however, that analytical methods (e.g., liquid chromatography with mass spectrometry) are already being developed with improved sensitivity and selectivity. Second, noting that perchlorate uptake by lettuce has been documented, the discussion leader (WA) noted that humans may be indirectly exposed to perchlorate in vegetables grown on land irrigated with perchlorate-contaminated water. Finally, another reviewer (TF) recommended that future studies characterize the mechanisms by which plants uptake perchlorate. With a mechanistic understanding of uptake, she noted, EPA may be able to predict uptake behavior in plant species that have not been sampled.

6.0 Responses to Questions in Topic Area E: Use of PBPK Modeling

This section summarizes the peer reviewers' comments on PBPK modeling for perchlorate, as presented primarily in Chapter 6 of the Revised ERD. AFRL developed the four PBPK models, and EPA applied them in its assessment approach. Dr. Kannan Krishnan moderated the peer reviewers' responses to charge questions E.1 and E.2, during which the reviewers critiqued the structure, parameterization, validation, and application of the four model structures. Detailed summaries of the peer reviewers' comments on the PBPK models follow; readers interested in the major findings for this topic area should refer to the Executive Summary of this report. (Note: Section 7.2 presents additional comments on the PBPK models, primarily on how EPA used these models for interspecies extrapolation.)

Summarizing the premeeting comments, the discussion leader (KK) indicated that the PBPK model structures are technically sound and accounts for the major anatomical compartments and that they are based on standard approaches and equations. However, he and other reviewers voiced several concerns about certain model representations, particularly that of perchlorate uptake into cells, and parameter selections. The reviewers' detailed comments, suggestions, and recommendations on these issues and many others are summarized below:

- *Representation of iodide uptake into thyroid cells.* Several peer reviewers (NC,KK,MK) questioned why the PBPK models consider passive uptake (i.e., diffusion) of iodide into thyroid cells. Suspecting that active iodide uptake into cells is the dominant transport process, these reviewers recommended that the model developers reconsider why passive iodide uptake is simulated. More specifically, one reviewer (KK) recommended that the PBPK models resolve the relative importance of these two uptake processes. Another reviewer (TF) commented further on passive uptake of chemicals into cells when discussing disposition of perchlorate (see next bulleted item).

  One reviewer (MK) indicated that the Revised ERD does not adequately justify the use of Michaelis-Menten kinetics to model iodide uptake into the thyroid. Another reviewer (NC), however, was not concerned with this aspect of the PBPK models, noting that several researchers have demonstrated that iodide transport via NIS follows Michaelis-Menten kinetics.

- *Representation of perchlorate uptake into thyroid cells.* The peer reviewers raised several issues when discussing perchlorate disposition: whether NIS translocates perchlorate into thyroid cells, whether perchlorate is translocated into cells by other mechanisms, and to what extent passive (i.e., diffusive) transport of perchlorate into cells needs to be incorporated into the PBPK models.

  Based on comments raised earlier in the peer review meeting (see the first bulleted item in Section 2.1), several reviewers (NC,KK,MK) recommended that EPA verify whether NIS actively transports perchlorate into thyroid cells—an assumption made in the four PBPK model structures. One reviewer (NC) noted that she is unaware of any research that unequivocally demonstrates that NIS translocates perchlorate into thyroid cells, while she has reviewed several papers that suggest such translocation does not occur. Regarding recent publications that report concentrations of perchlorate in the thyroid (e.g., Yu et al. 2001), this reviewer suspected that the perchlorate detected may be bound to cell membranes, rather than inside the thyroid cells. This reviewer indicated that researchers can readily design an experiment to determine the extent to which perchlorate interacts with NIS (i.e., whether it binds to NIS or is translocated by the protein), though she did not think such an experiment has already been conducted. The reviewers revisited the issue of active perchlorate transport when discussing the kinetic parameters used in the PBPK models (see the next bulleted item).

  Although several reviewers agreed that NIS apparently does not actively translocate perchlorate into cells, one reviewer (MK) cited evidence that perchlorate is likely entering cells by other mechanisms. Referring to the study that administered radioactive double-labeled perchlorate to humans (Anbar et al. 1959), this reviewer indicated that the presence of single-labeled perchlorate in the subjects' urine implies that perchlorate may be entering cells somewhere in the body. He indicated that various other anion exchange mechanisms may carry perchlorate into cells, even if NIS does not translocate the chemical. Based on these concerns, this reviewer noted that the Revised ERD does not provide a complete, convincing account of all cellular uptake mechanisms.

  Two reviewers (NC,TF) commented on whether the PBPK models need to consider passive transport of perchlorate into cells. Citing observations made for anionic transport in plants, one reviewer (TF) indicated that passive transport of anions into cells can be an important process, especially when concentrations of the anions in the extra-cellular matrix are extremely high (e.g., following an exposure). Another reviewer (NC) agreed in principle, but added that passive transport in humans would be relevant only when perchlorate achieves extremely high serum concentrations—concentrations that may not be physiologically relevant.

  Overall, the peer review panel indicated that the PBPK models should have more refined representations of active and passive uptake into cells, based on the comments summarized above. Two reviewers (NC,KK) clarified that they have no question that perchlorate

interacts with NIS, thus inhibiting iodide uptake: their primary concern is to what degree does perchlorate actually enter cells. Another reviewer (GW) agreed that this issue is important to resolve, because the extent of cellular uptake affects how EPA should approach other issues, such as the mutagenicity of perchlorate.

- *Mathematical representation of iodide and perchlorate uptake.* Given that NIS does not translocate perchlorate into thyroid cells, the reviewers questioned whether the PBPK models and the Revised ERD should describe the cellular uptake process via NIS as "competitive inhibition." The discussion leader (KK) explained that competitive inhibition generally implies that two (or more) molecules are substrates for a given protein or enzyme. Because perchlorate is not translocated by NIS, he said, it technically does not have a Michaelis-Menten constant (K_m). He questioned, therefore, how the model developers could derive a K_m for the PBPK models (see pages 6-23 to 6-25 in the Revised ERD). Another reviewer (NC) agreed, noting that perchlorate does not have a K_m, but it does have an inhibition constant that has been widely published. The reviewers recommended that the PBPK models include a revised kinetic description of iodide uptake inhibition.

- *Model parameterization.* Several peer reviewers evaluated the kinetic parameters assigned in the PBPK models. In addition to their concern that perchlorate does not have a K_m (see the previous bulleted item), the reviewers offered several comments. First, regarding the K_m value used for the active transport of iodide (4.0×10^6 ng/L), one reviewer indicated that decades of research have established that this K_m should fall roughly between 20 and 30 μM (micromolar). ERG notes that 4.0×10^6 ng/L is equivalent to 31 μM of iodine, though these figures were not mentioned at the meeting.

  Second, the peer reviewers discussed the derivation of other relevant parameters, including maximum velocity capacity (V_{maxc}) in various tissues, permeability area, plasma binding coefficients, and clearance values. One reviewer (MK) said that appropriate parameters were selected for iodide, but he indicated that the Revised ERD does not adequately describe how these parameters were selected for perchlorate. Another reviewer (KK) noted, however, the PBPK models were parameterized largely from experimental data for both chemicals, often by assimilating and integrating multiple data sets. He concluded that the approach for parameter selection was defensible, both for iodide and perchlorate, based on the data sets currently available. These reviewers identified types of additional data that would help improve the confidence in the parameterization (e.g., time-course data of perchlorate in multiple tissue types).

