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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.

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## 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).

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[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 ($4.0 \times 10^6$ 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 $4.0 \times 10^6$ 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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BRT-Burleson Research Technologies, Inc. 2000b. Addendum to study report: ammonium perchlorate: effect on immune function [with cover letter dated August 31 from G.R. Burleson]. Raleigh, NC.

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# Appendix A

## List of Selected Studies Performed Since the 1999 Peer Review

# Additional studies that have been performed since the 1999 peer review:


Argus Research Laboratories, Inc. (1999) Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-001.

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Argus Research Laboratories, Inc. (2001) Hormone, thyroid and neurohistological effects of oral (drinking water) exposure to ammonium perchlorate in pregnant and lactating rats and in fetuses and nursing pups exposed to ammonium perchlorate during gestation or via maternal milk. Horsham, PA: Protocol no. ARGUS 1416-003.

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# Appendix B

## List of Expert Peer Reviewers

United States
Environmental Protection Agency
National Center for Environmental Risk Assessment

# Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Holiday Inn Capitol Plaza
Sacramento, CA
March 5–6, 2002

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# Appendix C

## Premeeting Comments, Alphabetized by Author, and Charge to the Reviewers

# Peer Review on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

# Reviewers' Comments

**February 2002**

# Notice

Premeeting comments were prepared by each reviewer individually prior to the meeting. They are preliminary comments only, and are used to help reviewers become familiar with the document and charge questions, develop the agenda, and identify key issues for discussion. During the meeting, reviewers may expand on or change opinions expressed in their premeeting remarks and may introduce additional issues. For these reasons, premeeting comments should be regarded as preliminary and do not reflect the final conclusions and recommendations of individuals reviewers or the panel. These premeeting comments will be included as an appendix in the meeting summary report, along with other background materials.

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Charge to Reviewers

**Peer Reviewer Comments**

William Adams

Michael Aschner

Nancy Carrasco

Michael Collins

Thomas F.X. Collins

Anthony Cox

Teresa Fan

David Hoel

David Jacobson-Kram

Michael Kohn

Loren Koller

Kannan Krishnan

Merle Paule

Mehdi Razzaghi

Gary Williams

Ronald Wyzga

Thomas Zoeller

**Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"**

March 5–6, 2002

# CHARGE TO THE REVIEWERS

**Introduction and Background**

Perchlorate ($ClO_4^-$) is an anion that originates as a contaminant in groundwater and surface waters from the dissolution of its 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, the existing toxicologic database on perchlorate was determined to be inadequate for quantitative human health risk assessment by an independent non-EPA external peer review panel. A lack of data on the ecotoxicological effects was also noted. In May 1997, a perchlorate testing strategy was developed based on the known mode-of-action for perchlorate toxicity (the inhibition of iodide uptake in the thyroid and subsequent perturbations of thyroid hormone homeostasis), and an accelerated research program was initiated to gain a better understanding of the human health effects of perchlorate, examine possible ecological impacts, refine analytical methods, develop treatment technologies, and better characterize the occurrence of perchlorate in groundwater and surface waters.

In December 1998, the National Center for Environmental Assessment (NCEA) developed an external peer review draft document that assessed the human health and ecological risk of perchlorate ("Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information," NCEA-1-0503). This document presented a human health risk assessment that incorporated results of the newly performed health effects studies available as of November 1998 from the perchlorate testing strategy and a screening-level ecological assessment. The human health risk assessment utilized a model motivated by the mode-of-action that harmonized noncancer and cancer approaches to derive a single oral risk benchmark based on precursor effects for both altered neurodevelopment and thyroid neoplasia. A workshop was convened by the Agency in February 1999 in San Bernardino, California, to provide external peer review of that document. The external scientific peer review panel endorsed the conceptual approach proposed by NCEA, but recommended that new analyses be conducted and that several additional studies be planned and performed. NCEA has prepared a revised perchlorate assessment that addresses comments from the 1999 external peer review workshop and incorporates data from additional studies that have become available since the 1999 review. These supporting data and the revised draft assessment are the subject of the current external peer review.

Specific objectives of this draft assessment are to derive a human health risk estimate for perchlorate based both on its potential to cause noncancer toxicity or cancer, to provide a screening ecological risk assessment for perchlorate, and to evaluate the evidence for indirect exposures, i.e., those exposures not occurring by direct ingestion of contaminated water.

**Disclaimer**

This draft external review document is still undergoing scientific review and deliberations both by the external scientific community and within the Agency. As with any EPA draft assessment document containing a quantitative risk value, that risk value is also draft and should not at this stage be construed to represent EPA policy.

**Purpose of the Peer Review**

The Agency conducts external peer reviews of draft assessments to ensure that science is used credibly and appropriately in the derivation of human health and ecotoxicological assessments. After the scientific basis of these draft assessments has been peer reviewed, the documents are forwarded to the IRIS Consensus Process for final approval and adoption by the EPA. These hazard and dose-response assessments will then appear on IRIS and become available as Agency consensus risk information. You have been chosen to participate in the external peer review of the "Toxicological Review and Risk Characterization for Perchlorate" as an expert in a scientific discipline relevant to the perchlorate assessment, including reproductive and developmental toxicology, neurotoxicology, immunotoxicology, genetic toxicology, pathology, epidemiology, endocrinology, statistics, physiologically-based pharmacokinetic (PBPK) modeling, ecotoxicology, environmental fate and transport, or risk assessment. The charge to the external peer reviewers has two main components:

(1) To review the protocols, performance and results of studies that have been performed since the 1999 peer review that are not in the peer-reviewed literature (Note that these studies include PBPK models).

(2) To review the draft risk assessment and evaluate whether the data chosen and inferences based on the data employed in the derivation of the assessments are appropriate and scientifically sound.

Please note that you are *not* asked to review the recommended Agency testing or risk assessment guidelines or methodologies used to derive the human health or ecotoxicological assessments, because these have undergone independent review by external scientific peers, the public, and EPA Science Advisory Boards. However, we do ask that you comment on the application of these guidelines and methodologies within the assessment as you deem appropriate. For reference, the preface to the draft document lists the various Agency guidelines and methodologies that were considered when developing the perchlorate assessment.

**Instructions to Reviewers**

The peer review meeting will be structured around the charge questions that follow, which are organized into eight topic areas. The charge questions seek the panel's critical input on two topics:

- Studies published since 1999 that have not undergone peer review.
- EPA's interpretation of these and other studies in the perchlorate assessment.

Reviewers are not being asked to respond to every charge question, but instead have been assigned responsibilities based on their areas of expertise. Table 1 lists the reviewers' responsibilities. As the table indicates, reviewers are being asked to perform the following tasks:

- **Studies:** Almost every reviewer is being asked to review some of the studies published since 1999 that require peer review. Table 1 identifies the studies to which each reviewer has been assigned, and Table 2 gives the full citations for these studies. Copies of the studies were distributed to the reviewers, according to the assignments in Table 1. Attachments 1 and 3 present questions to guide your reviews of these studies. The questions in Attachment 1 pertain to human health, laboratory animal, and ecological studies. The questions in Attachment 3 pertain to PBPK studies. Please *consider* the questions in these attachments as you review the studies. *You do not need to answer every question in the attachments, rather use your professional judgment to address those that are most appropriate to the study in question.*

- **Perchlorate assessment:** Every reviewer is being asked to read the entire perchlorate assessment and review specific sections of the document. Table 1 identifies the specific sections that each reviewer has been assigned to review. It also lists the charge questions that you must answer, both in your premeeting comments and at the meeting. Attachment 2 provides a list of questions which give you the context for answering the charge questions B2, C2, D2, and E2. Please *consider* the questions in Attachment 2 as you review the document. *You do not need to answer every question in the attachment, rather use your professional judgment to address those that are most appropriate to the chapter in question.*

Though not required, you are encouraged to respond to charge questions other than those to which you have been assigned as time allows. At the peer review meeting, the reviewers will discuss their responses to the charge questions, with the goal of providing EPA with recommendations on how to improve the document. Table 1 identifies the peer reviewers who will serve as discussions leaders and moderate these discussions.

## SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

**Topic Area A:** **Hazard Characterization and Mode of Action**
**Designated reviewers:** **All reviewers (except William Adams and Teresa Fan)**
**Discussion leader:** **Thomas Zoeller**

A.1 Have all 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?

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

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?

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 for both.

**Topic Area B:** **Human Health Effects Data**
**Designated reviewers:** **Nancy Carrasco, Tony Cox, David Hoel, Mehdi Razzaghi, Ron Wyzga, Thomas Zoeller**
**Discussion leader:** **David Hoel, with Nancy Carrasco for clinical endocrinology and Mehdi Razzaghi for observational epidemiology**

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

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?

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?

**Topic Area C:** **Laboratory Animal Studies**
**Designated reviewers:** **Michael Aschner, Michael Collins, Thomas Collins, Tony Cox, David Jacobson-Kram, Loren Koller, Merle Paule, Gary Williams,Ron Wyzga, Thomas Zoeller**
**Discussion leader:** **Multiple reviewers (see Table 1)**

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

**Topic Area D:** **Ecological Risk Assessment and Evidence for Indirect Exposure**
**Designated reviewers:** **William Adams, Teresa Fan**
**Discussion leader:** **William Adams**

D.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

D.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in 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.

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.

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

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.

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

**Topic Area E:** **Use of PBPK Modeling**
**Designated reviewers:** **Michael Kohn, Kannan Krishnan**
**Discussion leader:** **Michael Kohn**

E.1 For each of the four models developed by the Air Force Research Laboratory (AFRL) listed below, consider the questions in Attachment 3 and comment as necessary. You do not need to answer every question in Attachment 3, rather use your professional judgment to address those that are most appropriate to the model and associated consultative letters/studies in question. Refer to Table 1 for all relevant citations. Note that the citations for the four models, which are contained in consultative letters, follow:

Adult Male Rat Model (Merrill, 2001c)
Adult Human Model (Merrill, 2001d)
Pregnant Rat and Fetus Model (Clewell, 2001a)
Lactating Rat and Neonate Model (Clewell, 2001b)

E.2 Please consider the questions in Attachment 2 to comment on how EPA applied and presented the models in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

**Topic Area F:** **Human Health Dose-Response Assessment**
**Designated reviewers:** **All reviewers (except William Adams and Teresa Fan)**
**Discussion leader:** **Thomas Collins**

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?

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

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?

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

**Topic Area G:** **Risk Characterization**
**Designated reviewers:** **Question G.1: All reviewers (except William Adams and Teresa Fan)**
**Question G.2: William Adams and Teresa Fan**
**Discussion leader:** **Ron Wyzga**

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

G.2 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the ecotoxicological risk posed by potential perchlorate exposures?

**Topic Area H:** **General Comments, Conclusions, and Recommendations**
**Designated reviewers:** **All reviewers**
**Discussion leader:** **Ron Wyzga**

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

**Table 1**
**Reviewer Assignments**

| Reviewer Name | Studies Published Since 1999 to Review | Chapters of the EPA Document to Review | Charge Questions to Answer | Discussion Leader Responsibilities |
|---|---|---|---|---|
| William Adams | Condike 2001<br>EA Engineering 1999<br>EA Engineering 2000<br>Parsons Engr. Sci. 2001 | Chapters 1–3, 8, 9, 10 | D1–D7, G2, H1-H2 | Topic Area D |
| Michael Aschner | Argus 2001<br>Bekkedal et al. 2000 | Chapters 1–3, 5, 7, 10 | A1–A4, C1–C4, F1–F4, G1, H1-H2 | Topic Area C (Neurotoxicity only) |
| Nancy Carrasco | Greer 2000<br>Lawrence 2001<br>Merrill 2001a | Chapters 1–3, 4, 7, 10 | A1–A4, B1–B5, F1–F4, G1, H1-H2 | Topic Area B (Clinical Endocrinology) |
| Michael Collins | Argus 2000 | Chapters 1–3, 5, 7, 10 | A1–A4, C1–C4, F1–F4, G1, H1-H2 | Topic Area C (Developmental only) |
| Thomas Collins | Argus 1999 | Chapters 1–3, 5, 7, 10 | A1–A4, C1–C4, F1–F4, G1, H1-H2 | Topic Area C (Reproductive only)<br>Topic Area F |
| Tony Cox | Greer 2000<br>Lawrence 2001<br>Merrill 2001a | Chapters 1–3, 4, 5, 7, 10 | A1–A4, B1–B5, C1–C4, D1-D7, F1–F4, G1, H1-H2 | Topic Area C (Statistical Issues) |
| Teresa Fan | Condike 2001<br>EA Engineering 1999<br>EA Engineering 2000<br>Parsons Engr. Sci. 2001 | Chapters 1–3, 8, 9, 10 | D1–D7, G2, H1-H2 | None |
| David Hoel | Greer 2000<br>Lawrence 2001<br>Merrill 2001a | Chapters 1–3, 4, 7, 10 | A1–A4, B1–B5, F1–F4, G1, H1-H2 | Topic Area B (Statistical Issues) |
| David Jacobson-Kram | None | Chapters 1–3, 5, 7, 10 | A1–A4, C1-C4, F1–F4, G1, H1-H2 | Topic Area C (Genetic Toxicity Issues) |

**Table 1 (Continued)**
**Reviewer Assignments**

| Reviewer Name | Studies Published Since 1999 to Review | Chapters of the EPA Document to Review | Charge Questions to Answer | Discussion Leader Responsibilities |
|---|---|---|---|---|
| Michael Kohn | Merrill 2001a<br>Merrill 2001c<br>Merrill 2001d<br>Merrill 2001e<br>Clewell 2001a<br>Clewell 2001b<br>Yu 2000, 2001, 2002<br>Yu et al. 2000<br>Mahle 2000, 2001 | Chapters 1–3, 6, 7, 10 | A1–A4, E1–E2, F1–F4, G1, H1-H2 | Topic Area E |
| Loren Koller | BRT Burl. Res. Tech. 2000a<br>BRT Burl. Res. Tech. 2000b<br>BRT Burl. Res. Tech. 2000a<br>Keil et al. 1999 | Chapters 1–3, 5, 7, 10 | A1–A4, C1–C4, F1–F4, G1, H1-H2 | Topic Area C (Immunotoxicity only) |
| Kannan Krishnan | Merrill 2001a<br>Merrill 2001c<br>Merrill 2001d<br>Merrill 2001e<br>Clewell 2001a<br>Clewell 2001b<br>Yu 2000, 2001, 2002<br>Yu et al. 2000<br>Mahle 2000, 2001 | Chapters 1–3, 6, 7, 10 | A1–A4, E1–E2, F1–F4, G1, H1-H2 | None |
| Merle Paule | Argus 2001<br>Bekkedal et al. 2000 | Chapters 1–3, 5, 7, 10 | A1–A4, C1–C4, F1–F4, G1, H1-H2 | None |
| Mehdi Razzaghi | Greer 2000<br>Lawrence 2001<br>Merrill 2001a | Chapters 1–3, 4, 7, 10 | A1–A4, B1–B5, F1–F4, G1, H1-H2 | Topic Area B (Observational Epidemiology) |
| Gary Williams | Argus 2001 | Chapters 1–3, 5, 7, 10 | A1–A4, C1-C4, F1–F4, G1, H1-H2 | Topic Area C (Pathology only) |

**Table 1 (Continued)**
**Reviewer Assignments**

| Reviewer Name | Studies Published Since 1999 to Review | Chapters of the EPA Document to Review | Charge Questions to Answer | Discussion Leader Responsibilities |
|---|---|---|---|---|
| Ron Wyzga | None | Chapters 1–3, 5, 7, 10 | A1–A4, B1–B5, C1-C4, F1–F4, G1, H1-H2 | Topic Areas G and H |
| Thomas Zoeller | Argus 2001<br>Bekkedal et al. 2000<br>Greer 2000<br>Lawrence 2001<br>Merrill 2001a | Chapters 1–3, 4, 5, 7, 10 | A1–A4, B1–B5, C1–C4, F1–F4, G1, H1-H2 | Topic Area A;<br>Topic Area C<br>(Endocrine and neuroendocrine only) |

**Table 2**
**Studies Conducted Since 1999 That Require Peer Review**

| Topic Area | Relevant Studies |
|---|---|
| Human health effects data: Topic Area B | Greer (2000). Does environmental perchlorate exposure alter human thyroid function? Determination of the dose-response for inhibition of radioiodine uptake. In: Abstracts of the 12th International Thyroid Congress; October; Kyoto, Japan. Endocrine J. 47 (suppl.): 146. |
| | Lawrence (2001). Low dose perchlorate (3 mg daily) and thyroid function [letter]. Thyroid 11: 295. |
| | Merrill, E. (2001a) Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit report for the study of perchlorate *pharmacokinetics and inhibition of radioactive iodine uptake (RAIU) by the thyroid in humans (CRC protocol #628)* [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 10. |
| Laboratory animal studies: Topic Area C (Reproductive Toxicity) | Argus Research Laboratories, Inc. (1999) Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-001. |
| Laboratory animal studies: Topic Area C (Developmental Toxicity) | Argus Research Laboratories, Inc. (2000) Oral (drinking water) developmental toxicity study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-003D. |
| Laboratory animal studies: Topic Area C (Neurodevelopmental Toxicity) | Argus Research Laboratories, Inc. (2001) Hormone, thyroid and neurohistological effects of oral (drinking water) exposure to ammonium perchlorate in pregnant and lactating rats and in fetuses and nursing pups exposed to ammonium perchlorate during gestation or via maternal milk. Horsham, PA: Protocol no. ARGUS 1416-003. |
| | Bekkedal, M. Y. V.; Carpenter, T.; Smith, J.; Ademujohn, C.; Maken, D.; Mattie, D. R. (2000) A neurodevelopmental study of the effects of oral ammonium perchlorate exposure on the motor activity of pre-weaning rat pups. Wright-Patterson Air Force Base, OH: Naval Health Research Center Detachment, Neurobehavioral Effects Laboratory; report no. TOXDET-00-03. |
| | Mahle, D. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0043, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; October 11. |
| | Mahle, D. (2001). Consultative letter, AFRL-HE-WP-CL-2001-0001, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; May 1. |

**Table 2 (Continued)**
**Studies Conducted Since 1999 That Require Peer Review**

| Topic Area | Relevant Studies |
|---|---|
| Laboratory animal studies: Topic Area C (Immunotoxicity) | BRT-Burleson Research Technologies, Inc. (2000a) Ammonium perchlorate: effect on immune function. Quality assurance audit: study no. BRT 19990524 -- plaque-forming cell (PFC) assay; study no. BRT 19990525– local lymph node assay (LLNA) in mice. Raleigh, NC. |
| | BRT-Burleson Research Technologies, Inc. (2000b) Addendum to study report: ammonium perchlorate: effect on immune function [with cover letter dated August 31 from G. R. Burleson]. Raleigh NC. |
| | BRT-Burleson Research Technologies, Inc. (2000c) Ammonium perchlorate: effect on immune function. Raleigh, NC: BRT 19990524 study protocol: plaque-forming cell (PFC) assay; BRT 19990525 study protocol: local lymph node assay (LLNA) in mice. |
| | Keil, D.; Warren, D. A.; Jenny, M.; EuDaly, J.; Dillard, R. (1999) Effects of ammonium perchlorate on immunotoxicological, hematological, and thyroid parameters in B6C3F1 female mice. Final report. Charleston, SC: Medical University of South Carolina, Department of Medical Laboratory Sciences; report no. DSWA01-97-0008. |
| Ecological risk assessment: Topic Area D | Condike (2001). Perchlorate data in fish and plants [letter with attachments to Annie M. Jarabek]. Fort Worth, TX: Department of the Army, Fort Worth District, Corps of Engineers; December 21. |
| | EA Engineering (1999). Results of algal toxicity testing with sodium perchlorate. Sparks, MD: EA Engineering, Science, and Technology, Inc. |
| | EA Engineering (2000). Results of chronic toxicity testing with sodium perchlorate using *Hyalella azteca* and *Pimephales promelas*. Sparks, MD: report number 3505. |
| | Parsons Engineering Science, Inc. (2001) Scientific and technical report for perchlorate biotransport investigation: a study of perchlorate occurrence in selected ecosystems. Interim final. Austin, TX; contract no. F41624-95 |

## Table 2 (Continued)
## Studies Conducted Since 1999 That Require Peer Review

| Topic Area | Relevant Studies |
|---|---|
| Use of PBPK modeling: Topic Area E | Merrill, E. A.(2001a) Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit report for the study of perchlorate pharmacokinetics and inhibition of radioactive iodine uptake (RAIU) by the thyroid in humans (CRC protocol #628) [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 10. |
| | Merrill, E. A. (2001c) Consultative letter, AFRL-HE-WP-CL-2001-0005, PBPK model for iodide kinetics and perchlorate-induced inhibition in the male rat [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 8. |
| | Merrill, E. A. (2001d) Consultative letter, AFRL-HE-WP-CL-2001-0008, PBPK model for perchlorate-induced inhibition of radioiodide uptake in humans [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; June 5. |
| Use of PBPK modeling: Topic Area E (Continued) | Merrill, E. A. (2001e) Consultative letter, AFRL-HE-WP-CL-2001-0010, comparison of internal dosimetrics using PBPK models for perchlorate induced inhibition of thyroid iodide uptake and sensitivity analysis for male rat model [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; December 20. |
| | Clewell, R. A. (2001a) Consultative letter, AFRL-HE-WP-CL-2001-0006, physiologically-based pharmacokinetic model for the kinetics of perchlorate-induced inhibition of iodide in the pregnant rat and fetus [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 10. |
| | Clewell, R. A. (2001b) Consultative letter, AFRL-HE-WP-CL-2001-0007, physiologically-based pharmacokinetic model for the kinetics of perchlorate-induced inhibition of iodide in the lactating and neonatal rat [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 24. |
| | Yu, K. O. (2000) Consultative letter, AFRL-HE-WP-CL-2000-0038, tissue distribution and inhibition of iodide uptake in the thyroid by perchlorate with corresponding hormonal changes in pregnant and lactating rats (drinking water study) [memorandum with attachment to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; June 28. |
| | Yu, K. O.; Todd, P. N.; Young, S. M.; Mattie, D. R.; Fisher, J. W.; Narayanan, L.; Godfrey, R. J.; Sterner, T. R.; Goodyear, C. (2000) Effects of perchlorate on thyroidal uptake of iodide with corresponding hormonal changes. Wright-Patterson AFB, OH: Air Force Research Laboratory; report no. AFRL-HE-WP-TR |
| | Yu, K.O. (2001). Consultative letter, AFRL-HE-WP-CL-2002-0001, intravenous kinetics of radiolabeled iodide in tissues of adult male Sprague Dawley rat dosed with $^{125}I^-$ plus carrier [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; December 21. |

**Table 2 (Continued)**
**Studies Conducted Since 1999 That Require Peer Review**

| Topic Area | Relevant Studies |
|---|---|
| | Yu, K.O. (2002). Consultative letter, AFRL-HE-WP-CL-2002-0002, intravenous kinetics of radiolabled iodide and perchlorate in tissues of pregnant and lactating Sprague Dawley female rats dosed with perchlorate and/or carrier free $^{125}I^-$ [memorandum with attachment to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; January 7. |
| | Mahle, D. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0043, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; October 11. |
| | Mahle, D. (2001). Consultative letter, AFRL-HE-WP-CL-2001-0001, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; May 1. |

**Attachment 1**

**General Considerations for Evaluating the Human Health, Laboratory Animals, and Ecological Studies listed in Table 2 (Note: Refer to Attachment 3 for general considerations for evaluating the PBPK models.)**

***Note to Reviewers:*** *These questions are being provided as general considerations for reviewing the studies published since 1999 that require peer review. You do not need to answer every question below when reviewing the studies that have been assigned to you; rather use your professional judgment to address those that are most appropriate to the study in question.*

1. Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

2. Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices or specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

3. Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe an effect?

4. Please comment on the strengths and limitations of the statistical analyses used to evaluate the study findings. What other statistical analyses, if any, should be performed?

5. Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

6. Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological / ecotoxicological risk characterization of perchlorate exposures? If so, indicate the extent to which it can be used for characterizing adverse effects.

7. Do the finding provide information relevant to the evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?

**Attachment 2**

**General Questions for Reviewing the Topic Areas**

***Note to Reviewers:*** *These questions are being provided as general considerations for reviewing how EPA interpreted and analyzed data from the various perchlorate studies. You do not need to answer every question below when answering charge questions B.2, C.2, D.2, and E.2; rather use your professional judgment to address the questions that you see being most relevant.*

1. Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

2. Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

   - Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

   - Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

   - Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

   - Are the assumptions and uncertainties clearly and adequately expressed?

**Attachment 3**

**General Considerations for Evaluating the Proposed PBPK Models Listed in Table 2**

***Note to Reviewers:*** *These questions are being provided as general considerations for reviewing the PBPK models contained in the consultative letters, and other documents published since 1999 that require peer review. You do not need to answer every question below when reviewing these models and associated materials and responding to charge question E.1; rather use your professional judgment to address those that are most appropriate to the model or consultative letter in question.*

1. ***Structure.*** Disposition is defined as absorption, distribution, metabolism and elimination (ADME).

   Does the proposed model structure contain the necessary anatomical compartments and physiological processes to accurately describe perchlorate disposition? Or iodide disposition?

   Uptake into the thyroid is described by an active (Michaelis-Menten) process and a permeability area for first-order movement of the anions between the subcompartments. Please comment on the advantages and limitations of this approach. Does it capture all the relevant behavior for the competitive inhibition of iodide uptake by perchlorate and distribution in the thyroid?

   Comment on the approach for describing perchlorate's plasma protein binding and dissociation.

2. ***Parameterization.*** Consider whether the experimental data or literature, fitting routines, and scaling assumptions were appropriate and adequate to support the values for the various species-specific and chemical-specific parameters used in each model structure. To describe perchlorate disposition? For iodide disposition? Are the parameters derived by fitting to available data reasonable and reliable?

   Comment on the "upregulation" adjustment of the Vmaxc_T*p* to represent upregulation of the NIS with increasing dose of perchlorate.

   Comment on the approach to growth of maternal and fetal parameters.

3. ***Validation.*** The models were validated to varying degrees with available data that were not used to estimate the parameters. Has sufficient validation of the structures been achieved?

4. ***Application.*** The models are being used to develop human equivalent exposures (HEE) for different dose metrics for dose-response modeling in Chapter 7.

   Comment on the utility of the proposed PBPK structures in the parallelogram approach.

   Comment on the advantages, limitations, and reliability of these models to describe an HEE for different dose metrics and the correlation between the two:

   Area under the curve of perchlorate in the blood (AUCB)
   Iodide uptake inhibition

5. ***Variability and Uncertainty.*** Comment on the variability in underlying data and resultant model structures. What are the uncertainties inherent in using these models for the applications to derive human equivalent exposures for interspecies extrapolation based on the different dose metrics? Are the uncertainties associated with the PBPK modeling similar to, or reduced, in relation to default approaches?

# William Adams

**Topic Area D:** **Ecological Risk Assessment and Evidence for Indirect Exposure**
**Designated reviewers:** **William Adams, Teresa Fan**
**Discussion leader:** **William Adams**

**Questions:**

**D.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.**

**Attachment 1**
**Questions & Answers** (for D.1)

1. Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

   *Condike Report: This letter report is informational only, is an in-progress report and was not intended as a definitive report. There is insufficient information on study design, methods or results to evaluate the quality of the data. The letter report does provide some interesting data on fish, plant, and sediment pore water concentrations for comparison with other field results.*

   *EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Tasting With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas*

   *The objectives of these two studies were stated and are clear. The strengths of the two experimental protocols are their simplicity and clarity. On the other hand, the protocol limitations include the fact that, with the exception of the fathead minnow early life stage study, the protocols followed were those designed for effluent toxicity tests and not protocols designed for product testing. The differences are fairly minor and deal primarily with the degree of documentation required, preparation of test substance, and reporting requirements. There are updated standard protocols available for fathead minnow early life stage studies and amphipod chronic studies that could have been cited. The reports appear to be draft reports as they were not signed and some of the appendices were missing.*

   *Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*
   *The purpose of this study was to determine the potential for perchlorate to accumulate in various environmental compartments and media at sites where perchlorate was known to be released. The study was intended to be a screening level study and not an in-depth assessment at any one site. The study design was adequate although it would have been helpful to have a more complete data set and consistency in the number of samples collected at each site. This would have required more intensive sampling, but would have improved the data set.* The primary limitations to the data set are: (1) lack of co-located

water-sediment-tissue samples and soil-tissue samples, (2) small sample sizes and lack of specific types of samples at each site and (3) insufficient sensitivity for perchlorate in tissue analyses (i.e., MDL = 400 ppb). These limitations limit the ability to correlate tissue levels to soil/sediment of water and a limit the ability to calculate defensible accumulation factors.

2. Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices or specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

*Condike Report: no comments - see response to number 1.*

*EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Testing With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas*

*The studies did not appear to be performed according to GLP practices, but rather following approaches developed for effluent testing and more in the spirit of GLP than actual compliance with GLP. QA audits and reviews were not documented in the report. Raw data and all data pertaining to statistical analyses were not included, i.e., the statistics for the IC25 calculation was included for the algal test and none of the statistics were included for the fathead minnow and amphipod studies. One could not determine if there were changes to the protocol or deviations from the protocol. The reports did not contain a discussion of water quality measurements and compliance with protocol requirements. No water quality parameters (pH, hardness, etc) were presented for the algal test. For the fathead minnow and amphipod test there was a summary table for water quality measurements. The pH variations in both studies were greater than one would expect, but not enough to compromise the study.*

*Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*

*The study was not performed to GLP standards, however, there appeared to be sufficient QC built into the analytical program to assure the analytical results.*

3. Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe an effect?

*Condike Report: no comments - see response to number 1.*

*EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Testing With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas.*

*Exposure levels for all EA studies were reported as nominal test concentration ($ClO_4$ mg/L) and were apparently not measured. There did not appear to be analytical confirmation of the stock solutions and stock preparation sheets were not included in the report. These are rather serious deficiencies. Standard endpoints and measurements were used and reported as well as a standard number of test vessel replicates and test organisms.*

*Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*

*The study was not set up with sufficient spatial and temporal replication to allow for an in-depth assessment of relationships between water and aquatic tissue levels or between soil and terrestrial organism tissue levels. Hence the study provides qualitative assessment of potential for perchlorate to be taken up by aquatic and terrestrial organisms.*

4. Please comment on the strengths and limitations of the statistical analyses used to evaluate the study findings. What other statistical analyses, if any, should be performed?

*Condike Report: no comments - see response to number 1.*

*EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Testing With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas.*

*The statistical analyses performed for the algal test appear acceptable, but as a general comment on the other studies, no data were provided to evaluate the statistical approach, the protocol was simply cited.*

*Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*

*The statistical analyses that were performed were primarily graphical representations of tissue, soil, sediment and water concentrations at the six sites of interest. Correlations/regressions were not performed nor were the data used to derive bioaccumlation/bioconcentration factors. The analyses performed were acceptable.*

5. Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

*Condike Report: no comments - see response to number 1.*

*EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Testing With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas*

*The results presented are consistent with the data obtained during the study. However, the data are summarized and presented as mean response values for growth, survival, etc. and data for the individual replicates were not provided. Hence, it was not possible to check the accuracy of the statistical output.*

*Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*

*The conclusions drawn from this report were appropriate for a screening level report. Sufficient samples were collected and analyzed to demonstrate that there is a general relationship between water concentrations and tissue levels and between soil and vegetation levels.*

6. Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological / ecotoxicological risk characterization of perchlorate exposures? If so, indicate the extent to which it can be used for characterizing adverse effects.

*Condike Report: no comments - see response to number 1.*

*EA Engineering 1999: Results of Algal Toxicity Testing with Sodium Perchlorate and EA Engineering 2000: Results of Chronic Toxicity Testing With Sodium Perchlorate Using Hyalella azteca And Pimephales promelas.*

*Overall, these reports met minimum requirements for whole effluent tests (WET), but would not meet minimum requirements for product registration tests under FIFRA, TSCA, or OECD guidelines. The overall quality of these reports is probably adequate for a screening level hazard assessment and due to the paucity of data on perchlorate, provide a contribution to the science. The studies are not of sufficient quality as presented in the reports to be published. However, there may be additional data in the raw data files that would supplement what is in the reports which could be used to improve their quality.*

*Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation*

*Overall, the study provides useful data for demonstrating that perchlorate can be taken up and stored in biological tissues in both aquatic and terrestrial organisms. It provides preliminary data which suggests that plant species accumulate greater amounts of perchlorate than other organisms. The study has several limitations which prevent the data from being used in a more definitive manner, for example, insufficient number of samples of each tissue and sample type at each site, lack of co-location of water/sediment/soil samplers with the tissue samples that were collected and lack of data on seasonal variability.*

**D.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.**

**Attachment 2**
**General Questions for Reviewing the Topic Areas**

1. Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both non-cancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

   *I am not aware of any other data. I searched some frequently used data bases and did not find any data of use for the ecological risk assessment.*

2. Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

   *The key aspects of the protocols and conduct of the studies were not discussed in much detail in the Toxicological Review and Risk Characterization Document. Additionally, for most of the studies the protocols were not available for review. The reference protocols were primarily those that would be selected for effluent testing except for the 28-day fathead minnow and Hyalella studies. The results of the studies were discussed although limitations to the studies were not pointed out (i.e., adherence to protocols, deviations in test requirements such as dissolved oxygen, pH, etc.). The lack of Agency discussion is primarily due to the fact that the studies were set up as screening level studies.*

3. Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

   *The risk assessment points out that most, if not all, of the ecological data has been collected as screening level data. There are some significant limitations to the ectoxicological data collected to date. First, it was not collected as part of an integrated well controlled risk assessment program, i.e., there was no overall Quality Assurance Protection Plan (QAPP). Second, most of the toxicity studies were performed using nominal test concentrations. While perchlorate is quite stable in water, confirmation of at least the stock solutions would provide assurance that the test solutions were appropriately*

*prepared. Third, the data base is very limited and not necessarily focused on key indicator species.*

4. Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

   *Regarding the question about statistical analyses performed beyond those in the original study, this does not appear to apply to the ecological assessment. Regarding endpoints, receptors/indicators or time points used, there was some inconsistency in the assessment program. For example, the use of 10-day fathead minnow study to estimate chronic toxicity (note a 28-day early life stage study was also performed). The algal test was a 96 hour test which provided a NOEC and LOEC, but did not provide a 96-hour LC 50 value. The Ceriodaphnia dubia acute toxicity value appears to be collected from the same study as the 7-day chronic value. Chronic tests are performed in the presence of food and acute tests are not, hence a small discrepancy. The primary limitation in this ecological assessment is the lack of toxicity data for key receptor groups. This is discussed more under D.3 below.*

5. Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

   *Clarity of the text is fine. Additional thoughts relative to improving the ecotoxicological effects is provided below.*

6. Are the assumptions and uncertainties clearly and adequately expressed?

   *The assumptions used in the risk assessment were clearly laid out. However, due to the lack of data numerous assumptions were used to derive conservative estimates of effects thresholds for aquatic and terrestrial organisms. Each of the assumptions were identified, supported and referenced. However, the use of extremely small data sets in conjunction with assessment/safety factors typically results in overly conservative threshold estimates. For example a single earthworm study was used to derive a threshold for terrestrial invertebrates. The 4450 mg/Kg value was divided by 242 to account for interspecies variability and 18 to account for the acute to chronic ratio resulting a chronic threshold value of 1.0 mg/Kg (a factor of 4450 below the only datum available). This value has a very low degree of accuracy. However, one might defend it by claiming it is very conservative. Likewise, for the aquatic data set the use of the Tier II Great Lakes Water Quality Initiative approach to setting water quality criteria (WQC) uses several assessment factors and is quite conservative. To that end an alternative approach is presented below for the acute and chronic aquatic data sets.*

**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.**

*The available acute and chronic aquatic toxicity data sets are listed below (Tables 1 and 2). For purposes of setting WQC, EPA recommends that 8 acute toxicity values be utilized representing different families. Additionally, a minimum of three chronic test results are recommended. Derivation of a WQC provides a reasonable aquatic toxicity threshold. Only three acute values were available and a fourth EC50 value for algae was estimated from the dose-response data for the one study that was performed. Acute data are relatively inexpensive and provide a reasonable way to evaluate the sensitivity of a variety of organisms. Typically, acute data are used to indicate which species should be tested on a chronic basis. This was not the case in this screening level study. Several key groups of organisms are missing from the acute and chronic data set, in particular, macrophytes, salmonids, aquatic insects, and bivalves. Additionally, select groups like the algae and macrophyte groups should be investigated in greater depth due to their ability to accumulate perchlorate. The lack of careful progression from acute to chronic testing for select sensitive trophic groups limits this assessment. The ability of vegetation to accumulate perchlorate to a greater extent than other species together with the fact that perchlorate appears to be released into small ponds or areas with riparian zones suggests that organisms which predominate in these areas should receive greater focus in the testing program. For example, greater emphasis might be placed on amphibians and herbivorous insects and fishes. See question D.5 for additional comments.*

Table 1. Perchlorate Acute Toxicity (mg/L)

| Species | 96 hr LC 50 |
|---|---|
| Ceriodaphnia *dubia* | 66 |
| *Daphnia magna* | 490 |
| Fathead minnow | 1655 |
| Selenastrum *cap.* | 1800* |

* value estimated from dose-response data.

Table 2. Perchlorate Chronic Toxicity (mg/L)

| Species | Study | NOEC | LOEC | Chronic Value |
|---|---|---|---|---|
| *Hyalella azteca* | 28-day chronic | 1000 | >1000 | >1000 |
| Selenastrum *cap.* | 96 hr | 500 | 1200 | 775 |
| Fathead minnow | 28-day ELS | 490 | >490 | >490 |
| Fathead minnow | 7-day-ELS | 155 | 280 | 208 |
| Ceriodaphnia *dubia* | 7-day chronic | 10 | 33 | 18.2 |

**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 goals and objectives of the ecological screening analysis were adequately described and are restated and discussed:

1. Are ecological risk best characterized as *de minimis*, *de manifestis* or somewhere in between?

*The analysis performed by EPA and the additional analysis performed for this review both support the belief that risk may be classified as de minimis. This is based on a comparison of chronic thresholds with environmental exposure levels reported in the environment. With the exception water concentrations measured at Mead, LHAAP - INF pond and perhaps LHAAP Site 2, water concentrations are well below predicted chronic thresholds for effects.*

2. Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is it likely that adverse effects occur at levels below current detection limits?

*Analytical detection methods, with the exception of water, have fairly high limits of detection. The detection limit for sediments is adequate, but the detection limit for fish and aquatic vegetation (400 ug/Kg) do not allow for the ability to demonstrate the relationship between water and tissue residues until residues exceed approximately 450 ug/Kg. At present, this does not appear to be a major concern. However, this does limit the ability to evaluate potential foodwebs in any depth.*

3. Is the available ecotoxicological information on perchlorate sufficient or are additional studies needed?

*The aquatic data set is quite limited (four acute and five chronic tests). The fathead minnow study may need to be repeated to evaluate the potential effects at concentrations below 28 mg/L where swelling and redness (presumably hemorrhaging) was observed in the fish. It is recommended that a broader array tests including algal species, aquatic macrophytes and amphibians should be tested. In general though, it would appear that based on environmental water concentrations of perchlorate that are typically less than 100 ug/L and the available toxicity data, the need for additional aquatic toxicity tests at present is limited.*

*Regarding the need for additional testing to assess perchlorate in terrestrial systems, there are almost no available data other than an earthworm study. It is recommended that a more extensive data set be constructed which would include herbivorous mammals, herbivorous insects and a variety of plant species including agricultural crops that are likely to be exposed to perchlorate.*

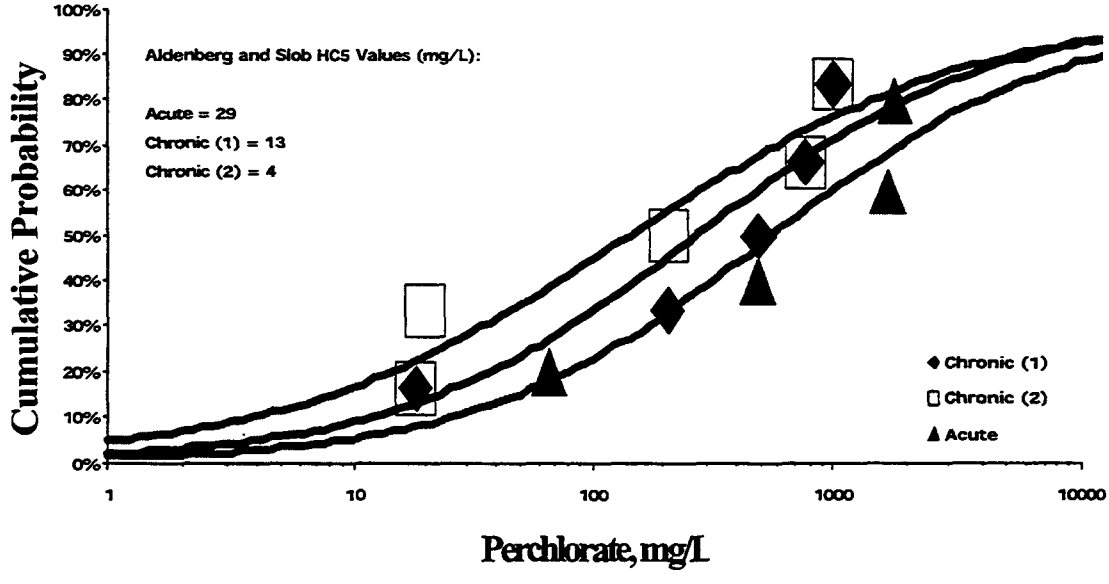
**D.5 Do the analyses support the summary and conclusions presented? Are relevant and important aspects of uncertainty addressed sufficiently?**

The analyses appear to support the conclusions drawn, however, the data available to assess potential effects in terrestrial systems is very limited and insufficient even for a screening level assessment as discussed above. A few studies with a focus on herbivores and plant species would dramatically improve the assessment. Soil concentrations at most sites do appear to be below the 1 mg/Kg threshold derived for assessment. In light of the 1.0 mg/Kg terrestrial effects threshold there appears to be only a few sites where high levels of soil-perchlorate contamination exists that would exceed this threshold.

Regarding conclusions drawn relative to aquatic ecosystems, a reassessment of the data was undertaken using species sensitivity distribution (SSD) techniques as opposed to the Tier II Water Quality Criteria approach develop by EPA for the Great Lakes Water Quality Initiative. The latter approach is one that is based upon empirical observations of interspecies toxicity and acute to chronic relationship in large data sets. The SSD approach is one developed by Aldenberg and Slob (1993) and is a statistical approach that is used to make predictions of species sensitivity distributions based on available data, the variance of the data and the shape of the distribution curve. The method incorporates an increase in the size of the extrapolation factors to account for small sample sizes. The approach of Aldenberg and Slob (1993) using a logistic approach to model the data was used to predict an acute and chronic threshold for aquatic species defined as the lower 95$^{th}$ percentile of the distribution of toxicity values (Figure 1). The 95$^{th}$ percentile for acute toxicity was determined to be 9 mg/L ( read directly from the Figure 1). This compares with a value of 5 mg/L calculated with the Tier II WQC approach.

The 95$^{th}$ percentile threshold developed for the chronic data set using the SSD approach lies between 1.1-4.1 mg/L depending on which data set is used (Figure 1). The chronic data were analyzed two ways: (1) using the chronic data provided in the EPA risk assessment report and including the algal datum in the SSD. And (2) additionally, the fathead minnow chronic study was included in the data set using a value of 14 mg/L as the chronic NOEC value. This value was obtained by dividing the lowest value reported where redness and edema occurred (28 mg/L) by 2 to obtain an estimated NOEC (14 mg/L). The SSD values of 1.1-4.1 compares with a Tier II WQC value of 0.6 mg/L. Considering that these alternative acute and chronic SSD values are higher than those calculated by the Tier II WQC methodology, no changes in the risk assessment is warranted. These additional analyses provide supportive information to that included in the EPA risk assessment report.

**Figure 1. Perchlorate toxicity to freshwater aquatic organisms. Curves were fit to the raw data using logistic regression.**



**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.**

There was very little data provided which would allow for an assessment of transport and transformation. The physico-chemical properties indicate that perchlorate is very stable, very soluble, has a low affinity for solids and unlikely to undergo degradation except in reducing conditions or in the presence of reductase enzymes that appear to exist in plants as well as bacteria. One can infer from available physico-chemical data and environmental monitoring data that perchlorate is extensively transported in groundwater and surface water and is attenuated primarily through dilution. Soil leachate studies were not provided, but perchlorate would be expected to have a low affinity for soils or sediments.

The available data suggests that primary route of exposure/pathway for exposure to humans and terrestrial mammals may be through terrestrial vegetation. This would only appear to be a concern where soil levels or water levels (used for irrigation are very high, i.e., ppm levels).

**Vegetation**

Some additional analyses in the available data were performed for the purpose of this review (Figure 2, Table 3). The data in Figure 2 (data from Parsons EA report) provide an indication that the relationship between soil and vegetative level can be fairly significant ($R^2$ = 0.79). The highest levels of accumulation by any group of organisms evaluated to date appears to be in the terrestrial plant group. The exposures were also fairly large at some sites. A calculation of soil to plant ratios for the data in Figure 2 reveals that the accumulation ratios range from about 7 at the lower end of the exposure concentrations to slightly less than 1.0 at the high end of the exposures. This inverse relationship between exposure and tissue concentrations is not unexpected and was recently reported by Efroymson et al. (2001) for other inorganic substances for terrestrial plants. It is also common in aquatic organisms (Brix and Deforest, 2000). The data contained in Figure 2 is primarily native (rooted) vegetation and the principal route of exposure would expected to be via root uptake and translocation to various portions of the plant. This would be highly influenced by both perchlorate concentrations on the soil and soil moisture/rainfall. By comparison, BCF values for terrestrial vegetation (water to plant tissue ratio) were calculated form the data of Susarla et al. (2000 and 2001?) provided with this review and from Hutchinson et al. (2000). In these studies the exposure pathway was assumed to be primarily from water to plant roots or water to soil to plant roots (Table 3).

Table 3. Water to terrestrial plant perchlorate concentration ratios with rooted plants

| Plant Species | Water Concentration (mg/L) | Plant Concentration (mg/Kg) | BCF |
|---|---|---|---|
| Lettuce-rooted | 10 | 300 | 10 |
| Parrot-Feather-rooted | 0.2 | 3.7 | 19 |
| Parrot-Feather-rooted | 2.0 | 46.5 | 23 |
| Parrot-Feather-rooted | 20 | 392 | 20 |
| Parrot-Feather-not rooted | 0.2 | 11.8 | 59 |
| Parrot-Feather-not rooted | 2.0 | 117 | 59 |
| Parrot-Feather not rooted | 20 | 1200 | 60 |
| Sweet gum-rooted | 0.2 | 2.5 | 12 |
| Sweet gum-rooted | 2.0 | 42.5 | 22 |
| Sweet gum-rooted | 20 | 145 | 7.5 |
| Black willow-rooted | 0.2 | 1.0 | 5.0 |
| Black willow-rooted | 2.0 | 4.6 | 2.3 |
| Black willow-rooted | 20 | 5.8 | 0.29 |
| Black willow-not rooted | 0.2 | 2.5 | 12.5 |
| Black willow-not rooted | 2.0 | 2.7 | 1.4 |
| Black willow-not rooted | 20 | 3.0 | 0.15 |
| Smartweed-rooted | 0.2 | 12.5 | 60 |
| Smartweed-rooted | 2.0 | 150 | 75 |
| Smartweed-rooted | 20 | 564 | 23 |
| Pickleweed-rooted | 20 | 305 | 15 |

The results of water to vegetative comparisons for terrestrial plants (Table 3) indicates that the tissue residues and BCFs were generally higher when the plants were cultured in water only solutions with no soil/sand. BCFs (tissue to water ratios) were similar to or slightly higher than observed under natural conditions at study sites where the soil was contaminated with perchlorate. The BCFs generally ranged from 2-20 (but reach 60) as compared with 1-7 at contaminated sites. There were differences between species in terms of total accumulation, distribution, and rate of degradation within the plant. As a general conclusion, the studies support the view that plants appear to be able to accumulate higher levels of perchlorate than other aquatic and terrestrial receptors. Under conditions of relatively high exposure, plants could provide a pathway of importance for terrestrial mammals including humans.

In comparison with terrestrial plants, the data for aquatic plants indicate that they do not accumulate perchlorate to the same extent as terrestrial plants. BCF values range between 1-2 as depicted in Figure 3 (data from Parsons EA report). It should be noted that most of the aquatic plant data is for algae and not rooted macrophytes. However, the lower accumulation by algae is noteworthy in that algal species typically have large BCF values for many inorganic substances due to their high surface area to volume. The hockey stick regressions performed with data from the Parsons EA report suggest that algal concentrations of perchlorate begin to increase when water concentrations exceed 458 ug/L. The inflection point (tau) is constrained by the analytical limit of detection of 400 ug/Kg.

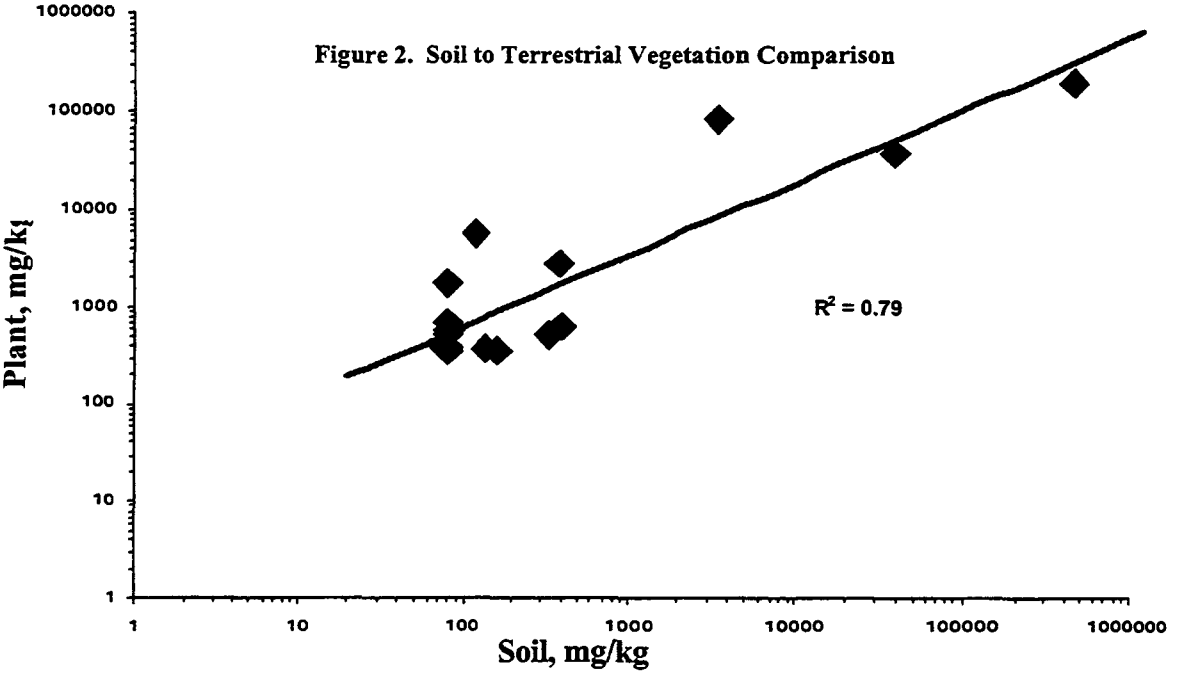
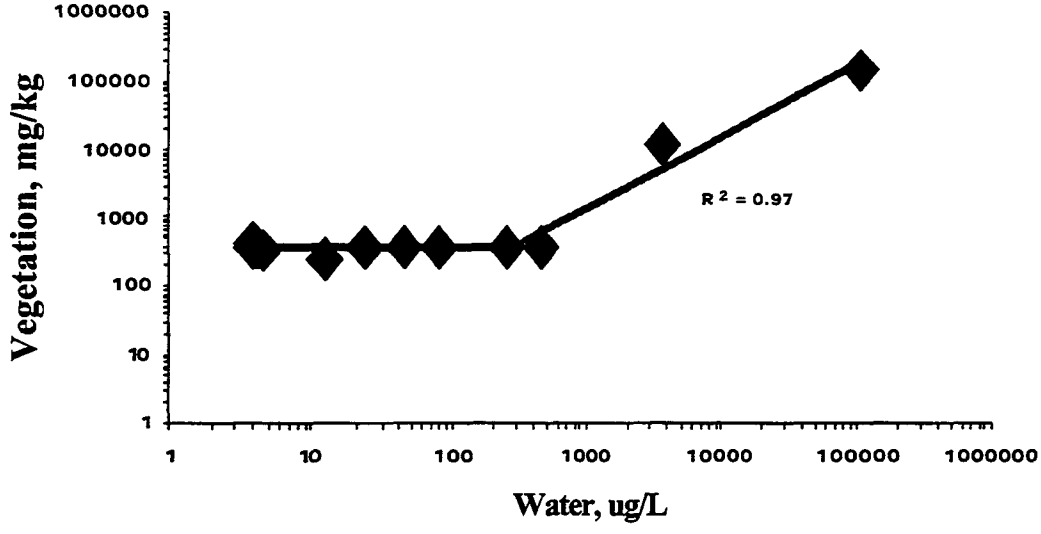



**Figure 2. Soil to Terrestrial Vegetation Comparison**

**Fish**

Accumulation of perchlorate by fish does not appear to be a critical issue in the risk assessment. Data analyzed from the Parsons EA report (Figure 4) indicates that fish tissues do not increase significantly until water concentrations exceed 646 ug/L. Once again the inflection point (tau) is constrained by the analytical limit of detection of 400 ug/Kg. Two additional data points from Smith et al. (2000) and from Condike (2001) were added to Figure 4 for comparison with the Parsons EA data. These data fit the general pattern of bioaccumulation observed by in the Parsons EA report. The Smith et al (2200) report had a perchlorate detection limit in fish of approximately 80 ug/Kg, hence this data supports an inflection point for accumulation( tau) at 80-100 ug/L. The tissue residue data in Figure 4 at tau or higher provides a

**Figure 3. Water to Aquatic Vegetation**




calculated BAF (bioaccumulation factor) of 0.23-0.61. These values are much smaller than calculated for plants. Once again, the highest fish BAF occurred a the lowest exposure concentration as would be expected based on kinetic models for uptake. In comparison, catfish bioconcentration data (laboratory 5-day exposure to 100 ppm) reported by Condike (2001) provides an average fish fillet BCF of 0.07 and a fish head BCF of 0.25. The lower values in the laboratory as compared with the field may reflect the short exposure duration, or more likely, a lack of dietary exposure as would have occurred in the field.

Figure 4. Water to Fish

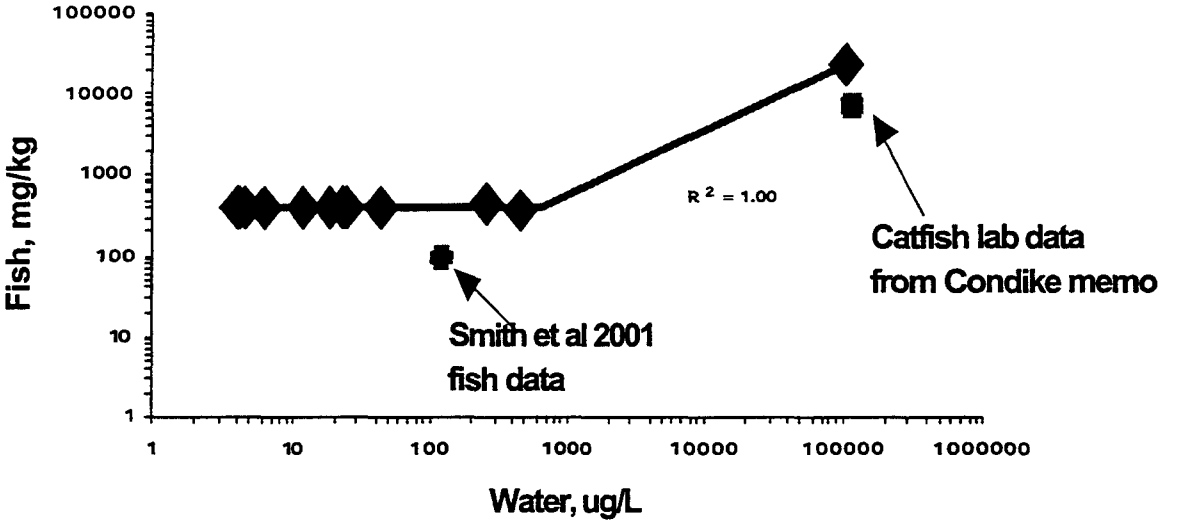
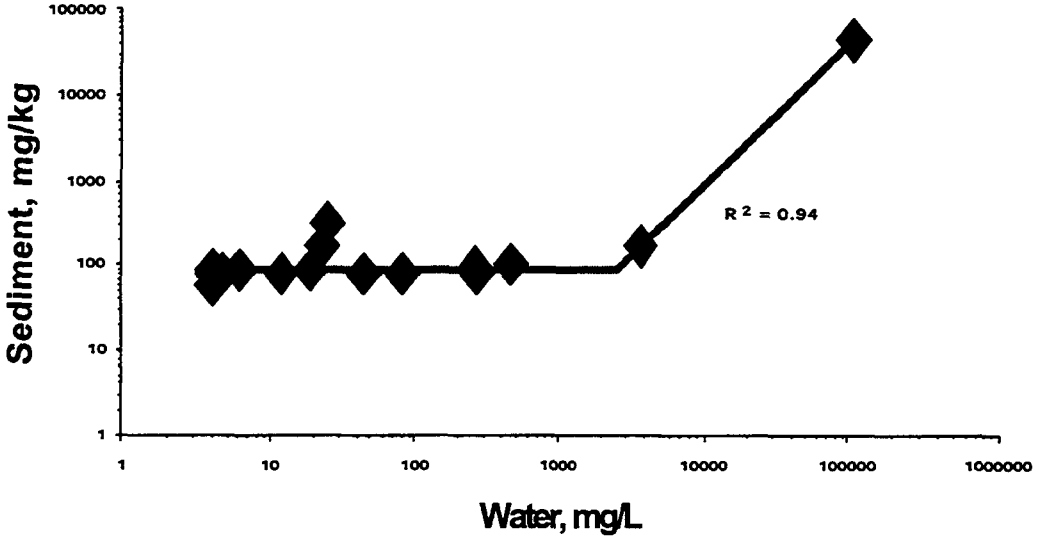



Figure 5. Water to Sediment



**Sediment**
Data from the Parsons EA report for sediment are interesting in that they show a general pattern of low sediment concentrations until water concentrations reach 2511 ug/L (Figure 5). This relationship is constrained somewhat by the detection limit in sediments (80 ug/Kg), but not as much as the plant and fish data. The data support a general conclusion that perchlorate has a low binding affinity for sediments. This conclusion is further supported by the pore water concentrations measured fo several sediments which were also generally quite low.

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

The most likely additional source of exposure other than drinking water would be from agricultural crops irrigated with perchlorate contaminated water. While the potential for perchlorate to enter the food chain either from fertilizers, rocket propellants, or unknown sources appears low do a limited use pattern of perchlorate, nevertheless the data available support the concept that perchlorate is taken up and stored/metabolized by plants. The extent to which this pathway is significant is unknown at present, but is suspected to be minimal. However, insufficient data are available to rule this pathway out. The known higher accumulation of perchlorate by plants rather than other organisms warrants some additional investigation.

**References Cited**

Aldenberg, T and W Slob. 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicol Environ Saf* 25:48-63.

Brix, K. V. and DeForest D. K. 2000. Critical Review of the use of bioconcentration factors for hazard classification of metals and metal compounds. OECD (Organization for Economic Cooperation and Development) Aquatic Hazards Extended Workgroup Meeting, May 15, 2000, Paris, France.

Efroymson, R. A, B. E.Sample, and G. W. Suter. 2001. Uptake of inorganic chemicals from soil by plant leaves: regressions of field data. Environ. Tox. And Chem., 20 (11): 2561-2571.

## Michael Aschner

# PERCHLORATE ENVIRONMENTAL CONTAMINATION: TOXICOLOGICAL REVIEW AND RISK CHARACTERIZATION

## Hazard Characterization and Mode of Action

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

There are specific issues that relate to the reliability of the data generated by the ARGUS 1416-003 Protocol (2001). These will be discussed below.

Specific comments for the Executive Summary and Chapter 1 (Introduction) are detailed below.

Executive Summary

A well-written and concise summary of the problem and the results.

E-9-10

"An administered dose of 0.01 mg/kg/day was supported as a lowest-observed-adverse-effect level (LOAEL) based on the effects on brain morphometry in pups from a PND21 sacrifice in a neurodevelopmental study that repeated similar observations made in a similar 1998 study..." I would have to disagree with this conclusion based on the problems that are inherent to both Argus studies. The studies are deemed inconclusive (see below).

Specific comments for the Introduction (Chapter 1)

The chapter provides background information and historical perspective on the evolution of perchlorate contamination, analytical detection methods, health effects and toxicity, and risk assessment, as well as an

exhaustive overview of ecotoxicology screening levels assessment. The layout is logical, and the chapter is easy to follow. It establishes the chronology of events, and provides essential background information to the reader. Overall, this is deemed an excellent introduction to the topic, and there are only a few minor comments or suggestions.

Given that recent publications have reported detection of perchlorate in tap water at levels as low as 0.1 ppb (Handy et al., 2000; Koester et al., 2000), it would be useful to provide additional data (and they likely exist) on the national occurrence of perchlorate in the drinking water. While Table 1-1 (Mayer, 2001) is helpful, the data seem restricted to the occurrence of perchlorate in suspected contaminated water sites. It should also be indicated what the parenthesis symbolize (presumably, lower detection levels). Information on the level of perchlorate in the general drinking water supply should be conducted and independently confirmed. If this has not yet been considered, an explanation should be provided.

Page 1-14-7 – The phrase "(3.7 million ppb)" should be corrected.

Page 1-15-29 – Insert "g" after "4.0 μ".

*A.2 The EPA has framed the conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium ($Na^+$)-iodide ($I^-$) symporter (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?*

The conceptual basis for perchlorate's effect on the NIS is well articulated. There are, nevertheless, deficiencies in the discussion on neurobehavioral and neurodevelopmental sequelae to hypothyroidism. While the general outcome of hypothyroidism is detailed, it is difficult to gauge similarities between the action of perchlorate and other anti-thyroid chemicals. The magnitude (on a quantitative basis) of hypothyroidism in other conditions (directly- and indirectly-induced thyroid dysfunction) is not well

described, making it difficult to compare with the effect of perchlorate. Some of the effects of hypothyroidism are embedded in various chapters, but nowhere is there a concise description in which hypothyroidism (either direct or indirect) is correlated with developmental CNS effects. A section describing these effects would be helpful, especially if it could be documented at the level of hypothyroidism produced by perchlorate.

Comments for Toxicokinetics/toxicodynamics and mode-of-action testing strategy (Chapter 3)
This chapter establishes the rationale serving as the basis of the testing strategy that was designed to evaluate the potential critical targets for perchlorate. The chapter is well written, and easy to follow. It is comprehensive in nature, and the initial part of the chapter lays out the knowledge basis that existed prior to the testing strategy that is discussed towards the end (Section 3.5). The discussions about ADME (both in humans and animals, Sections 3.1.1. and 3.1.2, respectively), iodide metabolism and physiology (3.2), toxicokinetics of perchlorate (3.3), and toxicodynamics of thyroid hormone perturbations upon perchlorate treatment (3.4) provide an excellent reflection on existing information prior to the testing strategy that is discussed, and there do not appear to be omissions of other relevant reports or published manuscripts. Figure 3-1 and 3-2, which depict thyroid hormone synthesis and hypothalamic-pituitary-thyroid axis, respectively, are deemed very helpful. The other Figures and Tables (Figures 3-4 through 2-12; Tables 3-3 through 3-8) are also extremely useful.

The relevance of the studies in which NIS is inhibited in the "cold" should be better explained. These studies are metabolic studies, which are conducted in vitro at 4°C to inhibit transport processes.

Table 3-1 on page 3-8 shows the percent inhibition of iodide uptake in the thyroid gland of adult rats dosed with perchlorate (Meyer, 1998). The concentrations overlap with those in the neurodevelopmental studies ranging from 0 – 3 mg/kg. They show that the percentage of $^{125}I$ uptake is affected dose-dependently. The time-course is different from the Argus 2001 study; nevertheless, the studies suggest a dose and time-dependent effect by perchlorate, making it difficult to correlate with the morphometric data, which do not show consistent effects across time.

Table 3-3 lists mechanisms of antithyroid-mediated neoplasia in rodents. An indirect mechanism that is invoked for neoplasia is chemicals inhibiting iodide uptake. It would be useful to explain what chemicals are included in this group, and detail the commonalities and differences in their effects vs. perchlorate.

Page 3-9-15 – Given the potential of perchlorate to affect CNS development and function, it would be useful to expand on the ability of the choroid plexus to concentrate iodide, and to consider the possibility that perchlorate can lead to a defect in the transport of iodide out of the CSF across the choroid plexus.

A goal of the discussion on Pages 3-21 to 3-24 is to establish a causal effect between chemical-induced alterations in thyroid hormone homeostasis and aberrant CNS development and function. Indeed, it is well established that adequate functioning of both the maternal and fetal thyroid glands are important to ensure that the fetal intellectual development progresses normally. The authors describe a number of clinical disorders potentially leading to impaired brain development: defective glandular ontogenesis (leading to congenital hypothyroidism), maternal hypothyroidism (usually related to chronic autoimmune thyroiditis), and finally iodine deficiency (affecting both the maternal and fetal thyroid functions). They cite a number of human studies where hypothyroidism caused by iodide deficiencies or a congenital condition lead to abnormal development, including mental deficiencies and hearing, speech, and motor deficits (Porterfield, 1994; Sher et al., 1998). Additional epidemiological and clinical citations are provided, in which maternal thyroid deficiency (Haddow wt al., 1999), and autoimmune disorders (high thyroid peroxidase antibody titers; Smit et al., 2000; Pop et al., 1999) during pregnancy result in decreased IQ scores in the offspring. The discussion fails, however, to correlate how both the severity and temporal occurrence of maternal thyroid underfunction will impair fetal neuronal development, and it does not provide sufficient information to gauge what magnitude of changes in maternal hormonal levels are associated with aberrant neurodevelopmental outcome in the offspring. Thus, it is difficult to contrast with the perchlorate studies.

*A.3 The1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inference 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?*

This question is beyond my expertise.

*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 for both.*

The approach is well justified, however, as will be discussed below, the brain morphometric analysis is fraught with significant problems, and, therefore deemed inconclusive.

*C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies?*

Yes. Argus Research Laboratories, Inc. (2001), Hormone, Thyroid and Neurohistological Effects of Oral (drinking water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or Via Maternal Milk. Horsham, PA: Protocol no. ARGUS 1416-003.

I will specifically comment about this study and the study performed in 1998 by Argus, given that my charge is related to the neurotoxicity studies. The comparison between the two studies is important for it highlights both methodological concerns, as well as divergent results. Discussion of these issues is also necessary to layout the problems with the utility and weight that these studies carry in the EPA's analysis, interpretation, and risk assessment (see C.2, below).

SPECIFIC COMMENTS RE ARGUS STUDY 2001

The objectives are clearly identified, and there is sufficient information to assess the adequacy of the experimental design. There appear to be no limitations in performance, and all experimental procedures appear to have been conducted in accordance with Good Laboratory Practices (GLP). The studies include QA/QC assurances, and there appear to be no occurrences that necessitated a change from the original protocol. Dosing and exposure measures to perchlorate are appropriately formulated and controlled. Overall, the study is extremely useful in enhancing the toxicological risk characterization of ammonium perchlorate, and as designed, performed and reported, it is of sufficient quality for use in hazard identification (note exceptions below).

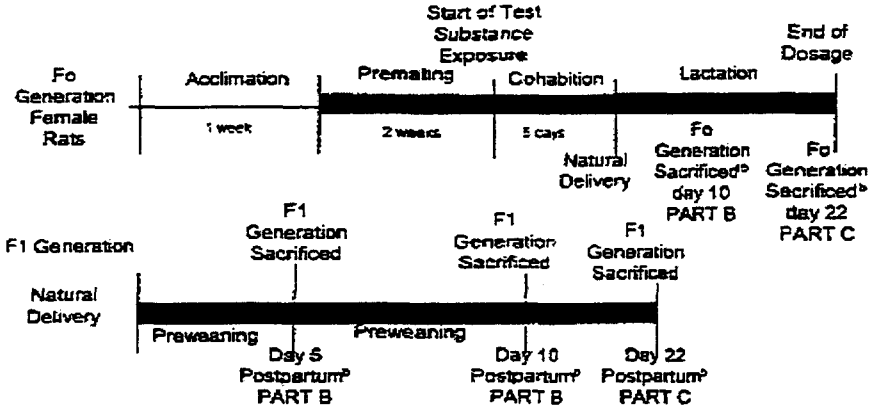
Notwithstanding the above strengths, there is major concern about the study design and methodology, including statistical analysis (multiple t-tests are flawed), raising questions about the validity of the conclusions and inferences, specifically those associated with the brain morphometry measurements. The evidence is suggestive of an association between exposures to ammonium perchlorate and changes in brain morphometry in the rat, but is limited because chance, bias, and confounding cannot be ruled out with confidence. The findings are not consistent in direction across dose and exposure time, they are inconsistent between studies performed by the same laboratory (Argus) and a consistent positive association cannot be ascertained. It cannot be concluded with certainty that the measured effects are within the range expected on the basis of sampling error and selection bias, and therefore, they are deemed inconclusive. The methodological issues that have led to this conclusion will be discussed below in greater detail.

Brief summary of the study

Fifteen or sixteen female rats were assigned to each of 5 exposure groups with ammonium perchlorate (AP) in the drinking water (0, 0.01, 0.1, 1, and 30 mg/kg body weight (bw)/day, I through V, respectively) beginning 14 days before cohabitation with males and continuing throughout pregnancy until postnatal day 22 (PND; PND 21 based on EPA's nomenclature).

The F1 generation was examined for brain morphometry on day of lactation 10 (DL 10; date of birth designated as DL 1, thus according to EPA's nomenclature it corresponds to PND 9) and DL 22 (EPA's PND 21), corresponding to study segments B and C, respectively (Argus, p.17; see Figure below). The neonates were not dosed with ammonium perchlorate, but were exposed to it via lactation.

**Study Segments B and C[a]**




Test Substance Exposure Period through Maternal Milk.
Test Substance Exposure Period.
a. For additional details see "Tests, Analyses and Measurements" section of the protocol.
b. Blood and tissue sample collection.

Methods for Brain Pathology Supplement (page 544)

No problems are identified for tissue collection and fixation [heads removed and placed in Bouin's fixative for >48 hours, rinsed x2 in 50% ethanol (EtOH) and placed in 10% neutral buffered formalin]. To date, brains have been analyzed for the pups sacrificed on LD10 and LD22. The original protocol called for initial studies to be conducted in the control and high dose groups (0 and 30 mg/kg ammonium perchlorate/day, groups I and V, respectively), with additional sectioning and analysis in the other groups should there be any significant changes in the high dose group vs. controls. The sections were cut at 60

micrometers (μm). In the LD10 pups, morphometric linear measurements were conducted bilaterally in 10 brain regions (excluding #9 and 10). In the LD22 pups, linear measurements were conducted bilaterally in 21 brain regions (2 gross and 19 microscopic, the latter representing 9 bilateral measurements plus one). The areas in which measurements were carried out are listed on page 901. All the measurements were carried out with a calibrated ocular micrometer. Full-face images of the coronal sections were also digitally captured and saved for future evaluation, should it be necessary.

Problems/clarifications

The protocol implies that sectioning of the brain blocks was not done at the same time, and it is unclear if the same individual carried out sectioning. It also appears that the DL10 and DL22 studies were performed at 2 different laboratories. Was this the original plan, and if so, why? It is not detailed. Although there is a detailed description of the "Histotechnology Procedures" (pages 818-820, and pages 901-903 for DL10 and DL22 studies, respectively), it is unclear what criteria were used to assure that the brain sections for each of the regions matched each other.

It is not sufficiently substantiated in the study (page 536) what the effects of hypothyroidism are vis-à-vis CNS development, as well as the rationale for the specific brain measurements that were carried out. For example, is there support in the literature for hypothyroidism at equivalent quantitative levels that is associated with hypermyelination (as measured here by increased thickness in the corpus callosum)? Intuitively, this seems to contradict an established literature that is consistent with reduced myelination in conditions of hypothyroidism.

Page 902 - There seem to be problems in positioning the metric ruler even in the sample provided on page 902, likely the best representation that there is (Figure 2, level of infundibulum; see below).

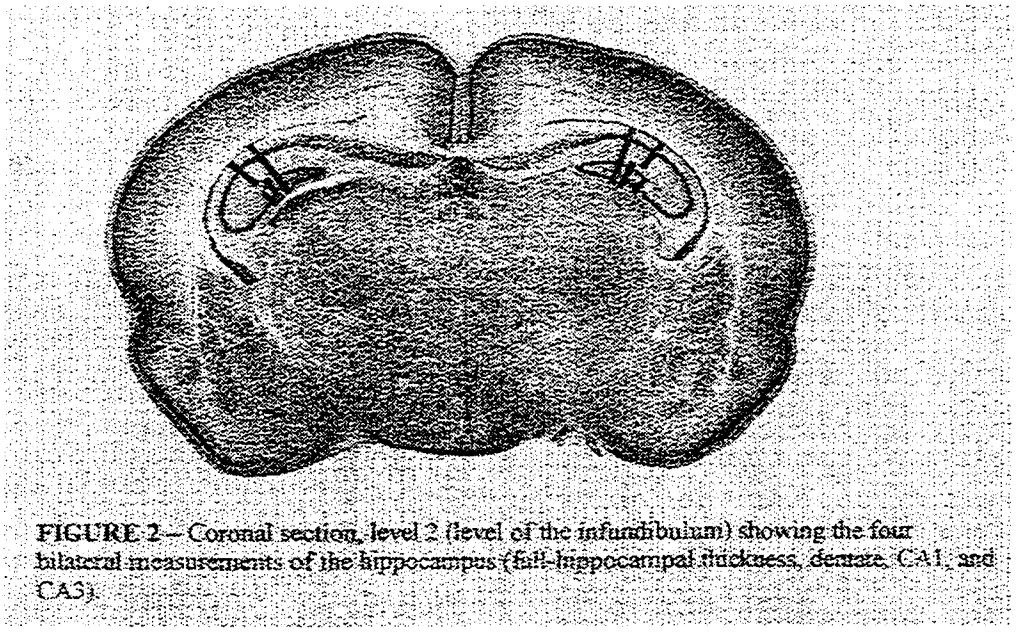


FIGURE 2 – Coronal section, level 2 (level of the infundibulum) showing the four bilateral measurements of the hippocampus (full-hippocampal thickness, dentate, CA1, and CA3).

The results for DL10 sacrifice suggest either significantly greater or lesser linear measurements in the thickness of various CNS areas in treated pups, with no apparent dose-response relationship. The authors suggest, "there was no evidence of any obvious treatment-related effects on male rat brain". Neither this reviewer, nor the EPA has accepted this conclusion. Significant differences were noted for some male structures in all treatment groups, excluding the lowest dose (0.01 mg/kg/day), with the most consistent change corresponding to increased thickness in the corpus callosum. The effect was bilateral and significant both in the 0.1 and 1.0 mg/kg/day group (Groups II and III, respectively), but not in pups in the 30-mg/kg/day group (Groups V). In female pups the trend was towards a decrease in linear dimensions on DL10. Statistically significant differences were noted only in the CA1 region of the hippocampus for female pups in the 0.1 and 1.0-mg/kg/day groups (II and III, respectively).

For the male pups sacrificed on DL 22, there was a trend (without statistical significance) for increased linear measurements in multiple brain regions, especially the corpus callosum (note difference from DL10). In the females, the pattern was reversed, with both increases and decreases in the linear measurements. For both the male and female pup groups, the cerebellar cortex was thicker compared with controls (in males 0.01, 0.1, and 1.0 mg/kg/day groups, II, III, and IV, respectively; in females 0.01, and 1.0 mg/kg/day groups, II, and IV, respectively).

Problems/clarifications

There is no support to substantiate that control morphometric measurements are comparable to other data sets in neonates of the same age (either males or females). Is it possible to compare the control values in this study with others to establish their validity with a high degree of confidence? There are major concerns with all the data sets regarding the tremendous variability inherent to many of the measurements. [Examples see Table below: Controls (Group I) male left corpus callosum 250 to 413 µm (mean ± SD 316 ± 48.6 µm; thicker than any on LD22 measurements at the same site). Group III (0.1mg/kg/day) male left corpus callosum 269 to 480 µm (mean ± SD 381 ± 79.2 µm). There are many more such examples]. There are inconsistencies in the data when compared to the Argus 1998 (see below) morphometric brain analyses. It is also unclear why the 2 studies were performed at different dosages as well as different sacrifice times.

**TABLE I**

**Brain Weights and Morphometry Data for F1 Generation Day 22 Postpartum Male Rats**

**Maternal Dosage Group I (0 mg/kg/day)**

| Rat Number | Right Striatum (µ) | Left Striatum (µ) | Rt. Corpus Callosum #1 (µ) | Lft. Corpus Callosum #1 (µ) | Right Hippocamp. (µ) | Left Hippocamp. (µ) | Right Dentate (µ) |
|---|---|---|---|---|---|---|---|
| 16731 | 3024 | 3024 | 317 | 346 | 1392 | 1368 | 792 |
| 16732 | 2688 | 2736 | 192 | 192 | 1512 | 1512 | 888 |
| 16734 | 2736 | 3072 | 240 | 269 | 1512 | 1488 | 864 |
| 16735 | 2688 | 2784 | 240 | 230 | 1392 | 1392 | 816 |
| 16736 | 3120 | 3072 | 269 | 259 | 1320 | 1344 | 744 |
| 16737 | 2784 | 2736 | 202 | 192 | 1368 | 1368 | 792 |
| 16738 | 2880 | 2976 | 182 | 202 | 1392 | 1416 | 768 |
| 16740 | 2832 | 2832 | 211 | 211 | 1296 | 1200 | 720 |
| 16741 | 2928 | 2976 | 307 | 365 | 1392 | 1344 | 720 |
| 16742 | 2592 | 2784 | 202 | 192 | 1512 | 1464 | 888 |
| 16745 | 2784 | 2880 | 211 | 211 | 1368 | 1200 | 792 |
| 16746 | 2736 | 2640 | 182 | 163 | 1272 | 1320 | 720 |
| 16747 | 2832 | 2736 | 221 | 288 | 1440 | 1320 | 816 |
| 16749 | 2688 | 2736 | 163 | 163 | 1440 | 1392 | 768 |
| 16750 | 2784 | 2784 | 202 | 192 | 1296 | 1392 | 744 |
| 16753 | 3024 | 3024 | 240 | 250 | 1440 | 1320 | 840 |
| MEAN | 2820 | 2862 | 224 | 233 | 1397 | 1365 | 792 |
| SD | 144.0 | 141.2 | 43.5 | 59.9 | 77.0 | 86.7 | 56.8 |

Neither the DL10 nor the DL22 studies substantiate cell death. Thus, the question is what mechanism might account for the increases in linear measurements of brain thickness. One potential mechanistic (physiologic) reason would be increased proliferation of astrocytes, commonly associated with brain injury, and referred to as gliosis. There are no measurements of gliosis (GFAP, vimentin etc.), hence it cannot be excluded nor verified. A second explanation would be reduced cell death, this would have to be ascertained with apoptotic markers, and to date it has not been done. A third potential mechanism is increased myelination (specifically vis-à-vis the increases in the thickness of the corpus callosum), either due to oligodendrocyte proliferation or hypertrophy. For the moment, evidence either in support or against this mechanism is also not available.

Lack of dose-response is problematic, as are the trends (sexual dimorphism). Most troubling is the lack of symmetry in the findings between left and right brain hemispheres (see Tables below). The most likely explanation is that these effects are attributable to artifacts associated with the cutting of the sections. Also troubling is the fact that there appear to be no consistencies between the DL10 and DL22 data. For example, the significant effects on the corpus callosum thickness in male pups treated with ammonium perchlorate (0.1 and 1.0 mg/kg/day; groups III and IV, respectively) disappear in the rostral measurements of corpus callosum in male DL22 pups, but persists in the infindibular area (becomes statistically significant also for 0.01 and 30 mg/kg/day groups, II and IV, respectively). It would be helpful to discuss the relationship between the regions vis-à-vis the measures that were conducted on DL10. Variations in measurements are troublesome, and go well beyond what one would consider "random variation". The only way to mitigate this problem is to compare the data to other studies or have the same lab perform additional studies to establish lack of intra-laboratory variability in the control values.

The sex-related differences in the thickness of corpus callosum are in disagreement with the findings from the developmental neurotoxicity study that was previously conducted by the same contract laboratory (1998). In the previously conducted study, thicker corpus callosum were noted for both sexes, albeit on a

different day (PD12) in the high dose group, which was 10 mg/kg/day (identical treatment paradigm), with more pronounced changes in the females.

Page 827 – "Detailed microscopic examinations of multiple coronal brain regions from postpartum day 10 rats in each of the treatment groups failed to indicate any evidence of treatment-related neuropathologic alterations or microscopic developmental anomalies". This statement is not supported by the data.

There are inconsistencies in measurements of thickness, even within regions. For example, in LD10 females left striatum ammonium perchlorate decreases the thickness (group IV vs. control), in LD10 males an opposite effect occurs (group V vs. I; see Table A below). Identical opposite trends are noted in the CA1 region of the right hippocampus.

**Text Table A**

**Measurements Differing Significantly for any Treatment Group vs. the Comparable Controls**

| Neuroanatomic Region | Day 10 Male Rats | Day 10 Female Rats |
|---|---|---|
| Right Frontal Cortex | IV > I @ $p \leq 0.01$ | IV > I @ $p \leq 0.05$ |
| Left Frontal Cortex | IV > I @ $p \leq 0.01$ | |
| Right Parietal Cortex | IV > I @ $p \leq 0.05$ | |
| Left Parietal Cortex | IV > I @ $p \leq 0.05$ | |
| Right Striatum | | III < I @ $p \leq 0.05$ |
| Left Striatum | IV > I @ $p \leq 0.05$ | III < I @ $p \leq 0.05$ |
| Right Corpus Callosum | III > I @ $p \leq 0.01$<br>IV > I @ $p \leq 0.01$ | |
| Left Corpus Callosum | III > I @ $p \leq 0.05$<br>IV > I @ $p \leq 0.01$ | |
| Right Hippocampus | IV > I @ $p \leq 0.05$ | III < I @ $p \leq 0.05$ |
| Left Hippocampus | | III < I @ $p \leq 0.05$ |
| Right CA1 of Hippocampus | IV > I @ $p \leq 0.05$ | III < I @ $p \leq 0.01$<br>IV < I @ $p \leq 0.05$ |
| Left CA1 of Hippocampus | | III < I @ $p \leq 0.01$<br>IV < I @ $p \leq 0.05$ |
| Left CA3 of Hippocampus | V > I @ $p \leq 0.05$ | |
| External Germinal Layer | | II < I @ $p \leq 0.01$<br>III < I @ $p \leq 0.05$ |

As suggested by the authors (page 908), those regions characterized by side variation or which may have been increased in dimension for some groups but decreased in others may represent a function of sampling/section level. I fully agree with the statement.

**Text Table A**

**Measurements Differing Significantly Between Treatment Groups and Control Group**

| Neuroanatomic Region | Day 22 Male Rats | Day 22 Female Rats |
|---|---|---|
| Right Frontal Cortex | V > I @ p ≤ 0.05 | |
| Left Frontal Cortex | III > I @ p ≤ 0.01<br>V > I @ p ≤ 0.05 | |
| Right Parietal Cortex | V > I @ p ≤ 0.05 | |
| Left Parietal Cortex | III > I @ p ≤ 0.01<br>V > I @ p ≤ 0.05 | |
| Right Striatum | V > I @ p ≤ 0.05 | II < I @ p ≤ 0.01<br>III < I @ p ≤ 0.01<br>IV < I @ p ≤ 0.01 |
| Left Striatum | | II < I @ p ≤ 0.01<br>III < I @ p ≤ 0.01<br>IV < I @ p ≤ 0.01 |
| Left Corpus Callosum, Level #1 (anterior) | | II < I @ p ≤ 0.05 |
| Right Corpus Callosum, Level # 2 (posterior) | II > I @ p ≤ 0.01<br>III > I @ p ≤ 0.05<br>IV > I @ p ≤ 0.01 | III > I @ p ≤ 0.01 |
| Left Corpus Callosum, Level # 2 (posterior) | II > I @ p ≤ 0.05<br>III > I @ p ≤ 0.05<br>IV > I @ p ≤ 0.05 | III > I @ p ≤ 0.01 |
| Right Hippocampus | V > I @ p ≤ 0.05 | |
| Left Hippocampus | IV > I @ p ≤ 0.05<br>V > I @ p ≤ 0.05 | |
| Right Dentate | V > I @ p ≤ 0.05 | |
| Left CA1 of Hippocampus | III > I @ p ≤ 0.01<br>V > I @ p ≤ 0.05 | II < I @ p ≤ 0.01<br>III < I @ p ≤ 0.05 |
| Right CA3 of Hippocampus | | III < I @ p ≤ 0.01 |
| Left CA3 of Hippocampus | III < I @ p ≤ 0.05 | III < I @ p ≤ 0.05 |
| Cerebellum | II > I @ p ≤ 0.01<br>III > I @ p ≤ 0.01<br>IV > I @ p ≤ 0.01 | II > I @ p ≤ 0.01<br>IV > I @ p ≤ 0.01 |

There is no indication of the laterality (symmetry) of effects in LD22 pups as well. There is no apparent consistency in the results within regions across the time of sacrifice (blue rectangles, Tables A above), nor across sex within given days of sacrifice (red rectangles in Tables A above).

**SPECIFIC COMMENTS RE ARGUS STUDY 1998**

This discussion is provided to allow for comparisons between this study and the Argus 2001 study. One hundred twenty-five female rats were assigned to 5 exposure groups with ammonium perchlorate in the drinking water (0, 0.1, 1, 3.0, and 10 mg/kg bw/day, I through V, respectively) beginning 14 days before cohabitation with males and continuing through pregnancy until postnatal day 10 (PD 9 according to EPA's criteria). The F1 generation subset 1 was examined for brain morphometry on day postpartum 12 (DP 12; corresponding to EPA's PD 11) and subset 4 on DP 82 (corresponding to EPA's PD 81). The neonates were not dosed with ammonium perchlorate, but were exposed to it via lactation.

Methods for Brain Pathology Supplement, page 22

Subset 1 was sacrificed and analyzed on DP12 (corresponding to PD11 using EPA's nomenclature).

Subset 4 was sacrificed and analyzed on DP 82 (corresponding to PD81 using EPA's nomenclature).

Problems/clarifications

The brains in this study and the one performed by Argus in 2001 were fixed using different procedures. The 1998 does not indicate that the brains were fixed in Bouin's fixative for >48 hours (as in the 2001 Argus protocol).

The reviewer disagrees with the conclusion that there were no neuropathologic alterations in the examined brains on PD12 (see Argus 1998, page 64). The mean value for the thickness of the corpus callosum in the high dosage ammonium perchlorate (10 mg/kg/day) female group was significantly greater vs. controls. There appeared to be no effects in the males at any of the doses tested.

In subset 4 of rats, PD82 analysis revealed significant increases in brain weights and in the frontal cortex and corpus callosum measurements of the high dose ammonium perchlorate adult male group only (page 68). The authors considered these effects "random variation" and not a neurotoxic effect, a conclusion that cannot be accepted by this reviewer. The studies lack description on the symmetry of these effects.

Specific comments on Bekkedal et al., 2000

I am not a behavioral pharmacologist/toxicologist. I have reviewed the document, and the study looks sound. The studies suggest that the offspring (both male and females) of female rats treated for 2 weeks prior to gestation through postnatal day 10 (PND 10) with 0, 0.1, 1.0, or 3.0, or 10.0 mg/kg/day do not show a change in the general locomotor activity (as tested on PND 14, 18, or 22). Variability seems extremely high, with no dose- or temporal-response effects. I will defer judgment on this topic to Dr. Merle Paule, the expert behavioral toxicologist on the panel.

*C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment.*

Problems/considerations regarding brain morphometry

The historical background and the necessity for additional studies to ascertain the effects of ammonium perchlorate are well developed. EPA's statements regarding the 1998 Argus study were meritorious, specifically the noted disagreement with Argus's conclusions that ammonium perchlorate-induced increases in the corpus callosum were "not suggestive of neurotoxic effect" and that the effects were "of an unknown biological significance'. Because of these and other concerns (per details on page 5-37), the EPA sought to use more rigorous experimental conditions, evaluate whether the previous corpus callosum finding could be replicated, and identify effects in other brain regions (page 5-60). These and other concerns identified both by the EPA, as well as a number of external peer-review panels laid the ground for the 2001 "Effect Study" that was conducted by Argus, and is detailed starting on page 5-53. The specific flaws associated with this study were enumerated above in Section C.1.

The rationale for the assessment of "other brain regions" (page 5-60-28) is not explicitly explained, and is unclear within the context of perchlorate's effects. The hypothesis regarding the actions of ammonium perchlorate in developing the rat must be stated because it determines the model and the method. The text about development on pages 5-61 is vague, and it does not identify specific issues relating to the corpus callosum and "other brain regions".

The notion that cardiac perfusion can produce artifacts and, therefore, immersion fixation is equally valid, needs to be reconsidered. If one has a clearly demonstrable fixation artifact in a case, it gets thrown away, not included in the mean. It could be that immersion fixation of very small animals with a relatively large volume of extracellular space is the optimal approach, however, the text makes no such distinction.

What is an edema artifact? Since the ventricle borders the ventral aspect of the corpus callosum, how is this "artifact" avoided? Changing the plane of section does not solve this problem.

Single section morphometric analysis is not acceptable for quantitative neuropathological studies. The sampling problems are clearly evident as most of the discussion of the data surrounds variations in the plane of section rather than demonstrating a volumetric change in a region of interest, as discussed above (C.1).

What is a site just off the midline? Where exactly in the anterior-posterior plane and how many mm of the midline in each hemisphere? On page 5-63, the authors highlight the flaws in the method, "given the variability of the plane of cut and the difficulty in examining brains of young animals..." Indeed, quantification of a region of interest should not depend on the plane of section. As mentioned above, the rationale for the sectioning areas is not well developed, and it is unclear how the sections were matched.

How is a major period of myelin protein and lipid synthesis defined? Are the authors referring to critical periods? This text is vague. Is there a peak in lipid synthesis between PND19 to 35? Is it in a plateau between two other phases? As noted previously in the text, myelin wrapping begins when the axon is present, why "may this period represent a critical period in myelin development?" The basic neurodevelopmental issues should be better communicated.

Figure 5-14 does not show landmarks on the ventral and dorsal surfaces of the brain. This is a single sagittal section through the rat brain. To show landmarks on these surfaces, one would require photographs of the dorsal and ventral surfaces.

Variability in the plane of section is the most prominent reason that this data cannot be trusted with confidence, and lack of a clear sampling procedure is one explanation for this variation. On page 5-67 the authors state that the samples "demonstrated some systematic variability in the sectioning resulting in differences in right versus left measurements in different brain regions". To circumvent this problem, they argue for simply averaging the right and left-brain region measurements. While, it is agreed that averaging could help reduce variability in the data due to sampling in one histological section, it is unclear to this reviewer that this represents a sound method, especially with n=1. The problems are further compounded by comments such as "Many sections in the PND9 brains also showed signs of disruption or damage that may have compromised the measurements." Page 5-67 refers to "evidence of hydration-related changes such as edema or other swelling" raising concerns that the brains might not have been fixed and processed correctly.

Page 5-71 continues to drive home the message that this study design is fundamentally flawed. "A post-hoc analysis of the planes of cut of the PND9 brain sections suggested that the 0.1 and 1.0 mg/kg-day dose groups were sectioned at a different depth than were the other dose groups (Harry, 2001). This likely contributed to the small but significant increase in size of the frontal, parietal, and striatum sections in the 1.0 mg/kg-day dose group and may have contributed to the large increase in size of the anterior corpus callosum seen in the PND9 males". These issues raise red flags, making the statistical approach irrelevant, for it deals with numbers for which it is difficult to assign a reasonable confidence level.

On page 5-73, the authors state that the brains were sectioned at different intervals. Histological artifacts do not affect a proper sampling methodology, plane of section, or number of sections.

Additional analyses were run to adjust the raw morphometry data. Why is it necessary to use a normalizing procedure? The absence of parallel profiles obviates further analysis for equal profiles.

Comparison between the Argus 2001 study and others is limited by differential dosing regimens and sacrifice times, but even with these differences, it is exceedingly difficult to reconcile the differential

responses (Note the differential responses in male CA3 hippocampus between LD10 and LD22 pups in Argus 2001 study, blue rectangles in Tables A). It appears that the EPA Profile Analysis of Brain Morphometry Effects (page 5-68) was applied only to the 2001 Argus study, and it is unclear how it compares with the data derived from the Argus 1998 study. The omission of the 1998 study appears unwarranted. Furthermore, close examination of the results suggests that there is no inter- or intra-study consistency between the effects, either temporally or with respect to specific brain regions.

Finally, the document makes no attempt to correlate and integrate the linear morphometric brain measurements with the behavioral results or the changes in thyroid hormones.

In conclusion, the brains were not fixed correctly, and single sections were analyzed instead of a preferable volumetric (stereology) analysis of a region of interest. There appear to be extensive histological artifacts, systematic variation in the planes and numbers of sections for one or more groups. An unbiased sampling methodology needs to be devised. The region(s) of interest need to be specified with specific developmental and toxicological hypotheses. Each region of interest must be identified with histological criteria, randomly sampled, and volumetric analyses (stereology) performed on the entire region of interest rather than single sections. A rigorous statistical standard with flawed numbers does not alleviate these concerns, and as evident by the results, even when employed, it still fails to account for lack of a dose response (page 5-69).

Comparison between the Argus 2001 study and others is limited by differential dosing regimens and sacrifice times, but even with these differences, it is exceedingly difficult to reconcile the differential responses. It appears that the EPA Profile Analysis of Brain Morphometry Effects (page 5-68) was applied only to the 2001 Argus study, and it is unclear why data derived from the Argus 1998 study were omitted in the final analysis.

Minor issues:

Page 5-53-20 and 23 – change "effected" to "affected".

Specific comments about thyroid hormone measurements

No other evidence is provided in support of hypothyroidism-induced increases or decreases in any of the reported morphometric brain measurements.

No correlations between the T3, T4, and TSH data and the linear brain morphometry measurements have been determined to confirm whether there is any trend or associations between the findings.

Page 5-18 - The historical data show that the group mean for females at the 14-day time point may be artificially low relative to other data (AFRL/HEST laboratory). How does this affect the confidence in the other control levels and measurements?

Thyroid hormone analysis

Argus 1998 study, page 23

"All dosages of ammonium perchlorate increased TSH, T3 and T4 serum levels for F0 generation dams at DL 10 over the control group values. Serum TSH was statistically elevated ($p<0.01$ or $p<0.001$) for the 1.0, 3.0, and 10 mg/kg/day groups, however, no dosage-response was evident between the 3.0 and 10.0 mg/kg/day dosage groups. Serum T4 values were greater than the control value in all groups exposed to the test substance, however, only the 0.1 and 10.0 mg/kg/day dosage group values were statistically significant ($p<0.001$ and $p<0.05$, respectively). Serum T3 values were significantly increased ($p<0.05$ to $p<0.001$) over the control group value in the 0.1, 3.0 and 10 mg/kg/day target dosage groups". No hormone measurements were carried out in the pups in the Argus 1998 study.

Argus 2001 study

EPA document page 5-59 refers to maternal hormone changes. "Exposure to perchlorate produced significant decreases in thyroid hormones and an increase in TSH in the dams. For effects on maternal T3, there was no age-by-treatment interaction and the NOAEL at all time points was 1.0 mg/kg-day. There was a significant age-by-treatment interaction for effects on maternal T4. Step down analysis resulted in a LOAEL at 0.01, 1.0, and 30.0 mg/kg-day at GD21, PN9 and PN21. The 0.01 mg/kg-day level is a

LOAEL for the dams at GD21. There was also a significant age-by-treatment interaction for the effects on maternal TSH. Step-down analyses resulted in LOAEL at 0.01, 0.01 and 0.1 mg/kg-day at GD21, PND9 and PND21. As for the effect on T4, there was no NOAEL at GD21 for the effects on TSH".

Problems/clarification

Though it is recognized that hormone measurements were carried out on different days it is puzzling that the results are essentially in opposing directions. Chapters 5 and 7 incorporate the results from Argus 2001, but fail to address the 1998 Argus study. Given the bi-directional differences in the effects on T3 and T4 (decreased in the 2001 study and increased in the 1998 study), there should be a serious concern about the validity of these data (maternal), as well as those presented for the pups.

Behavioral Evaluations pages 5-43 through 5-52

I do not have the expertise to assess the soundness of the statistical analyses (EPA and NIEHS analyses) and I will defer judgment to the experts.

*C.3 Are the toxicity data consistent with the proposed mode of action of perchlorate*

Yes.

*The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.*

The brain morphometric measurements are suggestive of an association between exposure to ammonium perchlorate and an adverse effect in the rat brain (morphometry), but are limited because chance, bias, and confounding cannot be ruled out with confidence. The findings are not consistent in direction across dose and exposure time; they are inconsistent between studies performed by the same laboratory (Argus) and a

consistent positive association cannot be ascertained. It cannot be concluded with certainty that the measured effects were within the range expected on the basis of sampling error and selection bias, and, therefore they are deemed inconclusive.

**Human Health Dose-Response Assessment**

*F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for the 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?*

Per above, the brain morphometric studies are inconclusive.

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

I feel unqualified to address this issue.

*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 brain measurement data are deemed inconclusive, and they do not accurately reflect the relevancy of the critical effects to humans.

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

Yes.

**Risk Characterization**

*G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of human health risk posed by potential perchlorate exposures?*

Yes.

Some calculations:
Revised RfD for perchlorate was established at 0.00003 mg/kg/day or 0.003 µg/kg/day.
Water at 100 ppb (100 µg/liter) will translate to 0.2 ppm or 200 µg/day based on a 2-liter daily consumption. A 70 kg human consuming 2-liters of water a day will consume 0.2 µg/day.

Water at 0.1 ppb (0.1 µg/liter) will translate to 0.0002 ppm or 0.2 µg/day based on a 2-liter daily consumption. At the proposed RfD a 70 kg man will be allowed to consume 0.003 µg/kg/day x 70 = 0.21 µg/day, corresponding to 2 liters of water with perchlorate concentrations of ~ 0.1 ppb.

## Nancy Carrasco

**Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"**

**A.1. Have all 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?**

**Perchlorate is not translocated into the cells.** Clearly, not all relevant data on toxicokinetics and toxicodynamics have been adequately identified and utilized in the draft document. One central point stands out: the fate of perchlorate upon its interaction with the $Na^+/I^-$ symporter (NIS) at the plasma membrane of the thyroid follicular cells. The text of the draft document and the interpretation of all the data presented are based on the notion that perchlorate (a competitive inhibitor of NIS) is translocated via NIS into the cytoplasm in these cells. This was indeed the generally held notion for decades. However, several recent studies [Yoshida, A. N. *et al* (1997) "Different electrophysiological character of $I^-$, $ClO_4^-$, and $SCN^-$ in the transport by $Na^+/I^-$ symporter". *Biochem. Biophys. Res. Commun.* 231: 731-734. Eskandari, S. *et al* (1997) "Thyroid $Na^+/I^-$ symporter: mechanism, stoichiometry, and specificity". *J. Biol. Chem.* 272: 27230-27238. Yoshida, A. N. *et al* (1997) "Differences in the electrophysiological response to $I^-$ and the inhibitory anions $SCN^-$ and $ClO^-_4$, studied in FRTL-5 cells. *Biochim. Biophys. Acta* 11: 231-237] have convincingly demonstrated that perchlorate is not translocated into the cells, although it is a competitive blocker of iodide translocation by NIS. This aspect of the draft document illustrates how in some fundamental respects the document is not up to date on the latest findings on NIS research and on the interaction of perchlorate and NIS. More importantly, the finding that perchlorate is not translocated into the cells is of considerable significance for a proper understanding of the mode of action of perchlorate and its toxicity.

Here is a summary of our observations on this issue: Eskandari *et al* examined the mechanism, stoichiometry, and specificity of NIS by means of electrophysiological, tracer uptake, and electron microscopic methods in *Xaenopus laevis* oocytes expressing NIS. Electrophysiological recordings were obtained using the two microelectrode voltage clamp technique, and showed that an inward steady-state current (i.e. a net influx of positive charge) is generated in NIS-expressing oocytes upon addition of $I^-$ to the bathing medium, leading to depolarization of the membrane. Similar steady-state inward currents were generated by a wide variety of anions in addition to $I^-$ (including $ClO^-_3$, $SCN^-$, $SeCN^-$, $NO_3^-$, $Br^-$, $BF^-_4$, $IO^-_4$, and $BrO^-_3$),

indicating that these anions are also transported by NIS. However, perchlorate ($ClO_4^-$), the most widely characterized inhibitor of thyroidal $I^-$ uptake, was surprisingly found not to generate a current, strongly suggesting that it is not transported. Yoshida *et al* (1997) have reported, similarly, that perchlorate did not induce an inward current in Chinese hamster ovary (CHO) cells stably expressing NIS, as measured using the whole-cell patch clamp technique. The most likely interpretation of these observations is that perchlorate is not transported by NIS, although the unlikely possibility that perchlorate is translocated by NIS on a 1:1 $Na^+/ClO_4^-$ stoichiometry cannot be ruled out. Therefore, perchlorate is a potent inhibitor of NIS most probably acting as a blocker, not as a substrate.