- *Overall conclusions.* The two peer reviewers who ERG assigned to critique the PBPK models summarized their overall comments. The discussion leader (KK) indicated that the overall value of the models depends largely on their ultimate application. For instance, he indicated that the models will not be useful for quantifying cellular concentrations of perchlorate until a greater mechanistic understanding of the relevant uptake processes is

achieved. Nonetheless, he noted that the PBPK models, largely because they adequately represent urinary excretion and estimate serum concentrations, are useful tools both for estimating internal doses from environmental exposures (or external doses) and for estimating human equivalent doses. Though he recommended that the models include more refined representation of uptake and inhibition processes in the thyroid, he stressed that these refinements likely will have minimal impacts on predicted serum levels—predictions that depend more on urinary clearance and volume of distribution than on uptake into a relatively small physiological compartment (i.e., the thyroid).

The other reviewer (MK) echoed many of these comments and added others. Regarding the cellular uptake processes, this reviewer recommended that the PBPK models include more refined representation of these processes based on comments raised earlier at the meeting. If this cannot be achieved, the reviewer suggested that EPA prominently acknowledge in the Revised ERD that the PBPK models are not based on a mechanistic understanding of the uptake processes.

7.0 Responses to Questions in Topic Area F: Human Health Dose-Response Assessment

This section summarizes the peer reviewers' comments on EPA's human health dose-response assessment for exposure to perchlorate. Dr. Thomas Collins moderated these discussions, which largely focused on the selected point of departure and uncertainty factors in EPA's proposed RfD derivation. The meeting chair (RW) indicated that peer reviewers expressed various, and often conflicting, opinions on many issues in this topic area: some reviewers recommended that EPA use human data from the Greer study for the point of departure, but other peer reviewers did not think this study is an adequate basis for the RfD derivation; some peer reviewers supported the decision to base the point of departure in part on the reported changes in brain morphometry, yet roughly the same number of reviewers did not think this study was an adequate basis for the point of departure; and the reviewers had various opinions on the proposed uncertainty factors, though nearly every reviewer agreed that the uncertainty factor of 3 for database insufficiency on immunotoxic endpoints was unnecessary.

A detailed summary of the peer reviewers' responses to the four charge questions in this topic area follow. Reviewers interested in a brief summary of the comments on EPA's human health dose-response assessment should refer to the Executive Summary of this report.

7.1 Charge Question F.1—*Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure? Should any other data be considered in arriving at a point of departure?*

Dr. Tom Collins facilitated the peer reviewers' discussions on point of departure. These focused on three general topics: the consistency between the mode of action and the toxic effects (see Section 7.1.1), the proposed use of changes in brain morphometry (0.01 mg/kg/day LOAEL)

when deriving the point of departure (see Section 7.1.2), and the proposed use of other data and endpoints when deriving the point of departure (see Section 7.1.3). Though much of the discussion focused on brain morphometry, EPA based the point of departure on other endpoints as well (e.g., changes in thyroid hormone levels and thyroid histopathology).

7.1.1 Consistency Between Observed Effects and Mode of Action

Summarizing the premeeting comments, the designated discussion leader (TC) indicated that a clear majority of the reviewers who responded to this charge question found the proposed mode of action consistent with the observed neurodevelopmental and neoplastic effects. Elaborating on this general response, one reviewer (KK) indicated that the Revised ERD clearly states how perchlorate exposure initiates the perturbation of the HPT axis, which leads to neoplastic and neurodevelopmental effects, thus supporting the harmonized approach to evaluating noncancer and cancer toxicity. Other reviewers, however, indicated that EPA could more convincingly link the mode of action to the observed effects:

- *Lack of pharmacodynamic modeling.* Two reviewers (AC,MK) acknowledged that the Revised ERD links perchlorate exposure to the key event, but they argued that the document does not provide specific details on any mechanisms linking the key event to the neurodevelopmental or neoplastic endpoints. One reviewer (AC), for example, indicated that the Revised ERD does not explicitly describe the full sequence of events between perchlorate exposure and neoplasia, particularly for how this is expected to occur in humans. He and another reviewer (MK) noted that the lack of pharmacodynamic modeling leaves the relevance of the mode of action uncertain. The other reviewer (MK) further explained that, with a detailed pharmacodynamic model, EPA could link iodide uptake inhibition to reduction in thyroid hormone production and circulation—parameters, he argued, that are more relevant indicators of whether toxic effects will occur. He said that EPA could have drawn from existing PBPK modeling applications (reference not cited) to characterize the pharmacodynamic mechanisms more effectively. This would have provided a more convincing link between the proposed mode of action and the observed toxic effects.

- *Comments on doses where iodide uptake inhibition occurs.* To link the proposed mode of action to toxic effects, one reviewer (GW) recommended, the Revised ERD should clearly indicate the doses at which iodide uptake inhibition have been observed in laboratory animals and humans. Focusing on the 2000 abstract of the Greer study (Greer et al. 2000),

three reviewers (NC,DH,RW) noted that the abstract reports measurable iodide uptake inhibition in humans dosed at 0.02 mg/kg/day; they also noted that Table 7-5 in the Revised ERD presents EPA's estimates of iodide uptake inhibition as a function of dose for the various PBPK model structures.[12] One reviewer (DH) indicated that EPA's estimates of iodide uptake inhibition in humans (in Table 7-5) appears to be quite consistent with the low-dose findings reported in the abstract of the Greer study. Noting that EPA's mode of action ultimately links toxic effects to iodide uptake inhibition, one reviewer (GW) recommended that the Revised ERD more prominently acknowledge the exposure doses at which this inhibition has been observed.

- *Inconsistencies of findings within studies on thyroid hormone levels.* Referring to Table 5-4 in the Revised ERD, one reviewer (GW) indicated that some groups of animals in the laboratory animal studies experienced decreases in circulating T3 levels, while no significant changes in circulating T4 levels were observed (e.g., see data for post-natal day 22 females). Given that T3 is formed by the deiodination of T4, this reviewer found such trends confusing and wondered if they suggest that perchlorate may affect thyroid hormone levels by some mode of action in addition to inhibiting thyroid iodide uptake. Another reviewer (TZ) agreed that some studies may have inconsistent results, but he noted that others (e.g., Argus 2001) have results quite consistent with expectations: T4 levels decrease, TSH levels increase, and T3 levels exhibit various changes. He noted that inconsistent findings in T3 levels might result from the fact that thyroxine-binding globulin (TBG) is found in lactating rats and pups, which could give some buffering capacity. No other reviewers commented further on this topic.

7.1.2 Comments on the Use of Brain Morphometry Effects as the Basis for the Point of Departure

The peer reviewers discussed at length whether EPA should use the brain morphometry data (Argus 2001) in deriving a point of departure for perchlorate. Their general concerns centered on the quality of the linear measurements of brain regions and the biological significance of any observed effect. The reviewers' comments spanned a broad range, from one reviewer (MA) finding the brain morphometry studies inconclusive to another reviewer (TZ) indicating that

[12] Though they clearly identified 0.02 mg/kg/day as a dosage where iodide uptake inhibition was observed in humans, the reviewers did not specify whether this comment considered the lowest dosage group (0.007 mg/kg/day) in the Greer study. This lowest dosage group was not documented in the abstract (Greer et al. 2000), but was documented in the subsequent quality assurance/quality control report (Merrill 2001a) and the final manuscript (Greer et al. 2002 - in press).

the multiple studies and multiple re-analyses of the studies all show brain morphometric effects occurring. The following bulleted items present a detailed review of these and other comments, culminating with the individual peer reviewers' final statements on the brain morphometry data:

- *Concerns about the study methodology.* Based largely on comments made earlier in the meeting (see Section 4.5.1), one reviewer (MA) reiterated that he found the brain morphometry studies inconclusive, due largely, but not entirely, to the methodologies used to measure the dimensions of brain regions. As a result, this reviewer indicated that EPA should not consider the results of the brain morphometry study when deriving a point of departure. Though not disagreeing that the brain morphometry studies have flawed designs, another reviewer (TZ) suspected that the methodological issues of specific concern (e.g., sectioning practices, use of linear dimensions) are expected to introduce random errors into the study, not systematic ones. He said random errors introduced by the study methodology would most likely make it impossible to detect statistically significant effects, not to detect effects that do not exist. Two reviewers (MP,TZ) noted that they found no evidence of systematic errors introduced by the study methodology (e.g., use of different section practices for different dosage groups), and therefore recommended that EPA not discard the data due to the random errors that the study design may have caused.

- *Comments on statistical re-analyses of the brain morphometry data.* The Revised ERD documents the results from two laboratory animal studies that evaluated changes in brain morphometry, as well as statistical re-analyses of these studies. One reviewer (TZ) was concerned about disregarding all of this information, which provides evidence—albeit with some inconsistencies between the studies—of brain morphometry changes in animals exposed to perchlorate. On the other hand, another reviewer (MA) did not find EPA's statistical re-analyses compelling, due not to any flaws in the statistical approaches but rather to his concern about the quality of the linear measurements of brain dimensions (see the previous bulleted item).

- *Mechanistic questions.* Two reviewers (MA,LK) noted that the changes in brain morphometry cannot be linked to perturbations in thyroid hormone levels, and presumably, therefore, to perchlorate exposure. This leaves questions about exactly what causes effects in brain structure and whether these effects are truly adverse or perhaps compensatory. One reviewer (MA) also questioned the relevance of brain morphometric changes in rats to humans. Though other reviewers (MP,TZ) agreed that the absence of mechanistic links is unfortunate, they did not think the brain morphometry findings should be criticized for this reason, especially considering there is no complete mechanistic understanding of how thyroid hormone levels affect all neurodevelopmental processes. Noting that the brain morphometry studies may be the first toxicological studies ever linking perturbations in thyroid hormone levels to changes in the sizes of brain dimensions, one reviewer (TZ) felt

uncomfortable disregarding the data because no previous studies have elucidated the mechanisms that may cause these effects.

- *Concerns about inconsistencies in the brain morphometry results.* Two peer reviewers discussed whether one should expect consistency among certain findings. One reviewer (MA) first listed various types of inconsistencies he observed, such as different results between the sexes, post-natal days considered, and the two brain morphometry studies (Argus 1998b; Argus 2001). Another reviewer (TZ) agreed that the lack of concordance across the two studies is troublesome, but he was not as concerned about the other issues raised. For instance, noting that certain neurodevelopmental events are known to take place over distinct (and sometimes narrow) windows of time, this reviewer indicated that it is not unreasonable to observe inconsistent brain morphometry effects at two different postnatal days. He further noted that perturbations in thyroid hormone levels may affect various brain regions differently, and one should not necessarily require that consistent effects be observed across multiple regions.

- *Comments on the shape of the dose-response curve.* The reviewers had various comments on the biological significance on the shape of the observed dose-response curve, which, Ms. Annie Jarabek (EPA) indicated, was an inverted U-shape for the corpus callosum (i.e., the smallest effects were observed at the lowest and highest doses and the largest effects were observed at the intermediate doses). For instance, one reviewer (GW) indicated that the dose-response curve implies that high doses of perchlorate may protect rats against neurodevelopmental effects.

  Other reviewers had different opinions. Because the mechanisms of thyroid hormone action on the reported brain morphometry changes have not been identified, one reviewer (TZ) indicated that he has no basis for dismissing the data because a linear or monotonic dose-response curve was not observed. Another reviewer (MP) agreed, saying that inverted U-shape dose-response curves have been documented, particularly in cases where increased effects initiate compensatory responses, similar to the upregulation of thyroid hormone synthesis observed following iodide uptake inhibition. This reviewer suggested that the Revised ERD include specific hypotheses about mechanisms that may account for the U-shaped dose response. Another reviewer (MA) also found no inherent problem with non-linear dose-response curves, but he was troubled by the fact that the dose-response trends are not consistently observed across both sexes.

- *Integration of brain morphometry data with other endpoints.* Given the proposed mode of action for perchlorate toxicity, one reviewer (GW) said, he would have expected that changes in brain dimensions in the brain morphometry studies would be accompanied by changes in thyroid hormone levels. But in the most recent brain morphometry study (Argus 2001), the dams dosed at 0.01 mg/kg/day showed no significant changes in TSH or T4 levels, and only marginal changes in T3 levels on gestational day 21. He suggested that

EPA consider whether such modest perturbations to thyroid hormone levels would result in altered brain structures.

Another reviewer (MP) was not convinced that the data currently available are sufficient for integrating the brain morphometry data with other endpoints, particularly thyroid hormone levels. Specifically, he indicated that the most recent brain morphometry study reported only "snapshots" of thyroid hormone levels, which may not be representative of the circulating hormone levels prior to the days when animals were sacrificed. He noted that the observed changes in brain morphometry may result from decreased thyroid hormone levels that occurred when these parameters were not measured.

- *Recommendations*. Summarizing the peer reviewers' discussions, the meeting chair (RW) noted that the reviewers' primary concerns about the proposed point of departure are whether the brain morphometry data are of acceptable quality and whether the reported effects are truly caused by perturbations in thyroid hormone levels. Individual reviewers then offered several additional insights. Two peer reviewers (AC,MP), for example, suggested that the raw data from the brain morphometry be re-analyzed by a party that is blinded to the dosage levels. Another reviewer (MA) supported this suggestion, but noted that such re-analyses would not address his underlying concern regarding the validity of the linear measurements of brain dimensions. A third reviewer (KK) indicated that EPA could defend use of the changes in brain morphometry as the point of departure, provided that it addresses the reviewers' concerns regarding the study methodology and inconsistencies in the findings. Finally, the meeting chair (RW) noted that EPA might consider the following three options when making its final decision on the point of departure:

  – Not consider changes in brain morphometry when deriving the point of departure.

  – Consider the changes in brain morphometry when deriving the point of departure, but address concerns about the quality of the underlying data.

  – Base the point of departure entirely on other endpoints, but perhaps account for database insufficiencies regarding neurodevelopmental effects using an uncertainty factor.

- *Final comments.* After discussing the various strengths and weaknesses of the brain morphometry study, the meeting chair asked the peer reviewers to give their final individual opinions on whether EPA should consider the reported changes in brain dimensions when deriving the point of departure. Table 2 (at the end of this section) summarizes the reviewers' final remarks: two reviewers supported EPA's proposed approach, three reviewers indicated that the brain morphometry findings were either inconclusive or not compelling, three reviewers offered conditional remarks on the use of the brain morphometry data, and the remaining nine reviewers did not comment specifically

on whether or not the brain morphometry data should be considered when deriving the point of departure.

7.1.3 Comments on Use of Data Other Than Brain Morphometry for the Point of Departure

Though their comments primarily addressed the proposed use of brain morphometry data for the point of departure, the peer reviewers also addressed EPA's use of other toxic endpoints in deriving an RfD. Examples of the peer reviewers' other comments follow:

- *Should human studies be used for the point of departure?* Though some peer reviewers suggested many times during the meeting that the Revised ERD more prominently acknowledge findings from human health effects studies, two peer reviewers (KK,TZ) questioned the utility of those studies for deriving a point of departure. One of these reviewers (TZ) gave an example to explain his feeling: he did not think the Greer and Lawrence studies, which examined iodide uptake inhibition and circulating thyroid hormone levels for a very small subset of healthy, euthyroid adults, offer any insights on potentially important sensitive populations (e.g., pregnant women, children, fetuses). The other reviewer (KK) agreed, and added that the human clinical studies are based on limited exposure durations and have not investigated important endpoints, such as reproductive toxicity, neurotoxicity, and developmental toxicity. Because of these data gaps, this reviewer supported an approach of evaluating the laboratory animal studies for insights on the endpoints that have not been examined in humans.