These results raise the question of whether or not perchlorate is indeed translocated into thyroid cells expressing endogenous NIS or into cells other than oocytes expressing transfected NIS, and this question has been the subject of some controversy. In the "Acknowledgments" section of his extensive review on perchlorate and the thyroid gland, [Wolff J. (1998) Perchlorate and the thyroid gland. *Pharmacol Rev.* 50: 89-105] Wolff asserts that "perchlorate accumulation in thyroid tissue has been repeatedly demonstrated", and suggests that the absence of perchlorate transport by NIS in oocytes reported by Eskandari *et al* may be due to differences between the oocyte system and thyroid tissue. However, Yoshida *et al* (1998) thereafter showed that perchlorate elicits no change in the membrane current in the highly functional rat thyroid cell line FRTL-5, as revealed by the whole-cell patch-clamp technique, thus strongly suggesting that perchlorate is not transported into FRLT-5 cells and supporting both their previous observations in CHO cells and Eskandari *et al's* results in oocytes.

Considering that the properties of NIS expressed in oocytes are virtually indistinguishable from those of endogenous NIS in thyroid cells, including FRTL-5 cells, it appears that earlier experiments ostensibly showing that [$^{36}$Cl]-labeled perchlorate enters the cell may have been misinterpreted. Because [$^{36}$Cl]-chlorate ($ClO^-_3$) is a $^{36}$Cl-labeled byproduct of the reaction employed to chemically synthesize [$^{36}$Cl]-perchlorate ($ClO^-_4$) for these uptake studies, it seems likely that [$^{36}$Cl] chlorate, rather than perchlorate, accounts for the presence of label in the cytosol of thyrocytes, given that chlorate is readily translocated via NIS into the cell (Esckandari *et al*). Current data, in conclusion, strongly indicate that perchlorate is not translocated via NIS into the cell. The authors of the draft document seem unaware of these key findings.

**Perchlorate metabolism.** The draft document indicates on page 2-8, line 25, that perchlorate is excreted virtually unchanged after absorption. However, on page 3-3, line 5, it points out that when double-labeled $K^{36}Cl^{18}O_4^-$ was administered to the human volunteers, total urine radioactivity was distributed among $^{36}Cl$, $^{36}Cl^{18}O_4^-$, $^{36}ClO_4^-$ and $^{36}Cl^-$. If perchlorate was reduced all the way to chloride it means that perchlorate was not excreted virtually unchanged.

**Locus of the toxic effect of perchlorate.** We read on page 7-29, line 4, that "there remains some uncertainty as to whether NIS is the only locus for the effect of perchlorate because of the efflux (discharge) phenomenon." This statement is incorrect. It is clear that the primary locus for the effect of perchlorate is NIS, and the discharge phenomenon, far from suggesting any uncertainty about it, actually confirms it. The mentioned statement indicates a lack of understanding of the mechanism of the discharge phenomenon. A test known as the "perchlorate discharge test" has long been carried out to ascertain a patient's thyroid's ability to organify iodide. Perchlorate administered to a healthy person who has previously received radioiodide will not exhibit radioiodie efflux (discharge) as a result of perchlorate administration, because intracellular radioiodide is organified and thus retained in the cell and the colloid. By contrast, if iodide organification is inhibited with PTU or MMI, perchlorate administration causes radioiodide efflux (or discharge), because perchlorate is inhibiting the influx component of the steady state reaction of iodide transport across the plasma membrane. Identical radioiodide discharge is observed in patients with impaired iodide organification, without inhibition by PTU or MMI. Therefore, the discharge phenomenon demonstrates that the effect of perchlorate occurs at NIS.

**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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?**

**Presentation of the molecular and functional characteristics and the structure-function relations of NIS.** The roles mentioned in the question above are not sufficiently articulated. A major weakness of the draft document as a whole is its lack of a thorough presentation of the molecular and functional characteristics and the structure-function relations of NIS, all of which

have been extensively investigated and reviewed in recent years. With NIS being the key molecule where perchlorate exerts its toxicity, a complete discussion of the latest data on NIS is indispensable to properly assess the mechanism of action of perchlorate and its toxicity. As it currently stands, the draft document includes only one short paragraph describing NIS (on page 3-9), but the description is incomplete and outdated, and is based on an indirect reference, written by an author who has not investigated NIS himself. In the draft document, the iodide transport system of the thyroid (i.e. NIS) is still often referred to as the "iodine pump", an outdated term now regarded as incorrect. The term pump actually describes ATPases (active transporters that are driven by hydrolysis of ATP). Since NIS is driven by the $Na^+$ electrochemical gradient (generated by the $Na^+/K^+$ ATPase), NIS is not a pump, but a $Na^+$-dependent $I^-$ transporter. A detailed discussion of the latest available data on NIS should be included.

**Missing key data on neurodevelopmental deficits and other potential adverse effects resulting from thyroid hormone disruption by perchlorate.** The issue of the effects of perchlorate on neural development in the fetus is addressed in relation to the interaction of perchlorate with the placenta. The document indicates (page 3-12, line 23) that perchlorate can cross the placenta, a conclusion apparently reached on the basis of a statement found in the figure legend of Fig. 3-7, namely that "given its physicochemical characteristics and similarity to iodide, perchlorate is anticipated to cross readily." However, no clear experimental evidence is provided or referenced to support such a conclusion. In any case, the only aspect being considered to ascertain the potential toxicity of perchlorate to the fetus is whether the anion crosses the placenta. Whereas this is obviously highly relevant, because perchlorate reaching the fetus would imperil iodide transport via NIS in the fetal thyroid and possibly cause hypothyroidism and its concomitant neural consequences (as severe as cretinism), a major and fundamental issue was overlooked: the effect of perchlorate on the function of placental NIS. The authors of the draft seem unaware that iodide is translocated across the placenta via placental NIS from the maternal to the fetal bloodstream. In other words, no iodide (or a markedly lower amount of iodide) would reach the fetus if placental NIS were absent or blocked. Therefore, perchlorate present in the maternal bloodstream would be potentially toxic to the fetus *even if it does not cross the placental barrier*, because it would inhibit placental NIS activity and could still lead to fetal hypothyroidism caused by insufficient supply of iodide. $T_4$ synthesized in the fetal thyroid is essential for the development of the central nervous system, as indicated in Fig. 3-8. The data on placental NIS should be included and carefully considered in any discussion of perchlorate toxicity to the fetus and the behavior of perchlorate in the placenta.

**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?**

The answer to this question is outside my area of expertise. Still, I might point out that the finding that perchlorate is not translocated by NIS into the cytoplasm further validates and supports the notion that perchlorate is not likely to directly interact with DNA.

**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 for both.**

Regarding the links between noncancer and cancer perchlorate toxicity, I can comment that important differences apparently exist with respect to the effect of chronic TSH stimulation in rats and humans. The draft document states that after 19 weeks of perchlorate treatment, rats develop cancerous tumors, seemingly as a result of persistent TSH stimulation However, patients with Graves disease, whose TSH receptor is constantly activated, don't develop tumors.

**B. Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.**

The mentioned studies were generally well conducted, comprehensive, and informative. However, some weaknesses and mistakes should be pointed out, as follows:

- In the study by Greer *et al* (page 6-20, line 25), each volunteer is said to have received a capsule containing 100 mCi of $^{123}I^-$ before thyroid scans were performed. This dose is extremely high; was it a typographical mistake?. Only therapeutical doses of radioiodide would be in this range.

- Dietary iodine intake by the volunteers in Greer's study should have been controlled and taken into consideration. In addition, the effects of perchlorate were followed for only a period of 14 days, a short period established to protect volunteers from the potentially serious effects of more prolonged exposure to perchlorate. Still, in spite of the demonstrable effect of perchlorate on NIS-mediated iodide uptake, no significant alterations were found in the concentrations of $T_3$, $T_4$ and TSH in the course of the 14 days. In fact, this is not surprising, because of the efficacy of the colloid to act as a thyroid hormone reservoir for over two weeks in the absence of *de novo* hormone biosynthesis.

- The data in attachment 6 would be much clearer if they were presented in graph form, in addition to being shown in a table.

- The data in attachment 7 are presented in a puzzling fashion. Why are the urinary concentrations of perchlorate, given in ppm, shown with as many as 9 digits to the right of the decimal point?

**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?**

Not all confounding factors were appropriately controlled. For example, as I suggested above, the dietary iodide intake of the subjects in Greer's study should have been controlled.

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

The values used to generate the PBPK models assume that perchlorate is transported into the thyroid cells and that the $K_m$ for iodide from the cell to the colloid is in the mM range, over 1000-fold higher than data reported more recently (9 μM) [Golstein P.E. *et al* (1995) "The iodide channel of the thyroid" *Am J Physiol* 268, C11-C118]. Furthermore, unlike the statement (p. 6-24 line 31) that this apical iodide channel seems to be very sensitive to perchlorate inhibition, Goldstein *et al* reported that the apical transporter is not sensitive to even a 1000-fold excess of

perchlorate over iodide. These data should have been taken into account when the PBPK model was generated.

**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?**

See H.1

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

It should be emphasized that under certain physiological conditions such as pregnancy and lactation iodide requirements increase and an therefore the effects of inhibiting NIS could have more pronounced consequences in those states.

**H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.**

**Considering NIS in the mammary gland.** The authors of the draft document refer to determinations of perchlorate in the milk. Initial attempts showed no perchlorate in the milk, but better detection techniques employed later demonstrated that perchlorate was actually present in the milk. Again, in a fashion similar to that discussed above in relation to the placenta, the authors are overlooking a fundamental consideration: NIS is present in lactating mammary gland, where it mediates the active transport of iodide from the mother's blood to the milk, thus supplying the nursing newborn with the precious and essential constituent of the thyroid hormones, namely iodide.

**H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.**

The data in the study by Merril (2001) should have been plotted (instead of only being presented in tables) to make the analysis clearer. Units should have been kept consistent throughout the document and relate them to the Ki perchlorate values.

**Minor points.**

On 3-5, line 21, it should say 14C-inulin instead of insulin.

# Michael Collins

A.1 Have all 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?

It is always difficult to answer affirmatively to a question that asks if "all" relevant data on anything have been identified and utilized appropriately. With that caveat, it appears that the significant information on toxicokinetics and toxicodynamics has been collected. I would like to have more information regarding the specificity of perchlorate for the NIS, and the relationship between NIS and the thyroid hormones.

Performing a relatively superficial literature search, the following articles (that are not referenced in the EPA report) have relevance to various issues that are significant for either toxicokinetics or toxicodynamics of perchlorate:

(a) Golstein, P, M. Abramow, JE Dumont and R. Beauwens (1992) The iodide channel of the thyroid: a plasma membrane vesicle study. Am. J. Physiol. 263(3 Pt 1): C590-597.

(b) Ganea, C., A. Babes, C Lupfert, E. Grell, K. Fendler and RJ Clarke (1999) Hofmeister effects of anions on the kinetics of partial reactions of the Na+,K+-ATPase. Biophys. J. 77(1): 267-281.

(c) Fernandez Rodriguez, A., H. Galera Davidson, M. Salguero Villadiego, A. Moreno Fernandez, I. Martin Lacave and J. Fernandez Sanz (1991) Induction of thyroid proliferative changes in rats treated with antithyroid compound. Anat. Histol. Embryol. 20(4): 289-298.

(d) Vijayalakshmi, K. and DB Motlag (1990) Effect of perchlorate on mitochondrial function. Indian J. Biochem. Biophys. 27(1): 48-51.

(e) Ben Hamida, F., L. Soussia, F. Guermazi, T. Rebai and N. Zeghal (2001) [Propythiouracil and perchlorate effects on thyroid function in young and lactating rats][In French]. Ann. Endocrinol. (Paris) 62(5): 446-453.

(f) Shennan DB (2001) Iodide transport in lactating rat mammary tissue via a pathway independent from the Na+/I- cotransporter evidence for sulfate/iodide exchange. Biochem. Biophys. Res. Commun. 280(5): 1359-1363.

(g) Schroder-van der Elst, JP, D. van der Heide, J. Kastelijn, B. Rousset and MJ Obregon (2001) The expression of the sodium/iodide symporter is up-regulated in the thyroid fetuses of iodine-deficient rats. Endocrinology 142(9): 3736-3741.

(h) Goleman WL, LJ Urquidi, TA Anderson, EE Smith, RJ Kendall and JA Carr (2002) Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in Xenopus laevis.

Environ. Toxicol. Chem. 21(2): 424-430.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

The role and relative importance of the key event are both clearly articulated and consistent with the majority of findings. There is a section in the EPA document (Section 5.5.3), where the results do not seem to be consistent with the general ideas. In this case, some thyroid and pituitary hormone changes are in the opposite direction of the anticipated results.

Furthermore, there are physiological issues which are relevant to the key event and remain uncertain to this reviewer, e.g. the specificity of perchlorate for the NIS and the alternate transport mechanisms for iodide. In general, specific inhibitors of transporters have been found in many instances to be less specific than originally thought, and the literature indicates that there is more than one transporter for iodide. However, the vast majority of the data seems to support the general theory of the key event.

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?

It seems logical that perchlorate will probably not interact with DNA given the genotoxicity results. This probably means that perchlorate toxicity has a threshold whether the toxicity is cancer or not. However, the EPA report argues quite effectively that there are a number of nutritional and physiological issues which may make specific individuals much closer to the threshold than others (and perhaps on a population basis there may be a very small threshold for some individuals), and if the key event is correct, then this variability in population susceptibility is probably also correct. If there are in fact a number of factors which all impact the mode-of-action, then it becomes quite difficult to perform low-dose extrapolation. With respect to the dose metric, it seems that there are some assumptions that accompany the choice of the AUC as the dose metric. Although it appears that this parameter is well correlated with inhibition of NIS, it is probably a combination of AUC and peak concentration, or at least there is some temporal limit to the accumulation of

the AUC. This has much relevance to environmental perchlorate which may be in the drinking water at ppb concentrations.

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 for both.

Providing that the basic assumption is correct, namely that both types of toxicity occur via the same mode-of-action through NIS inhibition, then the approach is protective for both forms of toxicity. However, in toxicity it is probably rare that only a single biochemical pathway is altered, and in this instance there may be a multitude of insults which can perturb this same pahtway. Thus, there could be tremendous variability in the human population regarding how much perchlorate is required to reach a threshold.

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies?

Regarding the Argus Research Laboratories Report (1416-003D) from 2000 and authored by Dr. R. G. York, the protocol uses a relatively common approach to assess developmental toxicity. The study follows GLP, the number of animals is sufficient to make conclusions, and the statistics are appropriate for this type of study. However, the following issues with this study are questionable:

(1) Starting the dosing 15 days prior to cohabitation of the animals could insure that inhibition of NIS was maximized, or, alternatively, that animals could develop an ameliorative response, e.g. induction of NIS protein.

(2) The doses used of 0, 0.01, 0.1, 1.0, and 30.0 mg/kg/day appeared to have a large difference between the highest and second highest dose. Thus, when some outcomes appeared only in the highest dose, it would have been beneficial to know the effect in some dose closer to the highest dose (e.g. resorptions, implants). The rationale for the chosen doses was not given in the study.

(3) It is stated that stained fetuses were examined for skeletal alterations and cartilage development, however, instead of double staining with alizarin red S for bone and alcian blue for cartilage, the fetuses were only stained with alizarin red S. This makes it difficult to visualize cartilage development.

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment.

(1) Regarding the interpretation of this study by the EPA document, there appeared to be disagreement between the EPA and Argus with respect to the issue in C.1 (3). In this instance, my interpretation corresponds to the criticism of EPA, but the issue is not well delineated in the EPA document. (2) There are differences of interpretation between EPA and Argus as indicated on pages 5-81 to 5-83 in the EPA document. It is this reviewer's opinion that the alopecia is not a significant finding (in agreement with Argus), but that the developmental endpoints may have relevance (in agreement with Argus).

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

The developmental toxicity neither supports nor refutes the proposed mode of action.

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

The NOAELs/LOAELs were correct in all instances except when describing the Argus study from 2000 as described in C.1. In Section 5.4.3.3 in the EPA document, it is stated that the NOAEL for developmental toxicity was 3 mg/kg/day and the LOAEL was 30 mg/kg/day. As for as this conclusion is concerned, it seems that the LOAEL is appropriate, but the NOAEL is inappropriate because the study did not use that dose. It is suggested that the NOAEL in that case should be 1.0 mg/kg/day.

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?

As mentioned previously, with the exception of the data in Section 5.5.3, the data for most of the animal experiments is consistent with the idea that the mechanism of perchlorate toxicity is mediated by the inhibition of NIS. Many different toxicological endpoints can be explained by the inhibition of NIS, which subsequently leads to a depletion of T4 or T3 or an increase in rT3, and then upregulates the TSH release from the pituitary leading to an alteration in the hypothalamic-pituitary-thyroid axis. The point of departure has not been well defined in the document in the section entitled "Point of Departure Analysis", and this should be rectified. The point of departure is an attempt to estimate the threshold for the most sensitive

endpoint, and in this case it has been chosen to be a LOAEL of 0.01 mg/kg/day. This is somewhat substantiated by the data, but there is frequently a nagging feeling that the derivation of the LOAEL is not really scientifically-based, but represents more of an art.

F.2 Comment on the use of PBPK models for interspecies extrapolation and the choice of the dose metric.

The advantage of PBPK models is the ability to perform interspecies extrapolation. However, since the tasks that were assigned to me did not include Chapter 6 regarding PBPK modeling and interspecies extrapolations, this question is somewhat beyond the scope of my responsibilities. Although pharmacokinetics is not my area of expertise, there are issues with the use of AUC as the dose metric that are disconcerting to me. If a bolus dose of perchlorate is injected into an animal and reaches a high peak concentration but is excreted with a couple of days, it is hypothesized that the toxicity will be greater than if the animal is exposed to one ppb over a period of years. This is because the one ppb is below the threshold for toxic effects and will remain below the threshold for as long as exposure continues. Alternatively, it is argued in the EPA document that perhaps the peak concentration should be the appropriate toxicolkinetic parameter because development is a series of timed events and the perturbation of one event could cause permanent deficits. This approach is also inaccurate because it is known that development can be perturbed adn undergo compensatory growth and development to make up for developmental delays. It is hypothesized that neither of these toxicokinetic parameters is a totally appropriate metric. The use of the toxicodynamic parameter, the percentage of inhibition of iodide transport in the thyroid, would be predicted to be more appropriate as a predictor of the key event in the mode-of-action of perchlorate toxicity.

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?

In general, the concept of uncertainty factors, namely determining the product of a series of factors which are based on an order of magnitude of uncertainty and then deriving a reference dose is not appealing from a scientific perspective. It is my opinion that the quantity of each of these factors could be debated. Surely the intraspecies variability having a factor of 3 is not consistent with the number of factors which can

make human variability so large. This factor should be 10. Also, the lack of any uncertainty factor for interspecies extrapolation seems to over-emphasize the current hypothetical idea of how perchlorate works. Deriving a value of 0.01 mg/kg/day seems equally suspect from my perspective as the LOAEL. It could be argued that there should be modifying factors predicated on the certainty of the data.

F.4 Have all of the factors influencing susceptibility been clearly described and accounted for in the assessment?

Some factors that will certainly increase susceptibility have been listed in Section 7.1.5.3. However, an exhaustive list of potential susceptibilities is not possible because there are innumerable potential interactions with this mechanism of toxicity. Some examples include individuals with kidney conditions that impact the excretion of perchlorate, or persons with occupational exposure to chemical agents that may have thyroid hormone disrupting capacity. Also, nutritional factors such as high exposure to retinoids might inhibit thyroid hormone activity by usurping the heterodimeric binding partner for both thyroid hormone and retinoic acid, retinoid X receptor (RXR).

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate expsoures?

This section of the document is only a couple of pages and represents an overview. I would remove the term "bright line". I believe that the section overemphasizes the portion on indirect exposures, but in general it is a relatively good attempt to summarize the issues.

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

(1) Affinity of perchlorate for the NIS.

(2) Specificity of perchlorate for the NIS

(3) Alternate routes of iodide transport into cells, and quantification of various routes.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

(1) Page 3-5, line 21. The use of three radiolabelled molecules to determine follicle volume and membrane potential is not clear.

(2) Pages 3-9 to 3-11, description of cellular processing is unclear.

(3) Concept of minimum reporting limit is not clear.

(4) Page 3-4, line 1: Radioiodide accounts for 30 % of thyroid gland volume?

(5) Page 3-4, line 4 and 3-9, line 14: Is there a contradiction regarding salivary glands?

(6) Page 3-13, line 31: Selectivity based on large cation?

(7) Page 3-16, line 12: "...antithyroid-mediated neoplasia...." Accurate wording?

(8) Page 5-49, line 16: Confusing.

(9) Page 5-86, line 16: Confusing.

# Thomas F.X. Collins

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Reproduction Study (Argus 2-Generation)**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.1** Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Sprague-Dawley rats (30/sex/group) were given continuous access to ammonium perchlorate at 0, 0.3, 3.0, or 30.0 mg/kg-day in drinking water. Concentrations were adjusted based on actual water consumption and body weights recorded the previous week. Feed consumption and water consumption were recorded at least 3 times per week. The animals were exposed for 70 days before mating. Estrous cycle of the P generation was evaluated daily for 3 weeks before mating and continued to GD 0. Dam viability, litter size, and pup viability were evaluated twice per day during LD 0 to 21. The pups were weighed on LD 1, 4, 7, and 21. F1-generation pups were weaned at LD21. Preputial separation was monitored in F1 males beginning on LD 39, and vaginal patency was monitored in F1 females beginning on LD 28. At LD21, F1-generation rats (30 rats/sex/group) were randomly selected, treated with perchlorate for 10 weeks, mated, and allowed to litter to produce F2-generation. F2-generation rats were sacrificed at LD 21. Estrous cycling, mating performance, duration of gestation, fertility parameters, maternal behavior, and litter data were recorded in the same manner for P and F1 animals.

All P and F1 adults were necropsied. F2 generation pups were sacrificed on LD21. At the time of sacrifice, blood was collected for the determination of TSH, T3 and T4. P and F1 male and female rat organs were examined histologically. The CASA was utilized for the evaluation of sperm motility and slides were examined for sperm morphology. At least 3 weanlings/sex/litter were necropsied and also examined histologically. They also collected blood from the pups for TSH, T3 and T4 evaluations. The data were analyzed for statistical significance.

No significant changes were reported in reproductive parameters except for a significant decrease in the percentage of F1 liveborn pups/litter at 30 mg/kg-day. No significant changes were observed in developmental parameters; litter size and pup weight were similar in all groups. In F1-generation adult rats, thyroid weights were significantly increased in all dose groups for females and in the 3 and 30 mg/kg-day dose groups for males. The weight at 0.3 mg/kg-day was increased but not significantly. Histopathological changes in the thyroid consisted of hypertrophy and hyperplasia that increased in incidence and severity in a dose-related manner in P and F1 rats. T4 levels were significantly decreased and TSH levels were significantly increased at 30 mg/kg-day in adult P and F1 males. T3 levels were not affected in adult P and F1 males. The investigators identified the 0.3 mg/kg-day dose level as the NOAEL.

**A1.1.** Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

The Argus 2-Generation Reproductive Toxicity Study is robust, was done according to the latest guidelines, and followed the protocol. It includes data on estrous cycles, sperm analysis, preputial separation, and vaginal patency. One weakness of the study is that the weighing and observation of the pups was done in 2 parts. The neonates were counted on LD 0 and weighed on LD 1. It is obvious that some pups are being lost due to maternal cannibalism. Mothers in nature cannibalize abnormal or underweight pups and this could be a compound-related effect that disappeared. I.e., the pups could not be evaluated. With that aside, however, there are no other serious problems with the study.

**A1.2.** Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices or specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

The study was conducted according to the FIFRA EPA GLP Final Rule and was evaluated for QA/QC compliance. Stability was determined by the sponsor (stable to 109 days). Concentrations were monitored according to GLPs. The study rooms were monitored for temperature and humidity. The feed was analyzed. Critical phases were monitored by QA and the raw data were audited by QA. AniLytics operates under GLPs.

The rats were acclimated to the environment and were placed on the study by stratified random procedure. Mating was done randomly within groups, and mating of siblings was avoided. One male and one female per litter were randomly selected for the next generation.

The 6 amendments to the study were either routine (e.g., AniLytics designated for analysis of

samples) or added to the robustness of the study (e.g., expansion of the list of tissues to be analyzed, and blood taken from pups not selected for mating provided for additional evaluation of TSH, T3 and T4 levels). The deviations listed did not affect the results of the study.

**A1.3.** Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe an effect?

Yes. Yes. Yes.

**A1.4.** Please comment on the strengths and limitations of the statistical analyses used to evaluate the study findings. What other statistical analyses, if any, should be performed?

Statistical analyses were done according to normal methods for analyzing reproduction data.

**A1.5.** Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

The study was published by York *et al.* in 2001 (citation on p. 11-22). In the thyroid table, for adult male and female P and F1 animals, the absolute weight of the thyroid showed a dose-related increase in all treated groups. With respect to the thyroid weight of the adults, there thus appears to be no NOAEL. Thyroid weight was significantly increased at 3.0 and 30.0 mg/kg-day in P and F1 males. In F1 females, the increases were significant and dose related at 0.3, 3.0, and 30.0 mg/kg-day. If an accumulation of effects occurs from a compound, effects can be observed in F1 animals, and there are dose-related effects in F1 females.

Dose-related decreases in sperm density, spermatid count, spermatid concentration, and spermatid density were observed at the 2 high levels in F1 males, where accumulation of the compound effects could occur. Although the decreases do not reach the level of statistical significance, there is cause for concern.

Otherwise, the data are accurately reported. In some cases, the TSH data do not follow the expected results if the thyroid is being inhibited. E.g., there is an increase in T3 values in P1 adults, where there should be a decrease. There is no effect on T4 values in F1 females, but there is an effect in males. In F1 weanling males, there is no effect on TSH, T3 or T4. In F1 weanling females, TSH was slightly increased, and T3 and T4 were decreased. In F2 weanlings, there were no effects except for a slight increase in T4 in males and females.

**A1.6.** Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological / ecotoxicological risk characterization of perchlorate exposures? If so, indicate the extent to which it can be used for characterizing adverse effects.

Yes, it can be used for hazard identification purposes. It was done according to the latest reproduction study guidelines, has extensive histopathology and hormone analyses, and it includes data on the males and females. It should be part of the toxicological profile of the compound.

**A1.7.** Do the findings provide information relevant to the evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?

Yes, obviously, being a reproduction study, it provides information concerning sexual maturation, mating, effects on neonates, adverse effects on lactation, and effects on developing offspring.

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

York et al. have published the developmental toxicity study by Argus done in rabbits (p. 11-22), and Siglin et al. have published the 90-day drinking water toxicity study in rats (p. 11-17). Both studies have already been cited in the report.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

No, the results were not adequately discussed. Greater emphasis should have been placed on the differences in thyroid weight and possible dose-related effects on sperm should have been mentioned and discussed.

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

The Argus study is discussed correctly and it does mention inconsistencies in thyroid and hormone analysis. The data from the study show the effects of the compound on TSH concentration and decreases in T3 and T4 values. The report addresses the inconsistencies. Having the thyroids re-evaluated with a consistent lesion grading system helped elucidate the thyroid-pituitary axis.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases

have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

They have calculated the Benchmark Dose, which gives a more accurate NOAEL. This was based on the additional pathology done by Wolf. The new statistics are based on the re-analysis of the thyroid. The actual reproduction data were not re-analyzed.

The incidence of tumors in the Argus study was compared to the incidence found in the NTP studies. However, the comparison is not exactly among equals, because NTP tested F344 rats whereas Argus tested Sprague-Dawley rats. Also, the Argus rats had been exposed *in utero,* whereas NTP rats had not, raising the concern for imprinting.

A Bayesian approach was used to assess the effect of the compound on the incidence of thyroid follicular cell adenoma in male rats. The resulting data supports the hypothesis that the compound at 30 mg/kg-day causes an increase in the incidence of thyroid follicular cell adenomas.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

Yes.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

Yes. There are effects on the NIS. In some cases, increased TSH levels were seen, as well as

decreased levels of T3 and T4. After the re-analysis of the thyroid samples, 2 pups (with 3 adenomas) were recorded. The proposed mode of action involves the gradual sequence of colloid depletion, hypertrophy, and hyperplasia of the thyroid.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

The study was published by York *et al.* in 2001 (citation on p. 11-22). Upon looking at Table 2 in the published document and Tables B11, C26, C30, and C31 in the report, there appears to be an increase in thyroid weight at the lowest dose (0.3 mg/kg), particularly in F1 males and females. The weight increase was dose-related in all doses and significant at 3.0 and 30.0 mg/kg-day groups. In females, there was a significant increase at 30 mg/kg-day, and in F1 there was a dose-related and significant increase at all doses. If there occurs an accumulation of effects, the effects would be more pronounced in the F1 animals. This is what was seen in the study. With respect to the thyroid weight of the adults, there appears to be no NOAEL.

There are also dose-related decreases in sperm density, spermatid count, spermatid concentration, and spermatid density at the 2 high levels in F1 males, where accumulation of the compound effects could occur. The male reproductive data were re-analyzed at FDA and the following questions and comments emerged:

**Questions: Sperm Evaluation:**

1. Does the percent motility presented in the report refer to "progressive motility" or to "motility"? If this value represents "progressive motility," how was "progressive motility" defined using the Hamilton-Thorne Sperm Analysis System? For example, Klinefelter et al., 1991 (Repro Toxicol 5(1):39-44), considered sperm progressively motile when their path velocity exceeded 20 µm/sec and their linear index (progressive velocity/path velocity) was greater than 40.

2. What embedding material was utilized for the testicular tissue? Paraffin? Methacrylate? How were the samples stained? PAS?, H& E?

**Comments (See Table 1 below):**

**Cauda epididymal weights and sperm density:**

Observation 1: For both the P and F1 generation animals there was minimal effect on absolute cauda weight, cauda weight expressed per gram of brain weight, or cauda weight expressed per gram of body weight. Additionally, significant changes were not observed for the parameter sperm density in the P generation when the control and the high (30 mg/kg) dose groups were compared. Surprisingly, in the F1 generation, there was a non-statistically significant decrease in cauda epididymal sperm density (concentration/gram cauda) in the high dose group when this group was compared to the control values.

Question: To what do the authors attribute this reduction in cauda epididymal sperm density in 30 mg/kg/day dose group of the F1 generation?

Observation 2: Cauda epididymal sperm numbers in the F1 control (1544 $\pm$ 521) were almost twice that of the P control (823 $\pm$ 285).

Question Why is the sperm density for the P controls so dramatically different from that of the F1 controls?

**Testicular Weight (Left) and spermatid density:**

Observation1: For both the P and F1 generation animals there was minimal effect on left testicular weights, testicular weight expressed per gram of brain weight or testicular weight expressed per gram of body weight. Additionally, there does not appear to be a significant change in sperm density in the P generation when the control and the high (30 mg/kg) dose groups are compared. However, there does appear to be a dose related non-statistically significant decrease in spermatid density in the F1 generation (see Table 1). A calculation of Daily Sperm Production (DSP; concentration/gram of testis/6.10; Robb et al., 1978) for the P generation did not reveal any dose related effect on DSP. In contrast, similar calculations for the F1 generation revealed a dose related decrease in DSP (See Table 1) ranging from 20.5 (which is comparable to historical values for the

rat) in the control group to 16.1 in the 30 mg/kg dose group.

Additionally if one counts the number of animals per total number of animals for a particular dose group with sperm density less than 110 one observes that the overall average number of animals having spermatid numbers less that 110 in the P generation are relatively similar. In contrast, the number of animal having spermatid densities below 110 increases in a dose dependent manner in the F1 generation from 11 of 29 in the control to 17 of 27 in the 30 mg/kg dose group (See Table 1).

Conclusion: It is possible that perchlorate exposure could have produced an adverse effect in the testis of the animals from the F1 generation. The absence of a reduction in testis weights in the high dose groups does not negate the possibility that subtle lesions could have occurred during spermatogenesis. It is possible to have no change in testicular weight but a slight increase in germ cell degeneration that would result in a decreased testicular spermatid count and a concomitant reduction in cauda epididymal sperm count. These lesions could have been missed during the histopathological evaluation of the testicular tissue if the testicular tissues were not embedded properly i.e. methacrylate embedding. It is also possible that perchlorate treatment made the condensed spermatids less resistant to homogenization. It is suggested that the histological slides be re-evaluated.

## Table 1

### Male Reproductive Organ Weights and Sperm Numbers Data

| | P Generation | | | | F1 Generation | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Cauda wt. | 0.3826 (0.0415) | 0.3872 (0.0378) | 0.3822 (0.0350) | 0.3740 (0.0396) | 0.3535 (0.0370) | 0.3702 (0.0390) | 0.3654 (0.0460) | 0.3774 (0.403) |
| Cauda wt./body weight | 0.061 (0.008) | 0.059 (0.007) | 0.057 (0.007) | 0.058 (0.006) | 0.057 (0.008) | 0.056 (0.009) | 0.057 (0.009) | 0.059 (0.007) |
| Cauda wt./brain | 16.26 (1.77) | 16.18 (1.64) | 16.14 (1.59) | 15.89 (1.68) | 14.86 (1.53) | 15.22 (1.70) | 14.86 (1.93) | 15.82 (1.62) |
| Sperm density | 823 (285) | 856 (299) | 770 (261) | 892 (264) | **1544 (521)** | **1572 (536)** | **1461 (439)** | **1373 (445)** |
| Testis | 1.89 (0.14) | 1.89 (0.20) | 1.92 (0.11) | 1.88 (0.15) | 1.85 (0.17) | 1.95 (0.17) | 1.93 (0.17) | 1.97 (0.17) |
| Testis wt./body wt. | 0.301 (0.028) | 0.294 (0.035) | 0.290 (0.026) | 0.298 (0.030) | 0.302 (0.033) | 0.294 (0.038) | 0.298 (0.039) | 0.310 (0.028) |
| Testis wt./ brain wt. | 80.53 (6.02) | 78.97 (9.00) | 81.04 (6.30) | 80.10 (7.19) | 77.68 (6.1) | 79.95 (6.80) | 78.62 (7.18) | 82.44 (6.76) |
| Spermatid count | 34 (11) | 33 (11) | 35 (14) | 33 (10) | **37 (16)** | **36 (14)** | **33 (9)** | **30 (12)** |
| Spermatid concentration | 2.0 (0.6) | 1.9 (0.6) | 2.0 (0.8) | 1.9 (0.6) | **2.1 (0.9)** | **2.1 (0.8)** | **1.9 (0.5)** | **1.7 (0.8)** |
| Spermatid density | 116 (37) | 109 (34) | 116 (43) | 113 (32) | **125 (44)** | **117 (46)** | **109 (29)** | **98 (41)** |
| DSP* | 19.0 | 17.9 | 19.0 | 18.5 | **20.5** | **19.2** | **17.9** | **16.1** |
| No. Animals per total no. animals with sperm density count less than 110 | 14/29 | 17/29 | 14/29 | 12/29 | **11/29** | **12/30** | **17/29** | **17/27** |

*DSP is determined by dividing the spermatid density (testicular spermatid concentration per gram of testis) by 6.10 days (Robb et al., 1978. Daily Sperm production and epididymal sperm reserves of pubertal and adult rats. J. Reprod. Fertil., Sep; 54(1):103-107)

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Cancer Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Kessler and Kruskemper (1966): Male Wistar rats were treated for 2 years with 0 or 1% potassium perchlorate (calculated to 1,339 mg/kg-day) in drinking water. Groups of 6-8 rats were sacrificed at 0, 40, 120, 220, and 730 days of treatment. Thyroid glands were examined histologically. Follicular cell hyperplasia was seen in rats treated for 40 days. Diffusely degenerative changes and increased colloid were seen after 200 days. The 1,339 mg/kg-day dose was considered a LOAEL.

Pajer and Kalisnik (1991): Female BALB/c mice (either 36/group or 12/group; 2 group sizes are given) were given 0 or 1.2% sodium perchlorate (calculated to 2,147 mg/kg-day) in drinking water. Thirty animals died of unknown causes. The At 46 weeks, 42 animals were sacrificed and thyroid and pituitary glands were examined. Increased TSH levels in the pituitary were observed, and thyroid follicular cell carcinoma was seen. The 2,147 mg/kg-day dose was considered a LOAEL.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No. In the studies reported, the mode of action is on the thyroid. The toxicology data are consistent.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

The studies have been discussed but neither study can be considered robust. The 1966 study has only one treatment level and 6-8 animals/group. The 1991 study also has only one treatment level, was conducted only for 46 weeks, and there are 30 animals that died without a report of what happened to them.

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

The studies are minimal studies. In the Pajer and Kalisnik (1991) study, the description of the study is very confusing. The treatment period is not adequately described, and number of animals started vs. the number of animals killed off is nonsense.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

Extra analyses were not done.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

The cancer studies are minimal studies. See the answer to A2.3.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

Yes. Increased TSH and thyroid follicular cell carcinoma were seen in mice.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

Each study had only one dose level, and each dose level was a LOAEL.

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Genotoxicity Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Man Tech (1998); Zeiger (1999a) [There are no 1999 references under the authorship of Zeiger in the list of references on p.11-22.] ; Springborn (1998): A battery of *in vitro* and *in vivo* genotoxicity assays were performed with ammonium perchlorate to determine its potential for interactions with DNA and insight into possible carcinogenicity. The results were confirmed by additional studies and evaluations. Ammonium perchlorate was neither mutagenic nor clastogenic.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

Yes, but a table of the results would help to clarify the results.

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately

evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

Yes. The studies were adequately performed by different laboratories, and repeated. The results were negative with respect to being a mutagen.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

No additional statistical analyses were done.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

The key issues, statements, and conclusions were stated clearly and were supported with sufficient data.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes.

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

It does not matter whether there is a direct effect on DNA or not for a response to occur in the thyroid.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

No effect.

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Short-Term and Subchronic Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Mannisto et al. (1979): Male Sprague-Dawley rats (5-6/group) were given 0, 10, 50, 100, or 500 mg/L of potassium perchlorate in drinking water for 4 days (0, 1.5, 7.6, 15.3, or 76.3 mg/kg-day, respectively). Perchlorate produced significant increases in serum TSH and significant decreases in serum T3 and T4 levels at 15.3 and 76.3 mg/kg-day. [The report does not state if they were dose-related.] At 7.6 mg/kg-day, T3 and T4 levels were decreased significantly and TSH was increased slightly (not significantly). No effect was seen at 1.5 mg/kg-day. With only 5-6/group, and only 4 days treatment, the study is not very robust. The NOAEL was considered to be 1.5 mg/kg-day.

Shigan (1963): Rabbits and rats were given potassium perchlorate at 190 mg/kg-day. [The following items were not identified: method of dosage, number of animals dosed, sex of animals, strain of animals.) Effects (cardiac, liver, immune, and adrenal) were not attributed to rabbits or rats. This study is very limited.

Shigan (1963): Rabbits and rats (number, sex, and strain not identified) were given potassium perchlorate for 9 months at levels of 0, 0.25, 2.0, or 40 mg/kg-day. The method of administration was not identified, and the effect (iodide excretion from the thyroid) was not attributed to rabbits or rats. This study is very limited.

Hiasa et al. (1987): Male Wistar rats (20/group) were given 0 or 1,000 ppm potassium perchlorate in the diet for 20 weeks (80.7 mg/kg-day). Absolute and relative thyroid weights were increased significantly, TSH levels were increased significantly, T4 levels were decreased slightly, and T3 levels were unchanged. The free-standing LOAEL was considered to be 80.7 mg/kg-day. Based on the single dose level tested, without body weights and feed consumption, the study is not very robust.

Gauss (1972): Female NMRI mice (number/group not stated) were given 0 or 1% potassium perchlorate via diet for up to 160 days (2,011 mg/kg-day). Feed consumption and body weights were measured. Thyroid glands were examined at 10-20 day intervals. The histological examinations showed a progressive change from colloid loss, nuclei volume expansion, and rising epithelium height, to hypertrophy and hyperplasia of the thyroid parenchyma. Later during treatment, hyperplastic follicles, areas of adenomatic tissue, adenoma complexes, and cystadenomas were observed, but no progression to malignancy was apparent. The free-standing LOAEL was considered to be 2,011 mg/kg-day.

Caldwell et al. (1995): Sprague-Dawley rats (6/sex/group) were given 0, 1.25, 5, 12.5, 25, 50, 125, or 250 mg/L in drinking water for 14 days (0, 0.11, 0,44, 1.11, 2.26, 4,32, 11.44, and 22.16 mg/kg-day for males and 0, 0.12, 0,47, 1.23, 3.06, 4.91, 11.47, and 24.86 mg/kg-day for females). Thyroids were weighed, histology and morphometry were performed, and thyroid hormone levels were measured. The EPA reanalyzed the T3, T4, rT3, TSH, and thyroglobulin (hTg) levels. The results were analyzed by sex. Relative thyroid weights were significantly increased in groups given the 2 highest doses , but the dose-response is not stated. Perchlorate decreased T4 in a dose-related manner in both sexes. Dose-dependent increases in TSH were observed for both sexes, but females appeared to be slightly more sensitive than males. Perchlorate exposure decreased circulating T3 and T4 and increased TSH. This is the only study in which rT3 and hTg were measured. The report indicates that perchlorate increased rT3 and significantly increased hTg. Free-standing LOAELs were found at 0.11/0.12 mg/kg-day for t3 in females, for T4 and hTg in both sexes, and for TSH in females. With only 6/sex/group and dosing limited to 14 days, this study is not very robust. [Note: On p. 5-20 (line 29), the doses were transformed from 0, 1.25, 5, 12.5, 25, 50, 125, or 250 mg/L to 0, 0.1, 1, 5, 10, 20, 50, and 100 mg/kg-day.]

Springborn Laboratories (1998): Sprague-Dawley rats were given ammonium perchlorate (0, 0.01, 0.05, 0.2, 1.0, or 10 mg/kg-day) by drinking water for up to 90 days. Rats (10/sex/dose) were sacrificed at 14 , 90, and 120 days (after a 30-day recovery). Clinical observations, body and organ weights, feed and water consumption, hematology, clinical chemistry, and ophthalmology were measured. Liver, kidneys, lungs, thyroid/parathyroid, and gross lesions were examined microscopically. No clinically remarkable findings were noted. Only animals in the 0, 0.05, 1.0, and 10 mg/kg-day groups were continued to 120 days. Absolute thyroid weight and thyroid weight relative to body weight and brain weight were increased significantly in males at 10 mg/kg-day after 14 and 90 days of treatment and in females at the 10 mg/kg-day group, indicating a LOAEL of 10 mg/kg-day. Thyroid weight was normal at the end of 120 days. Male rats showed follicular cell

hyperplasia by day 14 [at a dose not identified in the report] which was not fully recovered by day 120. On day 14, females showed decreased colloid and follicular cell hypertrophy at 10 mg/kg. By 90 days, colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia in both sexes were significantly increased at 10 mg/kg-day (LOAEL). By 120 days, thyroid histopathology had been reversed. Upon re-evaluation of the hormone analyses, the LOAEL for T3 effects in males and females was 0.01 at day 90, but there was recovery at day 120 (NOAEL at 10 mg/kg-day). There was a free-standing LOAEL of 0.01 mg/kg-day on day 90 for effects on T4 in both sexes. After 120 days, there was a free-standing LOAEL of 0.05 mg/kg-day in males and a NOAEL of 1.0 mg/kg-day in females for effects on T4.

Estrous cyclicity and sperm motility and morphology were also measured. Estrous cyclicity was evaluated for 3 weeks prior to sacrifice in all females of the 90- and 120-day groups. At 90 days, there was an inverted U-shaped, dose-related response for the absolute number and proportion of females with abnormal estrous cycles (<3 or >5 days). The proportion increased at 0.05 mg/kg-day, peaked at 0.2 mg/kg-day, then declined to 0 at 1 and 10 mg/kg-day doses. At 120 days, females not cycling were increased at 10 mg/kg-day. Sperm samples were obtained from all male rats terminated at 90 and 120 days for evaluation. No treatment-related effects were observed in sperm count, concentration, motility, or morphology.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

They were described, but in several instances, protocol information was missing or very limited. Some of the shortcomings are stated above. E.g., in Mannisto et al. (1979), was water consumption measured? That information is not stated. Treatment of 5-6/group for 4 days does not constitute a robust study. In Shigan (1963), many items are missing, such as number of

animals, sex, strain, and whether the effects were seen in rats or rabbits. The second study by Shigan (1963) suffers from the same problems.
Hiasa et al. (1987) studied a single dose and did not measure feed consumption and body weights. Gauss (1972) also studied a single dose. In the Springborn (1998) study, studies of estrous cycles and sperm parameters were done on a very small number of animals. This is the only study besides the Argus reproduction study in which sperm were studied. There are questions concerning the method of analysis of the sperm parameters; see the discussion concerning male *reproductive parameters in the reproduction evaluation.*

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

The report appears to be adequate, based on the fact that the early studies are less robust and difficult to analyze.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

The authors made additional analyses of the Caldwell (1995) data, but the re-analysis did not appear to change the results. Re-analysis of the subchronic study (Springborn) provided a smaller LOAEL; utilization of the BMD was very well done.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would

improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

Yes. No. No suggestions.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

The data are consistent with the proposed mode of action in that TSH was increased, and T4 was decreased. T3 was either decreased or unchanged. Gauss's study is important in that it showed the progressive change in thyroid histology. Springborn's study was a true subchronic study and a progression of thyroid effects was seen, from colloid depletion to follicular hypertrophy to follicular hyperplasia.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

They are appropriate and based on the available data. The only question involves the use of the 2 Shigan studies. They do not appear useful.

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Developmental Neurotoxicity Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Argus (1998a): Female rats (25/group) were given ammonium perchlorate at 0, 0.1, 1.0, 3.0, or 10 mg/kg-day in drinking water from GD 0 on. Animals were observed daily for clinical signs. Thyroids from all F0-generation rats were weighed and evaluated histologically. On PND 10, blood was collected from dams with no surviving pups or with litters of less than 8 pups for analysis of T3, T4, and TSH. Body weight of F1 animals was recorded on PND 1, 5, 8, 12, 14, 18, and 22, then weekly during post-weaning. Feed consumption was recorded weekly. Pups not selected for continued observation were necropsied on PND 5 or PND 10. [The report confusingly lists both dates for this measurement.] Post-weaning pups selected for continued observation were given ammonium perchlorate at the appropriate dose. Living pups were assigned randomly to one of 4 subsets for additional information: (1) brain weight and neurohistological examination; (2) neurobehavioral tests, and sacrifice at PND 90-92 with blood collection for thyroid and pituitary hormone analysis; (3) motor activity evaluation, and sacrifice at PND 67-69; (4) regional brain weight evaluation on PND 81-86 or neurohistological examination on PND 82-85. Female pups were evaluated for vaginal patency beginning on PND 28 and male pups were evaluated for preputial separation beginning on PND 39. There were no treatment-related effects on adult or F1 feed or water consumption, mortality, clinical signs, body weight, or pregnancy outcome measures. There were no treatment-related effects on F1 sexual development landmarks. There were no effects on brain weight or body weight of F1 pups in subset 1 or 3. Morphometric analyses of brains from subset-1 F1 pups at 10 mg/kg-day showed a 23.4% increase in the size of the corpus callosum in females and 30.2% increase in males (not significant). In subset 4 (PND 82), F1 male pups at 10 mg/kg-day showed 20.9% increase in corpus callosum size, 9.2% increase in frontal cortex size, and 10.2% increase in caudate putamen size, but no effect in females. Significant increases observed in brain components of F1 pups of the 3.0 mg/kg-day group were not considered treatment-related because they were not dose-related. The re-analysis of the data on corpus callosum size showed "normal"

range values according to Argus and a potentially adverse effect according to EPA. Additional analyses of brain morphometry by Geller (1999a) showed significant, dose-related effects in corpus callosum, hippocampal gyrus, anterior and posterior cerebellum, and caudate putamen of F1 pups of the 10 mg/kg-day group. [Note: A clear table of the results of analysis and multiple re-analyses would have been helpful.] F0 dams showed decreased colloid and increases in both hypertrophy and hyperplasia. At PND4, thyroid histology of F1 pups showed colloid depletion and increased hypertrophy at 0.1 and 3.0 mg/kg-day. Hyperplasia was seen at 3 mg/kg-day. Histopathology of animals from the PND 90-92 animals showed variable effects on colloid depletion, hypertrophy, and hyperplasia. T3 and T4 levels were significantly decreased at 3.0 and 10.0 mg/kg-day, and TSH levels were increased at 10 mg/kg-day. On PND 14, a delay seen in the onset of habituation was related to similar effects seen in thyroid hormones that induce delays in developmental landmarks such as eye opening. Behavioral evaluations showed no statistically significant effects, and EPA grappled with the issue of statistical vs biological effects.

Bekkedal et al., (2000): Female Sprague-Dawley rats (unmentioned number/group) were given ammonium perchlorate at 0, 0.1, 1.0, 3.0, or 10.0 mg/kg-day for 2 weeks prior to mating and through PND 10. The day the first pup appeared in the cage was considered PND 1. At PND 5, litters were culled to 8 pups. Litters <8 were eliminated. Motor activity testing (9 different measures) done on PND 14, 18, and 22 showed no effects. EPA re-analyzed the data and compared the results with those of Argus. The Bayesian method of analysis was used. A significant effect on habituation time was found, and a slight increase in motor activity with dose. Based on re-analysis of the data, a NOAEL for motor activity was placed at 1.0 mg/kg-day.

Argus Effects Study (2001): An unstated number of rats/group were given an unspecified perchlorate by an unspecified method for 2 weeks prior to cohabitation and then for an unspecified number of days. The dose levels for this study (0, 0.01, 0.1, 1.0, 30.0 mg/kg-day) are found in a table on p. 5-69, 17 pages after the beginning of the description of the results of the study. Evaluation of the results is difficult without the protocol. Thyroid and brain were evaluated for histology and morphometry on PND 1, 5, 10, and 22 (PND 0, 4, 9, and 21 according to EPA nomenclature). At 30.0 mg/kg-day, the GD 21 dams [from p. 5-53, should these be PND 21dams?] showed decreased colloid, increased hypertrophy and increased hyperplasia. Thyroid weight was significantly affected (increased or decreased?) at 30 mg/kg-day. At PND 21, there was a dose-related trend (increased or decreased?) in colloid depletion, hypertrophy, and hyperplasia in dams. Thyroid weight was increased at 1 and 30 mg/kg-day in PND 21 pups. At GD 21, the LOAEL for T4 and TSH was 0.01 mg/kg-day. At PND 10, the LOAEL for T3 in dams was 30.0 mg/kg-day; for T4,

it was 1.0; and for TSH it was 0.01 mg/kg-day. At PND 22, the LOAEL for T4 in dams was 30 mg/kg-day; and for TSH it was 0.1 mg/kg-day. At PND 22, the LOAEL in pups for T3 was 1.0 mg/kg-day; for T4 it was 0.01 mg/kg-day for males (only); and for TSH it was 0.01 mg/kg-day. Analysis of brain morphometry was done in an attempt to replicate the effects of the 1998 study. Two different analyses of the brain morphometry from the 2001 rat study yielded significant alteration of brain structures at PND 9 and 21 at doses of 0.01 mg/kg-day (LOAEL). The alterations included 23-39% increase in the size of the corpus callosum over controls. The 0.01 mg/kg-day dose was the lowest dose tested, therefore there is no NOAEL for the study. Alteration of brain structures in a laboratory animal is considered an adverse neurotoxic effect.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

The key aspects have been adequately discussed. The only question involves the statistical analysis of the motor activity which was not significant in the Argus (1998) study, and which was redone. In the Bekkedal et al. (2000) study, there was no significant difference in motor activity between groups. Concerning the analysis and re-analysis of the motor activity data, the following questions need to be answered:

1) There is some level of confusion as to the behavioral measure that was of concern. In the beginning, the discussion was centered around "habituation," but there was no information about how the parameter "habituation" was defined or calculated. Discussion then centered on level of measured activity (as "time spent in movement" or "total number of movements"). Discussion then periodically returned to habituation but with the use of varying undefined terms such as "rate of habituation," "habituation interval," and habituation period." A significant amount of attention was devoted to re-analysis of the % increase in number of ambulatory movements.

2) Elements of habituation can be viewed within session as well as between test sessions. If there was in fact a treatment-related effect on the subsystems underlying habituation, analysis of motor activity across test days may provide some additional information.

3) The statement was made that no treatment-related changes were detected in any other behavioral (i.e., other than the suggestive selective change in "motor habituation" of the male PND 14 offspring). Were there any other toxicological findings reported at any of the dose levels used? The Bekkedal study also found no clear statistically significant treatment-related effects, but did find the suggestion of a slightly slower rate of habituation. There is no mention as to whether Bekkedal reported any other behavioral to toxicological findings related to treatment.

In the absence of other signs of neurotoxicity or other toxic manifestations, the most parsimonious conclusion is that treatment did not appear to have a dramatic or robust neurobehavioral toxic effect under the treatment conditions used. If treatment did have any effect on the test for rate of motor habituation (a dose-related trend is apparent in Figure 5-10; was a similar trend noted in the Bekkedal study?), the effect was limited, highly selective, and apparently only suggestive. The EPA's rationale for emphasizing the biological relevance of such a suggested effect is not explained. The only statement with a reference is that an over 50% increase in motor activity in developing animals is not explained. The only statement with a reference is that over 50% increase in motor activity in developing animals is of concern from a biological perspective. This would a significant concern if there was more evidence that the effect was either debilitating, sustained, or accompanied by other associated toxicity/dysfunctional changes.

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

The section was difficult to understand. EPA appeared to be reaching for a positive effect and to be trying to find biological significance out of a non-statistically significant effect. If there had been no effect on brain morphometry, would there have been such interest in finding a neurobehavioral effect?

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

The section was difficult to understand.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

The text of the 2001 Effects Study was the sloppiest and most confusing of all the sections in the report. It is inexcusable to provide an analysis of the results without first providing the materials and methods, and to consistently report that an "effect" occurred without stating whether the effect was an increase or a decrease. It is also very annoying to see "effect" and "affect" used interchangeably; they have different meanings.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

See above.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

Yes.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

See above.

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Developmental Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

The early studies utilized small numbers of animals, brief dosage periods, and single dose levels.

Argus (1998c): Rabbits (25/group) were given ammonium perchlorate at 0, 0.1, 1.0, 10, 30, or 100 mg/kg-day during presumed GD 6-28. The does were assigned to groups by stratified random procedure. Viability was observed twice daily. Body weight, feed and water consumption, clinical observations, deaths, abortions, and clinical effects were monitored. Cesarean sections were performed on GD 29. Blood was drawn for evaluation of T3, T4, and TSH. Pregnancy status, gravid uterine weight, number of corpora lutea/ovary, implantations, resorptions, and live and dead fetuses were evaluated. The fetuses were examined for visceral and skeletal anomalies. The thyroids/parathyroids were evaluated histologically. No dose-related maternal effects were noted. Two does aborted at 1.0 mg/kg-day. One doe at 100 mg/kg-day delivered a full-term litter at GD 27, indicating an incorrect timing of mating. A dose-related but not statistically significant decrease occurred in doe thyroid weight. At 1.0 mg/kg-day and above, there was a clear dose-response for colloid depletion, hypertrophy, and hyperplasia. The fetal NOAEL was 100 mg/kg-day. Maternal levels of T3 and TSH did not differ significantly among groups. At 1.0 mg/kg-day and above, there was a significant decrease in maternal T4 level. No gross terata were reported, and no soft-tissues or skeletal development anomalies were noted. The decrease in thyroid weight and the lack of effects of TSH and T3 levels are difficult to explain.

Argus (2000): Presumed pregnant rats (24/group) were given ammonium perchlorate in drinking water at 0., 0.01, 0.1, 1.0, or 30 mg/kg-day for 15 days before cohabitation and continuing to sacrifice. The animals were assigned to groups by random stratified procedure. There were 19, 19, 17, 20, and 20 pregnant rats per group, respectively. All were sacrificed (cesarean section?) at

GD 21. Gravid uterine weight were recorded, and resorptions, corpora lutea, implantations, and live and dead fetuses were observed. The fetuses were evaluated for soft-tissue or skeletal development in approximately numbers. Dams in the 30 mg/kg-day group showed an increase in localized alopecia. EPA considers this a biologically significant reaction and disagrees with Argus which considers the value to be within normal limits. According to the report, there was a decrease in the number of live fetuses in 3 of the 4 treated groups that was significant at the highest dose. Ossification sites per litter showed reduced ossification at 30 mg/kg-day, which Argus dismissed as reversible delays. EPA disagreed. Without the actual values for the decreased number of fetuses and the delayed ossification, these statements cannot be evaluated.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

No. In the Segment-II study in rats, there is insufficient information and the results are not clear. Also, the wrong NOAEL is stated for the rat study (p. 5-83; the level should be 1 mg/kg-day instead of 3 mg/kg-day).

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

The inconsistencies are addressed. E.g., in the rabbit study, TSH and T3 are not affected, and thyroid weight is decreased. Part of the problem in evaluating the developmental toxicity studies is the lack of actual values and the lack of clarity of the description. The description does not include the specific statistical tests performed by Argus.

I disagree with EPA's statement that they consider the incidence of alopecia in 3 dams to be significant. Alopecia in 3 dams is within normal background limits in my experience. I also disagree with the statement that delayed ossification is always an irreversible effect. It is usually a delay in development, but in most instances the delay is overcome as the animal grows.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

In the analysis of preimplantation loss, it is not clear it the analysis was done on a litter basis or only on the number of implants. There could be clustering effects.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

The description of the rat developmental toxicity study should be rewritten with additional information, particularly with respect to dose response.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

There are few uncertainties. There were no effects on T3 and TSH in the rabbit study, and thyroid weight was decreased; these effects are difficult to explain.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

The rabbit data do not fit the proposed mode of action, in that they showed a decrease in thyroid weight and no effect on TSH and T3 levels.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

Yes.

**SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA**

**Topic Area C:** **Laboratory Animal Studies - Immunotoxicity Studies**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Multiple reviewers (see Table 1)**

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

**A2.1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**A2.2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.

Perhaps it is because I am not an immunologist, but I found the section very confusing. The reports have been adequately described in the chapter, however, from multiple studies and protocols by Kiel (all done in mice), the differences are confusing. It would have been helpful to have a table which shows the differences between the protocols.

**A2.3.** Indicate the strengths and limitations of the analyses performed on the data in Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the findings among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document

adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

In the studies, additional work has been done on both thyroid histology and hormones. What the investigators found is that for hormones, T3 differed from the controls at the 0.1 and 3.0 mg/kg-day levels, but not at the 1.0 and 30.0 mg/kg-day levels. For T4, there was no effect, with a NOAEL of 30.0 mg/kg-day at 14 days, but after 90 days of exposure the LOAEL was 0.1 mg/kg-day. The T4 recovered 30 days after exposure. They found also that there was no effect on TSH but in the histology of the thyroid they found decreased colloid, hypertrophy, and hyperplasia at 30 mg/kg-day.

**A2.4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

In one case, they found additional T3 data not reported in the original Kiel study. They analyzed the data.

**A2.5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusions supported with sufficient data and arguments? How would you suggest improving the clarity of the text. Please make specific recommendations or note revisions that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

In summary, we really don't know very much about the effect of ammonium perchlorate on immune function. In *in vitro* studies, there was suppression of macrophage phagocytosis, but in the *in vivo* studies, there was an enhanced response of the number of plaque forming colonies to sheep red blood cells, and there was also an enhanced response of the local lymph node assay to DNCB. In some cases, they were looking at cell-mediated response and were not looking at humoral immunology.

**A2.6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

Yes.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

They are appropriate.

All the immunological studies were done in female mice. Why not males? Why not rats?

**Topic Area A:** **Hazard Characterization and Mode of Action**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Thomas Zoeller**

**A.1** Have all 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?

They have adequately characterized the toxicity profiles across species (rats, rabbits, mice). Overall, there is good concordance among species, except for the rabbit (developmental toxicity study) which showed decreased thyroid weight and no effect on TSH or T3 levels. Also, some of the rat studies showed different perturbations than those expected.

In general, there is good concordance in histology. Progression is seen of colloid depletion, hypertrophy, then hyperplasia. The only missing studies appear to be 2 good chronic bioassays in 2 different species.

**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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

Yes. It is well known that depression in the thyroid during development can cause effects in neural development of the fetus and in some of these studies histological changes were seen in the thyroid as it moved through a series of stages toward malignancy.

The results also agree with those obtained after dosing with anti-thyroid agents. The agents have been known to affect the developing nervous system as well as affect the ontogeny of behaviors. These effects, however, can vary from increased to decreased depending on the chemical and the age of the animal being tested.

**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?

One obvious inference that can be made is that, if perchlorate does not react directly with DNA but does cause cell perturbations, then indirect effects (such as through RNA, proteins, etc.) are responsible for the cell perturbations that have been observed. The EPA has made significant progress in determining dose metric and in its approach for low-dose extrapolation.

**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 for both.

The approach appears to be protective for both. Both the adult and the susceptible population must be protected. In the adult, chronic exposure could lead to cancer, and the effects in susceptible populations, such as the fetus, the neonate, and the young child, must also be observed. Perturbation of the thyroid can cause *in utero* imprinting which could result in effects on the neonate neural system. In order to have a high confidence level, or RfD, data are needed from all aspects of development, reproduction, and lifetime assessment. This would reduce toxicological uncertainly.

**Topic Area F:** **Human Health Dose-Response Assessment**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Thomas Collins**

**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?

The data developed so far seem to indicate that the mode of action proposed by EPA is in line with the data so far developed on the various forms of perchlorate. The mode of action is mainly the inhibition of iodide uptake in the thyroid. The histological progression in the thyroid has been shown in many studies, including changes in total weight, and changes in histology, including colloid depletion, hypertrophy, and hyperplasia. Effects have been seen in hormones which result in decreased T3 and T4, and perturbation of the hypothalamus-thyroid-pituitary axis. As seen from some studies, there is progression in the thyroid that leads to hyperplasia and cancer. The potential in utero effects in 2 studies have shown that in utero exposure to perchlorate has led to changes in brain morphometry (corpus callosum, hippocampal gyrus, anterior and posterior cerebellum, and caudate putamen). There appeared to be no NOAEL in these studies.

Although the key event is discussed adequately for the known sequelae, the possible effect of the compound on male reproduction is not discussed. A closer, detailed examination and re-evaluation of the testes histology from a cytological point of view needs to be done. It is not clear that this was done in the Argus 2 generation study.

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

Several PBPK models that have been used: human, rat, lactating animal, and pregnant dam. Based on the mode of action and the available model structures, two dose metrics were used: (1) the AUC, which represents an average of the concentration of the serum associated with drinking water exposures, and (2) the percent of iodide uptake inhibition in the thyroid. The AUC appears to be a better measure than peak concentration which is transient and can be difficult to measure.

**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 extrapolation of perchlorate distribution and iodide inhibition at low doses has been adequately characterized by PBPK modeling. Uncertainty factors are applied to arrive at a reference dose. Interspecies and intraspecies uncertainty factors are applied. I think the uncertainty factor of 300 is adequate.

I think that a good chronic bioassay study is needed to clarify tumor development. It would help decrease the uncertainties and it would complete the toxicology profile of the compound. An even better choice would be a chronic bioassay study done in animals exposed in utero.

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

All the stages of cancer development have been observed and 2 studies have evaluated the effects on brain morphometry.

The possible effects on the testes in F1 generation rats have not been accounted for in the report. Dose level of 0.3 mg/kg-day was the LOAEL in the 2-generation study. Do doses <0.3 mg/kg-day cause any testicular effects? What is the NOAEL for testicular effects?

A very robust chronic bioassay needs to be done, as mentioned above.

**Topic Area G:** **Risk Characterization**
**Reviewer for Question G.1:** **Thomas Collins**
**Discussion leader:** **Ron Wyzga**

**G.1** Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

Yes. The chapter emphasizes what is known and summarizes how much is yet unknown about the distribution in the environment, uptake or not, concentration paths, etc.

The main problem with the compound is that of the thyroid effects. At the present time, it does not appear to be a large problem, but if perchlorate concentrations continue to build in the environment, the problem could become substantial. This is particularly important if it is shown that the compound is concentrated under specific conditions. Data are not yet available to validate or refute this possibility.

The risk of direct exposure via drinking water has been adequately characterized. The concentrations have been found in some public water supplies, but the known area of perchlorate distribution is limited at this time. Perchlorate has been detected in groundwater and surface waters in areas where there has been munitions manufacturing, solid rockets, etc. Indirect exposure via irrigated crops has not been completely characterized. Uncertainties remain in soil evaporation and concentration, uptake by aquatic organisms, uptake by vascular plants, effects in herbivores, and possible effects in carnivores.

**Topic Area H:** **General Comments, Conclusions, and Recommendations**
**Reviewer:** **Thomas Collins**
**Discussion leader:** **Ron Wyzga**

**H.1** Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Questions on the possible effects on male reproduction have been raised within the section on multigeneration reproduction. A review of the testicular slides for cytological differences should be done and there should also be a review of slides from other perchlorate studies in males (if available).

Studies of the long-term effects over several generations have not been done. The only chronic human studies available are flawed. Since we are concerned about *in utero* imprinting, it might be worthwhile to do a 2-year chronic study with adequate tests to complete the RfD for perchlorate. This study was introduced in the previous response to F.3. What is envisioned is an *in utero*/carcinogenicity study in which the lifetime effects of perchlorate are measured. The test animals are exposed from before birth to senility. This study includes measurement of effects in the older population, a susceptible population which is increasing in this country. This type of study is recommended by the Food and Drug Administration for some compounds (guidelines are found in the FDA's Redbook).

## Anthony Cox

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| TOPIC / CHARGE QUESTIONS | COMMENTS / RESPONSE |
|---|---|
| A.1 Have all relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized?<br><br>Have the similarities and differences in the toxicity profile across species been adequately characterized? | The toxicokinetics component looks relatively strong and well-validated (p. 6-30). Relevant toxicokinetic appear to have been identified and appropriately used in PBPK modeling.<br><br>Similarities and differences in the toxicity profile across species have *not* been adequately characterized. They are only briefly discussed in Section 3.4, without adequate detail or references, though their relevance and importance is recognized in qualitative statements (e.g., p. 3-18).<br><br>• Toxicodynamics and interspecies differences in thyroid development and responses are discussed qualitatively (e.g., pages 3-16, 3-19, but relevant citations should be added to the discussion.<br><br>• The paragraph on p. 3-18 ending "Any comparison of thyroid carcinogenic responses across species should be cognizant of all these factors" should be expanded to give more details and references. Specific references and additional toxicity profile information – especially comparing responses in humans and rates – should be added, starting around p. 3-18. (See e.g., McClain RM, Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat Res.* 1995 Dec;333(1-2):131-42.)<br><br>• Since Wistar rats are known for their high spontaneous rates of endocrine organ neoplasms (e.g., Bomhard E. et al., Spontaneous tumors of 2000 Wistar TNO/W.70 rats in two-year carcinogenicity studies. *J Environ Pathol Toxicol Oncol* 1986 Sep-Dec;7(1-2):35-52), it seems especially important to consider *differences in pharmacodynamic responses and toxicity profiles* in using rat data as a basis for human risk assessment.<br><br>• Toxicodynamic information does *not* appear to have been appropriately used in the quantitative risk modeling. Although Chapter 6 does a nice job on PBPK modeling, pharmacodynamic aspects that are crucial for understanding carcinogenesis seem to have been ignored in the quantitative risk modeling.<br><br>• The science policy position on p. 18 that "In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer caused by thyroid-pituitary disruption" is potentially inconsistent with available |

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| | data on perchlorate effects in humans and rats and with the statement on p. 3-19 that "There is evidence that humans may not be as sensitive quantitatively to thyroid cancer from thyroid-pituitary disruption as are rodents." |
| | |
| A.2 Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data | The roles of the key event and subsequent neurodevelopmental and neoplastic sequelae *are* clearly articulated (see e.g., Figure 7-2, p. 7-5) and seem consistent with available data in rats.<br><br>However, the relative importance of the key event and of subsequent neurodevelopmental and neoplastic sequelae are less clearly articulated, especially for purposes of comparing rat responses to human responses. (For example, do rat thyroids grow throughout life, while human thyroids do not, and does this create a potential for subsequent neoplastic development following perchlorate dosing in rats but not in humans?)<br><br>The key question of why "Acute exposure to ionizing radiation, especially in childhood, remains the only verified cause of thyroid carcinogenesis in humans" (Hard, 1998) while perchlorate and other chemicals cause thyroid carcinogenesis in rats, has not been explained in terms of clearly articulated roles for the key event and subsequent processes. |
| | |
| A.3 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? | At least for carcinogenesis, the AUC for dose is probably less relevant than the duration and magnitude of precursor responses (e.g., compensating hyperplasia) induced by the dose. Possibly, the AUC for precursor responses such as upregulation of TSH would be more predictive as an indicator of biologically effective dose than the AUC of perchlorate. |
| | |
| A.4 Is the harmonized approach to characterize the potential risk of both noncancer and cancer toxicity protective for both? | Yes. (It seems likely that the excess cancer risk will be zero or undetectable when there is no noncancer toxicity. The noncancer toxicity is addressed using "precursors" that, in fact, may not be associated with any increased risk of harm, i.e., it is not clear that they really are "precursors", in the sense of being on the causal path leading to harm. So, if anything, the harmonized approach may be over-protective for both noncancer and cancer end points.) |
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| B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? (See Table 2 and Attachment 1) | Yes. None of the studies published since 1999 that I have reviewed or am aware of has yet overcome the basic limitations on lack of good human exposure data, well-controlled confounding, adequate power, etc. Moreover, none of them has focused on the quantitative pharmacodynamic processes needed to complete the PBPK front end and form a full biologically-based risk assessment model for |

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| | perchlorate. The new studies and letter of Greer and Braverman are consistent with other literature in suggesting potential points of departure for human health risks that are much higher than the rat-based value; however, the QA/QC audit by Merrill reveals several potential weaknesses in the data. None of these studies changes the general point that rat-based studies suggest a point-of-departure for RfD calculations that may be much smaller than those from (limited and imperfect) human data. The new studies do suggest some possible numerical values that might be used in sensitivity analyses as possible values for a human-based point-of-departure (recognizing that there are many uncertainties and limitations of the human data.) |
| | |
| B.2 How well has EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment? (See Attachment 2) | The discussion for the most part seems fair and balanced. However, the contrary view of Soldin et al. (2001), see p. 4-30, deserves more discussion. The unexpected findings of Crump et al. (2000) also deserve more discussion and perhaps more weight in the quantitative risk assessment. Simply noting that there are unexplained contradictions and indications that risk is much lower in humans than might be expected based on extrapolation from rates does not fully interpret the significance of these results for quantitative risk assessment. As discussed later, the epidemiological studies might perhaps be useful, despite their strong limitations, to help develop a sharper plausible upper bound on human risks if Chapter 6 can be revised to include dose-response information about the predicted levels of exposures for which human health responses *are* predicted to occur. |
| | |
| B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization? | Yes. The summary of ecological studies (Section 4.1.1) seems generally well done. The fact that other reviewers find little cause for concern is acknowledged (p. 4-30).<br><br>However, although the epidemiological data are too weak to serve as the sole basis for an adequate hazard characterization, this does not mean that they should not be used at all. In general, the epidemiological studies do seem to be quite reassuring, showing that cancers and other adverse effects do not seem to occur at detectably elevated rates even at relatively high exposure concentrations. This suggests that the assumption that people are as sensitive as rates should probably be modified. It is consistent with the (animal?) evidence referred to (but not specifically cited) on p. 3-19. |
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| 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? | Perhaps some meaningful bounding could be done if the predicted dose-response estimates were more explicitly stated. While the limitations of ecological studies and lack of individual-level exposure data in normal human populations cannot easily be overcome, it does seem that humans are not developing thyroid tumors at the rates that would be expected if 0.00003 mg/kg-day (p. 10-3) were close to (i.e., within a couple of orders of magnitude of) exposure levels at which significant excess cancers occurred.<br><br>Despite their severe limitations, the data in the epidemiological studies might indeed be sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies *if* these predictions were clear and testable. But the numerical value of 0.00003 mg/kg-day given on p. 10-3 is a level at which appreciable risk is expected to *not* occur. It is notoriously difficult to test such a negative, even if epidemiological data were much stronger than they are.<br><br>To use the epidemiological data to provide a sanity-check on the risks extrapolated from animal models, it would be valuable to add to Section 10.1.2 some predictions about the lowest exposure levels for which detectable risks *are* expected to be seen in human populations. Then, these can be compared to realistic exposure levels (e.g., in California) to see whether the predictions are consistent with epidemiological observations.<br><br>In general, the proposed 0.00003 mg/kg-day level (p. 10-3) is much less than the estimated level of 35 to 100 mg/kg day "at which thyroid hormone levels may [begin to be] be reduced or thyrotropin levels increased" suggested by Soldin et al. (2001). Industry vs. regulatory perspectives aside, this gap is large enough to deserve some additional discussion. |
| | |
| 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? | The absence of positive associations observed in various (mainly ecological) epidemiological studies *is* consistent with the proposed (non-linear, indirect) model of action.<br><br>In general, power has *not* been sufficient to detect small effects (e.g., relative risks < 2). Confounders have *not* been controlled. |
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| C.2 How well has EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment? (See Attachment 2.) | The correlation analyses in Appendix 7A are a very blunt tool for analyzing these interesting data. The problem with correlations (ordinal or Pearson's) is that they consider only pair-wise associations. Multivariate interaction and response surface modeling techniques, such as classification trees (CART), MARS – or, better, dynamic models of the relations among T3, T4, and TSH over time – could add much more information and insight than correlation analyses. For an analysis that has been worked on so hard in many parts, it seems important to probe beyond mere correlations, which are necessarily at best quite superficial indicators of multivariate and dynamic relations. |
| | |
| C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate? | Yes, for toxicokinetics. Pharmacodynamics have not been modeled. |
| | |
| C.4 Are the NOAELs/LOAELs appropriate? Please explain. | The proposed 0.00003 mg/kg-day level (p. 10-3) is much less than the estimated level of 35 to 100 mg/kg day "at which thyroid hormone levels may [begin to be] be reduced or thyrotropin levels increased" suggested by Soldin et al. (2001). This gap is large enough to deserve additional discussion.<br><br>It is not clear that the NOAELs/LOAELs are appropriate *for human* risk assessment. Determining whether they are appropriate requires better quantitative modeling of relevant (or at least proposed) *pharmacodynamic* processes in rat and human thyroid glands. This is perhaps the most important gap in the current risk assessment.<br><br>The relevance of the 0.01 mg/kg-day LOAEL for *humans* is unclear. |
| | |
| D.7 Comment on the strengths and limitations of the available data to suggest sources of perchlorate exposure other than drinking water. | Data are very limited. The current report does a good job of documenting the limitations in the available ecotoxicity data and exposure data for non-drinking-water paths (e.g., via inhalation). |
| | |
| 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? | The proposed point of departure of 0.01 mg/kg-day (p. 10-3) is of uncertain relevance for *humans*. It may be too high by at least several orders of magnitude. |

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| 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? | It might be valuable to consider several other points of departure, including those suggested based on human data (while acknowledging their limitations). Presented as a form of sensitivity analysis and a guide to relevant uncertainties, a multiple starting-point analysis might be quite effective. |
| | |
| F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric. | Although the PBPK model in Chapter 6 seems to be well thought out and well developed, PBPK considerations alone do *not* justify the selected AUC dose metric, nor do they provide an adequate basis for interspecies extrapolation of risk (of RfD vales). The reason is that the toxico*dynamics* are also likely to be very important – enough so that they might dominate the analysis. Rather than using a PBPK-based AUC to accomplish interspecies dose conversion, it is worth asking first (in the hazard identification section) whether *any* HEE truly exists (e.g., do people develop thyroid tumors in response to perchlorate tumors at any level?) If it is assumed that human responses are similar to rat responses, then it may still worth using intermediate responses such as the AUC under the time course of compensating hyperplasia (plotting excess mitoses per unit time over time) as a more predictive dose metric than dose AUC.<br><br>In summary, the current quantitative modeling and interspecies extrapolation of doses essentially stops with PBPK results, but pharmacodynamics are likely to be crucial and should be considered in the quantitative modeling in order to better understand and appropriately represent interspecies differences. |

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| F.3 Are there other data which should be considered in developing the uncertainty factors?<br><br>Do you consider that the data support the values proposed or different values for each? | The epidemiological data suggest that uncertainty about pharmacodynamics may be important (i.e., people may be much less susceptible to various adverse health effects) than extrapolation form the rat data indicates.<br><br>Whether or not there are other *data* that should be considered in developing the uncertainty factors, other *modeling* techniques should definitely be considered. Model uncertainty seems very important (see e.g., p. 7B-6), and approaches such as model cross-validation and Bayesian Model Averaging (BMA) that account for it should be used in the uncertainty analysis.<br><br>At the risk of recommending something politically impossible, I believe it may make sense to consider allowing some uncertainties to *increase* the estimated RfD. For example, uncertainty about whether people can develop thyroid tumors at all in response to perchlorate exposures might be addressed by multiplying the point-of-departure RfD by a factor of 3 (or 10 or more). |

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| Do the confidence statements accurately reflect the relevancy of the critical effects to humans and the comprehensiveness of the database?<br><br>Do these statements make all the underlying assumptions and limitations of the assessment apparent? If not, what needs to be added? | More generally, the IRIS uncertainty factor methodology and confidence statements seem to me to obscure what is known and how well it is known (i.e., if the only possible responses to uncertainties are to divide by 3 or by 10, no matter what the evidence, then the resulting numbers don't indicate much about the evidence.) I would prefer to see an attempt to create a distribution for key quantities such as the NOAEL in humans, and then have this distribution used as the starting point for risk management decision-making. But this critique is really directed at the IRIS approach to expressing (or not expressing) uncertainty. It is not specific to perchlorate. Given that the IRIS methodology must be used, I think it is very hard (perhaps impossible) to make useful uncertainty statements or to give RfD values that are well supported by the data. It seems to me that the uncertainty factors tend to overwhelm the data with more or less arbitrary values.<br><br>No. One big uncertainty is how perchlorate acts on humans. (For example, are the "precursor lesions" summarized on p. 10-3 actually precursors of anything worse?) Because the available epidemiological data suggest an absence of some effects in humans that might be expected based on rat data, the confidence that 0.00003 mg/kg-day is unlikely to cause detectable harm in humans should probably be high.<br><br>The statement that "Confidence in the principal study is medium" (p. 7-30) does not indicate that human data suggest that humans may be much less responsive than rats – and that confidence that the RfD is protective should therefore be increased.<br><br>The statement on p. 7-26 that "a derivation based on the available human data would estimate the RfD.. in rather good agreement with that proposed based on the laboratory animal data. The consistency... is likely due at least in part to the use of AFRL/HEST PBPK modeling" seems to me to be quite a stretch. It would be better left out, as it indicates a form of corroboration that I think is not really there. When the numbers can be tweaked by a factor of $3^5$ to $10^5$ or so (or more if needed) in fairly ad hoc ways to "reflect uncertainties", it is not hard to get almost any two initial estimates (within about 10 order sof magnitude of each other) to "match". This should not be taken as an indication of validity in the estimated numbers. |
| | |
| F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment? | No. Do rat thyroids grow throughout life while human thyroid normally do not? How do normal tissue kinetics affect the susceptibilities of different species and of different sub-populations within species? |

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| G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures? | Unknown. The human health risk posed by perchlorate probably depends on pharmacodynamic and cell kinetics aspects that have not yet been modeled. The PBPK model and risk estimates based on it are probably *not* adequate to "clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures". Pharmacodynamics really must be considered as part of the quantitative modeling in ordre to satisfy this criterion – especially given the potential discrepancies between human epidemiology and risks extrapolated from rat data. |
| | |
| H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions. | Specific references and additional toxicity profile information – especially comparing responses in humans and rates – should be added, starting around p. 3-18. (See e.g., McClain RM, Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat Res.* 1995 Dec;333(1-2):131-42.) |

# Teresa Fan

Fan

D.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

I. Condike (2001). Perchlorate data in fish and plants [letter with attachments to Annie M. Jarabek]. Fort Worth, TX: Department of the Army, Fort Worth District, Corps of Engineers; December 21.

- No analytical detail was given to evaluate the accuracy and precision of the analysis. It is interesting to note that the detection limit in dry tissue was 50-170 ppb, which is lower than that (400 ppb) reported in Parsons Engineering Science (2001) document. Why was the reported detection limit varible for Table 1-3?
- It is interesting to note that the perchlorate concentration was consistently higher in head than fillet of fish (Table 1-3, X). This suggests that perchlorate may be more readily taken up by neural than muscle tissues.
- The perchlorate concentration in algae appeared to deviate from that in bulk water. Perchlorate concentration in algae was high (5.5 mg/kg dry wt) when no perchlorate was detected in the bulk water, while perchlorate concentration in the bulk water was 440 µg/L when no perchlorate was detected in the algae. This is contrary to the conclusion made in the Parsons Engineering report (2001), i.e. perchlorate concentration in bulk water is correlated with that in vegetation.

II. EA Engineering (1999). Results of algal toxicity testing with sodium perchlorate. Sparks, MD: EA Engineering, Science, and Technology, Inc.

- There was no statistics given for replicates in Table 1.
- There should be test done on other representative algal classes.
- Perchlorate burden in *Selenastrum* should be analyzed to determine the actual exposure level. It is possible that this alga is resistant to perchlorate toxicity by excluding it from uptake.

III. EA Engineering (2000). Results of chronic toxicity testing with sodium perchlorate using *Hyalella azteca* and *Pimephales promelas*. Sparks, MD: report number 3505.

- If disruption of material thyroid hormone production is the main mode of action of perchlorate, a more appropriate test would be to expose egg-bearing fish to perchlorate and observe the effect on subsequent embryonic and larval development.
- Why was the oxygen and conductivity range (2.2-8.8 mg/L) in the test for H. azteca more variable than that for P. promelas (Table 1)?

- How much perchlorate was adsorbed to the sediment in the H. azteca test? What was the property of the sediment used (e.g. organic content)? These factors may affect the availability of perchlorate to the organism.
- The perchlorate burden in the test organisms should be measured to assess the actual exposure level.

III. Parsons Engineering Science, Inc. (2001) Scientific and technical report for perchlorate biotransport investigation: a study of perchlorate occurrence in selected ecosystems. Interim final. Austin, TX; contract no. F

- This report did not include a site in California which is known to have major perchlorate contamination in public water supply and groundwater.
- The vegetation description was lacking for Site 5 (p. 2-17).
- Water samples were not taken for Location 1 or 2 of Site 5 due to no flowing water. Was there any soil sample taken? These two locations are the closest to the perchlorate source (LVOA) and their extent of contamination should be documented.
- P. 2-19 was missing.
- The decanting method for collecting pore water from the sediment (p. 2-23) is questionable. The pore water may largely consist of the overlaying water rather than the interstitial water.
- Root samples should be collected (p. 2-23) and analyzed since these tissues better represent perchlorate exposure to soil organisms.
- I am not sure that the assumption that perchlorate is very stable in the ecological media (p. 2-25) is justified with the present state of knowledge. If perchlorate can be transformed by plants and microbes, the possibility exists that it can also be altered in other biological systems.
- Please define "statistical limits" in Table 2.9 (p. 2-32). It appears that the perchlorate data on non-spiked and spiked pair were less reliable, which suggests matrix interference.
- It's unclear whether there was residual perchlorate in soils or sediments not released by the water extraction method. Both media should be subjected to a harsher extraction procedure (e.g. strong acid digestion) to see if additional perchlorate can be released, which would suggest strong adsorption.
- Some of the data entries should be considered as "detected", e.g. surface water at Location 5-7 had a mean of 400 µg/L (Table 3.6), which is well-above the 4 µg/L report limit.
- On p. 3-36, perchlorate concentrations in terrestrial vegetation and soil were 367 (instead of 36.7) and 138 (instead of 58.8) µg/kg, respectively at location 7. The values for aquatic vegetation and sediment at locations 7 and 4 were also inconsistent between the text and Table 3.7.
- The mobility of fish makes it difficult to interpret the body burden data since fish caught from a contaminated location may have been foraging in clean habitats elsewhere. Fish stomach content may provide some clue.

- The description on quality control regarding sample matrix spike is unclear and it is difficult to evaluate whether there was matrix interference or not.
- In data validation summary report, the sediment samples were reported to contain "an excessive amount of water". To obtain a more representative sediment sample, the water present in the sample should be removed by high-speed centrifugation which was not performed.

IV. Susarla et al, 2000:

- No pH adjustment of the plant growth solution was mentioned. If pH was not adjusted, plant growth can drive the solution pH acidic, which could in turn affect normal plant growth and ion uptake.
- It is unclear which treatment concentration resulted in depletion of perchlorate to < 10 ppb final concentration.
- Was the sand saturated with water? If so, the bottom sand layer could become hypoxic, which could in turn affect perchlorate transformation by anaerobic microbes.
- The conclusion that perchlorate was metabolized solely by plants is questionable since plant roots were not sterilized, which will be difficult to do in any rate.
- It is odd that perchlorate was not taken up as readily in aqueous treatment as in sand treatment. The authors did not have any explanation for this.
- Is it possible that a portion of the perchlorate was adsorbed onto the sand due to the influence of plant root exudate?
- Was the perchlorate depletion from the media mass-balanced by the amount accumulated in plant tissues? If not, it suggests that perchlorate could be lost to the sand matrix.
- How much was the transformation products present? It is difficult to judge whether these transformation processes were significant without quantitative information. In addition, chloride is a normal component of plant tissues and cannot, *a priori*, be assumed to be one of the transformation products.
- No statistics was given for replicate treatments.

V. Susarla et al, 1999:

- Continuous light was used for plant growth, which could cause abnormal physiology in plants.
- There was no control data shown for the 0.2 and 2 ppm perchlorate treatments. These data should be shown to help evaluate whether sorption process contributes to the depletion of perchlorate from the media.
- It is interesting to note that the perchlorate depletion time course of the media was similar for the sand and aqueous treatments at 20 ppm perchlorate concentration (Fig. 3) and yet the perchlorate concentration in the plant tissue was about 2 fold less for the sand than the aqueous treatments. This discrepancy was not explained in the paper and could result from perchlorate sorption to the sand matrix. The authors also indicated that 54-60% of

perchlorate in solution was adsorbed to the sand in a separate experiment.

- It is difficult to visualize that the rate constant for the 20 ppm/sand treatment (0.017) was less than 1/5 of that for the 20 ppm/aqueous treatment (0.09).
- There was no structure confirmation of the perchlorate metabolites. The sum of perchlorate in root, leaves, and stem (Table 2) did not add up to the perchlorate concentration of whole plant tissues in Table 1. Why not?
- It is difficult to reconcile that chloride was not detected in the sand cultured plants (Table 1) since chloride is a normal component of plant tissues.
- There was no consideration for foodchain transfer of perchlorate accumulated in parrot-feather to herbivores. If this plant were to be used for remediation purpose, such risk needs to be addressed.

VI. Nzengung, n.d.:

- The faster initial kinetics for perchlorate disappearnce in sand culture than in solution culture raises the question whether some perchlorate could disappear from water by adsorption to the sand, although the author concluded that perchlorate was not sorbed by the sand (with no data provided). On the other hand, another study (Susarla et al, 1999) indicated that 54-60% of perchlorate in solution was adsorbed onto the sand. This discrepancy needs to be addressed. It is possible that perchlorate may have different interaction with different types of sand. It is also possible that perchlorate does not interact strongly with sand in the absence of plant roots and associated microbes but gains affinity towards released organic matter (i.e. input of root and microbial exudates) when plants and microbes are present.
- It is still unclear regarding the extent to which plants degrade perchlorate. One cannot rule out the contribution of microbial activity to the perchlorate degradation observed by incubation with minced plant tissues or extracts, since microbial activity was not eliminated. Particularly, as the author indicated, microbes (possibly nitrate-reducing microbes) exhibit very high activity in perchlorate degradation.

VII. Smith et al., 2001:

- Insufficient details were given for the method developed for perchlorate analysis in biological samples, e.g. recovery, effectiveness of sample clean up, linearity of the analysis.

D.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

- The EPA document was generally well-written with appropriate presentation, analysis, and interpretation. I have listed below a few more aspects that should be discussed.
- Fish also has a hypothalamus-pituitary-thyroid axis and a similar mode of action of perchlorate on disrupting thyroid function could apply. There should be discussion on mechanistic study on representative fish species, similar to that on mammals to see if perchlorate could impact fish embryonic and larval develeopment via disruptiing maternal thyroid function. The gross effect observed on *Pimephales* lavae (described on p. 8-17) warrants this concern.
- Could perchlorate interfere with uptake and metabolism of anions other than iodide (e.g. sulfate, nitrate, or silicate)? For example, the presence of nitrate seemed to interfere with perchlorate degradation (Nzengung, n.d.). Interference with silicate uptake could preferentially impact plants and algae that have a higher requirement for silicate than *Selenastrum*. There should be discussion on these aspects and recommendation on broadening the test on plants and algal species.
- Perchlorate was found in seeds (Smith et al., 2001), which indicates that both xylem and phloem-mediated transport processes could occur
- The document assumed that perchlorate does not have a significant affinity to soils. This assumption needs to be verified. There is some indication that perchlorate is sorbed to soils and the extent of sorption appears to depend on pH and organic content (Susarla, S., Wood, G., Lewis, S., Wolfe, N.L. and McCutcheon, S.C. (1999) Adsorption characteristics of perchlorate in soils. Abstracts of Papers American Chemical Society, 218, ENVR 4.)

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.

- The assays selected were performed on standard test organisms under laboratory settings. Although they are useful in revealing the toxic threshold of perchlorate on the test organism, it is somewhat difficult to relate these test results to ecological effects. In particular, no perchlorate burden data was provided for the test organisms, which makes it difficult to compare the actual tissue exposure level in these tests to that measured in wild organisms. Organisms could exhibit varying toxicity threshold, depending on their ability to exclude (either via prevention of uptake or depuration) and/or transform perchlorate.
- These assays do not provide information on species difference in perchlorate sensitivity, nor do they reveal information on modes of action. For example, it is possible that perchlorate disrupts thyroid hormone production in egg-bearing fish, which can then lead to abnormal egg and larval fish development, as in the case for mammals. This aspect has not been tested.
- The route of exposure conducted in these assays is via water. There is good indication that the route of exposure in the actual environment is a composite of water, sediment or soil, and diet. Again, how this difference in exposure route affect perchlorate uptake and

metabolism is unclear.

- Moreover, different environmental factors such as pH, ion composition, redox state, richness of organic matter, etc. could affect perchlorate uptake and availability but this knowledge is currently lacking.
- The site-specific analyses are useful in identifying potential ecological effects of concern, although it would be difficult to quantitatively evaluate such effects from the laboratory-based assays (see above). More information on species-dependent perchlorate effect and mode of action is needed to conduct quantitative analysis.

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 goal of the ecological screening analysis along with the questions that may be answered is described on p. 8-1 & 8-2.
- The assessment endpoints for various ecological receptors are described on p. 8-5 to 8-6.
- The objectives and goals were met to some extent. In terms of perchlorate exposure, there were much more data (since 1988) on various ecological receptors for such ecological screening analysis. However, it is difficult to relate these field data to laboratory toxicity assays of perchlorate since no comparable exposure data was acquired for the former. In addition, for lack of understanding on the mechanism(s) of perchlorate uptake and effect, it would be difficult to extrapolate the laboratory assessment to ecological analysis.
- There was no literature cited to substantiate the assumption that perchlorate "absorbs weakly to most soil minerals" (p. 8-9). Whether perchlorate binds to soils appears to be controversial (see comments on study by Nzengung, n.d in D1).and premature to conclude. The question of whether perchlorate interacts with different soil or sediment systems is important to its fate, transport, and bioavailability, and therefore ecological effects.
- In terms of uptake by vegetation, all analyses were performed based on disappearance of perchlorate from aqueous phase. Such practice may be valid on solution cultures, but other confounding factors (e.g. sorption to sand matrix) may affect the accuracy of the uptake kinetics. Such limitation was not mentioned in the ecological screening analysis.
- There was no mention of perchlorate uptake into macro- and micro-algae, which would also be important for assessing exposure and effects in the aquatic foodweb.

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

- This is a well-written document with the analyses, summary, and conclusion clearly described. Many of the relevant and important aspects of uncertainty have been addressed sufficiently. Some of the limitations that have not been addressed in the document are stated in D.3 and D.4.
- The potential route of exposure through citrus (e.g. Sourthwestern region) and imported fruit and

vegetables was discussed but not indicated as an aspect needed to be investigated. Since little information on the perchlorate occurrence in these food items, they should not be discounted as a potential route of exposure to human.

- Since none of the ecological screening studies address perchlorate effect on species richness or population, it would be difficult to state that "the likelihood of effects on the richness and productivity of fish, aquatic invertebrates, and plant communities appear to be low".
- Since the knowledge on foodchain transfer potential of perchlorate is lacking, it is possible that herbivorous aquatic fish and invertebrates can bioaccumulate perchlorate from surface water via feeding on aquatic producers. If perchlorate has affinity for organic matter, the potential for bioaccumulation via detritus, particularly for detritovores, should be addressed. These aspects were not mentioned in the assessment of risks to consumers of aquatic life (p. 10-8).
- The uncertainty associated with dietary exposure was not mentioned (p. 10-11).
- Because of the uncertainty on the fate, transport, and foodchain transfer of perchlorate as well as the limitations in relating laboratory toxicity test to field exposure level, I don't think that the available ecotoxicological information on perchlorate is quite sufficient for screening-level ecological risk assessment.

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.

- There is good indication that perchlorate in water and soil is transferred to plants and other biota.
- More specific pathways of perchlorate biotransport is unclear.
- There is some indication that perchlorate is transformed by plants and microbes. However, the extent, generality, and the pathway of the transformation is less clear (see comments on the two Susarla's paper in D1).
- The transport from sediment to biota is presently unclear.
- Whether perchlorate is transformed in animals is unclear.
- One cannot exclude the possibility that perchlorate can be sorbed by soil, particularly by humic substances. There could be localized positive charge clusters on the surface of soil organic matter and minerals for perchlorate sorption to occur. It is interesting to note that the IC25 for lettuce was higher in soil than in sand (p. 8-22), which could be in part attributed to the influence of the higher organic content of the soil. Even in sand, perchlorate has been reported to be sorbed (Susarla et al., 1998). (see also comments in D.2. and D.4.).

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

- The report on a wide occurrence of perchlorate in various ecological receptors (e.g. Parsons Engineering report, 2000; Smith et al., 2001; Condike, 2001) in perchlorate contaminated sites indicate that there are exposure routes other than drinking water for wildlife.
- There is indication that vegetation is an important mediator of soil exposure.
- However, it is unclear how and to what extent these exposure routes affect human exposure, especially via citrus and imported fruits and vegetables.
- It is unclear regarding the spatial and temporal influence on these exposure pathways, nor is it clear on the interaction among individual components of the exposure pathway (e.g. diet-related transfer and transformation of perchlorate through different trophic levels).
- It is unclear on the effect and extent of exposure to perchlorate transformation products such as chlorate, chlorite, and hypochlorite.

G.2 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the ecotoxicological risk posed by potential perchlorate exposures?

- The risk characterization chapter clearly summarize the salient aspects of the ecotoxicological risk of perchlorate based on existing data. The one aspect that was not described adequately is the teratogenesis assay on *Xenopus*. It appears that perchlorate has effects on thyroid function, metamorphosis, and sex ratio in developing Xenopus laevis but no quantitative description of the data was supplied. This could be important for the ecotoxicological assessment since perchlorate has been detected in amphibian at a mean of 934 µg/kg at Site 6.
- There are, however, several risk factors (see D.5.) that are not discussed sufficiently, which cannot be addressed by the available data.

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

- The extent in which ecologically important aquatic macroalgae can accumulate perchlorate is unclear. This poses another uncertainty in the potential for indirect exposure in wildlife.
- The mechanism of perchlorate uptake and accumulation in primary producers was not discussed adequately. If better understood, this knowledge can help assess perchlorate exposure risk in ecological receptors.
- Abiotic transformation of perchlorate (e.g. under anaerobic conditions where hydride is abundant) is a possibility not discussed.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

- On p. 9-11, what does "6-6-18" mean?
- On p. 9-15, "the lettuce irrigated with 10.0 ppm perchlorate" appears to be in error. It should be 10 µg/L or 10 ppb.

# David Hoel

**Topic Area A: Hazard Characterization and Mode of Action**

**A.1** Have all 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?

Answer: I did not search for toxicology studies and thus have no comment here. With regard to characterizations it seems that similar experimental data should be compared and analyzed jointly. Specifically I think of the various studies in which thyroid hormone measurements are made in the SD rat at 14 days. With respect to the statistical methods normal theory is used with ANOVA and paired comparisons. Monotonic dose-response methods including nonparametric techniques should be considered. Also some of these methods allow for the estimation of the LOAEL level. In particular consider the Williams test, Jonckheere test, non-parametric trend test etc. As far as differences in studies consider Caldwell 1995 and Crofton and Marcus 2001 both using the SD rat. The control values for T3 are the same for male and female in one study and the males much greater than females in the other study. Also for me the standard errors on all the observations are surprisingly very small in these studies.

**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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

Answer: I am not expert on this issue but it seems to be correct. The neoplasia issue is somewhat confusing to me since my understanding is that medically a cancer is consider to be a malignant tumor and not a benign lesion.

**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?

Answer: Since perchlorate has not been shown to be a carcinogen (see A.2) or a mutagen there is not the need to assume a "linear no threshold" approach toxicity.

**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 for both.

Answer: I do not see evidence of cancer toxicity with this material.

**Topic Area B: Human Health Effects Data**

**B.1** Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: The Greer et al. study was the most relevant study published since 1999. The study was only published as an abstract in 2000 and as yet has not appeared in the peer reviewed literature. The study was, however, reviewed in detail by Merrill 2001 in a QA/QC analysis. In the review the actual data is given in great detail which allows for the agency to analyze the findings beyond the information given in the abstract and for that matter in a published paper. The details of the materials and methods are not given in the abstract. The data presented only included changes in $^{123}I$ thyroid uptakes levels after perchlorate ingestion but promised future analyses of the data on the measured levels of serum TT4, FT4, TT3 and TSH levels.

This study is important because it appears to be a carefully controlled human clinical trial. The main problem is with the limited number of subjects in the study and also with the wide age range of the subjects. The uncertainty is quantified and the basic data given in Merrill 2002 (attachment 7) allows for detailed dose response modelling. This should be carried out. Also it should be noted that more data is available than reported in the abstract. The exposure groups included 10 subjects although only 8 were reported. The reason being that only 8 had measurements at both 2 days and 14 days while all 10 had 14 day measurements. This is true for all 3 experimental groups. The subsequent exposures at the investigator's estimated NOAEL of 0.007 mg/kg/day were reported in the abstract to be 4 subjects. In the data base 7 individuals were exposed so that a more careful analysis is possible. Presumable in a published paper all of this data will be analyzed and discussed.

The other report is actually a letter (Lawrence et al. 2001) describing a continuation of their previously published study. In the new work they exposed 9 males to perchlorate at the lower dose of 3 mg/day for 14 days. They report no statistically significant effect although there was a decrease in TSH levels followed by a rebound after cessation of exposure.

**B.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: The EPA does not adequately describe the Greer findings. Several pages and tables are given for Lawrence study which is a single and less controlled dose study with fewer subjects. The agency does have the raw Greer data and as such should give it more prominence. In discussing the Greer study it is not mentioned that the authors estimate the NOAEL to be 0.007 mg/kg which was the reason for the subsequent tests on the 7 subjects at that dose. The agency mentions that there was a large range in percentage of effect. They should also indicate the large variability in baseline levels. For example the largest decrease as mentioned in the report was –38.6%. This occurred in an individual with a baseline level of 1.5 to 3 times greater than the other members of this exposure group. The single mention in the conclusions of this section of the report should not include the value –38.6% as was done since this is misleading at best.

The agencies' statements (4-24) about power at 0.007 mg/kg are not clear. What difference in response is desired to be tested and is it assumed that there is a biological threshold? It seems that the issue should be what is an appropriate dose-response function and what degree of effect is an appropriate acceptable level for a "NOAEL" and at what precision should it be estimated.

The discussion concerning the Beck 2001 study is peculiar. The study is a replication of the previous studies that were criticized as being small and for not controlling dosing. This study is criticized for not dealing with hypothyroxinemia or transient decrements in T4. It seems that all the clinical trials could be statistically combined for a more precise estimated NOAEL what ever it is defined to be.

In the discussion of the earlier Lawrence study and the subsequent NOAEL study it should be mentioned that for the degree of uncertainty observed in the first study the second study would not be expected to be significant given the experimental dose level and sample size. However as the authors mentioned the rebound effect helped to establish that there was an effect at the second experimental dose of 3 mg. Also for all the tables dedicated to this study (Why not some for Greer?) the 3 mg values should also be given.

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

Answer. The epidemiological studies have been adequately described. I would be more cautious in interpreting the causality and quantitative results of the ecological studies. One of the largest ecological studies is the one by Cohen which shows that environmental exposure levels to radon are protective for lung cancer which is counter to what case-control studies suggest. The analysis of the ecological studies was well done. The interesting and important study by Schwartz (2001) is not published (thesis) and thus cannot be commented on. It does not appear that it is used in the risk assessments and should be.

Two occupational studies with relatively high exposures have been published. The long discussion of the Gibbs et al. (1998) study 4-14 to 4-18 is not easily followed and should be summarized at its completion. Also it is referred to as both a cross-sectional study and as a case-control study the later being incorrect.

In the discussion of these two occupational studies it is mention that several confounders were not controlled for including temperature, socioeconomic status and body mass. There is no evidence given as to why these are strong confounders. Further these workers are likely to be of similar SES and also the temperatures would likely be similar. What is important is how high these exposures are and what is the implication for the apparent lack of adverse effects. What do the animal studies predict for these cohorts? I feel that as occupational studies, which are always difficult, these are reasonably well done.

**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?

Answer. The answers varying depending on the type of study. The controlled trials using euthyroid subjects did provide sufficient data to compare $^{123}I$ uptake with the animals. The measures of serum TT4, TSH etc. are not yet available. The recent ecological studies do provide fairly precise estimates that could be compared as long as the ecological nature of the studies is kept in mind. The occupational studies as typical have wide estimated exposure values. The effects or lack of should however have been compared with the animal studies to see if the results are actually consistent with the animal results considering the uncertainties in the worker studies.