  On the other hand, three peer reviewers (AC,DH,LK) indicated that EPA can better integrate data from human health effects studies into the Revised ERD, without necessarily using those data for deriving the point of departure. One of the three (LK) wondered if more insights can be drawn from effects observed among humans with Graves' disease who have been prescribed high doses of perchlorate (e.g., one patient received 3 mg/kg/day for 22 years), though he acknowledged that this dosing was necessary to treat hyperthyroidism. He also suggested that EPA consider basing the point of departure on data from the Greer and Crump studies. Reiterating a comment made earlier in the peer review, the second reviewer (AC) recommended that EPA use the human health effects data in a sensitivity analysis of the proposed point of departure. The third reviewer (DH) estimated an RfD based on human health effects data as 0.0001 mg/kg/day, which he derived using a point of departure of 0.001 mg/kg/day, combined with an uncertainty

factor of 10; he did not specify what the point of departure and uncertainty factor represent.[13]

- *Comments on basing the point of departure on changes in thyroid hormone levels.* The peer reviewers commented briefly on whether EPA should consider basing its point of departure on changes in thyroid hormone levels. One reviewer (GW) emphasized that EPA could choose this endpoint for the point of departure, but should carefully distinguish changes that are biologically significant from those that are simply statistically significant. Specifically, recognizing that thyroid hormone levels exhibit considerable diurnal variations, this reviewer recommended that EPA only consider measured hormone levels outside a "normal" range as being potentially adverse. Another reviewer (LK) agreed, indicating that statistically significant changes that fall within "normal" fluctuations should not be considered adverse effects.

  The reviewers briefly discussed whether decrements in thyroid hormone levels, specifically T4, can lead to adverse neurodevelopmental effects. In a general sense, one reviewer (TZ) said, decrements in T4 levels clearly can cause neurological dysfunction. This reviewer added that extensive dose-response data linking these decrements to adverse effects are not available, though some clinical thyroidologists have said that humans sustaining 10% to 15% reductions in circulating thyroid hormone levels may show symptoms of hypothyroidism. Another reviewer (NC) agreed, citing a study that found associations between pregnant mothers with lower levels of T4 during their first trimester (without an associated increase in TSH) and impaired intellectual function in their children. Moreover, she indicated that physicians evaluate babies for hypothyroidism very early in life to avoid potentially irreversible effects of decreased thyroid hormone levels.

- *Comments on basing the point of departure on thyroid histopathology.* Two peer reviewers (GW,TZ) addressed whether EPA should base the point of departure on observed thyroid histopathology, namely colloid depletion, hypertrophy, and hyperplasia. The first of these reviewers (GW) questioned, by way of an example, whether EPA should view thyroid colloid depletion as an adverse effect. He indicated that this effect is better characterized as adaptive. Another reviewer (TZ) made similar comments, noting that colloid depletion may demonstrate a perturbation of the HPT axis, but the biological significance of this perturbation is questionable in the absence of the reported changes in brain morphometry.

  The first reviewer (GW) did propose that 1.0 mg/kg/day may be an appropriate point of departure for thyroid tumorogenesis. He explained that this was the dosage required to observe signs (i.e., hypertrophy) that the thyroid was being stimulated and stressed, which

[13] This reviewer (DH) indicated that the RfD value he stated at the peer review meeting was only an estimate he quickly made during the discussions. This reviewer's post-meeting comments (see Appendix J) include a more detailed calculation he offered for an RfD based on the human health effects studies.

he considered a departure from homeostasis. This reviewer suggested that EPA apply an interspecies uncertainty factor of 0.1 to this point of departure, noting that perturbations of the HPT axis apparently have far different consequences in rats than in humans. As evidence of this, the reviewer noted that rats exposed to certain proton pump inhibitors readily develop gastric neuroendocrine tumors, whereas no evidence of such effects has been observed in humans.

- *Comments on basing the point of departure on iodide uptake inhibition.* The peer reviewers briefly discussed whether EPA should base its point of departure on any particular level of iodide uptake inhibition. One reviewer (AC) questioned this approach, wondering what specific adverse effects result at specified levels of iodide uptake inhibition and how these effects differ between rats and humans. Another reviewer (GW) agreed, noting that only marginal inhibition occurs among rats and humans dosed at 0.01 mg/kg/day, the inhibition appears to be reversible after short-term dosage periods end, and the thyroid hormone levels are not considerably altered at this exposure level. Moreover, he indicated that the existing data on short-term dosages are insufficient for evaluating iodide uptake inhibition over chronic exposure durations, unless EPA's models account for upregulating mechanisms.

  Another reviewer (NC) agreed with some of these comments, but cautioned about some of the inferences drawn. She indicated, for instance, that researchers have not yet established the extent to which NIS inhibition is reversible. Though she acknowledged that the kinetics of NIS active transport are strikingly similar across species, she was hesitant to make premature judgments on how iodide uptake inhibition affects humans and rats differently, especially considering that NIS is expressed in fewer thyroid cells in humans than in rats.

- *Other comments.* When discussing the appropriate derivation for the point of departure, the peer reviewers raised additional comments: apparent inconsistencies between thyroid upregulation in rats and humans, statistical analyses of thyroid hormone levels, and consistency between the proposed point of departure and findings from recent ecotoxicological studies.

  First, one reviewer (KK) indicated that the laboratory animal studies found evidence of upregulation, while the human studies did not. On the other hand, another reviewer (NC) noted that these apparent discrepancies are easily explained by differences in the thyroid hormone reservoirs in the species. Noting that humans have vast reservoirs of thyroid hormones compared to rats, this reviewer was not surprised that the 2-week dosage studies in humans (e.g., Greer et al. 2000; Greer et al. 2002 - In Press) found no evidence of upregulation.

Second, regarding benchmark dose calculations (see Appendix 7B in the Revised ERD), one reviewer (MR) questioned whether the Kodell-West algorithm is an adequate statistical methodology for evaluating the thyroid hormone data. Because this algorithm assumes that data are normally distributed, models means of distributions using quadratic functions, and assumes that variances are equal across dosage levels, this reviewer suspected that the algorithm is too restrictive to detect effects. He recommended that EPA instead use a more flexible model for evaluating the thyroid hormone data.[14]

Finally, one reviewer (KK) noted that the point of departure that EPA proposed is similar to the effects level reported in selected ecotoxicological studies—a factor the agency may wish to consider in the Revised ERD.

7.2 Charge Question F.2—*Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.*

The peer reviewers generally supported the use of PBPK models for conducting interspecies extrapolations, though some suggested that development of pharmacodynamic modeling may help identify dose metrics more closely linked to adverse effects than serum concentrations of perchlorate. Commenting specifically on the options EPA considered for dose metric, one reviewer (KK) indicated that AUC perchlorate in blood is the most reasonable measure of internal dose that allows for defensible extrapolations across species and different life stages. He added that other dose metrics (e.g., AUC perchlorate in thyroid, circulating thyroid hormone levels) would not be appropriate until the mechanisms of perchlorate uptake into cells and the kinetics of upregulation processes have been adequately characterized. Another reviewer (NC) agreed, and recommended that EPA verify that the K_m selected for translocation of iodide through the apical cellular channel is consistent with that documented in a recent publication (Golstein et al. 1995).

Though not disagreeing that AUC in blood allows for defensible interspecies extrapolations, two reviewers (AC,MK) advocated use of a dose metric more predictive of toxic

[14] Dr. Andrew Geller (EPA) explained that the statistical analyses in question were conducted by Toxicology Excellence for Risk Assessment and submitted to EPA for review. EPA did not use the Kodell-West algorithm in its statistical analyses.

effects. More specifically, one reviewer (MK) noted that decreases in thyroid hormone levels or increases in TSH may be better indicators of adverse effects than circulating perchlorate levels. Another reviewer (AC) agreed, adding that pharmacodynamic modeling can help differentiate metrics more related to adverse effects (e.g., excess cell mitoses per unit time) from those with no risk implications. Both reviewers, therefore, advocated pharmacodynamic modeling to predict the impact that perchlorate exposure has on thyroid function.

The reviewers' only other relevant comments addressed the use of PBPK modeling to interpret effects observed in rats on post-natal day 4. Noting that impaired thyroid function at this life stage would most likely result from decreased transport of iodide across the placenta, one reviewer (GW) wondered how the interspecies extrapolations with PBPK models accounted for any potential differences in placental physiology between rats and humans. One reviewer (KK) noted that human PBPK models were not developed to evaluate pregnancy, fetuses, or neonates and instead only a rat model was developed to evaluate these life stages. He indicated that EPA's approach for using outputs from the rat models to extrapolate between different human life stages is adequate, and he saw no other defensible approach for estimating human equivalent exposures for various life stages. Another reviewer (NC) noted that NIS is expressed in the placentas of both rats and humans, despite notable physiological differences across these species. Other reviewers did not comment on this issue further.

7.3 Charge Question F.3—*Are there other data which should be considered in developing the uncertainty factors? Do you consider that the data support the values proposed or different values for each? Do the confidence statements accurately reflect the relevancy of the critical effects to humans and the comprehensiveness of the database? Do these statements make all the underlying assumptions and limitations of the assessment apparent? If not, what needs to be added?*

The peer reviewers discussed the proposed uncertainty factors at length. They had widely varying opinions on the matter. The designated discussion leader for this topic area (TC)

indicated that the Revised ERD proposes a composite uncertainty factor of 300, which is derived from several individual factors. The peer reviewers' comments on the individual components of the uncertainty factor follow. Some of these comments addressed the uncertainty factors EPA used in deriving the proposed RfD, while others addressed appropriate uncertainty factors should the Agency base its RfD derivation on human health effects data. General comments on uncertainty factors follow, and the reviewers' final statements on the proposed uncertainty factors are listed at the end of this section.

- *Extrapolation from a LOAEL to a NOAEL (proposed uncertainty factor = 10).* The peer reviewers had few comments on this element of the composite uncertainty factor. Three reviewers (MC,KK,LK) acknowledged that EPA typically applies this factor when extrapolating from a LOAEL to a NOAEL. Aside from expressing concerns about the general practice of assigning exact numerical figures to this type of uncertainty (see the bulleted item below titled "General comments"), none of the reviewers questioned the proposed use of this uncertainty factor.

  In the event that EPA bases its RfD derivation on human data, one reviewer (DH) said this uncertainty factor may not be necessary, given that the authors of the Greer study report identifying a NOAEL.

- *Intrahuman variability (proposed uncertainty factor = 3).* The peer reviewers generally supported EPA's proposed uncertainty factor of 3 for intraspecies variability. One reviewer (KK) noted that the Agency often assigns a factor of 10 for interindividual uncertainty, but instead proposed a factor of 3 based on ". . . the variability observed in the data and PBPK modeling for the adult humans . . ." (see lines 5–6 on page 7-20 of the Revised ERD). This reviewer commended EPA for using the PBPK modeling to derive an appropriate uncertainty factor, though he noted that the Revised ERD does not describe exactly how EPA arrived at the factor of 3.[15]

  Regarding EPA's comment that data from human subjects ". . . do not represent kinetic data for the potentially susceptible populations of the hypothyroid and hypothyroxinemic pregnant women and their fetuses," one reviewer (GW) recommended that the Agency refer to recent publications from the National Academy of Sciences' Committee on Reference Dietary Intakes for alternate approaches regarding consideration of medically disadvantaged groups receiving treatment as potentially sensitive populations. The reviewers discussed susceptibility to perchlorate exposure in greater detail when responding to charge question F.4 (see Section 7.4).

[15] When reviewing a draft of this report, one reviewer (MC) indicated that EPA can rationalize using an uncertainty factor of 10 for intraspecies variability, but he did not make this comment at the peer review meeting.

- *Interspecies extrapolation (no uncertainty factor proposed).* The reviewers expressed differing opinions on whether an uncertainty factor for interspecies extrapolation is necessary. One reviewer (MP), for example, noted that EPA often applies a 10-fold factor for this element of uncertainty and commended the Agency for using the PBPK modeling results to justify its decision not to use an interspecies uncertainty factor. Another reviewer (KK) agreed, and supported EPA's proposed approach for interspecies extrapolation.

  Two reviewers (AC,GW) wondered if rodents are more sensitive to perchlorate exposure than humans; if so, they said an interspecies uncertainty factor less than one might be warranted. One reviewer (GW), for instance, said the rat is a poor model for humans in terms of thyroid physiology. Noting that thyroid hormone function in rats may be at least 10-fold more active than thyroid function in humans, this reviewer suggested that an interspecies uncertainty factor of 0.1, or even lower, may be defensible. Another reviewer (NC) acknowledged that rats and humans have notable differences in thyroid physiology, but she cautioned that researchers have not clearly established differential sensitivity to perchlorate exposure.

- *Database insufficiency (proposed uncertainty factor = 3, based on concerns of immunotoxicity).* The peer reviewer with expertise in immunotoxicity (LK) did not support EPA's proposed uncertainty factor of 3 to account for database insufficiencies relevant to potential immunotoxic effects, particularly effects of contact hypersensitivity. Because the LOAEL reported for contact hypersensitivity (0.06 mg/kg/day) is already more than 3-fold higher than the proposed point of departure (0.01 mg/kg/day), this reviewer saw no basis for applying the additional uncertainty factor.[16] Moreover, he questioned the relevance of the skin rashes observed in Graves' disease patients being treated with high doses of perchlorate, noting that the patients received extremely high doses of perchlorate and that their autoimmune condition may have contributed to the observed rashes. This reviewer concluded that the current database on the immunotoxicity of perchlorate is sufficient and application of any uncertainty factor due to database insufficiencies is unwarranted. As Table 7-2 shows, a majority of the peer reviewers agreed that this uncertainty factor is unnecessary.

  Another reviewer (KK) viewed two of EPA's proposed uncertainty factors—the factor of 3 for database insufficiency on immunotoxicity and the factor of 3 for lack of data on chronic exposures (see the next bulleted item)—as a single factor addressing overall database insufficiency. He said that, given the number of laboratory animal experiments that have now evaluated a variety of toxic endpoints (e.g., reproductive, developmental,

[16] Ms. Annie Jarabek (EPA) clarified that EPA proposed the uncertainty factor due to inadequate characterization on the immunotoxicity endpoints, primarily that for contact hypersensitivity. The factor is not based on consideration of the relative magnitude of the LOAELs.

neurotoxic), EPA should instead use an overall database insufficiency uncertainty factor of 1, if the point of departure is based on laboratory animal studies.

- *Subchronic to chronic exposure duration (proposed uncertainty factor = 3).* Some peer reviewers supported the proposed uncertainty factor for the lack of a chronic exposure study, while others did not. The discussion leader (TC), for example, indicated that the longest-duration exposure study (i.e., the 90-day study) has not provided convincing evidence that exposures over longer durations will not reveal additional effects. Another reviewer (DJ) agreed and added that the presence of tumors following a 19-week study was of concern. Though he was not concerned that the tumors observed in rats are relevant to humans, another reviewer (GW) indicated that no data have convinced him that *in utero* programming of the HPT axis does not occur. As a result, he indicated that EPA's proposed uncertainty factor may be justified.

  One reviewer (LK), on the other hand, was not convinced that the presence of tumors in two laboratory animals was biologically or statistically significant. Given that the tumors occurred at dosage levels (30 mg/kg/day) several orders of magnitude higher than the proposed point of departure (0.01 mg/kg/day), this reviewer questioned whether a 3-fold uncertainty factor for the thyroid tumors is meaningful. Finally, given that exposures occurred *in utero* during the study of concern, this reviewer suspected that further effects of *in utero* programming would not be identified if the study duration had been longer than 19 weeks. For these reasons, he concluded that the uncertainty factor for subchronic to chronic exposure duration is not justified.