**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?

Answer. The epidemiological results seem to go in the predicted manner from what is known of the animal results and mechanistic beliefs. The problem with the human data is precision, endpoints examined and possible design and confounding issues.

**Topic Area F: Human Health Dose-Response Assessment**

**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?

Answer: I do not feel that the data sources (i.e. animal studies, human trials, epidemiological findings etc.) have been integrated in a quantitative manner. They seem to be treated separately.

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

Answer: I did not review the PBPK work. I would however say that in general it is a critical component of risk estimation.

**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?

Answer: As mentioned elsewhere I feel that the values of the safety factors are fairly arbitrary and perhaps some effort could be made to estimate appropriate values for this particular risk estimation problem.

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

Answer: Although it does not apply to perchlorate, ionizing radiation which is both a mutagen and a carcinogen has only been shown to cause thyroid cancer in exposed children. To answer the question I feel that the Agency did a good job in discussing the possible susceptible subgroups although some were possibly speculative.

**Topic Area G: Risk Characterization**

**G.1** Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

Answer: The assessment of potential risks is well done. The problem I see is that as usual the human data is basically ignored when it comes to the quantitative estimation of effects. I would estimate the minimal effect level as measured in the human studies with the incorporation of the statistical precision of the estimate as well as an additional reasonable safety factor applied. For those endpoints not measured in man but seen in rats one could use a proportionality factor (i.e. parallelogram). The appropriate risk estimates that have thus been developed could be applied to the occupational studies to see how well they agree with the actual observed data. The large safety used by the Agency appear to be "policy" and not driven by scientific information for the particular problem at hand. Would the risk assessors change these default values for the perchlorate problem?

**Topic Area H: General Comments, Conclusions, and Recommendations**

**H.1** Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Answer: The only question I have is the curious statement concern using only government funded clinical data. (I assume this does not apply to FDA). I would hope that all available data would be used in public health decision making.

**H.2** Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Answer: What is not clear to me are the justifications for the use of a safety factor of 300 applied to the extensive amount of human data (7-26). Another question is the apparent distinction between various histopathology findings and the benign lesions (cancer?).

**David Jacobson-Kram**

**Topic Area A: Hazard Characterization and Mode of Action**

A.1 Have all 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?

*In my opinion, the Agency has done an excellent job in identifying and utilizing available toxicokinetic data. Naturally, information from human studies is more limited. Nevertheless, the most likely mode of action for perchlorate has been identified and appears to be consistent across species.*

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

*I believe the model the EPA has framed based on the inhibition of the NIS is logical and well supported by the data. I'm on much firmer ground in the area of carcinogenicity than neurodevelopmental toxicity. The model for perchlorate-induced carcinogenicity is logical and well supported by the data. In my opinion, perchlorate-induced thyroid cell proliferation and resulting neoplasia is completely consistent with other thyroid carcinogens having similar mechanisms of action.*

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?

*All available data continue to support the conclusions that perchlorate is not genotoxic and that the mode of action for inducing cancer is indirect. Data demonstrating anitthyroid activity are very convincing. While genotoxic carcinogens are conceptually thought not to have thresholds, nongenotoxic carcinogens with a well defined mode of action are expected to have no effect levels.*

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 for both.

*I believe this approach is protective for both endpoints because their induction is based on a common mehanism.*

**Topic Area C: Laboratory Animal Studies**

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

*No new studies fall into the area of my specific expertise. However, based on the summaries in the EPA document, I see no obvious limitations or deficiencies.*

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

*I am not aware of any other studies or data that are relevant for the assessment of adverse health effects of perchlorate. I believe that the studies are well described and the document is well written. The biggest limitation, in my opinion is the lack of human data. This of course is unavoidable since ethical considerations would preclude performance of such studies. Nevertheless the PBPK models that are used in the risk assessment appear excellent based on the good agreement between predicted and actual values. I believe the assessment has identified all health areas of concern and dealt with them as completely as the data and current science will allow. I believe the key issues and conclusions are clearly stated. There are some minor editorial observations listed below.*

*Page E-8, line 12. Change effected to affected.*
*Page E-9 line 24. Change effected to affected.*
*Page 4-29 line 7. Change effected to affected.*

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

*I believe the toxicity data are completely consistent with the proposed mode of action for perhlorate.*

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

*I believe the NOAELs and LOAEls are appropriate. In some cases I feel the Agency is being especially conservative and assigning LOAELs for effects which are reversible or have questionable biological significance. Nevertheless, I feel it is incumbent on the Agency to err on the side of safety. Further, by assigning NOAELs and LOAELs to precursor lesions and not to frank pathological lesions, it is again making conservative but reasonable assumptions.*

**Topic Area F: Human Health Dose-Response Assessment**

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?

*In my opinion, the conclusions regarding the key event and weight of evidence for effects after oral exposure to perchlorate are appropriate and completely consistent with the information on the mode of action. I believe the data have been integrated well and support the proposed point of departure. I know of no other factors which ought to be considered in arriving at a point of departure.*

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

*I believe the chosen dose metric is the most logical in light of the available data. This is one of the best interspecies extrapolations I have seen. The animal data is excellent and the human data, while limited is still consistent.*

F.3 Are there other data which should be considered in developing the uncertainty factors? *None that I am aware of*

Do you consider that the data support the values proposed or different values for each? *Although on the conservative side, I feel the data support the proposed values.*

Do the confidence statements accurately reflect the relevancy of the critical effects to humans and the comprehensiveness of the database? *Yes*

Do these statements make all the underlying assumptions and limitations of the assessment apparent? *Yes*

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment? *Yes*

**Topic Area G: Risk Characterization**

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

*I believe it does.*

**Topic Area H: General Comments, Conclusions, and Recommendations**

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

*While lengthy and certainly not an "easy read", I believe the review and risk characterization is well written and presented.*

# Michael Kohn

**Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"**

March 5–6, 2002

**Comments by:**

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**Prepared for:**

Kate Schalk
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**Specific Questions for Area A**

A.1 Have all 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?

- Generally yes. I would have liked to see use of data on hormone secretion by isolated pituitaries and thyroids. This enables adding regulation of hormones to the PD model.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

- The conceptual model attributes adverse health effects to altered circulating hormone levels. While this is likely to be true, there is no real mechanistic link, e.g. altered expression for $T_3$-sensitive genes, stated.

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?

- The notion that inhibition of NIS and the consequent reduction in $T_4$ production leads to chronic elevation of circulating TSH is credible. What is needed is an objective way of extrapolating serum TSH levels from those achieved at experimental doses of perchlorate to those expected from the much smaller environmental exposures. A NOAEL inferred by inspection is not convincing.

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 for both.

- The problem is that different endpoints probably show very different sensitivities to over-stimulation by chronically depressed $T_4$ or chronically evaluated TSH. If we knew the expression of which genes were being altered and by how much, we could avoid the "one size fits all" drawback of simply using serum TSH (worse yet is thyroid perchlorate) as the index of risk. Otherwise, we only have empirical correlations and limited predictive capability.

**Specific Questions for Area E**

E.1 For each of the four models developed by the Air Force Research Laboratory (AFRL) listed below, consider the questions in Attachment 3 and comment as necessary. You do not need to answer every question in Attachment 3, rather use your professional judgment to address those that are most appropriate to the model and associated consultative letters/studies in question. Refer to Table 1 for all relevant citations. Note that the citations for the four models, which are contained in consultative letters, follow:

- Why are the NIS kinetics represented as Michaelis–Menten? This would only be a good approximation if $Na^+$ were always saturating. But the model in the 8 May 2001 supplementary paper shows quite nicely how the kinetics are sensitive to the membrane potential. Does the Na+-dependent uptake of perchlorate tend to depolarize the thyrocyte? Use of this feature would make the representation of the kinetics more credible.

- The model gives the liver perfusion as 17% of cardiac output. But total liver perfusion is the sum of hepatic artery flow (~20%) and portal flow from the splanchnic circulation (~80%). The literature gives the splanchnic circulation alone as 18% of cardiac output. There are many PBPK models that treat these details of hepatic perfusion explicitly.

- Thyroid hormones are bound to proteins in the blood and some peripheral tissues. Are these considered in the modeling? $T_3$, produced by de-iodination of $T_4$, is the active form of thyroid hormone and binds to a nuclear receptor that regulates expression of genes for certain metabolic enzymes. Have these events been considered in the modeling?

- How is the use of allometric equations for extrapolating parameters among species justified? Why is a simple ratio sufficient to transform adult male rat exposure to equivalent effect exposure in pregnant rats? Given that O'Flaherty's pregnancy model is being used, can't those exposures be computed directly?

- Also, it is not clear if time courses from multiple doses were used to estimate parameter values or if only single doses were used. It appears that only the final state as a function of dose was used for multiple dosing scenarios.

E.2 Please consider the questions in Attachment 2 to comment on how EPA applied and presented the models in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

- The PBPK models are competently developed, though I would have liked to see the structure derived more from general principles than from *ad hoc* empirical relationships.

- The investigators refer to a previously published model that describes hormone secretion and metabolic clearance in detail. Why weren't those results used? Isn't this more appropriate given the hypothesized mechanism?

- The application of statistical tests of goodness of fit, reliability of predictions, and uncertainty of model structures seems very little, considering the potential regulatory impact.

**Specific Comments for Area F**

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?

- Yes, the data are appropriate, but I don't think they go far enough. I think the EPA needs to consider more carefully what measurable quantity(ies) should be the index of risk. I suggest altered steady-state circulating hormone levels.

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

- PBPK models are ideal for extrapolating responses among species and to doses lower than those used in experiments. But it has to be based a on a sufficiently rich set of data. I especially like the attempt to modify the basic model for pregnancy and lactation.

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?

- Inclusion of data on hormone secretion and metabolism would permit more complete modeling of hormonal responses.

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

- Not that I could tell. However, I'm not sure how one would relate the index of risk to the physiological response (disease state).

**Specific Comments for Area G**

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

- It succeeds on the basis of its limited view of the physiology. The use of sensitivity analysis is most welcome. The method of extrapolating between species is arbitrary and

unconvincing. To do this correctly, one requires the values of the model's parameters for the second species and the sensitivity of that species to the endpoint (e.g. .thyroid follicular tumors).

**Specific Comments for Area H**

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

- See above. Mostly I'd like to see a better rationale for the choice of calculated index of risk and a more credible method for extrapolating between species.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

- The document is quite clear, but the assumptions underlying the choice of risk assessment technique are arbitrary and inadequately justified.

Kohn

Date: 2/20/2002 7:45 AM
Sender: "Michael C. Kohn" <kohn@valiant.niehs.nih.gov>
To: Kate Schalk
Priority: Normal
Subject: Re: Additional information

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Dear Kate,

Thank you for passing the revised work of Merrill et al. to me. I did wonder at the HUGE values of the sensitivity coefficients. Correcting the calculation errors brought the coefficients to much more reasonable values. Also, The authors now state explicitly that perchlorate AUC is too prone to error and is not reliable as a means of extrapolating risk. I too was concerned about this in addition to its great distance from the actual toxiologic/carcinogenic event(s). So I approve of the investigators' changes.

Michael C. Kohn

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Date: 3/1/2002 3:24 PM
Sender: "Michael C. Kohn" <kohn@valiant.niehs.nih.gov>
To: Kate Schalk
Priority: Normal
Subject: Re: Reviewer's comments

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After having read the comments of other reviewers, I feel even more strongly about two points.

The choice of dose metric is really arbitrary and not well justified. The use of thyroid AUC for perchlorate sounds good but is ultimately without theoretical support. The intense search for a NOAEL makes unwarranted assumptions about the nature of the dose-response. The assumed safety (uncertainty) factors are without scientific support.

Use of PBPK modeling to arrive at a plausible dose metric is reasonable, but the models here do not go far enough. They really have to include the pharmacodynamics of hormone metabolism. Although I still believe that inhibition of NIS deprives the thyroid of iodine required to synthesis thyroxine, I'm troubled by the news that NIS is ineffective in transporting perchlorate. There are other anion transporters (e.g. those that swap anions such as chloride or bicarbonate for the perchlorate. So use of Michaelian kinetics for the addition of a passive diffusion is most likely totally unjustified. As doubly labeled perchlorate is eliminated in the urine a several oxidation forms with scrambled labels, the anion must undergo multiple redox reactions. What are they, and where do they occur?

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## Loren Koller

Loren D Koller

**Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization**

**Topic Area A: Hazard Characterization on Mode of Action**

Chapter 1 provides the necessary background information on the current status of perchlorate contamination in the United States and an historical perspective on how certain issues of concern have evolved. This chapter adequately identifies the various uses of perchlorate salts, sources of contamination, analytical methods with detection limits, and a background on health and ecotoxicological risk assessments, risk characterization and regulatory information. Chapter 2 presents the physiochemical properties of perchlorate which provides the foundation for pharmaco- and toxico-kinetics. Chapter 3 provides the basic information on absorption, distribution, metabolism, and elimination of perchlorate, a review of iodine metabolism and thyroid physiology, the mechanism of action of perchlorate, and the ensuing effects on the thyroid gland as well as other organ systems.

**A.1** The relevant data on toxicokinetics and toxicodynamics of perchlorate have been identified and appropriately utilized. It has been well established and stated in this document that differences exist in TSH activity and T3 and T4 half-life between rats and humans and that thyroid binding globulin that is present in humans, primates and dogs is absent in rodents and other vertebrates. Thus, T4 in rodents is bound to proteins with low affinity and therefore in highly susceptible to excretion. These features are important in making comparisons between rodents and humans. However, there does not appear to be species differences in the ability of perchlorate to inhibit iodine uptake by the thyroid gland.

**A.2** It is obvious that the primary target organ for perchlorate is the thyroid gland. Further, since perchlorate inhibits iodine uptake and interferes with active transport by NIS which reduces T3 and T4 thus stimulating TSH, the result is disruption of the hypothalamic-pituitary-thyroid axis. This mechanism of toxicity is clear. However, it remains unclear how disruption of this pathway by perchlorate may effect neurodevelopmental and neoplastic processes. Although there is a section describing the pathogenesis of neoplasia in the thyroid gland, the evidence is weak that perchlorate is a carcinogen at ppb doses. It is also stated (page 3-20) that human and rodents are presumed to be equally sensitive to thyroid cancer caused by thyroid-pituitary disruption. This statement is inaccurate as it has been stated appropriately in this document that rodents are more sensitive to this type of carcinogenic action than humans. The neurodevelopmental human studies described in chapter 3 are suggestive that thyroid deficiency contributes to neurodevelopmental effects but animal data from perchlorate exposure to support this concept is questionable.

**A.3** An appropriate dosemetric method to establish a high degree of confidence for low-dose extrapolation is based on data from animal dose-response studies. The animal data indicates a threshold level for toxicity, and carcinogenicity, for perchlorate. To use other methods or models for low-dose extrapolation lowers the level of confidence substantially.

Loren D Koller

**A.4** A harmonized approach to characterize potential risk of both cancer and noncancer toxicity based on iodine uptake inhibition is appropriate, but quite conservative. Many biochemical, molecular, immune, and hormone alterations serve as biological markers but do not necessarily represent adverse health effects. Good quality basic animal research and human epidemiology studies are required to demonstrate "cause and effect relationships" and "associations", respectively. Biological markers are of value once correlated to adverse health effects.

**C.1-1,2 &5.** The immunotoxicology studies that have not undergone peer review do have limitations and deficiencies. Most of these issues have been identified and adequately discussed in Chapter 5. The experimental protocols were designed to determine if perchlorate is immunotoxic. The studies use appropriate assays to evaluate both innate and acquired immunity as well as host resistance. The experimental design includes multiple doses for 14 and 90 day duration with a 30 day recovery period. The procedures used are current, validated, and widely accepted by immunotoxicologist. General toxicity, organ weights, and measurements of cellularity were also included in the studies as well as histopathology and hormone analysis of the thyroid and pituitary glands. It is interesting to note that effects on the humoral immune response are immunostimulating rather than suppressive;e.g. positive in nature. Effects on cell-mediated immunity (CMI) were normal while those on delayed-type hypersensitivity (DTH) were enhanced, although inconsistently. Phagocytosis by macrophages (in vitro) was decreased, but reversible, while the ability of macrophages to digest phagocytized material was not affected when cultured with interferon. Subsequent analysis indicated non significant differences, however, in nitrite production by macrophages. It is also interesting that perchlorate tended to increase host resistance to infectious agents with no effect on tumor induction. The summary of results section (5.6.6) does adequately discuss the limitations of the various assays, particularly the LLNA, and the lack of appropriate negative controls for the LLNA procedure necessary to accurately interpret the inconsistent results. Finally, it is stated that due to the uncertainties to attempt to extrapolate from the LLNA experiment that an uncertainty factor is recommended to be applied to this risk assessment. This approach is unnecessary for several reasons. First, the thyroid gland is the primary and most sensitive target organ for perchlorate toxicity. The effect on hormones resulting from that toxicity are at doses as low, or lower, than those observed from the LLNA assay. Second, the results from the LLNA assay are inconsistent and inappropriately controlled to definitively determine that low doses enhance contact hypersensitivity when high doses do not. These data do not conform to a dose-response pattern nor do they confirm that perchlorate sensitizes to contact hypersensitivity. Third, although "case reports" are suggestive of associations such as patients suffering from Graves disease and treated with potassium perchlorate presented agranulocytosis and/or skin rashes, skin rashes from "low dose" perchlorate exposures have not been confirmed in man and granulocytosis has not been identified, or even suggested, to occur in perchlorate treated animals. Fourth, the immune effects appear to be reversible and not permanent. Finally, it is unknown how much of an increase in the DTH response in rodents will translate into, if any, an increase in the sensitivity of humans to develop contact hypersensitivity when exposed to drugs or other chemicals. As stated in the document (pages 108 & 109), the LLNA data may represent a LOEL but not definitively a LOAEL.

Thus, there is no justification to add additional uncertainty for immunotoxic effects. There is no evidence that the immune effects compound the toxicity (additive or synergistic) produced in the primary target organ (thyroid), therefore, eliminating the need to add uncertainty for immunotoxicity.

**C.1-3** The dosing methods used in the studies were appropriately formulated and controlled with appropriate endpoints being evaluated. Sufficient numbers of rodents were used to observe an effect keeping in mind the 3–R's.

**C.1-4** The statistical analysis were of sufficient design to identify significant effects between treatment groups.

**C.1-5&6** The strength and limitations of the inferences made and interpretation of the results are discussed in C1-2,3,&5 above. The experiments as designed were "sound" in an attempt to provide basic immunotoxicological information following perchlorate exposure. The conclusions of the report are supported by the data but the recommendation to add an additional uncertainty factor based on the immune data is unfounded (see comments in C1-2,3,&5 above). The experiments evaluated the major compartments of the immune system using standard protocols and validated assays.

**C.1-7** These studies include one of the most sensitive, if not the most sensitive, populations;e.g. Prenatal, neonatal, and postnatal exposures.

**C.2-1** I am not aware of additional relevant perchlorate studies that have not been included in this document.

**C.2-2** The important data from the individual studies has been included and adequately described in the Toxicological Review and Risk Characterization Document and limitation of those studies has been adequately discussed.

**C.2-3** Comments on the strengths and limitations of the analyses performed on the data in the Toxicological Review and Risk Characterization Document are mentioned in C1-1,2&5 above. The document adequately discusses inconsistencies as well as data interpretation issues.

**C.2-4** The data was "messaged" by various statistical methods. Those procedures, for the most part, appeared to be complementary and supportive of each other in identifying statistical significance.

**C.2-5** The key issues, statements, and conclusions are clearly stated in the document and the conclusions, except for the recommendation of adding additional uncertainty for the immunotoxicity data, are sufficiently supported by the data. Chapter 5 is well written, self explanatory, and adequately presented, including the immunotoxicity section.

**C.2-6** The assumptions and uncertainties are clearly addressed but inaccurately applied (see comments in C1-1,2,&5).

**C.3** The toxicity data for most all studies in this chapter are consistent with the proposed mode of action of perchlorate;e.g. inhibition of iodide uptake at the NIS in the thyrod gland followed by decreases in T3 and T4 and increases in TSH.

**C.4** For the most part, the NOAEL's/LOAEL's for the immunotoxicity data are appropriate. The question to be asked, does the NOAEL/LOAEL represent an adverse human health effect. The only notable immune "adverse effects" in the rodent studies that could impact human health was the LLNA data that had flaws (see comments in C1-1,2,&5). That data is suggestive that humans could develop skin rashes (non-life threatening) but certainly is not definitive nor confirmed by other studies. Thus, the immune data which assessed the major compartments of the immune system, collectively, would suggest that the immune system is not the primary target organ of perchlorate toxicity and that the effects observed are questionable as a human health effect at equivalent doses.

**General Comments – Chapter 5** The introduction to chapter 5 (pages 5-1-3) discusses the problem of analytical variability between studies. However, analytical variability between studies can be minimized if the studies are appropriately controlled and standards are used allowing differences between test groups within a study to be adjusted to compensate for differences between studies. Under Section 5.2.3.1, page 5-26, why was a BMD and BMDL derived when a NOAEL had been identified? A question to be answered, what morphological change in the brain constitutes an adverse health effect if that change does not correspond with a functional change? Perchlorate is a promotor (nongenotoxic) rather than an initiator of cancer. Rats exposed to high doses of perchlorate for 2 years developed benign tumors of the thyroid gland. Higher doses resulted in thyroid follicular cell carcinomas. However, cancer occurred at doses extraordinarily higher than those that induce toxic effects. Although two rats in the F1 generation that were dosed from conception to 19 weeks of age developed thyroid adenomas, this incidence was not statistically significant (within the experimental design) and also was at the highest dose, 30 mg/kg//day, well above (~ 3 orders of magnitude) the doses that cause toxic effects. This dose could be approaching the "threshold" for adenoma development.

**F.1** The conclusions and conditions regarding the key event and the weight of evidence for effects after oral exposure to perchlorate are basically appropriate and consistent with the information on mode of action. The data for the most part support the proposed point of departure.

**F.3&4** Although it is stated in this document that transient drops in T4 can lead to permanent neurodevelopmental sequale, this feature should be unequivocally documented (referenced) in this document following perchlorate exposure; e.g. does this generalized assumption apply following perchlorate exposures? Also, how often (they are transient), how low a dose, and how long of a duration must these T4 deficits be to produce nerodevelopmental disorders, what are they, and what is their overall significance to

human health? It is stated that these deficits can result in permanent effects. "Can" deserves justification. The point of departure selected (0.01 mg/kg/day) is justified by the overall data as a LOAEL. Four uncertainty factors resulting in a composite factor of 300 are applied to calculate the RfD. The UF of 3 for intraspecies variability and UF of 10 to extrapolate from the LOAEL to NOAEL are appropriate. Adding an UF of 3 for a "significant increase" in tumors in the F1 generation pups at 12 weeks is inappropriate. First, benign tumors of the thyroid were observed in rats exposed to an extremely high dose (1,339 mg/kg/day) of perchlorate for 2 years. Secondly, benign tumors only occurred in 2 of 30 F1 mice which is hardly of biological or statistical significance, and at only the highest dose (30 mg/kg/day), indicating that a threshold exists for this promotor of carcinogenesis. Assumptions are made that in "utero programing" occurred, allowing for recalibration of the regulatory feed back system or changes in cellular sensitivity and demand for thyroid hormones with extended exposures. Regardless of these assumptions, the LOAEL used for noncancer toxic effects is 0.01 mg/kg/day, while the dose for cancer (F1 pups) was 30 mg/kg/day with no induction at lower levels, thus, demonstrating a definite biological threshold some where between 1.0 and 30 mg/kg/day, and well above the 0.01 noncancer toxic dose identified as a LOAEL. Thus, adding uncertainty for a cancer that is not induced except at approximately 3 orders of magnitude above the toxic level is unjustified.

Adding an UF of 3 for immunotoxicity data base insufficiency is also inappropriate. The immunotoxicology studies were well designed, used validated procedures, and the data was negative (actually immunostimulating rather than immunosuppressive) except for a decrease in in vitro phagocytosis and an increase in the contact hypersensitivity response at 0.06, 0.2, and 50 mg/kg/day but not at 2.0 mg/kg/day in the 14 day study and enhanced at 0.06 and 0.2 mg/kg/day but suppressed at the 50 mg/kg/day dose in the 90 day study. The inconsistencies, lack of a dose response, lack of negative controls, reversibility of the immune effects, and questionable relevance of these data to human health do not justify additional uncertainty for immunotoxicity, let alone using these questionable effects as a parameter in which to derive exposure limits for humans. Finally, the 0.06 mg/kg/day dose is larger than the 0.01 mg/kg/day selected as the LOAEL. Adding additional uncertainty factors for secondary effects when they occur above the most sensitive toxic endpoint and when they do not compound the toxicity of that endpoint (thyroid primary target organ effects) would be extremely difficult to justify and, thus, are not justified here.

**G.1** The first four pages of chapter 10 are a general summary of human health risks resulting from perchlorate exposure. The remainder of the chapter is mainly devoted to Ecotoxicologicology and Research needed in that arena. It is interesting that statements in this chapter recognize that the thyroid is the target tissue (organ) and that "other potentially adverse and permanent effects from decreased thyroid hormone" include developmental in utero effects (particularly the nervous system), possible immune effects, and tumors in young adults dosed in utero and during development which raise concerns about in utero imprinting of the regulatory system responsible for controlling thyroid hormone homeostasis. Recognition that the thyroid is the primary target

organ and the corresponding effects (hormone homeostasis and histopathological changes) are the most sensitive effects while the "other potentially adverse and permanent effects" (neurodevelopmental, immune, and cancer) are less sensitive, supports the contention that the RfD should be determined from the most sensitive primary health effects rather than the less sensitive secondary effects, thus, eliminating the additional uncertainty factors that are applied for those secondary, higher dose effects.

**H.1&2** Many of the general comments have been addressed in previous sections of this critique.. Overall, this document was well written, complete, and self-explanatory. Many statements were clarified based on previous reviews and comments making this document easily understood. Modeling was basically supportive of the "real animal data" but also illustrated that modeling can be manipulated according to input and assumptions. The data also illustrated differences in results comparing NOAEL's/LOAEL's to BMD and BMDL. Although biomarkers indicate effects, it was interesting that the biomarkers for the thyroid gland generally represented less sensitive functional changes. This raises an important issue, what constitutes an adverse health effect? Is it a change in a sensitive biochemical, molecular, or protein in the absence of obvious functional changes or should there be some obvious functional change identified to confirm adverse health effects? If the regulatory agencies move towards the previous, more conservatism will be built into the exposure standards (limits). This issue deserves to be appropriately discussed and resolved in the future.

Loren D Koller

**Title: Ammonium Perchlorate: Effect on Immune Function**
**Author: BRT - Burleson Research Technologies**

**Critique:** B6C3F1 mice were administered 0, 0.02, 0.06, 0.2, 2.0, or 50 mg/kg/day ammonium perchlorate for 14 or 90 days. Parameters evaluated were the plaque forming cell (PFC) assay, contact sensitization induced by DNCB – local lymph node assay (LLNA), hormone (T4 and TSH) analysis, and histopathology and S – phase labeling of thyroids. There was no significant effect on the PFC at all doses in the 14 day study and at 0.02, 0.06, and 0.2 doses in the 90 day study while a significant increase in PFC occurred at the 2.0 and 50.0 mg/kg/day in the 90 day study. In the 14 day LLNA experiment, a significant increase occurred at the 0.06, 0.2, and 50 mg/kg/day doses but
not at the 2.0 dose while in the 90 day study, significant increases occurred at 0.06 and 0.2 mg/kg/day but were significantly suppressed at the 50.0 mg/kg/day dose. These results produced an inverted U shaped curve. It is interesting that both the humoral and delayed – type hypersensitivity (DTH) responses were increased by perchlorate. An enhanced humoral response is considered to increase the resistance of a host's susceptibility to infectious agents (protective) while the DTH test performed suggests an increased susceptibility to contact sensitivity. It is unknown, however, if a significant increase in the DTH response in rodents actually equates to an increase in susceptibility in humans, and if so, how much of an increase is necessary to stimulate an hypersensitivity response? A disturbing feature of the LLNA (DTH) data is the inconsistency in the dose-response curve for both the 14 and 90 day study, and in particular, the inverted U shaped curve in the 90 day study ranging from a significant increase to a significant decrease. These discrepancies question the value of the DTH results for extrapolation to humans, particularly in the absence of other "negative" immune effects. The author does not adequately address this issue nor were negative controls included in the study to determine if this effect was a result of the perchlorate exposure. Even if it were, its relevance to human hypersensitivity is questionable. The design of the immune experiments were conducted in accordance with standard protocol and the assays have been validated for this species. The T4 and TSH hormones responded as expected in the 14 day study but in the 90 day study, the T4 levels were only significantly lower in the high dose while the TSH levels were elevated at a lower dose than in the 14 day study. Lesions in the thyroid gland typical of those produced by perchlorate treatment were observed in the 90 day study.

**Hitopathology Data for the 90 day 50 mg/kg/day Study**

The histopathlogy data for the BRT – Burleson Research Technologies, Inc. 90 day 50 mg/kg/day study is reported in this report. Hypertrophy was reported in the thyroid of 4/5 mice and colloid depletion in 5/5 mice. These lesions are consistent with those reported to occur from exposure to perchlorate. A slight increase in the labeling index was noted in the thyroids of the treated mice.

**Quality Assurance Audit**

A quality assurance audit was conducted on the PFC and LLNA assays. The audit for the PFC assay revealed that the data calculations were performed as described in the protocol and that the data shown in the graphs and tables were accurate. The findings of the LLNA data also were consistent with the protocol as were the overall findings. Deviations in the study protocol were noted, corrected, and did not affect the overall results of the study.

Loren D Koller

**Title: Effects of Ammonium Perchlorate on Immunotoxicological, Heematological, and Thyroid Parameters in B6C3F1 Female Mice**
**Authors: Deborah Keil, et.al.**

**Critique:** The research conducted and reported in this report is a rather comprehensive immunotoxicological investigation that includes body weights, thyroid histopathology, thyroid hormone analysis, organ weights, organ cellularity, CD4/CD8 thymic and splenic subpopulations, hematology, stem cell assay, natural killer cell assay, cytotoxic T cell activity, phagocytosis, nitrite production by peritoneal macrophages, IgM and IgG antibody titers measured by ELISA, delayed-type hypersensitivity (DTH), B19F10 melanoma tumor challenge, Listeria monocytogenes challenge, and antinuclear antibody screening. B6C3F1 mice were exposed to 0, 0.1, 1.0, 3.0, or 30 mg/kg/day in drinking water for 14 or 90 days. Another group was exposed for 90 days followed by 30 days of no exposure to perchlorate. The immune effects noted in this study were minimal. Some effects were noted at 14 days exposure but not after exposure for 90 days. The effects noted after 90 day of exposure included an increase in natural killer cell activity (30 mg/kg/day), a decrease in macrophage phogocytosis (0.1, 1.0, 3.0, and 30 mg/kg/day), and increased splenocyte proliferation (30 mg/kg/day). The suppression of macrophage phagocytosis is interesting since host resistance to listeria infection was normal. These two procedures usually parallel each other. The other two immune parameters which were altered, increased natural killer cell activity and splenocyte proliferation, are not considered to be detrimental to the host. There was no evidence of an immunostimulating disease (autoimmune) since autoantibodies were absent from the serum. However, a contact hypersensitivity procedure was not conducted.

These comprehensive studies indicate that perchlorate exposure results in minimal immunotoxic effects. The only immunosuppressive effect was macrophage phagocytosis which did not correlate with the effects of challenge by an infectious disease (L. monocytogenes) or suppressed digestive abilities (nitrite production). Thus, it can be concluded from this study that perchlorate has few, most likely, nonsignificant effects on the immune system; albeit, further studies to elucidate macrophage activity are warranted.

**Kannan Krishnan**

Dr. Kannan Krishnan

## Dr. Kannan Krishnan's Review of the Document entitled: Perchlorate Contamination: Toxicological Characterization and Risk Characterization

This document presents the toxicological profile of perchlorate in view of using that information to establish a reference dose. Available data on the effects of perchlorate collected in humans, laboratory animals, plants, invertebrates and in vitro systems have been analyzed in the context of this risk assessment. The establishment of the reference dose is based on recent animal studies, histopathology, exhaustive statistical analysis, PBPK and BMD modeling, as well as mechanistic considerations that permit the harmonization of cancer and non-cancer assessments within this paradigm. Whereas the interspecies uncertainty factor has been avoided due to use of PBPK models to derive human-equivalent dose, other factors totaling 300 are applied. The endpoint used in the assessment, point of departure, mechanistic considerations and modeling to derive human equivalent dose appear appropriate (see below for specific comments on concerns relating to the models). Even though the PBPK models are not sufficiently validated to predict the thyroid concentrations, they are in general adequate to predict the cumulative excretion profile as well as the plasma/blood concentrations. The number of fitted parameters in these models do raise a concern but the ability of the model to integrate a variety of PK data on perchlorate is significant. The use of area-under the blood concentration vs time curves (AUCs), generated by these models, for deriving the human equivalent doses and life stage equivalent doses are appropriately justified.

This reviewers' response to specific questions follow.

A.1 Have all 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?

There is sufficient, but an exhaustive or comprehensive, discussion of the toxicokinetic and toxicodynamic data relevant to the present exercise.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

The assessment in the present stage precludes the consideration of multiple mechanisms of action. The single, unifying mode of action used as the basis of this assessment,

however, is convincing, consistent with the available data, clearly articulated and scientifically-sound.

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?

Negative results have been reported in all genotoxicity assays, eliminating any consideration of the use of linear dose-response models. Threshold approaches, as suggested in this document, are appropriate.

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 for both.

The harmonized approach presented in the document is appropriate, on the basis of mechanistic considerations. However, if early effects in the continuum are used as a basis of the risk assessment, then why is there a need for the use of an additional uncertainty factor of 3 to account for potential carcinogenic effects.

E.1 For each of the four models developed by the Air Force Research Laboratory (AFRL), consider the questions in Attachment 3 and comment as necessary.

**1. *Structure.***

Does the proposed model structure contain the necessary anatomical compartments and physiological processes to accurately describe perchlorate disposition? Or iodide disposition?

The conceptual representation and the structure of the PBPK models for perchlorate and iodide appear to be appropriate. The separate representation and characterization of liver and fat compartments, despite the justifications provided, are unwarranted. The sub-divisions of skin, stomach and thyroid are acceptable based on the evidence of the presence of NIS-mediated transport.

Uptake into the thyroid is described by an active (Michaelis-Menten) process and a permeability area for first-order movement of the anions between the subcompartments. Please comment on the advantages and limitations of this approach. Does it capture all the relevant behavior for the competitive inhibition of iodide uptake by perchlorate and distribution in the thyroid?

The use of a mathematical description based on saturable uptake is consistent with the proposed mechanism of uptake and it allows to account for competitive inhibition by perchlorate. The NIS, localized in the basolateral membrane of the thyroid gland follicular cells, transports ions from extracellular fluid into the thyroid epithelial cell. As perchlorate is

not organified, what is the basis/mechanism involved in moving it across the cells through to the colloids ?

Active transport systems are saturable and susceptible to competitive inhibition, and typically move chemicals against concentration gradients. In the present case, it seems that the effect of the active transport of perchlorate or iodide into the follicular cells is offset by the simultaneous presence of the passive diffusion process which allows the re-establishment of equilibrium, on the basis of concentration differences and partition coefficients. What is the relative importance of passive diffusion and active transport for iodide and perchlorate ? The active uptake should be the key process here.

The use of a permeability area cross product is an acceptable way of describing the first order movement of anions. This approach presumably does not assume direct passive diffusion of ions, rather their movement in and out of cells through ionophores (such as carriers and channel formers). The value of PA should however be constrained by the Q for the tissue (i.e., PA should not exceed Q), an aspect that has not been respected in some cases.

Comment on the approach for describing perchlorate's plasma protein binding and dissociation.

The critical role of plasma protein binding in rats is justified with appropriate experimental observations and model simulations. The PBPK models appropriately consider the availability of the free anions in the plasma for diffusion and active uptake into tissues. However, it's unclear as to why a Michaelis-Menten type equation is used to describe plasma binding along with a first order clearance rate for dissociation. The exact equations used with the plasma, erythrocyte and whole blood compartments are not found in any of the documents provided to this reviewer (except the general clearance equation, which does not seem to specify the correct concentration term). The free concentration in the Michaelis-Menten equation and the bound concentrations in the clearance equation are probably used (should be verified).

**2. *Parameterization.*** Consider whether the experimental data or literature, fitting routines, and scaling assumptions were appropriate and adequate to support the values for the various species-specific and chemical-specific parameters used in each model structure. To describe perchlorate disposition? For iodide disposition? Are the parameters derived by fitting to available data reasonable and reliable?

*Species-specific parameters and modeling of the temporal change in maternal and fetal parameters:*

The compartment volumes and flow rates have come from standard references (O'Flaherty, Fisher, Brown). Thyroid volumes in almost all cases have been obtained for the appropriate age and group of animals simulated using PBPK models.

The temporal change in the weights of fat, placenta and mammary gland is accounted for adequately. The justification provided for not modeling uterus and liver growth is appropriate.

The use of a three stage growth model for fetus is consistent with available data. The adequacy of allometric scaling of tissue weights on the basis of data for adult male rat may be verified by comparing the calculated values with the available experimental data for fetus. Allometric scaling of fetal organ blood flows on the basis of adult male values may be inadequate but acceptable in the absence of relevant data.

Modeling the blood flow to tissues as a proportional function of volume is questionable if not incorrect.

*Chemical-specific parameters*

*Affinity constants for active transport*

The Km values for active transport of iodide were obtained from Goldstein et al. (1992) Gluzman and Niepomniszcze (1983), and these values were divided by about a factor of 10 to get the Km for perchlorate. These estimates are justified appropriately.

*Urinary clearance*

The use of kinetic data associated with the 10 mg/kg/d dose for estimating the urinary clearance values appears to be adequate, given the sensitivity of other relevant parameters to this dose level.

*Plasma binding constants (3), Maximal velocity for transport (4) and Permeability area constants (6)*

The whole set of these parameters are obtained by fitting model simulations to the experimental data, in most cases. The current state of knowledge does not permit the estimation of these parameters by other means. Normally, one would have anticipated the permeability constants to be identical or somewhat comparable between tissues. In general, these are too many parameters to be estimated given the limited exposure scenarios and differences in dose routes used in the various studies.

Comment on the "upregulation" adjustment of the Vmaxc_T*p* to represent upregulation of the NIS with increasing dose of perchlorate.

It is not unreasonable to do this, as an empirical means of representing the process.

*Partition coefficients*

The partition coefficients have been estimated either from tissue:blood ratios observed in previous or in-house experimental studies (without mention of the attainment of steady-state) or from the measured electrical potential differences in experiments.

The derivations of the thyroid follicle:lumen PCs and the thyroid follicle:stroma, based on electrical potential differences according to Kotyk and Janacek, are appropriate. However, the appropriate units and values of the constants in the equation should be provided (e.g., R = 8.314 J/mol°K, T = 312 °K, F = 96,494 C.mol$^{-1}$).

The use of the fat:blood data from hen, needs to be better justified or adjusted for appropriately since the authors report tissue:blood values whereas the model requires the specification of tissue:plasma values. Same comment for the Pearlman data.

*Other parameters*

Oral, sc and ip administrations are simulated, and there is no mention or indication of the absorption rate (and how it was obtained and used). In the main document, it is stated (page 6-15) that ip dosing was introduced in the same way as iv dose, which raises concerns.

***3. Validation.*** The models were validated to varying degrees with available data that were not used to estimate the parameters. Has sufficient validation of the structures been achieved?

The use of the models to simulate additional datasets must have required additional estimation of absorption rate constant (depending on the route of exposure). This information is not provided and therefore it is difficult to evaluate the level of confidence associated with these models. Despite the number of fitted parameters in these models, it is clear that the modeling framework has permitted the simulation and integration of a variety of data. There is sufficient simulations to show that the model adequately simulates the blood/plasma concentration and urinary excretion of perchlorate, two key aspects related to the human equivalence dose determinations.

***4. Application.*** The models are being used to develop human equivalent exposures (HEE) for different dose metrics for dose-response modeling in Chapter 7.

Comment on the utility of the proposed PBPK structures in the parallelogram approach.

The parallelogram approach is Ok except that it assumes linearity of the external dose – internal dose relationships, in both species or lifestages of interest.

Comment on the advantages, limitations, and reliability of these models to describe an HEE for different dose metrics and the correlation between the two:

- Area under the curve of perchlorate in the blood (AUCB)
- Iodide uptake inhibition

These aspects are adequately investigated and presented in the document. The use of AUC-blood is defensible. However, it is unclear as to whether the model simulations corresponded to AUC-blood or AUC-plasma.

***5. Variability and Uncertainty.***

Comment on the variability in underlying data and resultant model structures. What are the uncertainties inherent in using these models for the applications to derive human equivalent exposures for interspecies extrapolation based on the different dose metrics? Are the uncertainties associated with the PBPK modeling similar to, or reduced, in relation to default approaches?

The variability and uncertainty analyses are more appropriately done, using the oral and drinking water uptake scenarios. Simulations relating to the thyroid endpoints are likely to raise questions of uncertainty much more than those relating to plasma/blood concentrations. Basically the volume of distribution (i.e., tissue volumes times the tissue:blood partition coefficients) as well as the clearance rate (mainly the urinary clearance) are the likely to be only key determinants of the final outcome. Therefore, the uncertainty in the individual model parameters are unlikely to be propagated proportionately during repeated exposure scenarios, as long as blood AUC or concentration is used as the basis.

E.2 Please consider the questions in Attachment 2 to comment on how EPA applied and presented the models in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

See General comments above.

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?

Yes

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

PBPK model is appropriately used for interspecies extrapolation and the choice of dose metric. The use of these models for conducting the extrapolation of equivalent dose across the lifestages is also appropriate.

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?

Given the mechanism of action and that a physiological model has been used to establish the HEEs, an UF of 1 is acceptable for the interspecies extrapolation aspect. A part of the intraspecies extrapolation factor, as it relates to pharmacokinetics, is also not required since the modeling exercises comprised of the evaluation of HEEs for various lifestages (including potentially sensitive lifestages). The animals used for establishing LOAEL also were in hypothyroid state (2001 study). Therefore the use of 3 as inter-individual uncertainty factor is not totally justified but appears necessary.

The use of a 10 for LOAEL-NOAEL extrapolation is justified.

The use of 10 as the database uncertainty factor (3 for cancer effects and 3 for immunotox effects) is not convincing, given that the common mode of action (which is assumed to be part of the continuum of effects) forms the basis of the present assessment and in selecting the LOAEL.

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Sufficient well.

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

The profile of effects, the relevant mechanisms are considered in this assessment.

G.2 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the ecotoxicological risk posed by potential perchlorate exposures?

Yes, for a screening level assessment.

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

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H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

I have noted a number of minor comments (typos in the text and equations, lack of clarity in text, tables and figures) and will provide them at the workshop.

## Merle Paule

**Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"**

Comments by Merle G. Paule, Ph.D.

**Topic Area A: Hazard Characterization and Mode of Action**

**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 the sodium (Na+)-Iodide (I-) symporter (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?

The importance of the key event is plausibly and logically presented and it is adequately related to the neurodevelopmental and neoplastic sequelae shown to occur with exposure to perchlorate. The clear dose-response relationships between perchlorate exposure and inhibition of iodide uptake, increases in TSH, decreases in T3 and T4, colloid depletion, hypertrophy and hyperplasia of the thyroid are compelling. The U-shaped dose response curves for some metrics including rat pup brain morphometry are not atypical phenomena in biological systems, especially those that by their very nature are adaptive and plastic.

**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?

All noted effects of exposure correlate with the ability of perchlorate to alter thyroid hormone profiles and this effect is related to amount of compound at the target site (NIS). The use of AUC (area-under-the-curve) data as the dose metric is justified because it plausibly provides a good measure of average exposures likely to affect the NIS.

**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 for both.

The approach would appear to be protective for both toxicities since it seems clear that the elicitation of both the precancerous effects by perchlorate (colloid depletion, thyroid hypertrophy, hyperplasia) and the alterations in the concentrations/availability of TSH, T3 and T4 and the presumptive effects of these perturbations on neurodevelopment all show similar dose-responses and sensitivities.

**Topic Area C: Laboratory Animal Studies**

**C.1** Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgement to address those that are most appropriate to the study in question.

**Bekkedal et al., 2000.**

**1.** Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

The strengths of this study lie in the use of the litter as the experimental unit; the use of automated and, therefore, objective measurements of motor activity. While this approach to monitoring motor activity (Opto-Varimex) can be thought of as state-of-the science, the very large standard deviations associated with many of the measurements (often near or greater than 100% of the mean data) reported here, indicate an inherent lack of sensitivity of those measures. This finding suggests problems in the way the assessments are being carried out, the way they are being recorded, with the apparatus itself or some combination of the above. It is also not clear from this report, how the timing of the behavioral assessments were performed. While it is stated that the motor assessments occurred between the hours of 0830 and 1430, it was not stated whether any stratification was employed to distribute time of day assessments equally across treatment groups or if time of day was considered at all. It seems possible that the large variability noted in most of the assessment measures presented in this report might be due to changes in activity levels as a function of time of day.

It is unclear from the text (e.g., page 7) how the doses were calculated, but it appears from Table 1 that the doses were expressed as the ammonium salt and not the anion equivalent? This point needs to be clearly addressed.

It is somewhat confusing that the authors refer to 'habituation' (see page 8) both to describe aspects of the measured behaviors in the Opto-Varimex apparatus and processes that occur prior to beginning the Opto-Varimex test session. For purposes of clarity, perhaps 'acclimation' would better describe pre-test conditioning of subjects.

There is no reference for the Greenhouse-Geisser statistical test employed in the analyses of the data (page 9).

The Tables would all benefit from having a short legend/descriptive title.

The symbols used in the figures did not reproduce well and were often hard to read.

**2.** Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices of specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

As mentioned above, it is not clear how the timing of the behavioral assessments for each subject were determined. While it is stated that the motor assessments occurred between the hours of 0830 and 1430, it was not stated whether any stratification was employed to distribute time of day assessments equally across treatment groups or if time of day was considered at all. It seems possible that the large variability noted in most of the assessment measures presented in this report might be due to changes in activity levels as a function of time of day.
It would appear that appropriate QA/QC procedures were effected and that no significant deviations from the protocol occurred: however, the document attesting to this presumption is not signed.

**3.** Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe and effect?

Again, it is unclear from the text how the doses were calculated, but it appears from Table 1 that the doses were expressed as the ammonium salt and not the anion equivalent. The approach taken should be should be made very clear. Given the huge variation associated with the endpoints monitored, not enough subjects were used to provide adequate power to detect effects using the statistical approach employed by these authors. By re-analyzing the data using a different approach, the EPA was able to demonstrate significant findings in some endpoints.

**4.** Please comment on the strengths and limitations of the statistical analyses used to evaluate the study findings. What other statistical analyses, if any, should be performed?

See number 3.

**5.** Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

See also number 3.

**6.** Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological/ecotoxicological risk characterization of perchlorate exposure? If so, indicate the extent to which it can be used for characterizing adverse effects.

Given the re-analysis of the Bekkedal et al., 2000 data by the EPA, the study does provide information useful for hazard identification. This conclusion is afforded further confidence since a similar re-analysis of data from an earlier study of motor activity (Argus Laboratories, Inc, 1998a) showed similar findings. It should be noted that the discussion of the analyses of the Bekkedal data (page 5-49of the EPA Toxicological Review and Risk Characterization Document) is unclear in that it is difficult to know what the authors mean by 'first' and 'final' habituation intervals. Do these refer to intervals within a single test session or to the entire first session (i.e., on PND 14) versus the entire last session on PND 22?

**7.** Do the findings provide information relevant to evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?

Yes. The subjects in this study would serve as a model of developmental exposure. Even though dams were exposed before, during and after pregnancy, no data were obtained for these subjects, thus data pertinent to pregnant subjects or adult females were not modeled.

**Argus Laboratories, Inc., 2001:**

**1.** Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

The strengths of this study lie in the comprehensive period of exposure (2 weeks prior to mating up to lactational day 22 in some groups), use of the litter as the experimental unit; the use of targeted and comprehensive endpoints for elucidation of specific questions (clarity of objectives), objective measurements and adequate sample sizes. In addition, the analyses were comprehensive and thorough.

Data in the table on page 20 are inaccurate in several places: for Group III, rows 2 and 7 and for Group IV row 7 the data do not match that provided in Tables C1 and C3, pages 177 and 179, respectively.

Some text is unclear. For example, on page 23, 4$^{h}$ paragraph, see the first sentence and others. It is unclear just what this sentence means: "Numerous brain regions were slightly attenuated in comparison to DL 10 female pup control group values."

While maternal behavior was mentioned as a dependent variable (page 37), little presentation of those data was found in the report.

It is unclear how the perchlorate doses were administered/calculated: were they expressed as the ammonium salt or as the amount of anion available? This should be very clearly stated.

There appears to be a typographical error on page 40; first sentence under G.7.a.2 Scheduled Sacrifice – Part A. Should this not read "On DG 21" not "DL 21"?

In parts of the report (e.g., page 497) a study Part D is mentioned but it is never discussed in any meaningful fashion nor are data from it presented. Thus, reference to study Part D should be deleted.

There is a potential point of discord between the EPA's interpretation of some clinical observations (e.g., localized alopecia) and that of the study directors (Argus 2001). On this issue it would appear that the view of those who conducted might be the more informed in that they are likely to be more familiar with the occurrence of such findings in untreated subjects at their facility.

**2.** Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices of specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

It would appear that appropriate QA/QC procedures were effected and that no significant deviations from the protocol occurred. While certain procedures were changed (cardiac puncture to obtain blood samples rather than collection via the inferior vena cava) there is no indication that any would have impacted the outcome of the study.

As a point of clarification of the statement made on page 559, item 2, it would be helpful to know just how the dams and fetuses/pups were selected if it was not done randomly.

**3.** Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe and effect?

Again, it was not made at all clear just how the doses were calculated: were they expressed as the anion (perchlorate) equivalent or as the salt? The approach taken should be made very clear to the reader. Given the ability of the endpoints employed to detect significant effects, enough subjects were used to provide adequate power for the statistical approaches utilized. Additional statistical analyses were performed by EPA staff who correctly realized that simple t-tests were not adequate for the analyses of the brain morphometry data, particularly given the critical importance of that data set.

**4.** Please comment on the strengths and limitations of the statistical analyses used to evaluate the study findings. What other statistical analyses, if any, should be performed?

See number 3.

**5.** Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

One interesting statement can be found on the bottom of page 61 and the top of page 62 where the authors state " These significant increases" (in body weight) "were not considered treatment-related because the expected effect of a toxicant would be a decrease, rather than an increase, in the body weights." There is no

justification or support for this statement, particularly when increases in the weight of specific organs, such as the brain and the thyroid, are clearly taken as adverse events.

Another interesting observation surrounds the omission in the Summary of Results (pages 69-71), of the findings of significant increases in cerebellar and corpus callosal measurements seen in the males at the 0.01 mg/kg doses on DL22. Likewise for the significant findings at 0.01 and 0.10 mg/kg for female cerebellar (decreases), hippocampal (decreases) and striatal (increases) measures.

There seems to be an error in a standard deviation in Table B14 (PAGE 1) on page 94 where a value of 39.9 is reported under Group IV, days 1-14b. There also appears to be an error in Table C37 (PAGE 1) on page 278 where a value of "4." shows up in the last column for rat #16635.