  Three reviewers (TF,DH,KK) commented on whether EPA should consider an uncertainty factor for exposure duration if it chooses to base its point of departure on human health effects data. Two reviewers (TF,KK) indicated that a 10-fold uncertainty factor would be warranted if human data are used, given that the longest exposure duration in a controlled study was 14 days. Another reviewer (DH) noted that a lower uncertainty factor may be appropriate, particularly if occupational epidemiological studies provide perspective on the implications of chronic exposures.

- *General comments.* Several reviewers (AC,MC,MK,MR) indicated that they prefer approaches other than applying simple, multiplicative factors to address uncertainty in RfD derivations—a comment, they emphasized, that applies to all chemical risk assessments, and not only to EPA's perchlorate analyses in the Revised ERD. As an example of this concern, one reviewer (MK) thought use of generic uncertainty factors implies that risk assessors lack an understanding of the toxicity mechanisms. Two other reviewers (AC,MR) agreed, but added that they prefer more sophisticated uncertainty modeling, rather than application of default factors. For instance, one reviewer (AC) indicated that EPA could use Bayesian model averaging or Monte Carlo modeling to derive a probability distribution for the point of departure, rather than applying 10-fold and 3-fold factors that do not appear related to any physiological process. Another reviewer (MR) identified

additional approaches to consider, such as establishing confidence intervals for uncertainty factors so that the composite factor can be expressed as a range, rather than as a single number.

- *Final comments.* When the discussions on uncertainty factors ended, the meeting chair (RW) asked the peer reviewers to summarize their individual opinions on the composite uncertainty factor and its components. Table 3 summarizes the peer reviewers' specific comments. Every reviewer who specifically addressed the proposed uncertainty factors for deriving an RfD indicated that EPA should eliminate the uncertainty factor of 3 for database insufficiency, but some reviewers indicated that this uncertainty factor may be justified for database insufficiencies other than those relevant to immunotoxicity. No other clear trends emerged from this discussion, though 3 of the 17 reviewers also suggested that the uncertainty factor for subchronic to chronic exposure duration may be unnecessary.

7.4 Charge Question F.4—*Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?*

The reviewers had multiple responses to how EPA identified susceptible populations and whether additional ones should be considered. Regarding EPA's approach, two reviewers (AC,MK) indicated that they would have preferred identifying susceptibilities based on mechanistic arguments. One of the two (MK), for instance, suggested that EPA should have identified susceptibilities from insights on the most relevant biochemical events and how these differ among subpopulations. The other reviewer (AC) added that EPA's account of susceptibilities would have been more convincing if it were based on a systematic evaluation of specific factors (e.g., interspecies differences in TBG levels and thyroid tissue growth rates). Similarly, a third reviewer (MR) noted that EPA could address potential susceptibilities directly in its benchmark dose calculations by using mixture models that explicitly account for susceptibilities in their calculations.

Other reviewers (NC,TC,TF,DJ) identified the following potential susceptibilities for EPA to consider in the human health dose-response assessment: genetic variations in NIS across the population, the elderly, fetuses and neonates who depend on iodide transport across the placenta or into breast milk, smokers, and people with dietary iodide insufficiencies (particularly pregnant women). The reviewers briefly discussed the potential implications of developing health

guidelines that protect against all susceptible populations—a discussion that focused more on general risk management issues and is not summarized here.

Table 2
Peer Reviewers' Final Comments on EPA's Proposed Use of the Brain Morphometry Data as the Point of Departure

Peer Reviewer	Comment
Comments supporting use of the brain morphometry data as the point of departure	
Dr. Merle Paule	Agreed with EPA's choice of the point of departure.
Dr. Tom Zoeller	Agreed with EPA's choice of the point of departure.
Comments not supporting use of the brain morphometry data as the point of departure	
Dr. Michael Aschner	Found the brain morphometry studies inconclusive.
Dr. Loren Koller	Disregarded the reported changes in brain morphometry.
Dr. Gary Williams	Not convinced that the brain morphometry study identified adverse effects.
Conditional remarks on EPA's use of the brain morphometry data	
Dr. Michael Collins	Did not suggest that EPA reject the brain morphometry data, but was not convinced that the selected numeric value (0.01 mg/kg/day) was the actual point of departure. (Note: After reviewing a draft of this report, this reviewer indicated that he finds the brain morphometry data to be inconclusive based on the opinions that were expressed at the meeting. This reviewer did not make this comment at the peer review meeting.)
Dr. Kannan Krishnan	Suggested that EPA not base the point of departure on brain morphometry data, unless the Agency can adequately address the concerns raised at the peer review meeting.
Dr. Ronald Wyzga	Would have greater confidence in the study if a blinded re-analysis found the same effects, but did not specify whether the existing data are an adequate basis for the point of departure.

Note: This table summarizes the peer reviewers' specific summary statements made at the end of the discussions on the point of departure. The following peer reviewers either did not comment specifically on whether the brain morphometry data should serve as the basis of the point of departure or commented instead on whether human data should be used: Dr. William Adams, Nancy Carrasco, M.D., Dr. Thomas Collins, Dr. Anthony Cox, Dr. Teresa Fan, Dr. David Hoel, Dr. Michael Kohn, Dr. David Jacobson-Kram, and Dr. Mehdi Razzaghi. Refer to Sections 7.1.2 and 7.1.3 for a more complete discussion of the peer reviewers' specific comments on the proposed point of departure.

Table 3
Peer Reviewers' Specific Recommendations on Uncertainty Factors That EPA Proposed in the RfD Derivation

Peer Reviewer	Comment
Nancy Carrasco, M.D.	Did not support the uncertainty factor for database insufficiency.
Dr. Thomas Collins	Did not support the uncertainty factor for database insufficiency.
Dr. Anthony Cox	Noted that an interspecies uncertainty factor less than 1 might be defended. Did not support the uncertainty factor for database insufficiency.
Dr. David Jacobson-Kram	Did not support the uncertainty factor for database insufficiency.
Dr. Loren Koller	Did not support the uncertainty factor for database insufficiency or the uncertainty factor for subchronic to chronic exposure.
Dr. Kannan Krishnan	Did not support the combined uncertainty factor of 10 for database insufficiency with regards to immunotoxicity (factor of 3) and subchronic to chronic exposure duration (factor of 3).
Dr. Merle Paule	Did not support the uncertainty factor for database insufficiency, if it is based strictly on lack of information on immunotoxicity.
Dr. Gary Williams	Did not support the uncertainty factor for database insufficiency.
Dr. Ronald Wyzga	Did not support the uncertainty factor for database insufficiency.
Dr. Tom Zoeller	Did not support the uncertainty factor for subchronic to chronic exposure.

Note: This table summarizes the peer reviewers' specific summary statements made at the end of the discussions on uncertainty factors. The table identifies recommended changes in the uncertainty factors that EPA specifically proposed for deriving an RfD. The following peer reviewers did not comment specifically on the uncertainty factors EPA proposed in Section 7.1.4 of the Revised ERD: Dr. William Adams, Dr. Michael Aschner, Dr. Michael Collins, Dr. Teresa Fan, Dr. David Hoel, Dr. Michael Kohn, and Dr. Mehdi Razzaghi. Refer to Section 7.3 for a more complete discussion of the peer reviewers' specific comments on the proposed uncertainty factors.

8.0 Responses to Questions in Topic Area G: Risk Characterization

The peer reviewers briefly discussed the human health and ecological risk characterization statements that EPA presents in Chapters 10.1 and 10.2 of the Revised ERD, respectively. Their comments on these chapters appear in Sections 8.1 and 8.2, below.