This report was comprehensive in nature and provided adequate and sufficient data on a variety of measures likely to be affected by the mode of action of perchlorate. These included but were not limited to T3, T4 and TSH plasma levels; weights, pathology and morphometric measurements taken from a variety of brain areas; thyroid measurements that included weights and histopathological analyses. The data presented support the critical conclusions of the authors that "there is no clear-cut evidence of a no-effect level", thus, identifying the 0.01 mg/kg/day dose as a LOAEL.

**6.** Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological/ecotoxicological risk characterization of perchlorate exposure? If so, indicate the extent to which it can be used for characterizing adverse effects.

It was curious to see that the dose range used in this study included a 30 mg/kg/day dose given that previous studies had apparently shown 10 mg/kg/day to be a clear effect level.

This study was extremely comprehensive in a very targeted way and focussed on effects likely to manifest as consequences of the suspected mode of action of the perchlorate anion. Given the known trophic effect of thyroid hormones on brain development and the demonstrated ability of perchlorate to alter circulating levels of those hormones, it was logical to expend great effort on examining the effects of perchlorate exposure on a variety of measure of brain integrity, as well as on thyroid hormone levels and thyroid morphology and histology. The data obtained in this study replicate important earlier findings and provide clear evidence that, while generating a U-shaped dose-response curve, significant effects on important biological processes can be detected at doses as low as 0.01 mg/kg/day. Given that the rat appears to be less sensitive to the lethal effects of perchlorate than are other species, the data obtained in this study should be taken as reasonable evidence of likely effect at similar doses in humans.

**7.** Do the findings provide information relevant to evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?

Yes. The subjects in this study serve to model exposures in nonpregnant, pregnant and lactating females, and developmental exposures throughout gestation in the fetus (via maternal exposure) and postnatally to

offspring (via maternal milk and possibly via drinking water.). Exposures began prior to mating and continued throughout gestation and lactation to postnatal day 21.

**C.2** Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment... You do not need to answer every question in Attachment 2, rather use your profession judgement to address those that are most appropriate to the chapter in question.

**1.** Are you aware of any other data or studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of adverse health (both noncancer and cancer) or ecological effects of perchlorate? Note any references that have not been cited and their relevance to the hazard characterization.

No.

**2.** Have the key aspects of the protocols, conduct and results of each study been adequately described in the Toxicological Review and Risk Characterization Document? Where limitations exist in study reports or published papers, have they been adequately discussed? Please make specific recommendations on improvements to the discussion of the studies.
Key aspects of the protocols, conduct and results of each study have been well described and interpreted and important issues concerning the strengths and weaknesses of each study have been adequately addressed.

**3.** Indicate the strengths and limitations of the analyses performed on the data in the Toxicological Review and Risk Characterization Document, first of the specific toxicological studies and then of the overall toxicology database on perchlorate. Has the document adequately evaluated and integrated the results of all relevant studies to capture the biological relevance of the entire database? Where inconsistencies appear to exist in the finding among studies with respect to perturbation of the hypothalamic-pituitary-thyroid axis, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

**4.** Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these statistical analyses were performed, were they appropriate? Did they add to the overall understanding and relevance of the studies? Were the appropriate endpoints, receptors/indicators or time points used? Please make specific recommendations regarding data, methods and inferences.

In the case of the re-analyses of the motor activity data for the Bekkedal et al., 2000 and Argus Research Laboratories, Inc 1998a studies, the efforts appear justified and valid, given the extreme variability associated with the motor activity measurements as obtained in those studies. The fact that EPA's analyses showed the

same thing in both studies demonstrated replication of a perchlorate effect. The significant effects found in these data are all, however, above the doses shown to be 'active' in the Argus 2001 study, and therefore, do not directly impact the risk assessment. They do tend to show support for a functional effect of perchlorate exposure on the cerebellum, an association that would also tend to corroborate the morphometric findings reported in the Argus 2001 study.

For the re-analysis of the Argus 2001 brain morphometry data, EPA was clearly justified in the application of their approach, since earlier analyses employed only t-tests. The EPA's findings provided clear confidence in the significance of the effects reported in Argus 2001.

**5.** Are the key issues, statements, and conclusions clearly stated? Are the conclusion supported with sufficient data and arguments? How would you suggest improving the clarity of the text? Please make specific recommendations or note revision that would improve the usefulness of the document for the purposes of characterizing the human health and ecotoxicological effects of perchlorate.

The key issues, statements and conclusions are clearly stated and the conclusions are supported with sufficient data.

There are only minor examples of text that were difficult to follow:

On page 5-48, lines 6 and 7 should read "...dependent changes in the later portions (or 5-min blocks) of the 90-min sessions.."

Page 5-49, line 5. What is 'habituation interval'? Likely this refers to the first 5-min block of the 90-minute test session and the 'final interval' refers to the last 5-min block of the 90-min session. This should be clearly stated.

Page 5-75. Lines 8-19 are very unclear...it should be rewritten so that the reader can easily follow what is being described.

Page 5-82, lines 12-16. It is unclear why EPA would elect to consider the observation of transient, localized alopecia as a biologically significant effect of exposure when the finding is apparently relatively normal for animals in the Argus testing facility.

Page 5-84, line 17. What is the 'maternal behavior' that was observed?

Page 5-51, lines 7-9. These are unintelligible.

Page 7-14, Table 7-6. Legend indicates that human data are in the table, but there are none.

Page 7-17, line 8-9. This sentence seems out of context.

Page 7-17, line 26. There is reference to 'the battery' but there is no description or naming of it.

Paule

Page 7-18, line 24. There is reference to 'this effect' but it is unclear which effect is being referenced.

Page 10-3; line 9. Should not the word 'deviation' be 'derivation?'

**6.** Are the assumptions and uncertainties clearly and adequately expressed?

Yes and in appropriate detail; nicely summarized in Chapter 10.

**C.3** Are the toxicity data consistent with the proposed mode of action for perchlorate?

Yes, clearly.

**C.4** The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELS) or lowest-observed-adverse-effect levels (LOAELS) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

The EPA has adequately explained their description and identification of the NOAELs and LOAELs in these studies. In most cases, these are straightforward derivations.

**Topic Area F; Human Health Dose-Response Assessment**

**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?

The review document does a commendable job of addressing all of these issues and in sufficient manner to appropriately describe the approaches utilized and the findings and conclusions presented. It also appears that all of the appropriate data available to date has been considered during the process.

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

The use of AUC instead of peak values seems well justified and it is likely the better of the two metrics in this case. The application of the proposed PBPK models is reasonable and justifiable.

**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?

Clearly, additional data on the potential effects of perchlorate exposure on immune function would serve to decrease the uncertainty associated with database insufficiency. Additional studies could also serve to effectively establish a NOAEL and thus address the uncertainty associated with having to extrapolate using the LOAEL.

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

The potential increased susceptibility of subjects at greater risk, such as those who are hypothyroid or hypothyroxinemic, was clearly considered in applying the intraspecies uncertainty factor. In addition, it was acknowledged that, for this factor, there was uncertainty in the use of the parallelogram approach in extending the adult structure of the PBPK modeling to address different life stages.

**Topic Area G: Risk Characterization**

**G.1** Does the risk characterization chapter adequately and clearly summarize e the salient aspects of the human health risk posed by potential perchlorate exposures?

Yes, the known potential effects are brought together well in a concisely informed manner.

**Topic Area H: General Comments, Conclusions, and Recommendations**

Please provide comments on additional topics relevant to the perchlorate assessment but not explicitly addressed in the previous charge questions.

The obvious occurrence of a U-shaped dose-response curve in a variety of endpoints associated with the effects of perchlorate exposure is intriguing. While such phenomena are not unusual, this issue could be a point of concern for the non-science public, in that the data would seem to suggest that it would be better to be exposed to the higher doses of perchlorate and avoid any of the problems with lower dose exposures. In anticipation of such concern, some discussion of this issue seems warranted.

**H.2** Please identify specific section of the document you find unclear or difficult to understand and explain why.

These were listed earlier.

**Mehdi Razzaghi**

Reviewer Comments

**A.Studies**

1. **Greer, M.A. et al (2000). Does Environmental Perchlorate Exposure Alter Human Thyroid Function? Determination of the Dose-Response for Inhibition of Radoiodine Uptake.**

A.1 Have all 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?

Answer: No. The study is based on contaminated drinking water and is performed on 24 volunteers.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

Answer: No, only iodide thyroid uptake in adult humans is considered. No neurodevelopmental or neoplastic consequences are studied or discussed in the paper.

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?

Answer: N/A

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 for both.

Answer: The study is not concerned with cancer or noncancer effects, but only the iodide uptake.

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: There is insufficient information in this study to enable one to assess the reliability of the results. First, it is the design. No information is provided about the subjects selected for the study other than gender and having a normal thyroid. Information regarding their health status, smoking habit, weight, ethnicity, etc. are missing. This information could be crucial in the evaluation of the effect. Although, it is mentioned that the subjects were all in the 18-57 yr old age group, no further information is given about the distribution of age for each gender and in each dose group. Probably a randomized block design would have been more appropriate in this regard. No information regarding the choice of the dosage levels is provided. The experiment is conducted at three dosage levels, and therefore the results could have important impact in the understanding of the mechanism of perchlorate, but unfortunately with such limited information, it is hard to evaluate the study. Another problem is the way the paper analyzes the data. They use a 3-dose linear regression model on log-dose to compare effects. This is a very simplistic approach and consequently raises several questions about the results. How was the goodness-of-fit assessed? Were other models considered? The investigators conclude that a dose of 0.5 mg/day has no-effect and therefore water supplies containing less than this amount should have no effect on human thyroid function. In my opinion this is a very strong conclusion from this study and because of the above problems in design and analysis, such conclusion is not completely reliable.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: N/A

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

Answer: This is not an epidemiological study.

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?

Answer: N/A

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?

Answer: A regression model with three points in a 1-way lay out is probably not an adequate way to analyze the data. Although there appears to be a clear trend in the data in most cases, this trend does not seem to hold for the post exposure day 15. And to control confounding factors such as age, health status, etc., a randomized block design would be preferred.

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?

Answer: N/A

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

Answer: N/A

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?

Answer: N/A

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Answer: No. Human susceptibility has not been addressed.

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

Answer: N/A

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Answer: No more comments

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Answer: N/A

**2. Lawrence, J. et al (2001). Ltr to editor – low dose perchlorate (3 mg daily) & thyroid function (Thyroid, v11, #3, 295).**

A.1 Have all 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?

Answer: No. The experiment is on a group of men and thyroid functions (TSH,T3 and T4) as well as free thyroxine index, urinary iodide, creatinine measurements, and thyroid iodine uptakes are measured.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

Answer: No. Only iodide thyroid uptake is considered. No neurodevelopmental or neoplastic effects are considered.

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?

Answer: N/A

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 for both.

Answer: N/A

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: This study is conducted on only one dose, arbitrarily determined, on eight volunteer male subjects. Although no information about the method of data analysis is provided, apparently one-way ANOVA is used to compare the effects. Since no information about the background of subjects is given, it is hard to determine if the correct methodology has been used. Moreover, with only eight subjects in the experiment, there is probably not sufficient degrees of freedom to detect an effect. No analysis of the power of the test is provided.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: N/A

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

Answer: N/A

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?

Answer: N/A

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?

Answer: The confounding factors are not even addressed. No information about the subjects (age, weight, health status, smoking habits, etc...) is provided. Although no significant effect is found at the dose

examined, there are other factors that one should consider before one can conclude that environmental exposure to perchlorate would not affect thyroid function.

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?

Answer: There is no analysis of point of departure in this study.

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

Answer: N/A

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?

Answer: N/A

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Answer: Susceptibility is not an issue in this study.

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

Answer: N/A

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Answer: None

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Answer: None. The document is clear.

3. **Miller, E. (2001a).Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit report for the study of perchlorate pharmacokinetics and inhibition of radioactive iodine uptake(RAIU) by the thyroid in humans (CRC protocol #628) [Memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB,OH:Air Force Research Laboratory; May 10.**

A.1 Have all 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?

Answer:

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

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?

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 for both.

## EPA DOCUMENT

**B. Perchlorate Assessment**

A.1 Have all 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?

Answer: Not qualified to answer.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?

Answer: Not qualified to answer.

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?

Answer: Not qualified to answer.

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 for both.

Answer: Not qualified to answer.

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: Studies were reviewed before.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do

not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

Answer: The summary of the eight epidemiological studies performed since the 1999 external peer review clearly reveal that there is an effect due to exposure to perchlorate. However, as noted in the document, most of these studies have limitations due to uncontrolled confounders and hence the utility of these studies for characterization of risk becomes questionable. For example, the only study on the general population (Li et al, 2000) did not control several important confounding effects and risk factors. Other studies also ignored some of the confounding effects. According to the EPA document, of all the studies on children, the study of Schwartz (2001) is by far the most convincing of the neonatal studies. However, there are some ambiguities in the way the results are reported. It is not clear why the thyroid hormone T4 declined at four of the perchlorate exposure levels with age until about 18 hours and then increased over the next 30 hours. Does this mean that there may be a hormetic effect due to exposure to perchlorate? A clarification and a better description would be useful in this case.

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?

Answer: In general, there appears to be a good analysis of the epidemiological studies. The report summarizes the important results and findings of the studies and points out the weakness of each in some detail. The point-of-departure analysis of chapter 7 is particularly interesting as it emphasizes the use of model approach to risk assessment to determine the benchmark dose and hence reduce the influence of experimental conditions such as dose-spacing, sample size and variability of the NOAEL. Since the ultimate goal is to obtain an acceptable exposure level for humans, it is more reasonable to take a unified approach for cancer and noncancer endpoints. Gaylor, et al (1999) discuss this issue in great depth and propose the use of a statistical lower confidence limit on the dose estimated to produce an excess incidence of adverse health effects in 10% of the animals in bioassay experiments as a point-of-departure.

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?

Answer: The weakness here is mainly in the clinical studies. Although the EPA report provides a good

summary of the available studies, unfortunately the studies are not sufficiently informative to warrant reliable conclusions. Generally, they are based on small samples with insufficient power, ignore confounding factors, and therefore one cannot confidently derive a NOAEL for humans from such studies. Although the studies are very informative in displaying the some mode-of-action aspects of perchlorate, as pointed out in the EPA document, they fail to address some potentially important aspects of mode-of-action for perchlorate.

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: Discussed in the review of studies.

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: In the review of the Argus Research Laboratories (1998a), which examines the developmental neurotoxicity of ammonium perchlorate (page 5-34 of EPA document) the litter information is not clearly stated. For example at the bottom of page 5-34, it is stated "Other pups (F1-generation) were assigned to four different subsets for additional evaluation." And from then on no information is provided about the litters. Litter information is crucial in all of reproductive and developmental studies because of the existence of intra-litter correlation.

In the evaluation of developmental neurotoxicity of ammonium perchlorate, it is particularly interesting to note the application of Bayesian hierarchical models to assess the weight of evidence of a dose-response trend in motor activity (Dunson, 2001a) applied to the data of Bekkedal et al (2000), a study that evaluates motor activity of rats in both sexes. It would probably be useful to give brief information about the choice of level 1 and level 2 variables in the hierarchical model. Dunson (2001a) also uses a modification of the model to perform a combined analysis of data from Bekkedal et al (2000) and Argus Research Laboratories (1998a) studies (pp. 5-48 to 5-52 of EPA document). This kind of analysis is remarkable, essential and highly useful in better understanding of the dose-response trend. A widely used software package for Bayesian inference, BUGS, is utilized for the analysis. BUGS is a software for full Bayesian inference and it uses the Markov Chain Monte Carlo (MCMC) algorithm to generate posterior samples, i.e. a set of correlated draws from a sequence that converges to the exact posterior distribution. A set of independent draws from the exact posterior distribution (see Everson and Morris, 2000) would

probably improve the analysis. Moreover, one should be aware that BUGS does not support some of the prior distributions (e.g. uniform) on the level-2 covariance matrix.

Page 5-90 line 15, 1923 should be 1852.

On page 5-90, it is explained that "To account for the fact that the Argus(1999) study recorded thyroid incidence at 19 weeks and not at the time of natural death or sacrifice at two years, a prior...." . Although the analysis is correct, alternatively one could consider an age-adjusted trend test (see Kodell and Ahn, 1997) for this analysis.

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

Answer: No particular comments.

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

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?

Answer: In Appendix 7B of the EPA document, a very thorough and interesting discussion of BMD for hormone analysis is presented. The Kodell-West procedure is used to derive BMDs for quantitative responses. The fits to the data did not reach statistical significance and lack of fit raised difficulties with interpretation suggesting that the estimates should not be used as the basis for risk assessment. The Kodell-West procedure is based on the assumption of normality of responses with equal variances at all dose levels, and uses a quadratic polynomial as dose response model. Although the model may be very attractive in some cases, the reason why it has not produced satisfactory results may be that the model is not flexible enough to provide adequate fit to the data. Perhaps a more flexible model which allows for bimodality and bitangentiality such as a mixture of two normal models (see Razzaghi and Kodell, 2000) would provide a better description of the data.

According to the EPA document (page 7B-4) The rabbit developmental studies of Caldwell et al (1995) subchronic hormone data were "best fit" by unrestricted power functions. The hormone data from developmental neurotoxicity study and mouse immunotoxicity study were fit by either unrestricted power or polynomial (linear or quadratic) functions. One must be warned that simply because a model provides a good statistical fit to the data does not justify the use of the model in risk assessment. A model should also be biologically interpretable. Surely, if a model is based on the biological mechanism of toxicity, it would lead to more reliable estimates. To this end, the use of biologically based dose response (BBDR) models should be encouraged.

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

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?

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Answer: The issue of susceptibility is of crucial concern. There are several models that incorporate susceptibility in the analysis. Recently, mixture models have been proposed for consideration of susceptible subpopulations (see Razzaghi and Kodell, 2000). It would probably be worthwhile to use such models for identifying factors influencing susceptibility.

## Gary Williams

# Review of

# Perchlorate Environmental Contamination:

# Toxicological Review and Risk Characterization

prepared for

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by

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February 22, 2002

## I. REVIEW OF STUDIES PUBLISHED SINCE 1999

### 1. Argus 2001 Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or via Maternal Milk

a) Study Objectives

The objectives of this study were:

i) to determine the teratogenicity of perchlorate in the rat;

ii) to measure thyroid stimulating hormone (TSH), thyroxine ($T_4$) and triiodothyronine ($T_3$) changes in developing rats (fetal and neonate) and in adult female rats during pregnancy and lactation;

iii) to measure neurohistological and thyroid effects at the same time points; and

iv) to correlate these effects with hormone concentration changes at different stages in development of the rat.

b) Study Design

A good description of the study design (A.5 p 26) is lacking.

A range of doses of ammonium perchlorate was administered to female Crl: CD® (SD) 1GS BR VAF/Plus® rats during breeding, pregnancy and up to 22 days post partum. After 2 weeks on test, female rats were mated. Inseminated females were designated gestation day (DG) 0. The study consisted of 3 parts:

A. On DG 21, P generation females were killed, uteri were excised and fetuses examined.

B. 4 pups of each sex per litter were continued on study until postnatal day 10 (DL 10) when they and dams were killed. Remaining pups were killed on DL 5.

C. 4 pups of each sex per litter were continued on study until postnatal day 22 (DL 22) when they and dams were killed. Remaining pups were killed on DL 5.

c) Performance of Study

The study was conducted according to GLPs and no significant deviations occurred.

The criteria for histopathology of the thyroid are not given in the report, but are stated to be those developed for a PWG review of a previous 90 day study (Wolf, 2000). The histopathology was performed at Environmental Pathology Laboratories by K.A. Funk, who is said to have been a member of the PWG (5-53).

The histopathology of brain was performed at Consultants in Veterinary Pathology by R.H. Garman.

d) Dosing of Test Substance

Ammonium perchlorate (99.8% pure) was administered in drinking water at target doses of 0., 0.01, 0.1, 1.0 and 30 mg/kg/day. Actual achieved doses in the precohabitation exposure period were 0.00, 0.01, 0.08, 0.77 and 23.93 mg/kg/day, respectively and in the gestation exposure period, 0.00, 0.01, 0.10, 0.98 and 28.73 mg/kg/day, respectively.

e) Data Presentation

The nomenclature used for days of life differs from that of EPA (5-52).

Text tables are not numbered.

In Part A, fetuses showed no abnormalities (p 46), except thyroid colloid was decreased 1.0 and 30.0 mg/kg/day (p 48). Dams showed dose-related changes in TSH and and $T_4$ beginning at 0.01 mg/kg/day (p 48) and changes in thyroid weight and histopathology only at 30.0 mg/kg/day (p 47).

In Parts B and C, pup body weights were significantly increased in treated groups (p 61), but this was not considered treatment-related because "the expected effect of a toxicant would be a decrease." Could this effect be due to hypothyroidism?

In Part B, in DL 10 dams, thyroid weights were increased at 30 mg/kg/day (p 51) and decreased colloid was evident at 1.0 and 30.0 mg/kg/day (p 53). In DL 10 pups, thyroid weights were increased in males at 0.01 mg/kg/day and above and in females at 30 mg/kg/day (p 52). Decreased thyroid colloid was found in high dose males and females and in females at 1.0 mg/kg/day (p 53). Changes in TSH and $T_3$ were also found (p 55).

In Part C at DL 22, dams showed an increase in hyperplasia at 1.0 mg/kg/day and a decrease in colloid and hypertrophy at 30 mg/kg/day (p 63). Pups had decreased colloid at 30 mg/kg/day.

f) Statistical Analyses

Appropriate parametric and nonparametric tests were used (p 42).

g) Study Conclusions

The report has a Summary (page 69), but no conclusion.

Neuropathology: no evidence of treatment-related neuropathologic effects (p 66). Morphometric differences, however, were found even in the pups of dams receiving the lowest exposure.

Thyroid histopathological changes were found in both dams (p 69) and pups (p 70, 71).

h) Overall Assessment

This study is of sufficient quality for hazard identification. The relevance of thyroid effects in rodents to human hazard, however, is questionable because of species differences.

II. REVIEW OF DRAFT RISK ASSESSMENT

1. Chapter 1

No comment

2. Chapter 2
No comment

3. Chapter 3
3.9 line 29 needs authoritative reference for thyroid physiology

4. Chapter 5
5.1.2.3 No studies on genotoxicity in thyroid are available.

5-53 lines 23 and 28, "effected" should be "affected."

5-53, line 29 states that in PND 21 (DL 22) dams there was a clear dose related trend in colloid depletion, hypertrophy and hyperplasia.
This is not evident in the table on p 63 of the Argus report.

5-73 two different analyses of brain morphometry from the 2001 Argus
study yielded significant effects resulting from developmental exposure of rats to perchlorate at doses of 0.001 mg/kg/day and higher.

5. Chapter 7
The toxicity data support the proposed mode of action of perchlorate as an inhibitor of iodide uptake in the thyroid (7-3)

6) Chapter 10
10.1.1 does not mention goiter hazard

III. ANSWERS TO CHARGE QUESTIONS:

A.1 Available data are appropriately utilized. Species differences in toxicity have not been examined.

A.2 Yes. It could be noted that the mode of action is not unique to perchlorate, but is also produced by thiocyanates.

A.3 A non-DNA-reactive (epigenetic) agent should have a threshold at which the no-effect-level for the mode of action would be a nontumorigenic exposure.

A.4 The approach is protective for both.

C.1 The Argus 2001 study does not have a NOAEL.

C.2 see Section II

C.3 Yes

C.4 Yes

F.1 The conclusions are consistent with the mode of action.
However, in applying a point of departure, it should be taken into account that humans regularly consume thiocyanate goitrogens in food at low levels without adverse effects.

F.2 PBPK models are useful for interspecies extrapolation. The key metric is the thyroid exposure.

F.3 Species differences in sensitivity of the NIS to perchlorate should be considered.

F.4 No. Susceptibility of the NIS should be considered as well as species differences in requirement for thyroid hormones.

G.1 No. No discussion of goiter.

H.1 Iodine deficiency is not established as a cause of thyroid cancer in humans.

H.2 see Section II

# Ronald Wyzga

Comments on "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Ronald E. Wyzga, Sc. D.

General Comment: It would be helpful to know something about the levels of environmental contamination and of personal exposure. These would help place the contents of this report in perspective and allow us to make some judgment about how important the various uncertainties are. In other words do they really make much of a difference with respect to current exposure levels?

Chapter 4: By and large I found this chapter to be very well written. The various studies are comprehensively described along with their strengths and weaknesses in a most objective way.
There could be more discussion about the problems/uncertainties associated with ecological studies at the beginning of the section on page 4-3. .

pp.4-12-4-13: Has the Schwartz study been published in a peer-reviewed journal? Has it been submitted? Are EPA Staff following the disposition of the results of this study? If it will not soon be published, what is the EPA policy about citing/using studies that have not been published in the peer-reviewed literature.

p. 4-15: I worry a bit about the calculation of inhaled "dose" for 2 reasons. I would be surprised if there were not considerable variation in some of the variables in (4-1) over time and across individuals. Has there been a good investigation of this issue? I also am concerned about the reliance on total dose. Alternative measures of exposure (e.g., peak concentrations or exposure to concentrations greater than some fixed level) may be more important. I am struck by the non-linearity of some of the results presented in this chapter. This could suggest that alternative exposure measures are more relevant than total or average exposure.

p. 4-17, ll. 6-7: this is one example of non-linearity.
ll. 23-26: or that there is no effect.

4-21-4-23: The data are interesting and could be used to support some non-linear dose-response functions.

Chapter 5: Extensive toxicological data were available for consideration in the development of the draft RfD for the perchlorate anion described in this document. The testing strategy developed in 1997 and augmented again in 1999 in response to a second external peer review addressed target organs and tissues other than the thyroid, augmented the thyroid data to allow quantitative dose-response assessment, and assessed the effects of perchlorate on reproductive capacity and in potentially susceptible populations. Endpoints considered included cancer and genotoxicity; general toxicity (short-term and subchronic);

developmental neurotoxicity; developmental toxicity; two-generation reproductive toxicity; and immunotoxicity. Given perchlorate's mode of action as a competitive inhibitor of iodide uptake in the thyroid, with resultant disruption of the hypothalamic-pituitary-thyroid axis, the endpoints selected for study were appropriate. Furthermore, because perchlorate's carcinogenic effects are due to its effect on iodide uptake and not due to genotoxic effects, one harmonized risk estimate for both noncancer and cancer sequelae could be derived.

Chapter 7: The introductory material is particularly useful and well-written.

p. 7-6: I'm not sure whether this is an appropriate citation of Weiss. I though his comments applied to IQs and the overall shift in a known distribution function. It is unclear whether such a shift would occur here.

p. 7-15:, ll. 1-4: It would be interesting to consider several alternative dose metrics. E.g., how would the results change if the peak dose level were used?

Point-of-departure analysis showed the exposure dose considered to be a level of concern for the adverse effects of perchlorate to be 0.01 mg/kg-day, based on effects on brain morphometry (increased corpus callosum size), motor activity, thyroid histopathology, and alterations in thyroid hormone levels. However, importantly, this dose level was the lowest tested and is therefore considered the LOAEL. A human exposure equivalent of 0.01 mg/kg-day was derived, which was less conservative than the HEE for iodide inhibition in the dams of 0.002 mg/kg-day. Four uncertainty factors were applied: a factor of 3 for intraspecies variability; a full factor of 10 for LOAEL-to-NOAEL extrapolation due to the shallow slope of the response curve; a factor of 3 to extrapolate to longer duration exposures; and a factor of 3 for database insufficiency, largely in light of uncertainties with respect to immunotoxicity. Notably, the interspecies UF was omitted because the PBPK modeling was deemed to adequately address this extrapolation. The composite UF was thus 300, and the derived RfD estimate is 0.00003 mg/kg-day after adjusting for the percent of the molecular weight of the perchlorate salt from the cation. The RfD that would have been derived from the available human data was estimated at a maximum of 0.00007 mg/kg-day; this estimate is one-half as conservative as the proposed toxicology-based value; if one compares absolute values, there appears to be agreement between the approaches.

p. 7-22: I would have liked to have seen a greater justification for the use of the 10-fold factor associated with extrapolation from the LOAEL.

p. 7-26, ll. 23-26: The presentation of real-world exposure data would help one judge whether the difference in the RfD calculations from animal and human data were significant. There might also have been some consideration of using the Schwartz data to caluculate an RfD.

Dose-response analysis of thyroid neoplasia results in an RfD derivation of 0.005-0.0002 mg/kg-day. It should be noted that the neoplastic effects of thyroid disruption (i.e., elevated TSH levels) are more significant in rodents than in humans as the human thyroid is much less sensitive to this pathogenetic phenomenon than rodents. However, this species difference does not affect the interpretation or regulatory

implications of this quantitative risk assessment because the RfD derived for non-cancer effects was more conservative than that derived for cancer effects. Moreover, the similarity in the RfDs derived for cancer and non-cancer outcomes appears to support the mode-of-action concept.

In summary, the draft RfD derived in this quantitative risk assessment appears to be a conservative estimate based on extensive, multi-endpoint toxicological data and incorporating PBPK modeling for inter-species extrapolation. Notably, the fact that the LOAEL was the lowest dose tested (0.01 mg/kg-day) precluded the determination of a NOAEL for perchlorate. However, this uncertainty appears to have been addressed through the application of the full factor of 10 uncertainty factor in the RfD derivation. Additional studies at lower doses would verify the appropriateness of this assumption. In addition, uncertainty remains regarding the immunotoxic effects of perchlorate, particularly with respect to possible contact hypersensitivity, and additional investigation should be conducted to this end.

Responses to Charge Questions
Ronald E. Wyzga, Sc. D.

A.1. Since I am not an expert on this literature, I cannot comment on the first part of this question. The similarities and differences in toxicity profiles across species have been reasonably well-characterized. They are certainly systematically addressed.

A.2. Yes, this is particularly well explained.

A.3. This issue needs to consider experimental data as well as mode-of-action data. Consonance between the two need be assured. I would like to see more discussion here. I note, for example, that some of the experimental data suggest non-linearity of response; this could imply that alternative dose metrics need be considered.

A.4. There are relatively few data on cancer endpoints; hence it is unclear whether the approach taken for non-cancer events is protective. Indeed the lack of genotoxicity data and the absence of malignant tumors (in the studies for which there is documentation) raises the question about whether there is any current evidence for carcinogenicity. What are needed are more studies of the carcinogenic potential of perchlorate. The existing data need be scrutinized to determine whether any malignant tumors were found in studies to date.

B. 1. I would like to see further discussion and analysis of the Schwartz (2001) study and data. I don't understand why this study is not in Table 2. Since it is only a dissertation at present, I don't consider it to be a peer-reviewed study per se. The author should be contacted to determine whether a peer-reviewed publication of this study is likely soon.

B. 2. I am not aware of additional studies. I found the description of the various studies to be clear; in addition, I appreciated the limitations listed for the various studies. By and large, the discussion of the various studies was objective and well-balanced.
I worry a bit about the calculation of inhaled "dose" (p. 4-15) for two reasons. I would be surprised if there were not considerable variation in some of the variables in equation 4-1 over time and across individuals. Has there been a good investigation of this issue? I am also concerned about the reliance on total dose. Alternative measures of exposure (e.g., peak concentrations or exposure to concentrations above some fixed level) may be more important. I am struck by the non-linearity of some of the results presented in Chapter 4. This could suggest that alternative exposure measures are more relevant than total or average exposure. Examples on non-linearity are p. 4-17, ll. 6-7 and p. 4-21-4-23.
I would have liked to have seen more discussion of how consideration of the Schwartz study results would have changed the overall conclusions of the report.

B.3. Yes, but see above comments about consideration of the Schwartz results.

B. 4. This has no straightforward answer – partly because we don't know the correct dose measure. See response to B.2. above.

B. 5. The associations are broadly consistent, but there are results that suggest non-linearity. (See above.) I would have liked to have seen more discussion of these results. Several studies were based on small populations or were ecological studies. The latter always raise confounding issues. They were well-described, but it is difficult to control for confounders definitively in this type of study design.

C. 1. –

C. 2. By and large the studies were well described with limitations clearly stated. What is particularly useful was the framing of the various studies on the context about what we know about mode-of-action.

C. 3. This depends upon the endpoint. The relationship is clearer for some endpoints than for others. For example, the relationship for developmental toxicity endpoints involves considerable conjecture.

C. 4. NOAELS/LOAELS appear to be reasonably defined.

F. 1. Given perchlorate's mode of action as a competitive inhibitor of iodide uptake in the thyroid, with resultant disruption of the hypothalamic-pituitary-thyroid axis, the endpoints selected for study were appropriate. Point-of-departure analysis showed the exposure dose to be considered to be a level of concern for the adverse effects of perchlorate to be 0.01 mg/kg-day, based on effects on brain morphometry (increased corpus callosum size), motor activity, thyroid histopathology, and alterations in thyroid hormone levels. This dose level was the lowest tested and is considered the LOAEL. A human exposure equivalent of 0.01 mg/kg-day was derived which was less conservative than the HEE for iodide inhibition in the dams of 0.002 mg/kg-day. These point of departures appear to be appropriate.

F. 2. I would have liked to have seen consideration of alternative dose metrics. Some kind of sensitivity analysis would have been useful here.

F. 3. I would have liked to have seen a greater justification for the use of the 10-fold uncertainty factor associated with extrapolation from the LOAEL. I also worry about the argument about the need for an uncertainty factor associated with an incomplete database. Databases will never be complete; I personally dislike the use of this safety factor. Presumably the effects most likely expected have been considered. Certainly those associated with the described mode of action have been considered. Hence I would reject that argument.

F.4. No comment here.

G. 1. The presentation of real-world exposure data would help one judge whether the difference in the RfD calculations from animal and human data were significant. I'm also uncomfortable about making any statement about carcinogenicity of perchlorate. The data base does not allow any statement to be made. The data are either negative or not interpretable. In addition, it appears as if perchlorate is not genotoxic.

H. 1. See comments on G. 1 about exposure data.

H. 2. See previous comments on specific sections of document.

## Thomas Zoeller

PREMEETING COMMENTS

## Topic Area A: Hazard Characterization and Mode of Action

**A.1 Have all 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 data on perchlorate toxicokinetics has been largely identified. These issues are discussed in Chapter 3 and in Chapter 6. However, the uptake of iodide and/or perchlorate into milk does not appear to be well described or considered in Chapter 3. Specifically, it seems fundamentally important to highlight the data showing that iodide in breast milk is concentrated 20-30 fold over that in maternal serum (reviewed in ). Although measurements of perchlorate in milk do not appear to have been performed, iodide uptake into milk is inhibited by perchlorate and perchlorate is likely to be concentrated in milk as it is in the thyroid gland. This inference is supported by the observation that the sodium/iodide symporter (NIS) that transports iodide (or perchlorate) into the thyroid gland is the same NIS protein expressed in mammary tissue , and its expression is induced and enhanced by prolactin . The residence of perchlorate in milk likewise would be important to identify and evaluate. Considering that milk is the sole, or main, food source for infants, and that the recommended adequate intake of iodine for infants is 110 µg/day for infants 0-6 months and 130 µg/day for infants 7-12 months , it seems important to consider this issue. Finally, perchlorate in milk would both reduce dietary iodine and inhibit iodine uptake into the infant's thyroid gland.

It is somewhat inaccurate to state that rats do not have Thyroid Binding Globulin (TBG). In fact, rats do produce TBG , but its abundance in serum during the life cycle (e.g., pregnancy and lactation) are not well studied.

Aside from these shortcomings, the EPA review of perchlorate toxicokinetics is generally thorough and logical.

The toxicodynamics of perchlorate also is well-characterized in chapter 3. The EPA document clearly identifies that perchlorate inhibition of iodide uptake into the thyroid gland and subsequent inhibition of thyroid hormone synthesis is the mode of action of perchlorate toxicity. In addition, two classes of adverse effects are cited as deriving from this effect. First, the

incidence of thyroid cancers may increase as circulating levels of TSH rise in response to reduced thyroid hormone concentrations. Second, reduced circulating levels of thyroid hormone may impact brain development. These two categories of potential adverse effects are conspicuously absent general physiological effects, but there are no validated measures of adverse physiological consequences of thyroid hormone deficits in animals. Moreover, both cancer and neurobehavioral deficits represent permanent adverse effects.

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 the sodium ($Na^+$)-iodide ($I^-$) symporter (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?**

The EPA has clearly identified iodide uptake into the thyroid gland as the mode of action of perchlorate toxicity. This concept is fully consistent with the available literature as reviewed by the EPA, and there is little evidence that perchlorate exerts direct actions on physiological systems.

Identification of neurodevelopmental sequelae as an important category of adverse effects is fully consistent with the literature. This literature is appropriately reviewed in the EPA document, but focuses mainly on the clinical literature. The literature on congenital hypothyroidism and gestational hypothyroxinemia is accurately reviewed in the EPA document . However, few details are provided about the role of thyroid hormone in brain development in experimental systems. For example, thyroid hormone exerts a variety of effects on the developing striatum that may, in principle, account for changes in the linear dimension of striatum size in the ARGUS, 2001 study. Likewise with the corpus callosum as well as the other brain areas.

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?**

The EPA document clearly articulates the choice of dose metric and approach for low-dose extrapolation in Chapter 7. It is reasonable to conclude that perchlorate does not exhibit direct genotoxic effects. Moreover, it is reasonable to conclude that the primary mode of action of perchlorate is its interaction with the NIS which reduces iodide uptake into the thyroid gland (and into any other tissue that expresses the NIS and actively takes up iodide). Considering this mode of action, several inferences can be made about characteristics of the most reliable dose

metric. First, the choice of dose metric should provide a reliable index of the inhibition of iodide uptake into the thyroid gland, since suppression of thyroid hormone synthesis and subsequent reduction in circulating levels of thyroid hormone represents the mechanism by which perchlorate would produce adverse effects. The EPA chose the area under the curve (AUC) of perchlorate in serum because it provided the best index of the inhibition of iodide uptake – the direct mechanism of perchlorate toxicity. Moreover, the AUC is an integrated measure of the product of time and concentration, which appears to provide a much more reliable index of toxicity compared to other measures (e.g., peak levels).

The approach for low-dose extrapolation requires either a linear or non-linear model. Considering the mode of action, the relationship between perchlorate exposure and adverse effects must certainly be non-linear. There are several reasons for this. First, the interaction of perchlorate with the NIS certainly follows Michaelis-Menton kinetics. Thus, the mode of action itself is governed by non-linear interaction. The relationship between iodide uptake inhibition and circulating levels of thyroid hormone will also certainly be non-linear. This is a complex issue that includes a variety of compensatory mechanisms, the sum of which will exhibit complex relationships. Finally, the relationship between circulating levels of thyroid hormones and adverse effects – whether they be changes in thyroid histopathology or changes in neuroanatomy – will likewise be complex and non-linear.

The EPA document emphasizes the importance of structural changes in thyroid histopathology whether they are changes in colloid, hypertrophy or hyperplasia. The logic justifying this interpretation does not integrate, or harmonize, cancer and non-cancer endpoints. Specifically, the justification presented is that sustained activation of the thyroid gland by TSH, as evidenced by changes in colloid, can lead to cancer. This reviewer concurs with the conclusion that any change in thyroid structure should be considered adverse, but using an integrated logic. First, because thyroid hormone directly suppresses circulating levels of TSH , TSH is a direct biomarker of thyroid hormone action. In fact, it is the only biomarker of thyroid hormone action identified in the entire database. However, it is not traditional to consider changes in circulating levels of TSH to be considered adverse *per se*. In fact, changes in circulating levels of TSH associated with changes in circulating levels of thyroid hormones are usually considered to be "compensatory" . However, there is no evidence that the developing brain can "compensate" for transient deficits in thyroid hormone; in fact, there is considerable evidence to the contrary . Thus, the critical question, for which we presently have no answer, is whether the

hypothalamic-pituitary-thyroid axis is more sensitive to changes in circulating levels of thyroid hormone than are tissues that require thyroid hormone to function properly, such as the developing brain. Therefore, any change in thyroid histopathology secondary to elevated TSH due to reduced thyroid hormone demonstrates that thyroid hormone levels have been altered enough to affect TSH for a period sustained enough to produce structural changes in the thyroid gland. Likewise, it is reasonably inferred that this degree and persistence of change in thyroid hormone would have consequences in vulnerable tissues such as the developing brain.

Low-dose extrapolation was calculated using the NOAEL for thyroid histopathology and for neurodevelopmental measures and this was compared with the finding of Greer et al. . Interestingly, the two approaches yielded nearly identical results. Given the uncertainties associated with the study by Greer (e.g., variability among subjects, relationship to vulnerable subpopulations), the EPA focus on the experimental literature is warranted.

**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 for both.**

The EPA document clearly considers both cancer and non cancer toxicity and articulates how both of these categories of effects are caused by the same mode of action of perchlorate. This harmonized approach is protective for both.

**Topic Area B: Human Health Effects Data**

**B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.**

Greer 2000

This study is reasonably well designed, and is clearly focused on attempting to estimate the risk of thyroid hypofunction resulting from environmental perchlorate exposure. Twenty-four healthy adult volunteers were recruited for this study in which they were given one of three doses of perchlorate in water consumed at 4 set times each day. Measurement of 8- and 24-hr iodide uptake was then performed both prior to perchlorate exposure for baseline estimates and on exposure days 2, 14, and also at 15 days after

exposure was terminated. A linear log-dose relationship was observed between perchlorate exposure and iodide uptake inhibition. Extrapolation toward 0 using this linear function indicated that a no-effect level would be achieved at 7 µg/kg (assuming a 70 kg healthy adult). This was confirmed in a separate study.

The authors estimate that the no-effect level of 0.5 mg/day would be consumed in drinking water containing perchlorate at 250µg/L and that, therefore, "...water supplies containing less than this should not affect human thyroid function". This conclusion, without qualification, is clearly not supported by the study for two types of reasons. First, the authors do not report on the post-exposure iodide uptake in the 4 volunteers provided with the putative no-effect level of perchlorate at 7 µg/kg. Others (Lawrence 2001) have shown that a dose of perchlorate that does not produce statistically significant effects on iodide uptake nonetheless produce a very significant rebound following the exposure period. This rebound is a clear and sensitive indication of a biological effect of perchlorate on thyroid function, but the Greer study does not report on this in the population tested with the "no-effect" level of perchlorate. Second, the authors fail to consider the variability in the human population when they refer to the "human thyroid function". Specifically, people with the variety of thyroid disorders, infants, pregnant women and their fetus.

Overall, the study appears to provide reliable information within the main study. The QA/QC audit did not appear to reveal problems that would further limit the reliability of those data. However, the anecdotal information provided on the group of 4 volunteers exposed to 7µg/kg perchlorate are not reliable.

Lawrence 2001

This study, like that of Greer *et al.*, was conducted to further study the effects of perchlorate on thyroid function in an attempt to find a NOEL. Eight healthy male volunteers with normal thyroid function were provided with 3 mg perchlorate in 1L of spring water daily for 14 days. Thyroid function tests, 24-hour urinary diodide, and 8- and 24-hour iodide uptake measurements were taken at baseline, on day 14 of exposure, and 14 days after perchlorate exposure was discontinued. No statistically significant effects were observed in any of these measures after 14 days of exposure. However, 14 days after exposure was terminated, the RAIU was significantly elevated. No mention was made of the measures of thyroid function in the post-treatment samples.

The conclusion that 14 days exposure to 3mg perchlorate did not significantly affect thyroid function is mostly supported by the data, though the post-exposure rebound demonstrates that there was a biological effect. The data appear to be reliable and justify the conclusion that perchlorate given to healthy adult males for two weeks at 3mg/day does not significantly alter circulating levels of thyroid hormone. Given that the half-life of $T_4$ in humans is nearly a week, and that the majority of circulating $T_3$ comes from peripheral deiodination of $T_4$, the duration of the experiment does not appear to warrant extrapolation to life-time exposures.

**B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.**

The EPA treatment of the new studies by Lawrence, Greer and Merrill appears thorough and reasonable. The interpretation is clear and conclusions warranted.

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

**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?**

**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?**

The epidemiological studies appear to be thoroughly summarized in the EPA document, and their inclusion as a basis for hazard characterization is clear and logical. A severe weakness in all of the epidemiological studies is that measures of exposure are not adequate to identify specific dose-effect relationships. This point is perhaps made best by Li et al. when they state that, "This study was sufficiently sensitive to detect the effects of gender, birth weight, and the day of life on which the blood sample was taken on the neonatal $T_4$ level, but it detected no effect from environmental exposures to perchlorate that ranged up to 15 µg/L (ppb)." Specifically, if population measures of gender (proportion), birth weight (average), and day of life (average) had been used instead of individual values, no relationship with $T_4$ levels would have been observed. Thus, the failure to observe a significant shift in average monthly $T_4$ levels in a population of newborns living in a geographic location in which perchlorate has been reported in the water supply is not, in itself, convincing.

The study by Schwartz, as reviewed in the EPA document, appears to be somewhat more sophisticated in its estimate of exposure, but still lacks the power of individual measurements of perchlorate and its relationship to thyroid function.

Finally, the studies by Crump *et al.* in which the reference population exhibited goiter in 30% of the population is also difficult to make or defend conclusions.

The EPA document evaluated each of epidemiological studies in detail; this reviewer has nothing additional to add.

**Topic Area C: Laboratory Animal Studies**

C.1 **Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.**

Argus 2001

*Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.*

This is a very large study that appears to be well designed and clearly presented. The objectives are clearly articulated and the methods employed are clearly described. A logical weakness in the study design appears to be the following. A great many linear measurements of brain areas were incorporated as endpoints of perchlorate toxicity. However, when changes were observed, the interpretation was complicated because it was not known whether these were specific endpoints of thyroid hormone action. Thus, it would appear to have been more appropriate to build valid endpoints of thyroid hormone action into the developmental neurotoxicity endpoints.

*Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices or specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?*

The study included pathological observations of brain sections, but the conclusion was that processes such as cell proliferation and apoptosis were not affected by treatment. For example, on page 815, the authors write, "As would be expected, the brains from the day 10 postpartum rats were characterized by active cellular migration and cell death (i.e., physiologic cell death or "apoptosis"), as well as by ventricular remodeling. However, no differences were found between the teest substance-treated and control group rats in the degrees of cell death or in the overall pattern of brain morphology." These conclusions are simply without foundation. No formal measures of these processes were taken. Biologically important treatment effects on the rate of cell proliferation, migration, or apoptosis are simply not observable without formal quantitative analysis and, ideally, with the use of specific markers (e.g., BrdU for proliferation, TUNNEL or activated caspase-3 immunocytochemistry) . Considering that thyroid hormone can affect rates of proliferation in some but not all neuronal populations , and that thyroid hormone can affect the rate of apoptisis in some, but not all, populations of neurons , these measures would have been important to include in the study.

The radioimmunoassay for $T_4$ is of also of some concern in this study. This assay uses standards that range from 1 µg/dL on the low end, but levels in some of the animals was clearly below this as evidenced by the mean ± SEM (see page 782, Table 1). Thus, it appears that the standard curves may have been generated using the "0-tube". This would not be a valid approach . Alternatively, they may have generated the standard curve properly, but simply extrapolated between the low standard and 0, which is also invalid. These values draw into question whether these assays were properly conducted, but sufficient information was not available to conclude this.

*Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe an effect?*

Dosing appeared well formulated and controlled. Sufficient numbers of animals were included. Endpoints were discussed above.

*Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.*

As discussed above, there is no evidence that perchlorate treatment did or did not affect processes such as cell proliferation or apoptosis. These processes simply were not evaluated.

Likewise, the conclusion that perchlorate did not produce adverse effects on neural endpoints appears to be unsupported by the data. A good example of a common flaw in logic is illustrated on page 817. Specifically, the comment is that, "Although the mean thicknesses of the external germinal (granular) layer of the cerebellum were significantly thinner for the Group II and III females in comparison to the female control group, this layer is highly variable in thickness and, therefore, difficult to assess accurately with only six linear measurements. The inter-group differences in thickness of the external granular layer are, therefore, considered to be of no biologic significance." Clearly, the variability in this measure (i.e., thickness of external granular layer) would serve to make it more difficult to obtain statistical differences among treatment groups. Thus, if the treatment produced effects great enough to overcome this variability, it is more likely that these observed effects are not spurious. There is no discussion of the effects of thyroid hormone on these measurements in the brain.

*Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological / ecotoxicological risk characterization of perchlorate exposures? If so, indicate the extent to which it can be used for characterizing adverse effects.*

Overall, the study is of sufficient quality, given the qualifications listed above, for use in hazard identification purposes. This study represents the only analysis of the effects of perchlorate in the neonatal brain; thus, it provides important information for evaluating adverse effects.

*Do the finding provide information relevant to the evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?*

See above.

**C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.**

The EPA document is very thorough in its description, review, analysis and interpretation of the experimental studies on perchlorate. The EPA has extensively reevaluated data generated by other parties and, generally, these reevaluations have been clearly described and reported. However, reanalysis of the RIA for thyroid hormones ($T_4$, $T_3$, TSH) was confusing. It appeared that raw data (i.e., cpm) generated from three different locations (i.e., scintillation counters)

were pooled. However, this is not at all clear. Moreover, the statistical reanalysis of the RIA data was not clear.

Aside from this weakness, the EPA document appears to very clearly describe the various analytical strategies employed to evaluate all data it reviews. This is true for published data (e.g., epidemiological data) as well as unpublished data (e.g., Argus, 2001). The analysis and reanalysis of data – whether published or unpublished – appeared warranted and well justified. Conclusions, in general, appear to be logical and to be supported by the data.

**C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?**

**C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.**

The toxicity data are largely consistent with the proposed mode of action for perchlorate. The only departure from this are the linear measurements of brain region in the Argus, 2001 study (see above). These measures are not well known to be sensitive to thyroid hormone. It may be that they are, and if one were going to test this hypothesis, perchlorate would be a good drug to use to control thyroid function. However, as a set of endpoints with known relationship to the mode of action of perchlorate, the ones chosen are weak. This is not reviewed in the EPA document. That is, the relationship between mode of action and neurotoxicity endpoints, is not evaluated. It may be that the endpoints used are, in fact, endpoints, but this is not known

An important weakness in the database on perchlorate that the EPA reviews in the document is that of the effect of perchlorate on human infants. There are simply no studies that focus on the infant. Iodide (and perchlorate) is likely to be somewhat concentrated in milk (20-30 fold over maternal serum) , and infant milk consumption is very high on a body weight basis compared to adult water consumption. Therefore, this issue might be more thoroughly developed in EPA document. For example, in developing the implications of the Greer study on establishing an RfD, the EPA might have articulated that perchlorate levels in breast milk taken from lactating women consuming 7µg/kg perchlorate (0.5 mg/day) might be expected to be higher than her serum levels. Perchlorate may not be 20-30 times higher in milk than in serum, but there are no data to refute this possibility. Therefore, a 10 kg infant drinking 1L/day of milk containing perchlorate that may be 10-fold or more higher in concentration than the NOEL in maternal

serum, could be receiving perchlorate at a concentration considerably higher than the NOEL in a healthy adult male.

Despite this arguable weakness in the EPA document, the description of how the EPA established NOAEL's and LOAEL's was very clear and thorough. Also, the relative importance of various endpoints used to identify these characteristics was clear and thorough.

**Topic Area F: Human Health Dose-Response Assessment**

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?**

It is clear that perchlorate inhibits the Na/I-symporter and that this event is the key mechanism by which perchlorate potentially produces adverse effects on human health. The observation that perchlorate causes iodide release from the thyroid gland is similar to the observation that iodine itself causes iodide release from the thyroid gland – the so-called Wolff-Chaikoff effect . Thus, perchlorate-induced iodide release does not conflict with the conclusion that perchlorate inhibits the NIS.

The mode of action of perchlorate indicates that potential perchlorate-induced adverse effects on human health would be mediated by a reduction in circulating level of thyroid hormone. Therefore, key effects of perchlorate exposure to consider as the point of departure would be measures of thyroid function and measures of thyroid hormone action. The EPA document reviews the LOAELs, including circulating levels of thyroid hormone, changes in thyroid histopathology, and various linear measures of neuroanatomy reported in Argus, 2001. These are important endpoints that reflect changes in circulating levels of thyroid hormone subsequent to perchlorate action.

The EPA document reviews a number of key events considered in identifying the point of departure. However, their arguments for choosing those described above are compelling. It is this reviewer's opinion that it is also essential to consider endpoints affected by perchlorate during postnatal development; therefore, the chosen endpoints around which to build their assessment is logical and important. The assumptions and limitations considered in the assessment appear reasonable. However, it may be reasonable to consider the prevalence of thyroid disorders in the human populations in perchlorate contaminated regions. Some studies indicate that the prevalence of various disorders is quite high, even among young pregnant women in California and Nevada .

**Topic Area H: General Comments, Conclusions, and Recommendations**

**H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.**

**H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.**

Overall, the EPA document is clear, thorough and logical. Conclusions are based on clear reasoning. Perhaps most importantly, measures of thyroid hormone action are emphasized in establishing the point of departure. Perhaps the least clear issue is the description of the reanalysis of RIA data reported in the Argus 2001 study.

# Appendix D

## Index of Public Comments Submitted before the Peer Review Meeting

United States
Environmental Protection Agency
Office of Research and Development

# Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Holiday Inn Capitol Plaza
Sacramento, CA
March 5–6, 2002

# List of Pre-workshop Public Comments

**(Received as of 3/6/02)**

Acculabs, Inc.
The Analysis of Perchlorate in Groundwater and Soil by Electrospray LC/MS/MS

Alliance for Responsible Water Policy
Written Oral Comments

American Council on Science and Health (ACSH)
Written Oral Comments

American Water Works Association (AWWA)
Letter

American Water Works Service Company, Inc.
Letter

The Boeing Company and The Perchlorate Study Group
Assessment of the Potential Human Health Risk Caused by Exposure to Perchlorate

CF Industries, Inc. (CF)
Letter

Consultants in Epidemiology and Environmental Health

- Letter
- Analysis of T4/TSH vs. Iodine in NHANES III Data
- Benchmark Doses for Perchlorate Obtained from Lamm et al. (1999) Study of Thyroid Function in Perchlorate Workers
- Cancer of the Thyroid Perchlorate
- Comments on Brechner et al. (JOEM, 2000)
- E-mail from Steve Lamm (data)
- Exploration of the Jackie Schwartz Dissertation
- Fetal and Neonatal Human Hormone Changes
- Goitrogens in the Environment
- Human Exposure to Environmental Perchlorate and Iodine – A Public Health Perspective
- Lack of relationship between neonatal T4 and neurobehavioral disorders
- Neurobehavioral Diseases in Nevada Counties with Respect to Perchlorate in Drinking Water
- Neonatal Thryoxine Level and Perchlorate in Drinking Water
- Newborn Thyroxine Levels and Childhood ADHD

- Occupational and Environmental Health Aspects of Perchlorate
- Perchlorate and Human Health: Literature Summary
- Perchlorate Clinical Pharmacology and Human Health: A Review
- Perchlorate – Overview of Human Data
- Review: Therapeutic Drug Monitoring in Pediatrics
- Similar Effects of Thionamide Drugs and Perchlorate on Thyroid-Stimulating Immunoglobulins in Graves' Disease: Evidence Against an Immunospressive Action of Thionamide Drugs
- Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study

Crump, Casey
Addendum to submission by Kerr-McGee Corporation

Department of Defense Perchlorate Working Group
Comments on the U.S. Environmental Protection Agency's Draft Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (NCEA-1-0503, 16 January 2002)

Department of Defense - Office of the Under Secretary of Defense
Letter

Department of Health Services (State of California, Health and Human Services Agency)
Letter

Environmental Working Group
Letter

Fisher, Jeffrey, University of Georgia
Letter

Goodman, Gay
Thyroid Function, Perchlorate Mode of Action, and Interspecies Differences: Comments on the EPA/NCEA External Review Draft of January 16, 2002

Greer, Monte
Why it is essential to use human dose-response data to evaluate the human health hazard from perchlorate concentrations in drinking water

Greer, Monte; Goodman, Gay; Pleus, Richard; Greer, Susan
Health Effects Assessment for Environmental Perchlorate Contamination: The Dose- Response for Inhibition of Thyroidal Radioiodine Uptake in Humans

Intertox
- Assessment of Neuropsychlogical Studies by Haddow et al. (1999) and Others Cited by U.S. EPA to Support Their Concerns for Developmental Deficits Related to Maternal Thyroid Deficiency – Executive Summary
- Assessment of Neuropsychlogical Studies by Haddow et al. (1999) and Others Cited by U.S. EPA to Support Their Concerns for Developmental Deficits Related to Maternal Thyroid Deficiency
- Assessment of the Validity of U.S. EPA's Interpretation of an Effect of Altered Neurobehavior in Offspring Treated with Perchlorate *in Utero*: A Critical Review of the Argus (1998) and Bekkedal et al. (2000) Studies – Executive Summary
- Assessment of the Validity of U.S. EPA's Interpretation of an Effect of Altered Neurobehavior in Offspring Treated with Perchlorate *in Utero*: A Critical Review of the Argus (1998) and Bekkedal et al. (2000) Studies

- Review and Assessment of TSH and Thhyroid Hormones during Pregnancy in the Rat and Human and Comparison to Hormone Values in the 2001 Effects Study – Executive Summary
- Review and Assessment of TSH and Thyroid Hormones during Pregnancy in the Rat and Human and Comparison to Hormone Values in the 2001 Effects Study
- Summary of the Expert Review of the Argus, 2001 ("Effects Study") Evaluation of Perchlorate Effects on Brain Morphometry in Neonatal Rats – Executive Summary
- Summary of the Expert Review of the Argus, 2001 ("Effects Study") Evaluation of Perchlorate Effects on Brain Morphometry in Neonatal Rats
- Summary of the 1999 External Peer Review Panel Workshop – Executive Summary
- Summary of the 1999 External Peer Review Panel Workshop

Kerr-McGee Corporation
Assessment of the Potential Human Health Risk Caused by Exposure to Perchlorate

Koren, Gideon
Letter

Ladd, Larry
Letter

Lockheed Martin Corporation
Comments on: Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (External Review Draft January 16, 2002)

Lockheed Martin Corporation
Comments on EPA's Draft "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Los Alamos National Laboratory
Letter

McNabb, Anne
Letter

Parsons Engineering
- Letter
- Technical Memorandum

The Rooney Group
Written Oral Comments

Schwartz, Harold
Thyroid Hormone Effects on the Developing Brain: Critical Review of Data Presented in a Neurodevelopmental Study in Rats by Argus Laboratories (the 2001 "Effects Study") with Reference to an Earlier Neurodevelopmental Study by Argus Laboratories (the 1998 Developmental Neurotoxicity Study) and a Subchronic Study by Springborn Laboratories (the 90-Day Testing Strategy Bioassay in Rats): Comments on the EPA/NCEA External Review Draft of January 16, 2002

Schwartz, Jackie
Gestational exposure to perchlorate is associated with measures of decreased thyroid function in a population of California neonates.

Toxicology Excellence for Risk Assessment
- Quantitative Evaluation of Perchlorate Risk Assessment
- Use of Human Data in Perchlorate Risk Assessment

Texas Natural Resource Conservation Commission (TNRCC)
Letter

The Fertilizer Institute (TFI)
Letter

Vinson & Elkins L.L.P.
Letter

Wahlsten, Douglas
- Perchlorate effects on neonatal rat brain morphometry: A critical evaluation
- Perchlorate effects on rat motor activity: A critical evaluation
- Summary and Re-Analysis of Data: Brain Morphometry Results from a Perchlorate Toxicity Study (Primedica 2001)

White, La Donna
Written oral comments

# Appendix E

## Index of Public Comments Submitted after the Peer Review Meeting

United States
Environmental Protection Agency
Office of Research and Development

# Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Holiday Inn Capitol Plaza
Sacramento, CA
March 5–6, 2002

# List of Post-workshop Public Comments

**(Comments received between 3/7/02 and 4/5/02)**

Aerospace Corporation
Assessment of Perchlorate Releases in Launch Operations

Alliance for Responsible Water Policy
Letter

American Water Works Association
Letter

Association of California Water Agencies
Letter

California Public Interest Research Group
Letter

California Cancer Registry, Region 5
- Community Cancer Assessment in Redlands California, 1988-1998
- Community Cancer Assessment in Response to Long-time Exposure to Perchlorate and Trichloroethylene in Drinking Water (Abstract)

Cocopah Indian Tribe
Letter

Consultants in Epidemiology and Occupation Health, Inc.
- Letter
- Comments on Brechner et al. (JOEM, 2000) [Note: Previously submitted, submitted here as a reference to above letter]
- Perchlorate Effects on the Thyroid – Clinical Laboratory Confirmation of Occupational Epidemiology Findings [Submitted here as a reference to above letter]
- Benchmark Doses for Perchlorate Obtained from Lamm et al. (1999) Study of Thyroid Function in Perchlorate Workers [Note: Submitted here as a reference to above letter]

Department of Defense
Consultative letter

Environmental Working Group
EPA's Proposed Perchlorate RfD: Not Good Enough

Goodman, Gay
Graphical Analysis Reveals Anomalies in the Thyroid Hormone Results of the Developmental Toxicity Studies in Rats Performed in Support of the EPA Risk Assessment for Perchlorate: Comments on the EPA/NCEA External Review Draft of January 16, 2002

Intertox
Letter

Kerr-McGee Corporation
Assessment of the Potential Human Health Risk Caused by Exposure to Perchlorate – Supplemental comments submitted to the Environmental Protection Agency and The External Peer Review Panel

Leighton, Patrick
Letter

Los Alamos National Laboratory and Los Alamos County
Los Alamos National Laboratory and Los Alamos County Comments on Perchlorate Rulemaking

Lockheed Martin Corporation
Comments on U.S. EPA's Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

Metropolitan Water District of Southern California
Letter

Parsons Engineering Science, Inc.
Letter

Perchlorate Study Group
Letter
CD

*Note: Many of the documents on the CD were perviously submitted. New or revised documents that are not listed above include:*

- Chronic Environmental Exposure to Perchlorate and Thyroid Function During Pregnancy and the Neonatal Period (Tellez et al.)
- Does Perchlorate in Drinking Water Affect Thyroid Function in Newborns or School Age Children (Crump et al.)
- Evaluation of a Population with Occupational Exposure to Airborne Ammonium Perchlorate for Possible Acute or Chronic Effects on Thyroid Function (Gibbs et al.)
- Letter to Goehl (Goodman)
- Written oral comments (Guth)
- Letter (Intertox)
- Public Health Goal for Perchlorate in Drinking Water (Office of Environmental Health Hazard Assessment, California Environmental Protection Agency)
- Thyroid Function, Perchlorate Mode of Action, and Interspecies Differences: Comments on the EPA/NCEA External Review Draft of January 16, 2002 (Goodman) [REVISED]

Pingaro, Daniel
Letter

Southern Nevada Water Authority
Letter

State of Nevada, Department of Environmental Protection
Letter

Wahlsten, Douglas
Perchlorate effects on brain morphometry

# List of Public Comments Submitted After Deadline

**(Comments received after 4/5/02)**

*Note: Comments were submitted to EPA, but were not forwarded to the peer reviewers*

Consultants in Epidemiology and Occupation Health, Inc.

- E-mail [Subject: Perchlorate and the Mammary Gland]
- Effect of prolactin on sodium iodine symporter expression in mouse mammary gland explants
- Enhanced iodine concentrating capacity by the mammary gland in iodine deficient lactating women of an endemic goiter region in Sicily
- E-mail [Subject: Response to Comments on Lawrence Studies]
- Letter-to-the editor (received by Thyroid Journal)
- Response to Bruckner-Davis *et al.*

SQM North America
Letter

# Appendix F

## List of Registered Observers of the Peer Review Meeting

United States
Environmental Protection Agency
Office of Research and Development

# Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Holiday Inn Capitol Plaza
Sacramento, CA
March 5–6, 2002

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# Appendix G

# Agenda for the Peer Review Meeting

United States
Environmental Protection Agency
Office of Research and Development

# Peer Review Workshop on EPA's Draft External Review Document "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Holiday Inn Capitol Plaza
Sacramento, CA
March 5–6, 2002

# Agenda

**Workshop Chair:** **Ronald Wyzga, Electric Power Research Institute**
**Facilitator:** **Jan Connery, Eastern Research Group, Inc.**

## T U E S D A Y , M A R C H 5 , 2 0 0 2

| | |
|---|---|
| 8:00 AM | **Registration/Check-In** |
| 8:30 AM | **Welcome and Announcements**<br>*Jan Connery* |
| 8:35 AM | **Opening Remarks**<br>*Herman Gibb, Acting Associate Director for Health, U.S. EPA, ORD/NCEA, Washington, DC*<br>*Jane Diamond, Superfund Deputy Director, U.S. EPA, Region 9* |
| 8:50 AM | **Reviewer Introductions/Conflict-of-Interest Disclosure**<br>*Jan Connery* |
| 9:10 AM | **Background on EPA's Revised Draft, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"**<br>*Annie Jarabek, U.S. EPA, ORD/NCEA, Research Triangle Park, NC* |
| 9:30 AM | **Charge to the Reviewers**<br>*Ronald Wyzga* |
| 9:45 AM | B R E A K |
| 10:00 AM | **Topic Area A: Hazard Characterization and Mode of Action**<br>*Discussion leader: Thomas Zoeller* |
| 11:00 AM | **Topic Area B: Human Health Effects Data**<br>*Discussion leader: David Hoel* |
| 12:00 Noon | L U N C H |
| 12:30 PM | **Observer Comment Period** |

## TUESDAY, MARCH 5, 2002 *(continued)*

1:30 PM **Topic Area C: Laboratory Animal Studies**
*Discussion leaders: Michael Collins, Developmental Toxicity; Thomas Collins, ReproductiveToxicity; Thomas Zoeller, Endocrine and Neuroendocrine; Gary Williams, Pathology; Michael Aschner, Neurotoxicity; Loren Koller, Immunotoxicity*

3:30 PM BREAK

3:45 PM **Topic Area D: Ecological Risk Assessment and Evidence for Indirect Exposure**
*Discussion leader: William Adams*

5:15 PM **Closing Remarks**
*Ron Wyzga and Jan Connery*

5:30 PM ADJOURN

## WEDNESDAY, MARCH 6, 2002

8:00 AM **Observer Comment Period**

8:30 AM **Review of Day One Discussions and Charge to Reviewers**
*Ron Wyzga*

8:45 AM **Topic Area E: Use of PBPK Modeling**
*Discussion leader: Michael Kohn*

9:45 AM BREAK

10:00 AM **Topic Area E: Use of PBPK Modeling** (continued)
*Discussion leader: Michael Kohn*

11:00 AM **Topic Area F: Human Health Dose-Response Assessment**
*Discussion leader: Thomas Collins*

12:30 PM LUNCH

1:30 PM **Topic Area G: Risk Characterization**
*Discussion leader: Ron Wyzga*

3:00 PM BREAK

3:15 PM **Topic Area H: General Questions, Conclusions, and Recommendations**
*Discussion leader: Ron Wyzga*

4:15 PM **Closing Remarks**

4:30 PM ADJOURN

## Appendix H

## Public Comments Provided Orally during the Peer Review Meeting

Note: The peer review meeting included three designated observer comment periods. This section includes verbatim transcripts of the observer comments, in the order the comments were given.

**Larry Glidwell, U.S. Department of Defense (DoD)**

Good afternoon everybody. I hope everybody had a great lunch. More detailed discussions of the following, as well as any additional DoD comments on the Agency's risk assessment methodology and conclusions, is provided in the comments submitted by the Department of Defense perchlorate docket. In the area of human health and toxicology, although the Department of Defense contributed data used by EPA in the risk assessment, DoD does not support all the conclusions as stated in the document, nor does DoD support EPA's proposed revised RfD value. However, we do believe the harmonized assessment based perchlorate's inhibition of iodine uptake as the mode of action represents the major EPA chemical risk characterization. In addition, we believe that the careful evaluation and use of availability dosimetry modeling, all to perform cross-species dosimetry in lieu of defaults, as well as to evaluate the potential for age-dependent sensitivity differences, is a strong point of the EPA risk characterization.

In regards with risk assessment characterization, the use of analysis of epidemiological studies, EPA's perchlorate risk characterization puts too much weight on the results of certain animal studies. This data selectively creates a biased, or fatally flawed, perchlorate risk characterization. While human studies data, including the epidemiological, occupational, and clinical information, were presented and discussed in Chapter 4, given the perception that the data were factored into EPA's decision, the data and the studies were not used to derive the draft perchlorate RfD.

In addition, it is apparent that the Agency has provided an unbalanced consideration of available epidemiological studies. The Agency dismissed several well-conducted, published studies that were negative, while giving great credence to one unpublished graduate study that was positive. The fact that the two studies have demonstrated that workers exposed to very high concentrations of perchlorate do not display alterations of thyroid function is an important piece of information for evaluating the risk for perchlorate exposure. In the graduate study, the odds ratios for perchlorate exposure as a presumptive positive for congenital hypothyroidism were in the opposite direction compared to increase in exposure. Therefore, the odds ratios for the high exposure group were likely due to chance. Thank you.

**Marni Bekkedal, Naval Health Research Center Toxicology Defense**

Implementation of a weight-of-the-evidence approach for the selection of the point of departure is potentially more subjective than the traditional approach of separate calculations on each candidate critical study or endpoint. The Agency selected a point of departure in the rat of 0.01 mg/kg/day. The basis is provided by multiple analyses over many studies and endpoints. Analyses include conventional significance testing, benchmark analysis, Bayesian statistical analysis, ANOVA, and profile analysis. These endpoints reflect exposures ranging from a few weeks to a large fraction of a lifetime, from gestation to adulthood. The validity of characterizing all these results with a single point of departure must be questioned.

There's no evidence to support immunotoxicity as a more sensitive critical effect than the developmental endpoints used as the basis for this assessment. The difficulty of implementing EPA's weight-of-the-evidence approach is illustrated by the Agency's rationale for calling the 0.01 mg/kg/day point of departure a LOAEL based on four class of endpoints: (1) profile analysis of brain morphometry effects in neonatal rats; (2) increased motor activity in neonatal rats; (3) thyroid histopathology; and (4) thyroid hormone changes in a number of studies. Only two of these—brain morphometry and hormone analyses—actually demonstrated effects at the cited point of departure. Both of these classes of endpoints are highly inconsistent, suggesting a problem identifying this dose as a LOAEL or NOAEL.

EPA's policy on changes in brain morphometry is that, in the absence of data that would prove otherwise, changes in the size of a particular brain region are considered adverse. However, in the absence of a consistent dose-response or data that would support the assertion that the observed responses could be the result of compensatory mechanisms, there is no conclusive evidence that demonstrates changes in brain region size were exposure related. Given the uncertainty associated with the small sample size, considering these changes a LOAEL may not be justified. The ability of humans to more easily maintain blood thyroid hormone levels is important with regard to the developmental toxicity of perchlorate, where decreases in thyroid hormone in fetal and neonatal rats are believed to influence brain development and perhaps induce changes in brain morphometry.

The other basis for describing the point of departure as the LOAEL is the results of hormonal analysis in several studies indicating changes in T4, T3, and TSH at doses as low as 0.01 mg/kg/day. However, these changes are not consistent and, as with the brain morphometry data, should be considered an equivocal LOAEL/NOAEL, justifying an uncertainty factor of at most 3. Chemicals which inhibit thyroid hormone synthesis would not result in the dramatic changes in blood T4/T3 levels in humans that have been reported in the rat. Doses that result in alterations in blood thyroid hormone levels in rats, and consequently produce the developmental effects observed in the brain of rats, would not be expected to produce similar disruption in humans, due to the presence of TBG, which is also present in the developing human fetus and neonate.

**Mike Dourson, Toxicology Excellence for Risk Assessment**

Mike Dourson, Toxicology Excellence for Risk Assessment. We are a non-profit group in Cincinnati, Ohio, with a mission to protect public health. We're funded on this activity by the Perchlorate Study Group, but the comments we're going to make are those of our own, and not theirs. We appreciate EPA's accommodation of public comments and really all their hard work. We applaud the partnership of federal, state, industry, consulting, academic, and non-profit scientists that helped make this evaluation possible.

TERA scientists generated the first two perchlorate reference doses and had one of them externally peer reviewed. It was appropriately critiqued, principally due to the lack of data. Our

role since that time has been to monitor toxicity studies and to share information with public and private groups, when requested. The key findings that we have here is that decreased serum T4 should be designated as the critical effect because it's a known precursor to other adverse effects in the thyroid and brain. Pregnant animals or women are a sensitive sub-population. EPA's weight-of-evidence analysis and support of a point of departure should be rethought, because it ignores data that does not support its position and fails to evaluate the adverse nature of each of the endpoints discussed. Furthermore, we agree with several reviewers that human data should be used as a reality check. We would focus on the critical effect of decreased T4.

Moreover, we feel that the quality of the human database is sufficient for deriving a human-based reference dose. Dramatic, dynamic differences between the rat and humans in their responses following iodide uptake inhibition suggests that using the animal data as the basis of the RfD will introduce an unnecessary degree of uncertainty and excess conservatism in the assessment. For example, EPA's RfD is approximately 230-fold lower than the threshold for inhibition of iodide uptake found in the Greer et al. 2001 study.

We feel the most appropriate point of departure for a perchlorate reference dose is a benchmark dose analysis on the data from Greer et al. 2002. The study followed the common rule. A benchmark dose lower limit of 0.02 mg/kg/day, based on the 20% inhibition of iodide uptake, was identified by us as an appropriate point of departure since no effect was observed on serum T4 levels in Greer 2002 and other chronic human exposures. In fact, doses up to 70% inhibition appear to be without hormone changes.

So, in conclusion, we again thank EPA for accommodating our comments. We recommend that the perchlorate reference dose be based on human data. This RfD has more confidence than that based on rats and is possibly more protective of human health.

**John Gibbs, Kerr-McGee Chemical LLC**

For over 10 years, I've been responsible for medical surveillance at Kerr-McGee's Nevada facilities, where perchlorate was produced. In 1997, there were no studies of health effects from chronic low-level exposure to perchlorate in humans. We used the then newly available analytical technique to measure levels in air, studied employees in our Nevada facilities, and found no thyroidal or other health effects related to chronic or single-shift exposures up to 30 mg/day.

In 1998, Dr. Lamm studied the only other U.S. workforce with significant exposure to perchlorate. He measured perchlorate in urine and calculated absorbed doses across work shifts, which correlated well with doses estimated from simultaneous airborne exposures, confirming the rapid systemic absorption to the respiratory route. Dr. Kenny Crump analyzed the Lamm data set and determined that the BMDL was 44–58 mg/day for hormonal effects in healthy working adults.

We found perchlorate present naturally in groundwater in northern Chile, where nitrate fertilizer was applied; located three coastal cities with water supplies where well-defined, containing non-detectable, 6, and 110 parts per billion perchlorate. We studied approximately 50 first graders with lifelong residence in each city and obtained neonatal TSH data from these same cities for a 3-year period. We found no decrease in T4 or increase in TSH associated with perchlorate in water supplies. Frozen urine and serum samples are currently being analyzed by the Air Force Research Laboratory. We expect to have those values very soon.

Until very recently, we were unaware of the shifting concern regarding the most sensitive subpopulation, from infants and children to the first trimester fetus. We are convinced that northern Chile is the best laboratory in which to evaluate this concern. Accordingly, a study of pregnant women in the same three cities has been commissioned. We expect to follow approximately 50 pregnancies from the first trimester through delivery in each city and have the study completed in 2003.

Upon reviewing some of the early literature on perchlorate, such as Stanbury and Wyngaarden, it is apparent that perchlorate is not unique in its ability to block iodide uptake by the thyroid. Several other inorganic anions share the same pharmacology, only with differing potencies. Two of those—thiocyanate and nitrate—are present in many of the foods we consider healthy, such as broccoli, lettuce, spinach, cabbage, and milk. The relative impact of NIS inhibition from normal dietary sources overshadows the possible contribution from perchlorate in drinking water in most areas where perchlorate is detectable. Furthermore, in the past few years, [inaudible] has been demonstrated that there is dramatic synergism between soy in rodent diet and iodine deficiency. By inference, a soy diet will be synergistic with perchlorate in causing thyroid hormone effects. In the influential rodent studies referenced in the draft assessment, the rats were fed diets consisting of approximately 25% soy. This unrecognized confounder renders the "Effects Study" inappropriate as a departure point for risk assessment at this time.

I've included full-text copies of the specific studies to which I've just referred in written comments. I encourage members of the panel to read them. Thank you.

**Richard Pleus, Intertox, Inc.**

Thank you very much for the opportunity to speak with you. I'm here to more or less to give a reality check on the neurodevelopmental studies. I'm going to be talking a little bit about, and my papers are about, the behavior from both an animal and human standpoint. I have basically three questions and I've outlined them in green here to help point them to you. In addition, if you look on the top side of this, you'll see the papers we have presented to you and they are identified by reference here so you will have an opportunity to go back to those papers and take a loot at them. So, hopefully that will make it a little bit easier. In addition, I've got a couple of things in lighter green to help you orient to some of the questions that may also be brought up.

The three questions I have are, were the perchlorate doses high enough to cause any adverse health effects in laboratory animals. I think that's a very fundamental question and I would request that you take a look at the study that we wrote. Also, you might look at the study by Dr. Goodman and also a study by Dr. Wahlsten. Why is that important? Well, if you don't have a high enough perchlorate dose, you're not going to get any effects.

Were the studies conducted correctly? On two of those aspects, both the behavior and also the morphometry, the question is, I think, valid. On the morphometry, we had five experts take a look at the way that the process was done for brain morphometry and their conclusions are both summarized here on the front page as well as in the documents. Was the behavior analyzed correctly? And I think if you take a look at the documents that were provided in the CD by the authors, you'll find that when they did statistical analysis using repeated measures ANOVA, in fact, there were no significant effects. Also, I want to point you to the fact that we have set up a Web site. For those that are not neuro-anatomically inclined, if you want to take a look at what we have—an animation that might help you understand a little bit more about the process. Please take a look, it's there. The logon is "rat" and the password is "brain."

Lastly, did the EPA document correctly assess the literature, both from an animal and a human standpoint? I think there are some interesting questions here. Number one, for example, in the literature, it is very clear that, from a behavioral aspect, auditory startle habituation is a documented sensitive indicator of neurodevelopmental effects by reduction of hypothyroidism. So I would encourage you to take a look at that, what is listed in the literature versus what was actually reported in the risk assessment. And then lastly on the [inaudible] studies, Haddow and a bunch of other documents have been cited, and I would say take a look at the document that we wrote on that, which we say effectively information was selectively taken. Thank you.

**Gay Goodman, Human Health Risk Resources, Inc.**

The first tabled item is actually Monte's presentation. The second one was supposed to be my presentation, but then I was asked to talk about something else. I was asked to talk about the iodine in the human study, and so I tacked on that page 7 that you see, which really doesn't belong with the presentation but I had to stick it somewhere so that's what the last page is. So first I'm going to talk about the iodine. Then, if I have time, I'll go back to my actual presentation, which is related to the interpretation of the animal data.

Basically I wanted to say whoever earlier, it was said that, it was a weakness of the human study; I was the co-investigator on the study. Dr. Monte Greer, who is the next speaker, was the principal investigator. A weakness of that study is that the dietary iodine was not controlled in these people. And yes, that's a difficulty; it makes very difficult to tease out the influence of iodine on the inhibition for two reasons. One is because we didn't control it. And second is, even though we measured iodine throughout the study, on exposure day 1 and 2, certainly the highest dose and possibly the next highest dose, there was an iodine excretion was increased in these people. And so it's very difficult to look at the relationship. However, I'm very much

involved in analyzing these data, and I've done a lot already, and you can expect within a couple of weeks to receive a manuscript that describes the relationship of the [inaudible] uptake on the iodine, as best as we can analyze it in these subjects. So basically what I'm saying is expect a thorough analysis of the subject. To give you a heads up, there is a dependence and it doesn't depend on the percent; if you analyze it in terms of percent inhibition of uptake, it doesn't work. In other words, you have to factor in something that accounts for low iodine versus high iodine. You can't just look at percent inhibition of uptake and expect that to apply over the whole range of iodine. So there is a nuance to these data and you can look for the data.

Now I have 1 minute to talk my regular presentation. Basically I wanted to say that I do not believe that the mode of action proposed by EPA applies very well to these animal data, if at all, because these animals were extremely over-sufficient in iodine and they were able to escape from the inhibition by up-regulating in a manner that does not go through the pattern that is described in the EPA document. What happens in over iodine sufficiency, and there is plenty of data, if you look at the data, and I don't have time to describe it, but there are plenty of data showing that after the initial acute phase, these animals had up-regulated and were no longer inhibited after a couple of days. So, certainly in the 14-day studies, 90-day studies, developmental studies, there was no inhibition of iodide uptake, by any criteria. Period. End of story. There is no inhibition of iodide uptake, then there must be some mechanism for why T3 and T4 were down, TSH was up.

I have plenty more to say on this. Some of it's in there. Look for another two presentations from me, written presentations, one on the human, one on the animal.

**Monte Greer, Oregon Health and Science University**

Good afternoon. Can you hear me OK? I'm Monte Greer. My name has been mentioned a couple of times today. I thought you'd like to see what I look like, but those of you in the back of the room are out of luck. I am a physician and scientist who has studied thyroid function, including the effect of iodine deficiency and the actions of perchlorate and similar drugs in the rat and the human for more than 50 years. My strong conviction is that evaluation of the risk of perchlorate-contaminated drinking water to human health should be based primarily on data obtained in human subjects. Although the changes that occur during adaptation to iodine deficiency or anti-thyroid drugs are qualitatively similar in rats and humans, the potency of anti-thyroid drugs in humans is not always predicted by their potencies in rats. Perchlorate inhibits the thyroidal uptake of iodide through the sodium-iodide symporter. However, in both humans and rats, there can be no depression of thyroid hormone formation secondary to iodine deprivation if there is no inhibition of iodide uptake.

I and my co-investigator, Gay Goodman, performed a 14-day exposure study of perchlorate in 37 human volunteers. You may want more, but you have got to be realistic. We measured the dose-response for inhibition of thyroidal iodide uptake at daily doses of 7, 20, 100, or 500 μg of perchlorate per kg body weight. Each subject served as his or her own control.

Inhibition of uptake was linearly related to the logarithm of dose over the dose range tested, a span of two orders of magnitude. There was no build-up of effect between exposure days 2 and 14, indicating that once steady state was reached, no further accumulation occurred. We found no sex difference in the perchlorate inhibition.

Based on the observed dose-response relationship, we extrapolated that the true no-effect levels is 5 to 6 μg/kg/day, which is the amount that would be ingested from drinking water containing approximately 200 ppb perchlorate, one or two orders of magnitude greater than the contamination reported for drinking water supplies throughout the southwest. Doses below the threshold for inhibition of iodide uptake will have no effect on thyroxine synthesis. Further, there is no reason to expect that the pregnant or lactating woman, her fetus, or her infant would have a different dose-response relationship for perchlorate inhibition of the NIS than adult men and women. It may happen, but that's never going to be studied. It thus seems possible that current levels of perchlorate contamination of water supplies pose any thyroid-related human health risk.

**Dan Guth, Boeing Company**

I have a handout that was distributed to the peer reviewers earlier, you can distinguish by being plain white paper with plain black print. I am with the Boeing Company. Actually, if you can pull that out, I want you to refer to a figure in there, but not to the text. I'm with the Boeing Company, and I'm speaking for the Perchlorate Study Group. I'm really speaking for myself, though, because I've been doing chemical-specific risk assessments for 15 years, and really the Greer data is as good as I've ever seen it get and I want to address three issues that occur in using that data for risk assessment.

First, the intraspecies uncertainty has to be addressed. Iodine deficiency has to be considered, but the NHANES data shows that there is no iodine deficiency in the United States. Steve Lamm did a multiple regression analysis on the NHANES data and submitted it in the public comments, and it shows down to well, well below the range of what's normally considered iodine deficiency there is no effect on hormones. Secondly, thyroid disease must be considered, but the major cause of thyroid disease in the U.S. is auto-immune disease. It is not contributed to by iodine levels, especially in an iodine-sufficient population. Third, the gestational and post-partum maternal hypothyroidism is caused by several factors, two of which are increased iodine loss to the fetus and in the urine. Both of those suggest that there may be some impact of iodine insufficiency, which an uptake inhibitor might affect. The concern is reduced by the fact that the NHANES data shows essentially no iodine insufficiency in the U.S.

The second point is that the fact that this inhibition is a very sensitive precursor to the hormone change has to be addressed in the risk assessment. The figure that I have in my handout on page 2 is essentially a plot of the Greer data. It's a dose versus percent inhibition, and it also has the occupational and the therapeutic dose ranges shown with the predicted inhibition based on the Greer data. Essentially what these data show is that there has not been a

hormone change observed in the Greer study or in the occupational studies with exposures up to 0.5 mg/kg/day. So although the lowest dose that causes an effect on hormones in humans is not known, the data that we have suggest that it is probably 100-fold or so higher, or up to 100-fold higher, than the no-effect level for the iodine uptake inhibition. So, in summary, there is nothing in the literature that supports or justifies an RfD that is 100 to 200 times below the no-effect level for iodine inhibition and 10,000-fold below the effect that could have an effect on hormones.

My final point, in 10 seconds, there's a list on Table 3 in here of about a hundred different things that influence the thyroid. The biggest issue or reason for this orders-of-magnitude difference between the EPA and my conclusion is the fact that the EPA sees any change in the thyroid as being subclinical disease, when in fact the thyroid adapts to all these changes and perchlorate is an insignificant contributor.

**Steve Lamm, Consultants in Epidemiology and Occupational Health**

My name is Dr. Steven Lamm. I'm a physician, pediatrician, and occupational health specialist. I am the medical consultant for American Pacific, currently the only manufacturer in the United States of ammonium perchlorate. I also do contract work for the Perchlorate Study Group. Thank you very much for giving me the opportunity to try and summarize 5 years of research in 3 minutes.

I've given you a handout. The front page tells you what I'm going to say. The next thing is to tell it. Page number two demonstrates the model of human health effects over perchlorate ranges. To be understood is that perchlorate has been used for 50 years as the treatment of choice for certain types of thyroid disease, and we know a lot about it that's been published in the peer reviewed literature, on which the medical community depends.

The toxic level for perchlorate is 1,000 mg/day, which is about equivalent to the 30 mg/kg/day in the highest exposure doses. The therapeutic range, the range at which you have an effect on the thyroid, is from about 100 mg/day to 1,000 mg/day. Now, most interesting, and I submitted to you a copy of the paper by Wenzel and Lente, is that the standard way for treating hyperthyroidism is that you give a high dose to begin with, and then you try and gain control of the thyroid, and you back off to a maintenance dose. When you look at the literature, you find that the typical maintenance dose is 85 mg/day, and I hold that to be the level at which you're just having an adverse effect of thyroid hormone output. So I give you that 85 mg/day as a very important number for you to be looking at. The pharmacology group is the iodine level at which you have an effect, and you have from the Greer data that it goes down to 0.5 mg/day. In comparison, you see that the occupational exposure zone is the same as the pharmacological and does not reach the adverse effect level, and environmental is well below that.

Let's turn to the second figure. The second figure now deals with the issue of what is the effect of iodine levels in the United States on serum thyroxine levels, and you see from the

NHANES study that the thyroxine levels are steady throughout, even the low urine exposure. The third picture now deals with some of the ecological studies on newborns. It shows the relationship of the T4 level to birth weight. The reason we've done our studies at 2,500 to 4,000 is because that's where thyroxine is stable.

**Offie Porat Soldin, Soldin Research and Consultants, Inc.**

Thank you very much for giving me the opportunity to speak. My name is Offie Porat Soldin. I actually represent myself, but I do work with Steve Lamm and he introduced me so beautifully. I have three comments. One of them is about the TSH surge, and basically it relates to the Schwartz study. The Schwartz study gets all its data basically from the first 24 hours after birth. After birth, immediately after birth, there is a huge surge of TSH and other thyroid hormones. Therefore, the presumptive positive is actually a false positive. If you look at the bottom line, there is no increase in congenital hypothyroidism. So I would urge everybody to reconsider the data, although the study was beautifully done and the statistics, I'm sure, are great. Considering the TSH surge area of the first 24 hours is absolutely not useful. Secondly is about measurement, and I can't urge enough that, in any study, EPA studies or others, the way of measuring anything is very important, and when you pull data from several labs, if it's not the same lab that did it, it's very important to say different labs did it; one lab did it in one method, and the other lab did it in another method. If you pull all the results, it doesn't necessarily mean they are all the same. And the third thing, I was in Las Vegas myself just a month ago, two months ago, and I had no hesitation in drinking the water. I've had kids, and if I'll be pregnant again, I will not hesitate to drink the water, straight tap water. I do not believe that the levels of perchlorate in the water will do anything, not to me, and not to my baby.

**Larry Ladd, Community Advisory Group, Aerojet Superfund Group**

Hi, my name is Larry Ladd. I live about 15 minutes down the highway. I'm the interim chair of the Community Advisory Group for Aerojet Superfund site issues, which is an EPA-sponsored forum where Aerojet employees, regulators, and concerned members of the public get together and share concerns about whatever chemicals happen to be emanating from the Superfund site, and so the information I have to offer is in the realm of concerns, questions, and anecdotes. I do have one specific concrete correction to make to the draft risk assessment. My authority comes from having participated in creating the first document cited in the fourth chapter on ecological effects and that is the original health consultation. It states on page 4-4 that there are four cases of congenital hypothyroidism out of 11,814 births. That's inaccurate. All four of those were in the exposed zip code, and the other three zip codes basically have no perchlorate measurable at 4 ppb. And in Table 1-A of the September draft, it points out that the ratio is actually 5,217 births. That would give an incidence of 1:1,300. California is about 1:3,000; other states up to 1:5,000. You can narrow that further because in that zip code there are three separate water systems and that wasn't applied, too.

The other concerns we had were what was guaranteed to us is that the first step of the evaluation would be a fine-grained map of the thyroid data, and that map was generated. Every kid born in Rancho Cordova for the last 20 years is on a map, but unfortunately the fellow who was doing the study, Dr. Martin Hill, formerly of Public Health at UC Berkeley was laid off right before he could paste the data. So we're disappointed that that analysis wasn't here. Finally, another concern in any public issue raising thyroid questions, predominantly your concerned audience is going to be elderly women with auto-immune thyroid problems. To help address that public concern it would have been useful if some of the studies involved strains of test animals that are susceptible to the experimental auto-immune thyroid disease. Finally, the weakness of this particular study was that there was absolutely no health survey done. I went down to what I considered the apex of perchlorate exposure, knocked on the very first house, and the neighbor across the street had had [an inaudible type of] thyroid cancer. Another case of the exact same type of cancer also occurred amongst another gentleman who had moved away, who had the well in his backyard. And another family some point distant, where they grew all their own vegetables, and so the issue of bioaccumulation comes in, also suffered from the same cancer. That, of course, is just anecdote and it is not a testable hypothesis, but if you look at the incidence of that cancer in California, it's 31% in Los Angeles County, 22% in the rest of the state, and 19% in San Jose, which has comparable demographics.

**David Garrison, U.S. Air Force**

Hi, I actually go by Mike Garrison, and I work for the Air Force Center for Environmental Excellence, and I'm a member of the Perchlorate Working Group. Under the topics of ecotoxicology and ecological risk, I'd like to make a comment about the endpoints used to derive ecological screening benchmarks. The goal of EPA is to assess potential effects on receptors at the community or population level. The DoD's primary concern over the eco-risk component of the document centers on the choice of endpoints used to derive the screening benchmarks. In deriving an ecological screening benchmark, a requirement for endpoint selection is that it's based on an ecologically relevant effect, such as survival, reproduction, or growth. In fact, within the eco-risk arena, effects that are not clearly related to survival, growth, and reproduction of an organism are frequently argued as being irrelevant and unsuitable for benchmark derivation.

In the current document, ecological screening benchmarks for perchlorate appear to be based on endpoints with no known or implicated ecological relevance. For example, the apparent alteration in thyroid function, which serves as the basis for the herbivore dietary screening benchmark, has not been shown to result in any ecologically relevant effect. In fact, data suggests that, at the levels where the thyroid effects occur, there are no effects on development, growth, or reproduction. Another example is the use of redness and swelling as an ecologically relevant endpoint for the chronic fish assay is unjustified. At the very least, the effects of perchlorate chosen as endpoints for screening benchmark derivation should be adequately supported. In the current draft document, support of the choice of benchmarks is inadequate. If convincing justification cannot be made for current benchmark values, it's

suggested that the screening benchmarks be revised using endpoints with known ecological relevance. If no endpoints with ecological relevance are known, then there is not sufficient evidence to support the current ecological screening benchmarks.

One final comment I'd like to make on another topic, on interspecies variability, comparing rat and rabbit development studies show evidence for the magnitude of rat sensitivity to the inhibition of iodide uptake by perchlorate. However, in the rabbit developmental toxicity study, there was no statistically significant difference in the levels of T3 or TSH in dams that received up to 100 mg/kg/day. The EPA paid little attention to the dissimilar results between rats and rabbits in the perchlorate document.

**Penny Newman, Center for Community Action and Environmental Justice**

For the record, for those who might be curious, Roman Racca had given me his time from the California Department of Toxic Substance Control, but I want to make it very clear I am not speaking on behalf of DTSC, representing their views in any way, shape, or form, as will be very apparent, I think, as I talk.

My name is Penny Newman. I'm with the community organization in Glen Avon, California, which is the host to the Stringfellow Acid Pits, one of California's top priority Superfund sites. Even though we have over 200 major corporations that were dumpers at this site, EPA, being involved, and DTSC for well over 24 years that I've been involved with the project, not one of them have raised the issue of perchlorate. Aerojet was one of those dumpers and certainly knew about what they dumped there and that perchlorate would have been one of the contaminants to be tested for, along with the other 200. It wasn't until last summer that DTSC did any sampling for perchlorate, and have no found it throughout the community. While the plume was originally defined by TCE, which we thought we were getting pretty much under control, the area around that plume people have been hooked into an alternate water system and off of their private wells. We now find that, because of the perchlorate, that plume is more than three times what was known before. So we have an entire new population of people who have been exposed to the chemical.

In all of these instances, the corporations, EPA, DTSC have failed this community once again, and I'm wondering how many other communities around this nation are also being failed by the agencies not taking aggressive action to test for perchlorate. I wanted to kind of give a little bit, again, a reality check. I've heard that mentioned a number of times. And why I don't see the need for establishing what we consider a safe level of exposure to perchlorate, and I raise that because perchlorate is not a chemical that is added to our drinking water for any socially advantageous reason. It doesn't purify the water. It doesn't add anything to public health protection. It is simply a pollutant. It does not belong there. It clearly has identical sources of polluters. It is rocket fuel. It can be traced back to where it comes from, and under our current, which may be changed in the near future, but our current standard is that polluter pays. Or, as my mother always told me, if you make the mess, you clean it up.

I think we have enough information to know that this is a very potent chemical, and that it does not belong in our drinking water. I would hope that, as we're looking at the stakeholders in this discussion, that we include those people who you all want to study. I know human data is very appealing, but that's my family and that's my neighbors that you want to study. So please at least let us at the table, when you're discussing, at an equal level, as all of the polluters that you have listed up here, the DoD, Aerojet, and all the rest of them. We belong here to have a discussion, and you're deciding what we should be exposed to. Thank you.

**Dan Rodgers, U.S. Air Force**

My name is Lieutenant Colonel Dan Rodgers, and, since January of 1998, I've been the DoD team lead and a member of the Interagency Perchlorate Steering Committee executive. I like to begin my comments this afternoon by going on record recognizing the professionalism, dedication, and courage of a number of public servants, and the teams they represent. Without their vision and effort, this process would not have been possible: first, Dr. Bill Farland and his team in EPA, ORD; second, Dr. Annie Jarabek and her team throughout EPA, but especially at RTP; Kevin Mayer and his team in Region 9; Dr. Cornell Long and Ron Porter and their team at Brooks Air Force Base; Dr. Mattie and his team at Wright Pat; Jim Hurley, [inaudible], Major Jeff Cornell from Tyndall and Brooks; Larry Glidewell, Catherine McCracken, and Rachel Secada, our public relations team; Mike Girard, the chairman of the PSG and the associate members; Dr. Klaassen, and his team of professional, independent experts from the February 1999 peer review; Dr. Steve Lamm and his team at CEOH; and our state partners, including Brenda Pohlman, Bill Wallner, and Mike Honeycutt.

Throughout these last 3 years collectively, we, EPA, the member states, DoD, the PSG, and our stakeholder colleagues have taken the path least traveled in making decisions throughout the process that some may consider controversial and, more times than not, outside the partnership and research box. Our focus to get the most information about perchlorate to the American public and the relevant decision makers. We have not been afraid to make the controversial call by taking the challenge of Dr. Klaassen and the 1999 EPA external peer review, extending the information database on perchlorate by adding almost $12,000,000 in requested research. We brought the study directors for all the principal perchlorate studies, or representatives, here to answer any questions that you on the panel may have. On behalf of DoD, we prepared additional comments that we distributed to you this afternoon. DoD is also submitting more written comments to be forwarded later.

Within the partnership, our goal has never been focused on the ultimate number, but in directly making sure that, in protecting the nation from suspected pollutants, credible science becomes and remains the primary catalyst for credible decision making. Your role, as outlined in the EPA charge, is to review the Draft Risk Assessment and evaluate whether the data chosen, and inferences based on the data employed in the derivation of the assessments, are appropriate and scientifically sound. I'm proud to be in a country where two federal agencies can disagree on both scientific and policy recommendations. While our men and women in arms are fighting

a war against terrorism at home and abroad, you're here with an eye towards recommending a course of action for EPA and the nation. Do not accept the status quo. Be critical; be cautious; but regardless of any preconceived notions, apply your collective wisdom and scientific direction to this important process.

**Randy Palachek, Parson Engineering Science, Inc.**

Thank you for staying extra for hearing the comments. Unfortunately, just to talk about ecological, that's what I would like to focus my issues on now. A couple of quick clarifications that have come up in the recent discussion: In the Parsons study, we had over 700 or 800 tissue samples. We do have dry-, wet-weight, and percent moisture all in the original study, so that information can be pulled out. I think EPA, when they summarized it, just ended up putting in wet weights for comparisons, but the whole data is available. The other one issue when we were talking about the plant accumulation issue, I know at least in the Vegas site, where we saw the highest plant accumulation in the leaves there, the groundwater is very shallow there—and it's within 6 inches of the surface. So, you have to know and look at the soil concentrations to see what the concentration is in the tissues, but also the groundwater should be looked at as well.

A couple other quick comments I'd like to make is, we are currently—to let everybody know, all the interested parties—working on an Air Force study, where we are collecting seven additional species for ecological toxicity. One of those is an earthworm study, and that is just about wrapped up. Our preliminary numbers indicate that they do not bioconcentrate perchlorate, either, having less than 1 BCF. In addition, we have [inaudible species name] clam, for a bioconcentration study from the aquatic side, and [inaudible species name] sunfish species; and both of those indicate that perchlorate is not bioconcentrated in the tissue in the laboratory studies. In addition, we are accomplishing with those two species and four other species, including [inaudible species names], rainbow trout, and [inaudible species name], additional aquatic toxicity studies. And those should be finished up within the next few months. We hope to have a report out in the June–July timeframe that will have all the data in it. It will be GLP, and it will be measured concentrations and not just nominal.

All this data will be used to develop a surface water quality standard, using the EPA protocol. We are working with several state agencies, as well as a few EPA regions, to develop that surface water quality criteria document that will be available for all parties to use in the risk assessment purpose. With that, I'll just wrap up and let Ron Porter, who collected some of the data in the Parsons study, address anything else that might come up.

**Richard Garrett, City of Waco**

Thank you. I'd like to applaud the efforts on the committee and EPA on this issue. I'm with the City of Waco water utility department, and we provide water to about 130,000 people, and there's just a couple of points I want to make. The Texas Tech study, a lot of the work that

was done, is in our watershed. We are growing at about 9% per year, and our watershed, along with that of Lake Belton, which serves another 300,000 or so people in total, is also affected. So, the two points are: while we're deliberating on what is acceptable risk and making sure the science is sound, the exposures are continuing. Through groundwater and surface water, we have some exposure from a former munitions plant that is being decommissioned, and so this work—if I don't have to tell you all—is very critical. The other issue is the lack of data in the study on the ecological assessment. I think the Tech work, if you haven't reviewed that, I think it fills a lot of the gaps that were identified. It certainly makes it a lot more comprehensive, and I just appreciate your work, and give your time back.

**Douglas Wahlsten, University of Alberta**

First of all, I'd like very much to thank the peer reviewers for this opportunity to address you in person. I know several of you all read some of the comments I've made. My specialty is the study of genetic variation and sex differences in the corpus callosum of mice, rats, and humans. I've worked on this for about 30 years. I've looked at thousands of corpus callosums of these species. I've also created strains of mice that have no corpus callosum—it's a hereditary condition. My current work, I'm working on a mouse [inaudible] project, and my laboratory in Alberta has been chosen as the site to develop or to obtain standard morphometric data on the mouse brain, including the corpus callosum and other fore-brain commissures, for a large number of inbred strains of mice. So, this is sort of the main thing we do.

I have to tell you, tell the committee, that I do have prior involvement with perchlorate. After the Sputnik went up, I, and a lot of the students in my neighborhood, we actually began to build small rockets and launch them, and a number of these were actually fueled by perchlorate. That's the extent of my knowledge, and I ask that you not tell my parents about this. They might be a bit upset.

So, onto the brain morphometry, Toxicology Excellence for Risk Assessment asked me to review the data from the Argus 2001 Effects Study. I was provided with the raw data and also with scanned images of the brains. In my opinion, the morphometric measurement methods that were used in these studies are seriously flawed and very much prone to artifacts in the way they made the measurements. Linear measures of the corpus callosum in the hippocampus simply do not achieve the acceptable standards in neuroscience today. What we need are areas of these structures measured in cross-sections. In the case of the corpus callosum, it's very simple. You cut the brain right down the middle, and then measure the size of that structure in one section. That's all you need, one.