8.1 Comments on the Human Health Risk Characterization

Summarizing the relevant premeeting comments, the designated discussion leader (RW) indicated that the peer reviewers generally thought the human health risk characterization adequately summarizes the information originally presented in the Revised ERD, though he noted that EPA should eventually revise the risk characterization to reflect the peer reviewers' various findings listed throughout this report.

The peer reviewers made relatively few specific suggestions for improving this section. To ensure that the risk characterization reflects the current understanding of perchlorate toxicity, one reviewer (RW) recommended, Chapter 10.1 should acknowledge the diversity of opinion regarding how to interpret key toxicity studies, particularly for the studies reporting changes in brain morphometry in rats. For greater perspective on whether perchlorate-related toxicity is believed to occur in humans, two reviewers (KK,RW) suggested, Chapter 10.1 should include more information on current human exposure levels, trends in these exposures (e.g., are levels of perchlorate in drinking water supplies increasing or decreasing?), and relevant effects observed in humans at various dosage levels. Another reviewer (GW) suggested that Chapter 10.1 document data on the prevalence of goiter, noting that an increased prevalence of goiter would likely be one of the first detectable thyroid effects in populations exposed to perchlorate.

8.2 Comments on the Ecological Risk Characterization

Two reviewers (WA,TF) addressed EPA's characterization of ecological risks, drawing mainly from comments they raised earlier in the peer review meeting (see Section 5). Though both reviewers initially found EPA's screening-level risk assessment adequate, their views changed upon reviewing an ecotoxicological study published after the release of the Revised ERD (Goleman et al. 2002). The two reviewers' comments addressed three general issues:

- *Comments on exposure data.* One reviewer (WA) indicated that, although the Revised ERD correctly focuses on environmental media where perchlorate is expected to occur, the data available for evaluating environmental exposures are limited. He suggested that future research efforts focus on characterizing potential exposures more broadly, particularly exposures in the range over which ecotoxicological effects are observed. Another reviewer (TF) recommended that EPA clarify its statements on chemical transformation of perchlorate, rather than asserting that the contaminant is extremely stable, and that more detailed information on biological transformation may be necessary, particularly as it applies to potential phytoremediation strategies. Two reviewers (WA,TF) suggested that EPA characterize the extent to which humans are exposed to perchlorate by consuming agricultural produce grown in areas with perchlorate contamination, whether domestically or abroad.

- *Comments on aquatic effects assessment.* Two reviewers (WA,TF) questioned the adequacy of EPA's screening-level ecological risk assessment for perchlorate, given that a recent study (Goleman et al. 2002) suggests that adverse effects may be occurring at exposure concentrations considerably lower than the threshold (0.6 ppm) EPA originally proposed for aquatic toxicity. The reviewers recommended that EPA critically review potential limitations of this study (e.g., implications of the extended duration of the experiment, presence of considerable adverse effects in the control groups, and relevance of de-ionized water as an exposure matrix) to determine if its proposed toxicity threshold is scientifically sound. Based on concerns raised by the recent study, one of the two reviewers (WA) recommended that EPA's ecological testing strategy focus on life stages and organisms that may be affected by changes in iodide uptake inhibition. This reviewer specifically suggested that EPA shift its focus in future studies from invertebrates to vertebrates. The other reviewer (TF) agreed, and recommended that future studies examine rooted macrophytes and detritus, which she indicated may be important for dietary exposures in the aquatic food chain.

- *Comments on terrestrial effects assessment.* Two reviewers (WA,TF) indicated that the Revised ERD lacks extensive detail on ecological exposures and risks associated with soils contaminated with perchlorate, and with the contamination of plant tissues that may result.

These reviewers recommended that future studies focus on dietary exposure routes for terrestrial organisms that feed on aquatic vegetation and that have developmental stages influenced by thyroid hormone production (e.g., mice, voles, ducks).

9.0 Responses to Questions in Topic Area H: General Comments, Conclusions, and Recommendations

In the final discussions, 14 peer reviewers presented their overall impressions of the Revised ERD. Three peer reviewers (Dr. Michael Aschner, Dr. Anthony Cox, and Dr. Michael Kohn) were not present during these final discussions. A summary of the peer reviewers' final comments, organized by topic area, follow:

- *Representation of perchlorate uptake and metabolism.* Nancy Carrasco, M.D., suggested that EPA further research two specific issues regarding perchlorate toxicokinetics. First, she questioned the validity of EPA's assumption that NIS translocates perchlorate into thyroid cells. She noted that none of the references cited in the Revised ERD provide compelling evidence that active translocation of perchlorate occurs. Dr. Carrasco indicated that even the most recent studies reporting concentrations of perchlorate in the thyroid (e.g., Yu et al. 2001) are not convincing, largely because the studies do not distinguish whether perchlorate detected in the thyroid is inside cells or simply bound to them. Second, Dr. Carrasco recommended that EPA reconsider its assumption that perchlorate is not metabolized and is "excreted virtually unchanged," because studies that administered double-labeled radioactive perchlorate to humans suggest that some of the amount ingested is metabolized.

- *Human health effects data.* Dr. David Hoel made several recommendations for how EPA can better integrate findings from human health effects studies into the Revised ERD. First, he recommended that EPA more thoroughly evaluate data from certain human clinical studies (Greer et al. 2000; Greer et al. 2002 - In Press; Lawrence et al. 2000, 2001) and perhaps use these data as a basis for the proposed point of departure, provided that it adequately addresses concerns regarding confounding factors (e.g., dietary iodide levels, smoking, body weight) and notes the limitations associated with the 14-day exposure duration. Second, Dr. Hoel recommended that EPA determine whether the remaining epidemiological studies (i.e., ecological and occupational studies) offer further insight into the clinical studies, particularly regarding long-term exposures. Finally, Dr. Hoel suggested that EPA calculate human equivalent exposures for the effect levels observed in the laboratory animal studies and assess whether humans experience comparable effects at these equivalent exposures.

- *Laboratory animal data: immunotoxicity.* Dr. Loren Koller indicated that the two immunotoxicology studies conducted since the 1999 peer review used standard protocols and validated assays to evaluate both the innate and acquired immune responses, considering most compartments of the immune system. Dr. Koller noted that the effects

observed in these studies were generally immuno-stimulatory, or protective, with regards to host resistance to infectious disease and neoplasia. Though he acknowledged that two separate experiments (i.e., 14-day and 90-day evaluations) identified contact hypersensitivity effects, Dr. Koller noted that the observed effects did not follow a clear dose-response signal. He questioned whether these observed effects are expected to occur in humans, especially considering that skin rashes have only been observed in patients being treated for Graves' disease with high doses of perchlorate. Dr. Koller concluded that the immunotoxicity studies do not provide an adequate basis for deriving a point of departure or for applying an uncertainty factor for database insufficiency.

- *Laboratory animal data: thyroid hormone levels.* Dr. Thomas Zoeller indicated that the data on thyroid hormone levels generally support EPA's proposed mode of action for perchlorate toxicity, even though consistent effects (i.e., decreases in T4 and increases in TSH) are not observed in all studies. Dr. Zoeller emphasized that the laboratory animal study with the most rigorous design (Argus 2001) reported the most consistent, dose-dependent changes in thyroid hormone and TSH levels. For instance, he said, the study reported hormone levels within typically reported ranges and the researchers properly applied the diagnostic kits for measuring the serum concentrations. Dr. Zoeller acknowledged that decrements in T4 were not always observed. He attributed this unexpected outcome to the measurement limitations of the diagnostic kits: T4 levels in control groups were near the lowest testing standard, where measurement variability is greatest. Dr. Zoeller supported EPA's use of ANOVA in its statistical analyses of the thyroid hormone levels, but questioned the validity of pooling results from separate assays in the data analysis.

- *Laboratory animal data: brain morphometry.* Dr. Thomas Zoeller indicated that the recent study of brain morphometry is an important element in EPA's RfD derivation, because there is only limited evidence that adverse effects occur at the lowest dosage levels in the other endpoints (e.g., perturbations in thyroid hormone levels, thyroid histopathology, inhibition of iodide uptake). Dr. Zoeller indicated that most reviewers questioned the integrity of the brain morphometry data, with overall impressions generally falling into two categories. Some reviewers concluded that linear measurements of the brain sections will never be an adequate basis for the analyses of brain morphometry. Other reviewers agreed that the linear measurements and other aspects of the study design introduce error into the raw data, but they believed these errors would be evenly distributed among treatment groups, without any systematic biases; these reviewers believed that a blinded re-analysis of the linear measurements of the brain slides may resolve the issue.

- *Laboratory animal data: neurotoxicity.* Building on the comments summarized in the previous bulleted item, Dr. Merle Paule acknowledged that one peer reviewer (Dr. Michael Aschner) had serious concerns regarding EPA's proposed use of the brain morphometry data for the point of departure. EPA's and other peer reviewers' comments on the study

methodology, however, convinced Dr. Paule that the brain morphometric results are valid. Further, he noted that EPA's Bayesian analyses of the data on spontaneous motor activity behavior identified effects in both studies that evaluated this endpoint (Argus 1998; Bekkedal 2000).

- *Laboratory animal data: thyroid pathology.* Dr. Gary Williams indicated that administration of low doses of perchlorate to rats and rabbits produced adaptive changes in thyroid histopathology, namely colloid depletion and epithelial hypertrophy, while administration of higher doses (i.e., 1.0 mg/kg/day and higher) produced hyperplastic responses to TSH stimulation. Dr. Williams added that dosage at much higher levels (30 mg/kg/day) produced follicular cell neoplasms in rats. He noted that the thyroid physiology in rats renders the species particularly susceptible to such metabolic perturbations. Dr. Williams concluded that these various observations indicate that perchlorate is an anti-thyroid agent that induces thyroid neoplasms in rats—an effect that he believed has a LOAEL of 1.0 mg/kg/day. Noting that other agents with comparable anti-thyroid effects have induced thyroid tumors in rats but not in humans, Dr. Williams found no compelling reason to believe that the currently reported environmental exposure levels to perchlorate would cause thyroid cancer in humans.

- *Laboratory animal data: reproductive toxicity.* Dr. Thomas Collins indicated that a two-generation reproduction study (Argus 1999) had been completed since the 1999 peer review—a study that he found scientifically sound, except for some methodological concerns (see pages 39–41 of the premeeting comments in Appendix C). Dr. Collins supported EPA's interpretations of the study, but suggested that the Agency further evaluate the apparent dose-dependent decreases in sperm density and daily sperm production levels.

- *Laboratory animal data: developmental toxicity.* Dr. Michael Collins briefly summarized the two studies completed since the 1999 peer review that examined developmental toxicity. Dr. Collins indicated that the authors of one study reported a NOAEL of 30 mg/kg/day for developmental toxicity in rats (which EPA interpreted as being a LOAEL) and the authors of another study reported a NOAEL of at least 100 mg/kg/day for developmental toxicity in rabbits. Dr. Collins noted that the NOAELs for these studies are orders of magnitude higher than the doses EPA considered as possible bases for the point of departure. More generally, he emphasized that the available studies suggest that fetuses are more susceptible to perchlorate toxicity than are the maternal organisms.

- *Laboratory animal data: genetic toxicology.* Dr. David Jacobson-Kram indicated that no new genotoxicity studies have been published since the 1999 peer review. He agreed with the findings of the previous peer review panel, which concluded that the battery of available genetic toxicology tests suggests that perchlorate is not genotoxic. Because of this finding, he supported EPA's nonlinear model for evaluating perchlorate carcinogenicity.

- *Ecological risk assessment and evidence for indirect exposure.* Dr. Teresa Fan indicated that the data currently available on perchlorate exposure and effects are not sufficient for conducting a screening-level ecological risk assessment. She listed several issues that require further research before EPA can state its conclusions (e.g., toxicity thresholds) with confidence: EPA must further evaluate dietary exposures, particularly from aquatic macrophytes to herbivorous aquatic organisms and from terrestrial vegetation to herbivorous mammals; focus ecotoxicological studies on species expected to develop adverse effects resulting from thyroid iodide uptake inhibition (e.g., amphibian metamorphosis studies and studies of egg-bearing female fish); gather data on perchlorate body burdens to allow for extrapolations between laboratory toxicity tests and field studies (this would enable EPA to resolve apparent discrepancies in the plant bioaccumulation data); and revise text implying that biological and chemical transformation of perchlorate does not occur. Because of these and other data gaps, Dr. Fan did not think EPA could conclude that perchlorate is not expected to have effects on populations and species richness. Dr. William Adams agreed with Dr. Fan's final comments.

- *PBPK modeling, selection of dose metric, and interspecies extrapolations.* Dr. Kannan Krishnan indicated that the structure, basic equations, and physiological parameters EPA used in the PBPK models are generally appropriate. However, the peer reviewers questioned three of the models' assumptions regarding cellular uptake processes and their associated kinetics: Is passive diffusion of iodide a relevant process? Does perchlorate actively translocate into cells, or does it simply bind to them? Are the kinetics of active transport of iodide best described as competitive inhibition? Dr. Krishnan noted that errors in the representation of these cellular uptake processes and their kinetics may have only marginal impacts on the predicted serum concentrations of perchlorate. Nonetheless, he recommended that EPA refine the PBPK models to address the reviewers' concerns regarding cellular uptake processes. Dr. Krishnan indicated that the AUC of serum perchlorate is an acceptable dose metric, though he noted that other reviewers were not convinced that this dose metric is the best predictor of adverse health effects.

- *Human health dose-response assessment.* Dr. Tom Collins said the majority of reviewers agreed that EPA's proposed mode of action is consistent with the observed effects in laboratory animals and humans. He also said the reviewers had differing opinions on the proposed point of departure of 0.01 mg/kg/day and whether it should be based on human health effects data. Dr. Collins noted that the peer reviewers also had various opinions on the appropriate selection of uncertainty factors for deriving an RfD from the brain morphometry studies, though nearly every reviewer agreed that application of an uncertainty factor for database insufficiencies in immunotoxicity was not warranted. He listed many additional specific comments that the reviewers raised on finer points of the dose-response assessment.

- *Recommendations for further action.* The reviewers listed several recommendations for future research on perchlorate toxicity:

  - Conduct a blinded re-analysis of the brain sections from the brain morphometry studies (multiple reviewers).

  - Develop valid and validated endpoints of thyroid hormone action on brain development (TZ).

  - Evaluate the relative impacts of anti-thyroid dietary components, the effects of exposures to lower perchlorate doses (0.001 mg/kg/day), the potential for progression of tumors induced by exposures to ammonium perchlorate, and the potential impacts of *in utero* exposure (GW).

  - Conduct replications of laboratory animal studies during the same time of year to prevent seasonality in rodent physiology from masking notable results; incorporate more sophisticated neurobehavioral endpoints into future developmental studies (MP).

  - Conduct an additional multi-generational developmental study that evaluates a full suite of neurobehavioral, neurodevelopmental, and thyroid histopathological endpoints (LK).

  - Consider conducting an epidemiological study on the prevalence of goiter among populations exposed to perchlorate (DH).

  - Conduct a more extensive chronic exposure study (i.e., with a "womb-to-tomb" design) and another study of potential *in utero* programming (TC).

  - Characterize the pharmacodynamics by which iodide uptake inhibition leads to neurodevelopmental and neoplastic sequelae (MC).

  - Ascertain unequivocally whether active translocation of perchlorate occurs, and characterize potential adverse effects resulting from prolonged exposure to perchlorate (NC).

  - Verify whether ecological effects recently reported in a laboratory study (Goleman et al. 2002) are expected to occur in the field (WA).

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