I would not rely on a coronal section—a single coronal section—even to tell me if a corpus callosum is 50% of its normal size. If it's absent, sure; but if it's reduced by 50%, I would not rely on a single section. You have to cut it down the middle, and everybody in the field does that. I do feel that there has been more time and money spent in debating this issue than would have been required to do the whole thing correctly in the first place. When you look

at the data, and I went back and looked at it recently, sadly there is a serious bias in those data, and the EPA conclusions about the post-natal day 21 rats are sadly based upon an artifact and I urge the EPA strongly to remove that part from their report. The plane of sectioning is not comparable, and it occurs in particular groups that leads to the inverted U-shaped function.

**Ronald Porter, Mitretek Systems**

Thank you very much. My name is Ron Porter, and I'm a biologist with Mitretek Systems. My conflict of interest is that I'm a former employee of the U.S. Air Force and was one of the principal architects and field ecologists for the bio-transport studies. So, I'll take any applause or blame for the outcomes of those studies. Earlier in the day, I heard some discussion about how to weight the human health studies and human health outcomes with that of the laboratory data, and I think we see a similar circumstance with ecological data, because if you are in the field and you are at locations with known perchlorate contamination, you do not see the types of perturbations that you might expect with similarly exposed laboratory animals.

I would like to report some of the observations that were made at Lake Mead. It's our most contaminated site. The contamination at Lake Mead began probably 50 or 60 years ago, at the end of World War II, with the manufacture of perchlorate. By today's measurements, if you look at concentrations of perchlorate in Lake Mead and in the Colorado River as far south as Yuma, and you do the mass calculations, it would have to be literally tons of perchlorate that would have to pass through the shallow groundwater and into the Las Vegas Wash. And our observations there, and Dr. Adams presented some of the data, the highest concentrations in soil, the highest concentrations in groundwater, the highest concentrations in tissue coincidentally were also found at the sites where our trapping success and collection success was the highest.

For mammals collected in the area, our trapping success was absolutely the highest. You could walk down the trap line, bait the traps, and, on the way back, you would hear the traps slam shut. Coincidentally, that's also the sites where there were highest concentrations of perchlorate in the plants and in the soils there. Likewise, for [inaudible species name]—the mosquito fish—Annie always likes for me to have at least one genus and species in my talks—for the mosquito fish, in the areas of the seeps where the concentrations of perchlorate were the highest, so high in fact that the electroshockers could not generate a current in the water, the numbers of [species name] that were there were also the greatest. So you could actually take a dip-net and scoop those things up.

I would also like to comment on some of the data that was discussed earlier related to the *Xenopus* assays. I know that Jim Carr's done some great work with some of that and provided some great information. There's also some data out of Jim's lab that reports that site-contaminated water that's brought into the lab and the *Xenopus* assay used with that has not been able to duplicate the results that are seen in the lab using regular lab water and the regular *Xenopus*. Thank you.

**LaDonna White, Capitol Medical Society**

Good evening. Thank you very much for your time and attention. My name is Dr. LaDonna White, and I represent the Capitol Medical Society, an affiliate of the Golden State Medical Association. We are the largest association of African American physicians in California. I am here today because the Capitol Medical Society and Golden State Medical Association are vitally interested in ensuring that appropriate risk assessment methodology is used to establish a reference dose of perchlorate.

Our concern stems from observations as physicians and as committed members of an underserved community. What we are seeing is extremely conservative risk assessment practices that result in very costly treatment and remediation actions. Far too often, these risk assessment practices and findings are distorted by various interest groups who deliberately mislead and scare the public. The result is the diversion of public and private dollars into unnecessary risk management efforts and away from more immediate, real and dangerous health related programs.

Yes, I am aware that your task is to look only at the science behind the draft reference dose for perchlorate. Yet we must consider the impact of our decisions and actions on health challenges that face us today, at this moment. As detection technology improves, more resources are devoted to removing smaller and smaller amounts of elements from our drinking water, yet at a price that takes dollars away from our communities and our ability to address very real health concerns. In a time of severely restricted local, state, and federal budgets, we must be very careful on choosing those problems upon which we commit our limited financial support. Indeed there is a cost of being extra safe by adding uncertainty factors and deciding to provide overly stringent parameters on our findings of no observable adverse effects. Let us not create phantom health threats where valuable resources provide no real reduction in risk to public health.

Here are a few examples of real health issues affecting the African American community—issues that can reap substantial public health benefits if significant public financial resources are directed their way. The infant mortality rate is more than twice as high for African Americans than for whites. The African American death rate due to diabetes is more than twice that for whites. African Americans are 30 percent more likely to die from heart disease than white Americans and 30 percent more likely to die from cancer than are whites.

There is only so much money, and there are so many needs. I hope you will keep in mind the high cost of being extra safe, and the missed opportunity to spend money on health issues that will have the most impact on the health of the minority community in California. Thank you.

**Jonathan Borak, Yale University**

I'm Jonathan Borak. I'm a physician and a DABT toxicologist. In the school of medicine, I teach toxicology, and I teach risk assessment. I am here on behalf of Lockheed Martin, and, in the interest of everybody else, I'm going to make only one point. I would like to first, however, thank EPA and ERG for the opportunity to be here, and I thank all of you for your incredible patience and tenacity, not only for sitting here all day, but for also the amounts of reading I know you've all done, because many of us have done a good bit also.

I would like to talk to one particular issue that was raised earlier, but I'd like to reinforce it, if I may. The risk assessment relies on a LOAEL generated out of four rodent studies. Those were the Argus 2001, the Argus 1998a, the Springborn 1998, and the Bekkedal 2000. I apologize if I've mispronounced your name. There's a critical issue here, which has to do with confounding. I would rather be talking about human health, instead of rodent health, but the fact is, when speaking of human health studies, I heard a great deal of concern about confounding—confounding lack of information on cigarettes, or lack of information on birth temperatures, and lack of other kinds of concerns (body weight).

The effect of confounding by the choice of chow that was used—the dietary basis of these studies—is so extraordinary, and the fact that it has not been brought up more, and more succinctly and clearly, I feel that an enormous error and an enormous need for you to address it. Argus' two studies and Springborn used a Purina chow 5002, and the Bekkedal study used Techlab-certified rodent diet. Both of those are proprietary and it was not possible for me to get the actual breakdown of constituents, though, if you call or ask, you can easily get the quantity of isoflavones in them. Both studies, particularly the Purina chow, has been specifically studied because of its endocrine disruptive effects, not because of its thyroid disruptive effects. It is also a phytoestrogen.

And it is a concern, however, and in my discussions with the manager of specialty research at Purina, [name inaudible], that the concern about the potential thyroid interactions is something that Purina is aware about, and they have simply said, and I have offered you the cite in my submission that was written, that synergism with perchlorate and that diet should be expected. Soy is goitrogenic. It's been known since the early 1950s that pure soy diets cause hypothyroidism and goiter, but the most impressive data is actually from [name inaudible], published in *Carcinogenesis* in the year 2000. We are looking at a diet of gluten versus soy. Gluten has no isoflavones. We are looking with and without iodine. The effect of soy and iodine depletion is enormous. It is more than additive; it is multiplicative. And I believe this data invalidates the extrapolations made in the four studies that are the relying point for the LOAEL, and it would be so readily simple to simply reproduce, with these dietary manipulations, to determine whether that data is usable. I thank you very much. I appreciate the opportunity to be here.

**Sujatha Jahagirdar, CALPIRG**

Thanks very much. My name is Sujatha Jahagirdar, and I am CALPIRG's safe drinking water advocate. CALPIRG stands for the California Public Interest Research Group. We are one of the largest environmental and consumer watchdogs in the state of California. We have 70,000 members and are part of a national network of state PIRGs.

The purpose of today's proceedings, in my view, is to try to decide how much rocket fuel is safe to drink. My critique of the proceedings today rests on those who haven't been at the table to help make this decision. CALPIRG believes that the most important constituency in making this decision is the millions of men, women, and children that have been unknowingly drinking rocket fuel for decades across the country. As the proceedings went on inside this hotel today, communities across California, including Rancho Cordova, the San Gabriel Valley, San Bernardino, Chino Hills, the list goes on, are grappling with the consequences of this massive public health and regulatory debacle by going to the doctor for thyroid problems, rare forms of cancer, and aplastic anemia.

CALPIRG does not believe that those entities that have been identified as responsible polluting parties, and those entities that they have funded, should have an equal voice at the table in deciding the outcome of these proceedings. Since public representation has been so lacking today, I would presume to speak for the average community member with whom I've been working with for the past several years in answering what I see as the fundamental questions that have been raised here today. Question number one: Are there major health concerns from drinking perchlorate raised in this EPA draft toxicological assessment? The answer, in my view, is yes. Do I want to be drinking it? The answer is no. Do I want my unborn child exposed to it? The answer is no.

CALPIRG believes that the EPA and relevant regulators should move as quickly as possible, based on the answers to these questions, to [a] get rocket fuel out of our drinking water supply completely, [b] make sure that polluters pay completely for cleanup, and [c] prevent further contamination. Thanks very much.

**Paul Winkler, Acculabs, Inc.**

Good morning. My name is Paul Winkler. I'm the director of specialty analytical services at Acculabs. Acculabs is a contract analytical laboratory in Golden, Colorado. Our interest in perchlorate analysis came about because we have several clients who had a need for perchlorate analysis, but their data quality objectives were not being met due to limitations with the current ion chromatography method. These limitations were primarily based on a lack of sensitivity, rendering high detection limits, and a lack of specificity, giving rise to potential for false positives.

This led us to get involved in a study with the Department of Energy, in the Albuquerque operations, where we studied water that was spiked at 4 ppb. Using the ion chromatography method, we found that, at 4 ppb in the blanks, we got a 20% rate of false positives, which is an indication of a lack of specificity. Then, in the waters that were spiked at 4 ppb, we got a 20% false negative rate, and that's due to a lack of sensitivity in the method and a difficulty in actually identifying a peak. This led us to develop an LC/MS/MS method—that would be liquid chromatography/mass spectrometry/mass spectrometry. This method is far more sensitive and far more specific than the current ion chromatography method. With our method, we were able to get a detection limit of 50 parts per trillion for perchlorate. That was arrived at by spiking an actual groundwater sample from a deep well from west Texas at 250 parts per trillion, and we analyzed seven samples and received 112% recovery, which gave rise to the calculated MDL of 50 parts per trillion. Similarly, we spiked a sandy soil at 5 µg/kg, and we ran seven samples with an average recovery of 114%, which gave rise to an MDL of 2 ppb.

So, yesterday's speaker, the last speaker of the day, indicated that he wanted a method with 1 µg/L for waters and 10 µg/g for soils, and I would say that that method is here today. We have fully validated the method from 0.25 ppb up to 20 ppb. And the last comment I'd like to say is that it's not only more sensitive than the IC method, but it's also more specific. For us to have a false positive identification, the compound would have to have the same retention time as perchlorate, form a negative ion at the molecular mass of perchlorate, fragment to the daughter ion of perchlorate, and also form a negative ion. And that's not very likely. So, in conclusion, I'd like to say, I believe that we have the analytical methodology present today to do these analyses at lower levels for environmental monitoring. And I would like to thank ERG and the Agency for allowing me to speak this morning. Thank you.

**David Mattie, Air Force Research Laboratory**

First of all, I'd like to thank you for the opportunity to make these comments. First of all, on the transport of perchlorate into thyroid cells, AFRL HEST would like to point out that evidence to support the uptake of perchlorate into the thyroid exists, and the AFRL studies by Yu et al.2000a and 2000b, which are iv and drinking water studies, when NIS was up-regulated in rats, thyroid perchlorate concentrations also increased, as well as iodide. Cold perchlorate was measured in the thyroid of rats in these studies using ion chromatography, a modification of the EPA Method 314. This method is selective for perchlorate. We checked for chlorate and chloride in the thyroid and confirmed we were looking at perchlorate and not metabolites.

Perchlorate is concentrated in thyroid. We saw thyroid to serum ratios between 10 and 30 in the male rat in drinking water studies at doses from 0.1 to 10 mg/kg/day. In fact, PBPK models can predict these up-regulated levels quantitatively, when the model assumed that NIS upregulation maintained the iodide uptake in the presence of perchlorate competitive inhibition. In a 3 mg/kg study, 93% of the dose was excreted in 24 hours. On analytical issues, the matrix is an issue with HPLC method for perchlorate. The sensitivity is reduced in blood, urine, milk, and rat tissues, but you can still separate the perchlorate peak without any interference, because the

sample preparation we developed for biological matrices. In the 90-day perchlorate study by Springborn Labs, it was a Hamilton-Thorne IVOS-10 semen analyzer that was used for the sperm parameters.

On the use of human data, we've heard a lot about the inconsistencies in the animal data, especially because of the soy products in diet. This further supports the use of human study. There are concerns about the use of the Greer study. However, the Crump study in Chile looked at the critical effect: T3/T4 in children and TSH in newborns. A reference dose could be developed from the Crump data that would be similar to the RfD for the Greer study, showing that both the human clinical and epidemiological studies are mutually supportive. We recently received the blood and urine samples from the Crump study. In an initial analysis just completed, we can detect perchlorate in the blood and urine. Blood levels are approximately what the PBPK model predicted. Furthermore, a follow-on study will start shortly looking at pregnant women in the same three cities in Chile. Perchlorate will also be measured in blood, urine, and milk. I feel you don't need to wait for this study to use the Crump study now for the development of an RfD. The results will serve as further validation of the human data.

To end with some questions for the panel: Is the Greer study compromised by scientific limitation, such that it can't be used for risk assessment? Is it reasonable to conclude that the rat data is a more reliable basis for human health risk assessment for perchlorate? Is there any reason that a dose below the NOAEL for iodide uptake inhibition would cause any risk? And finally, we would ask that you provide definitive comments and clear conclusions back to EPA to help us solve controversial scientific issues, rather than simply identifying the issue. Thank you very much.

**John Gaston, Alliance for Responsible Water Policy**

Thank you very much. Good morning. My name is John Gaston. I believe you've got a copy of the comments that we've made here. I'm here today in my capacity as the Executive Director of the Alliance for Responsible Water Policy, a coalition of water-related institutional and business organizations that's been engaged in the area of drinking water quality issues in California since 1995. I've got almost 40 years experience in the drinking water and public health area—20 as a regulator with the United States Public Health Service and the California Department of Health Services, and 20 as a consultant. Along the way, I served for 12 years on the U.S. EPA National Drinking Water Advisory Council, 5 years as chairman.

The alliance that we represent here today exists for the purpose of ensuring drinking water safety standards are based on the best available science. I'm here today because the Alliance for Responsible Water Policy is vitally interested in ensuring that appropriate scientific methodology is used to determine the U.S. EPA's reference dose for perchlorate, and that's where we're going to end up is with a drinking water standard. As an aside, not in the remarks, there are two sea changes that we have seen in the drinking water utility business that dramatically affect what we will do here today. Number one is that consumers are increasingly concerned and increasingly

aware of drinking water quality issues and that includes the realization that drinking water actually does contain compounds other than hydrogen and oxygen. And the second is that lawsuits involving drinking water utilities are increasing exponentially. This magnifies the need for good science and balanced interpretation on your part, and those that will follow after you.

Throughout my professional life, I have represented drinking water quality professionals and advocated the furtherance of public health in the water utility industry. These are the men and the women on the front line that have to implement the standards and have to protect the public from water-borne disease or water-borne illness. What concerns more and more the drinking water professionals is the use of ultra-conservative risk assessment practices that are not necessarily based in sound or complete science and that may result in extremely cost treatment and remediation burdens. Far too often, these risk assessment practices and findings are misunderstood by the public and, worse, distorted by various interest groups. This is often a regrettable and an entirely avoidable and uncalled for loss of confidence in the public drinking water systems and diversion of public health and private dollars.

I'm well aware that your task today is here to look at the science, but you must also consider the policy implications involved in that, and that comes at a high price. Interestingly enough, of the 14 people that spoke yesterday, I thought that it was very interesting that, of the 11 that had what I consider to be credible scientific experience, they were relatively unhappy with some parts of the document, and I would share that there are some scientific concerns involved with that. I'm going to conclude at this point to urge the peer review panel to take a close look at the human data—we, too, believe the human data is important—reported by Drs. Greer and Goodman at Oregon State and others in the review of the reference dose. The outcome of your deliberations here will have an enormous impact on the lives of everyday Californians and, sadly, we are not sorry about being overly cautious in this matter. Thank you very much.

**Gilbert Ross, American Council on Science and Health**

Good morning. I'm a physician and Executive Director of the American Council on Science and Health. ACSH is a consumer education organization—a consortium of 350 physicians and scientists from throughout the country concerned with issues related to nutrition, chemicals, pharmaceuticals, lifestyle, and the environment. We were founded in 1978 by a group of scientists who had become concerned that many important public policies related to health and the environment did not have a sound scientific basis. It is with this mission in mind that we turn to the perchlorate issue in an attempt to help guide the EPA to adopt a scientifically sound and appropriate reference dose for perchlorate and to address misrepresentations that have been associated with this debate.

In January, we published a special report. We commissioned a toxicologist, Dr. Daland Juberg, to write this report for us. We were attracted to this issue because of certain alarmist scares that had been released into the media. The American Council on Science and Health is

vitally interested that an appropriate risk assessment methodology be used to establish a reference dose for perchlorate, because too often overly stringent regulatory approaches to trace levels of environmental contaminants place onerous financial burdens on local governments and industries, with little or no public health benefit ensuing. We must avoid diversion of scarce health resources away from areas where they are otherwise desperately needed. We believe that a thorough review of the available science will demonstrate that the draft RfD of 1 ppb is far too conservative.

The collaboration among the EPA, the Department of Defense, and the interindustry Perchlorate Study Group is unique over the past several years. Their findings ought to be given great weight as you evaluate the adequacy and the scientific soundness of the EPA's conclusions and draft RfD. Because of our mission to identify significant public health threats and to distinguish these from non-significant to non-existent scares, using sound scientific analysis, we highlighted some of the recent scientific studies that have further characterized the toxicity of perchlorate in animals and humans. We wish to avoid what we believe to be ultra-conservative environmental exposure levels and give due weight to extensive amount of toxicological data, including reliable human and animal studies that have been generated in the last few years. We anticipate the final RfD will be scientifically supported, as well as any other regulated chemical in commerce today.

In conclusion, I urge the peer review panel not to be overly cautious in evaluating the newly-proposed RfD for perchlorate, bearing in mind the ramifications and unintended consequences of doing so. We have accumulated much new data on perchlorate over the last few years, so the RfD, we think, should be higher than the prior mark. The latest human studies especially support a higher RfD as a safe exposure level. Unnecessary expenditures and needless remediation of a non-problem is unwise policy. As our analytic techniques get more and more sophisticated, we will be able to detect thousands of chemicals at trace levels. If we attempt to purge them all down to zero, under the [inaudible] of better safe than sorry, we will have precious few resources left for other urgent public health needs. Thank you.

**John Gibson, American Pacific Corporation**

Thank you for the opportunity to comment. My name is John Gibson, and I am CEO of American Pacific Corporation. We are a small business manufacturer of perchlorate chemicals. We have made perchlorates since 1958. Therefore, we have a vital interest in the outcome of this risk assessment and the subsequent regulatory process.

I recognize that this exercise today is a technical peer review of an assessment which will form the basis for determining justification for regulation. I should not be here to question the process or the administrative decisions which have resulted in the content of this document. However, the administrative process has, in fact, determined what you will now review and the emphasis given the various scientific studies contained therein. Sadly, the review and risk assessment is incomplete and dismissive.

In mid-December of last year, the Agency adopted an interim policy which states in part that "the Agency will not consider or rely on third-party studies involving deliberate exposure of human subjects." This policy unnecessarily restricts your ability to fully assess the risk of perchlorate exposure on human health. For example, the study authored by Greer, Goodman, Pleus, and Greer, the so-called Greer study, although referenced in the risk assessment, is effectively dismissed: "the human clinical studies have significant scientific technical imitations that preclude their use as the basis for a quantitative dose-response assessment." We respectfully disagree and find this conclusion surprising in light of the fact that the Agency assisted in the design of the study protocol.

Other work by Lamm, Braverman, Crump, Gibbs, Lawrence et al. is similarly dismissed. Since the Greer study identified a no effect level in water (concentration of 200 ppb) for the key event in mode of action analysis, we are more than just disappointed with the risk assessment's treatment of this and similar work. In fact, K.S. Crump calculated in an occupational study of the highest exposure group in the country that a safe work place exposure level would be 50 to 60 mg/day, which is equivalent in water concentration to 20,000 to 30,000 ppb. In plain words, the interim policy adopted by the Agency is dumb, and it is dumb because it deprives the EPA of the best available information—information that other agencies, such as the FDA, use in their deliberative processes.

Since 1997, our company alone has spent over three million dollars in funding human health studies and in characterization work. Always it has been our objective to protect our employees, preserve the environment, and assure our 211 employees, their families, our customers, suppliers, shareholders, and the community in which we work that the product we make is safe. The data and conclusions of these human health studies support our belief that the reference dose could be safely set at a higher level than indicated in the subject review and risk characterization. Thank you.

**Mick Major, U.S. Army**

Good morning. I'm with the U.S. Army, USA CHPPM. We're a health branch of the Army. We're not in restoration. We report directly to the Surgeon General. We try to assist in things like this when we can. I read this risk assessment for the first time about 4 or 5 weeks ago, and I thought it really was a beautiful piece of work. I thought it was elegant. I thought it was well conceived. I liked the idea of planning ahead, getting the tests done. I liked the idea of including the cancer with the noncancer endpoints. There was an awful lot that I really liked about it. I didn't realize there were some problems with the rodent data until I came to this meeting, and I realize that it's still going to be a toss up and that data may still be used.

So what I'd like to talk about just for a second is the use of the 3 uncertainty factor for duration, the cancer effect. I understand why using the rat data for morphometry was necessary. Obviously, we can't be cutting up human brains. And I understand why a 3-fold factor would be used for duration if you only used the rodent data, because the NOAELs of the developmental,

cancer, and the morphometry were very close—the LOAELs, excuse me, were both very close—there wasn't NOAELs. So, the NOAELs may actually have been overlapping, so I understand why the 3 was used. However, there's no reason not to use human data for the cancer effect. We have an awful lot of human studies and also epidemiological studies. They're negative for the cancer effect. Now, if we look at the human populations who are receiving perchlorate and they're not having sustained increases in TSH—I understand that there's a transient increase, but if the increases are not sustained—they're not getting the hypertrophy and the hyperplasia, we can safely say that these populations are not going to have a significant increase in the incidence in the thyroid cancers.

Now, because we have the human data, there isn't an effect, and I think if we look very closely at the human data now and see if the TSH is elevated and see if we're getting the hyperplasias, and if we're not, I think we ought to absolutely eliminate the factor of 3 from the risk assessment. Thank you for your attention.

**Renee Sharp, Environmental Working Group**

I'm here representing the Environmental Working Group and also the California Public Interest Research Group, which are both research and advocacy organizations working on public health issues. Perchlorate was detected in groundwater in 1957 for the first time. Forty-five years later, contamination has been found in 20 states, and the true extent is still unknown. The first perchlorate reference dose was proposed in 1992. Ten years later, we are still waiting for an enforceable drinking water standard.

The EPA's newest proposed reference dose is a step in the right direction, but it is still not sufficiently protective. Briefly, EWG and CALPIRG have concerns about the use of a composite safety factor of 300, given the high incidence of hypothyroidism in the general population, the progression of effects seen at longer exposure durations, a lack of any truly long-term studies, and the use of a LOAEL versus a NOAEL for RfD derivation. EWG also notes that an equivalent drinking water standard of about 0.2 to 1 ppb would provide only a minimal margin of safety, if any, given Schwartz's finding of a thyroid hormone change in California infants born to mothers who consumed water with only 1 to 2 ppb perchlorate during pregnancy. Furthermore, EWG continues to object to the use of adult drinking water consumption values and body weight values for the calculation of a hypothetical drinking water standard, and would like to see some discussion of this among the peer review panel.

It is clear that many scientific questions remain about the toxicology of perchlorate, and there probably always will be, but the people and communities who are being affected by contamination, knowing or unknowingly, cannot afford to wait another decade for further study. There is a sufficient body of research now, and EWG and CALPIRG encourage the EPA to expedite the standard setting process. Finally, I'd like to take a moment to remind the peer reviewers that, in matters of public health, if we are to err, let it be on the side of caution. The people and communities with perchlorate contamination don't care that a tumor is reversible,

that their babies' thyroid hormone levels may only be affected a little bit, that the iodine uptake by their thyroid is depressed by just 20%, or that the changes in brain section sizes—I've seen it in rats—may not have any effects that are immediately apparent. These people do not choose to be drinking rocket fuel and, to them, any amount of risk is too much. Thank you.

**Peter Rooney, Rooney Group**

Good morning. My name is Peter Rooney, and I'm the former Secretary for Environmental Protection in the state of California. I'm appearing here today at the request of several business clients. My experience is not in the area of science, but rather in the realm of public policy. Your task today crosses both fields. Many will say your task is purely scientific, but I submit that the document that you are asked to review is flawed because of public policy positions incorporated by U.S. EPA.

EPA adamantly complains about the lack of knowledge and about the acts of this chemical, yet they refuse to incorporate the data from human studies, such as that of Drs. Greer and Goodman. I submit as your role to separate science from these public policy positions, and determine if the conclusions reached are scientifically sound in light of the body of knowledge that does, in fact, exist at this time. EPA would generate greater public confidence in their work, if they would emulate President Bush. The President was faced with a similar ethical decision concerning research based on stem cells. He chose a more sensible course. He did not bar the use of the existing stem cells. He decided the existing stem cells should be used to further the world's knowledge base, but then no further stem cell lines should be developed with federal funding.

U.S. EPA, in this case, made a different decision. They chose ignorance over knowledge. They decided to ignore existing human data. They then decry the lack of knowledge and call for an increase in the previous 100-fold uncertainty factor to the proposed 300-fold uncertainty factor. I call upon you to consider the short-sightedness of this approach. I call upon you to ask that EPA consider the body of the world's knowledge in determining a realistic reference dose.

EPA further tells us that this proceeding is not about management. They assert, and I quote: "An RfD would only be one step in the future regulatory process of determining, based on a variety of elements, whether a drinking water standard for perchlorate is appropriate." I suggest that this proceeding, in fact, is the very foundation of risk management. If policy makers are given a flawed number, they will devise a flawed response.

Subsequent proceedings must start with confidence in the expression of hazard. You are being asked to add your voice to confirm the public policy determination that a 300-fold uncertainty factor is necessary. You are being asked to confirm two public policy decisions: one, that some existing human research is inappropriate; and two, that this self-inflicted ignorance results in the need for a 300-fold safety factor. If you are wrong, if the number is overly conservative, public policy is not well served. Affected parties will be subject to

increased anxiety, and responsible parties will be called upon to meet overly-ambitious remediation goals. Over the long term, numbers that have no grounding in reality tarnish the credibility of the full range of the program. And let me conclude by saying a house that is built on a shaky foundation will surely fall, and I'm afraid you are being led down to that shaky foundation. Thank you very much.

**Gideon Koren, Hospital for Sick Children (Comments faxed to the meeting and read by Steve Lamm)**

Hello. First of all, I'd like to thank you for giving Dr. Koren the opportunity to be spoken for. He says: I speak to you in the context of the EPA review of environmental perchlorate exposure, as it may affect thyroid function *in utero* with potential long-term neurobehavioral effects on children. I'm a pediatrician toxicologist at the University of Toronto. In 1985, I founded the Mother Risk Program, which councils pregnant women, their families, and health professionals on the risks of drugs, chemicals, radiations, and infections during pregnancy and lactation. In addition to conducting our human research, we systematically review the cumulative world knowledge as pertain to human teratogenic exposure. Presently, we council up to 200 cases a day.

I am concerned with EPA putting a lot of weight of the recent PhD thesis of Schwartz. From a clinical standpoint, it is not reasonable to compare indices of thyroid function during the only day in life when they are known to be all over the place. Any statistical attempt to correct for different times of obtaining these tests is bound to make assumptions on mean changes over time. This is especially critical here, because the groups are not evenly distributed. This is important because papers looking at later measurements come to different conclusions.

Not surprisingly, there is no correlation in Schwartz's work between presumed intake of perchlorate, based on postal code, and congenital hypothyroidism. At exposure levels presumed to happen in these regions of California, there are no known adverse effects in animal models tested. Human studies of thyroid drugs—PTU, methimazole, [inaudible drug name]—show effects in newborn thyroid function and also, in some instances, on neurodevelopment. As expected, exposure levels in these human studies are those causing measurable effects in the mother. However, studies showing *in utero* thyroid hyperplasia from anti-thyroid drugs failed to show long-term effects on neurodevelopment (see *Prenatal Diagnosis* volume 220, etc.).

Additionally, there are three neurobehavioral studies, now in various stages of publication, that have been submitted to you in the public comments. All of these failed to show neurobehavioral effects of environmental perchlorate in typical levels encountered in the environment. I am sure you receive input from different experts. I hope you will have the wisdom to listen to people whose expertise in human *in utero* exposure, so that there is a meaningful context for the animal data. Sincerely, Gideon Koren, M.D., Professor Pediatrics, Pharmacology, Pharmacy Medicine, and Medical Genetics, University of Toronto. Thank you.

# Appendix I

## Copies of EPA's Opening Presentation Materials

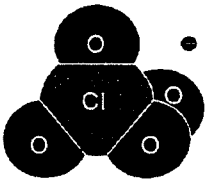
EPA United States Environmental Protection Agency

Office of Research and Development

## *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization*

**External Peer Review Workshop**
**Sacramento,CA**
**March 5 & 6, 2002**

**Opening Remarks**
Herman J. Gibb, Ph.D.
Acting Associate Director for Health
National Center for Environmental Assessment




## Perchlorate Contamination

- Issue identified by Region 9 in 1985
- Appreciation of widespread nature emerging with improvement in analytical methods and occurrence surveys
  - Method 314.0 for detection in water developed
  - Placed on CCL and UCMR
- Integrated approach required:
  - Analytical detection methods in various media
  - Occurrence / exposure / transport & transformation
  - Health and ecological risk assessments
  - Treatment technology
- Revised risk assessment in response to 1999 external peer review

## Pro-Active Partnership

**Department of Defense**
**AFRL**
**Perchlorate Study Group**

**PSG**

**ORD**
**OW**
**OSWER**
**Regions**

**NIEHS**

**NIOSH**

**Interagency**
**Perchlorate**
**Steering**
**Committee**

I P S C

## IRIS Peer Review

- Integrated Risk Information System (IRIS) is Agency database for risk assessment estimates
- Independent contractor (ERG) charged with finding scientific experts in needed areas
- Document provided on web January 18, 2002 with an accompanying CD of gray literature available upon request
- This is a ***DRAFT*** risk assessment and not a rulemaking
  - Premature to interpret these draft risk estimates as final EPA conclusions
  - Ample future opportunity to comment in proposed rulemaking process according to legal, regulatory or policy requirements of programs

*Perchlorate Environmental Contamination:*
*Toxicological Review and Risk Characterization*
External Scientific Peer Review
March 5 & 6, 2002 Sacramento, CA

## Purpose of IRIS Peer Review

- Provide peer review of protocols, performance, and results reported in studies since 1999 that have not appeared in the open literature
- Provide individual expert comment on EPA external review draft regarding approach, analyses, and inferences used in the human health and ecological risk assessments
  - Panel is ***NOT*** charged with arriving at a consensus opinion or conclusion
  - Public and observer comments incorporated according to professional judgement of panel
  - Comments related to EPA policy or potential rulemaking are ***NOT*** relevant to scientific review

## Risk Assessment Closure

- Public comment period extended until April 5, 2002
- Draft peer review report back to the panel and to the Agency on April 22, 2002
- Final external peer review report May 22, 2002
  - Posted on the EPA web in June
  - Agency is responsible to respond to comments
  - Disposition of major comments will be indicated in final document
- Submit revised final draft document to IRIS Agency consensus review in summer 2002
- Final changes in response to Agency review
- Expect IRIS clearance with final document posted to IRIS in fall 2002

## Future Steps: Regulatory Readiness

- No National Primary Drinking Water Regulation (NPDWR) at this time
- Draft ORD 1999 guidance will stand until new assessment finalized
- CCL Research Priority in All Areas:
  - **Health:** Develop reference dose (RfD) as risk estimate
  - **Analytical:** Method 314.0 for water, extend to other media
  - **Treatment Technology:** Cost and efficacy by end use (e.g., drinking water versus agricultural)
  - **Occurrence/Exposure:** UCMR and other surveys
- Near term: Use "RfD" to develop a health advisory (HA) under SDWA general authority
- Evaluate progress in each area for "go" on maximum contaminant level goal (MCLG)

## Future Steps: Regulatory Readiness

- Determine to regulate perchlorate as a CCL contaminant under SDWA
- Timing based on evaluation of "meaningful opportunity for health risk reduction" based on sufficient health effects and occurrence data
- Development time frame
  - If determination to regulate is made, the agency has 24 months to propose a NPDWR and 18 months to finalize
- Considerations of additional risk management factors:
  - Analytical methods
  - Treatment technology capabilities
  - Cost and benefits

# The Perchlorate Contamination Challenge

## Credible Science

## Credible Decisions

- Accurate risk characterization
- Appropriate management strategies

EPA United States Environmental Protection Agency

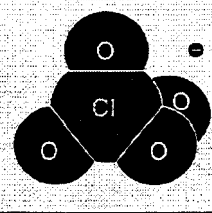
Office of Research and Development

## *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization*

**External Peer Review Workshop**
**Sacramento,CA**
**March 5 & 6, 2002**

**Background and Assessment Highlights**

Annie M. Jarabek
Special Assistant to the Associate Director for Health
National Center for Environmental Assessment




## 2002 Risk Assessment Authors

**Randy Bruins, Ph.D.***
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**Kevin Crofton, Ph.D.***
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"The Right Stuff"

*Perchlorate Environmental Contamination:*
*Toxicological Review and Risk Characterization*
External Scientific Peer Review
March 5 & 6, 2002 Sacramento, CA

## 2002 Overview

- Background
  - 1999 External peer review recommendations
  - Conceptual Mode-of-Action Model
  - New studies and results
- Assessment approach highlights
  - Human health
  - Ecotoxicological
- Public comment issues and clarifications
- Summary

## 1999 External Peer Review

- Basis of health assessment
  - Thyroid histopathology in PND5 rat pups
  - Histopathology used as biomarker for adverse hormonal changes *in utero*
- Screening level ecotoxicological assessment
  - Agreed with characterization
  - Identified additional data gaps
- Scientific expert peer findings
  - Concurred with conceptual model and nonlinear approach
  - Supportive of concern for neurodevelopmental
  - Provided recommendations

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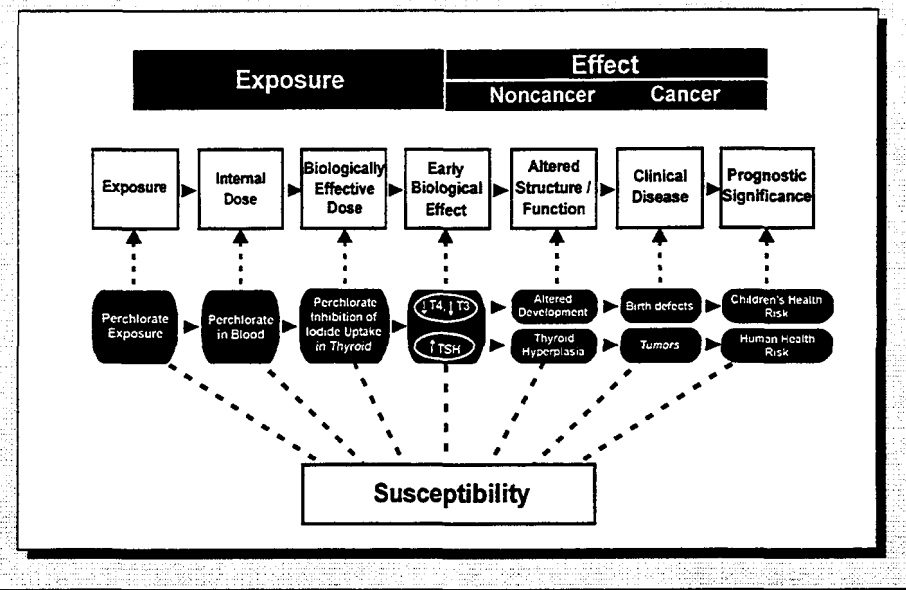
## Proposed Mode-of-Action Model for Health Risk Assessment of Perchlorate




## 1999 Peer Review Recommendations

- Evaluate variability in RIA kits across laboratories
- Pathology Working Group of thyroid histopathology
- Additional brain morphometry if material available
- Developmental study in rats
- Repeat motor activity study in rats
- Repeat and additional immunotoxicity studies in mice
- Pharmacokinetic information in humans and rats
- Alternative statistical analyses for hormone data
- Chronic ecotoxicological studies
- Additional ecotoxicological receptors
- Data on transport and transformation

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## Recommendation Responses

- EPA and NIEHS performed PWG on thyroids
- Protocols reviewed by EPA
  - Not all protocols were EPA guideline studies
  - Not all suggestions from EPA taken
  - EPA testing guideline studies or modifications
    - Developmental study in rats
    - Brain morphometry and motor activity in rats
- Performed and reported by DOD, PSG, or their contractors
- EPA and NIEHS independent analysis
  - Additional statistics as deemed appropriate
  - Peer-review of those data not submitted to scientific literature

## New Studies: Humans

- Observational (ecological) epidemiological studies
  - Not part of testing strategy
  - Limited exposure measures, demographic data, population size and outcome measures
  - Lack of control for confounding
- Clinical studies
  - 3 different laboratories
    - Greer et al. (2000)
    - Lawrence et al. (2000) and (2001)
    - Unpublished data from Drs. H. Leitolf and G. Brabant
  - EPA had limited input on one (Greer et al. 2000) at outset; designed with intent to provide pharmacokinetic information and not to designate effect levels
  - Those that underwent QA/QC used to develop human PBPK model and others to support validation

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## EPA Interim Human Study Policy

- Federal agencies adhere to "common rule" guidance that includes informed consent
- Agency has long-standing concern for "third-party" human data
  - Intentional dosing with toxicant to determine effect levels
  - IRB information often unavailable
  - Issue is how to ensure adherence on *post hoc* basis
- Moratorium issued on December 14, 2001 re: use of this type of data **in the future** until the NAS determines criteria for acceptability
  - Human studies were considered and shortcomings noted in assessment
    - *Studies not used to determine hazard based on human NOAEL*
    - "What if" calculation was provided
  - Human data were used to support the AFRL PBPK model

## New Studies: Laboratory Animals

- Pathology Working Group (PWG) of previous data
  - Thyroids: colloid depletion, hypertrophy, hyperplasia
  - Brains: Insufficient materials
- AFRL interlaboratory study of RIA kits to measure hormones evaluated across 3 laboratories
- Argus 1999 two-generation reproductive study in rats
- Argus 2000 developmental study in rats
- USN (Bekkedal et al., 2000) motor activity study in rats

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## New Studies: Laboratory Animals

- "Effects study" protocol in rats (Argus, 2001)
  - Hormones and thyroid histopathology in pups and dams
  - Brain morphometry
- Immunotoxicity study in mice
  - Repeat macrophage phagocytosis
  - Sheep red blood cell (SRBC) assay of humoral immunity
  - Contact hypersensitivity

## New Studies: Ecotoxicology & Exposure

- Acute (EA Engineering, 1999)
  - *Selanstrum caprinconutum* 96-hr
- Subchronic ecoxoticity (Block Env. Svcs., 1998)
  - Pimephales promelas 7-day
- Chronic ecotoxicity (Block Env. Svcs., Inc., 1998; EA Engineering, 2000)
  - *Pimephales promelas* 35-day Early Life Stage
  - *Hyalella azteca* definitive 28-day study
  - *Ceridaphnia dubia* 6-day
- FETAX studies
  - Dumont and Bantle, 1998
  - Goleman et al., 2002

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## New Studies: Ecotoxicology & Exposure

- Six site-specific occurrence & biotransport studies (Parsons Engineering, 2001)
  - Site media
  - Various ecological receptors @ each site
- Phytotransformation and plant uptake studies
  - Nzengung et al., 1999; Nzengung and Wang, 2000
  - Susarla et al., 1999; 2000
- Occurrence & biotranport studies
  - US Army Corps of Engineers (Condike, 2001): fish
  - Smith et al., (2001): water, sediments, vegetation, fish, mice
- Indirect exposure characterizations
  - EPA Fertilizer study with The Fertilizer Institute (US EPA, 2001a,b)
  - Wolfe et al., 1999; Ellington et al., 2001; Urbansky, 2000

## Designation of Effect Levels

- Thyroid histopathology
  - Benchmark response @ 10%
  - BMDL used as NOAEL surrogate in RfD derivation
- Thyroid hormones
  - Response level @ 10%
  - Analysis of Variance (ANOVA)
- Brain morphometry
  - Repeated measures issue — T-tests inappropriate
  - Profile analysis
    - Mulitvariate analysis of variance
    - Vector does not require expectation on magnitude or direction
  - Issues on sectioning addressed with restricted analyses
    - PND21
    - Sidedness, normalization, region and level

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US EPA ORD NCEA

## Designation of Effect Levels

- Motor activity data from Argus 1998 DNT and USN
  - Bayesian hierarchical analysis with linear mixed-effects regression
  - Individual studies and data combined
  - Results indicate effects @ 1 mg/kg-day
- Thyroid tumors in Argus 1999 two-gen study
  - 3 tumors in 2 animals @ 19 weeks in F1 adults
  - Compared to incidence of all thyroid tumors in NTP archives for SD-rats @ 2-year bioassay terminal sacrifice
  - Bayesian analysis
  - Results indicate concern for *in utero* programming
    - Latency
    - Incidence

## Point of Departure

- Key event defined as an empirically observable precursor step that is a necessary element or marker for mode of action
- Identified as iodide uptake inhibition @ the $Na^+$-$Iodide^-$ Symporter (NIS)
  - Reinforced by repeat studies showing neurodevelopmental effects
  - Precursor for thyroid hormone perturbations
  - Allows harmonization in approach to address neurodevelopmental and neoplastic sequelae
- Weight of evidence for 0.01 mg/kg-day LOAEL
  - Thyroid and pituitary hormones
    - Dams on GD21
    - Pups on GD21, PND4 and PND9
    - 14-days and 90-day for T4 and TSH
  - Thyroid histopathology
    - Pups on PND4 in 1998 and 2001 and weanlings in 1999
  - Brain morphometry in pups on PND21

Annie M. Jarabek
US EPA ORD NCEA

*Perchlorate Environmental Contamination:*
*Toxicological Review and Risk Characterization*
External Scientific Peer Review
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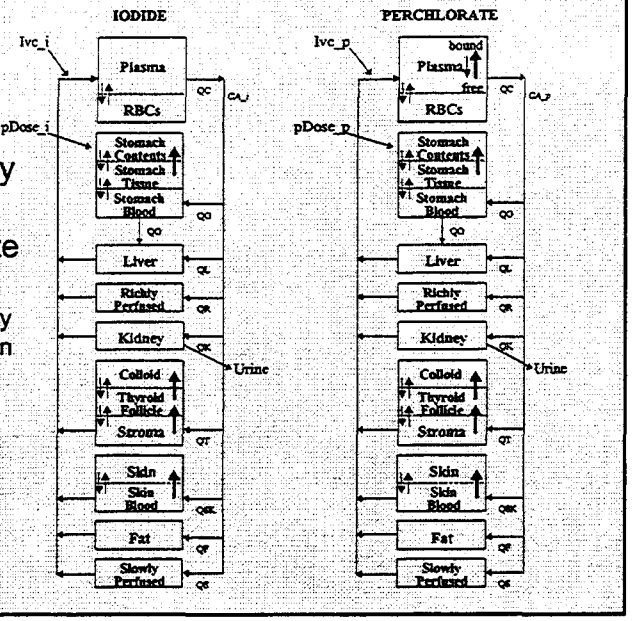
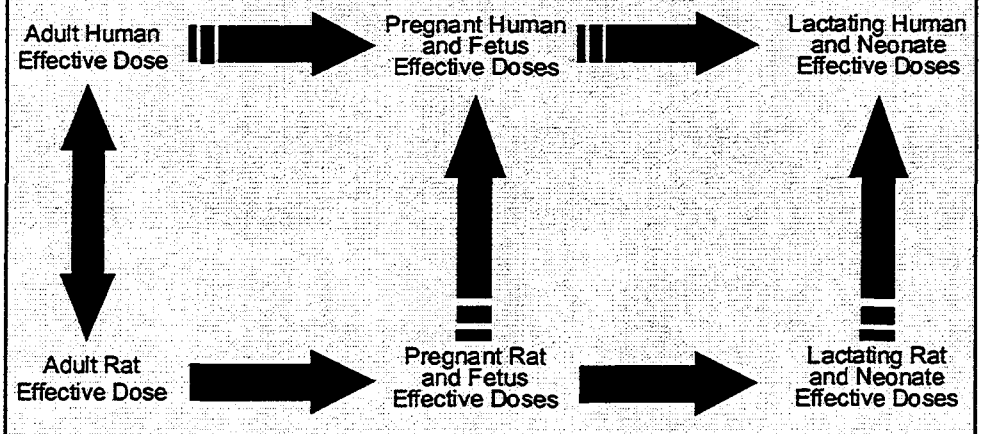
# AFRL Dosimetry Model Structures

- 4 Model Structures
  - Adult male rat
  - Adult human
  - Pregnant rat & fetus
  - Lactating rat & fetus
- Compartments for key tissues
- Iodide and perchlorate disposition
  - Active uptake described by Michaelis-Menten saturation
  - Permeability areas cross products and partitions
  - Passive diffusion
  - Plasma binding
  - Urinary elimination
- Growth




# Parallelogram Extrapolation

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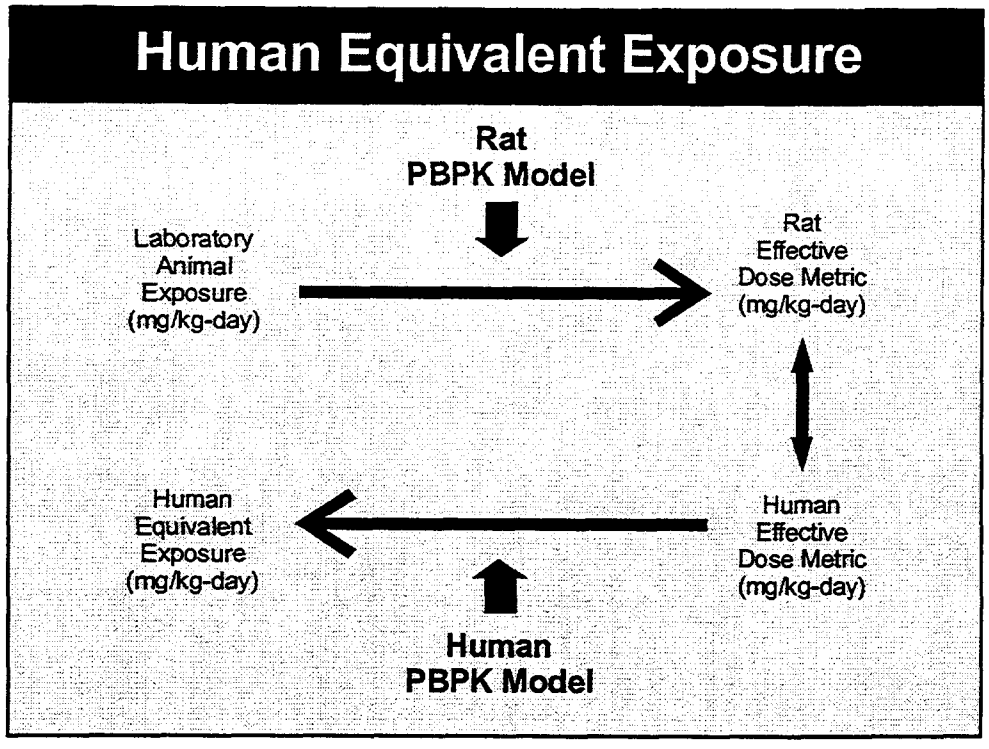
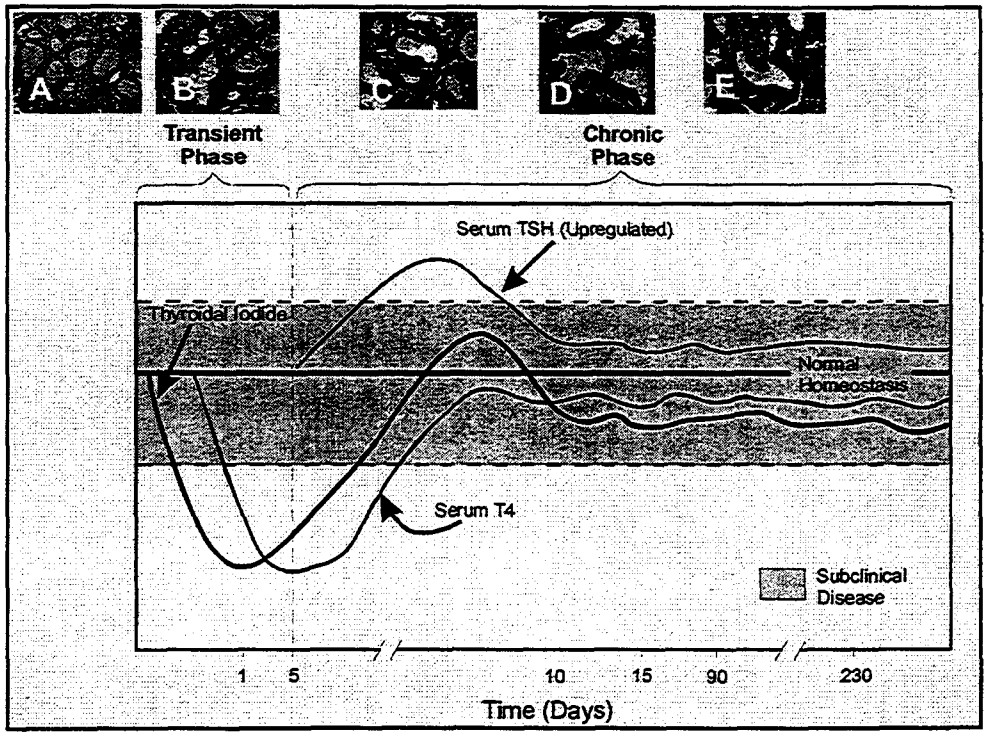
*Perchlorate Environmental Contamination:*
*Toxicological Review and Risk Characterization*
External Scientific Peer Review
March 5 & 6, 2002 Sacramento, CA

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## Choice of Dose Metric

- Internal perchlorate concentration as metric associated with key event of iodide inhibition
  - iv data in rats ("acute")
  - Drinking water in humans
- Area Under the Curve in (AUCB) blood versus peak
  - Good correlation with iodide inhibition
  - Average of serum and thyroid
- EPA agreed with DOD re: uncertainty in and lack of validation of thyroid parameters notably in fetus and neonates for iodide inhibition description
- HEE based on maternal AUC in blood at GD21

## Uncertainty Factors

- Composite factor of 300 parceled into components
  - Intrahuman: 3
    - Pharmacokinetic variability
    - Not representative of sensitive populations
  - Interspecies: None
    - PBPK dosimetry model for extrapolation
  - LOAEL to NOAEL: 10
    - Hormones (slope), thyroid histopathology and brain morphometry
    - Interdependence with lack of interspecies and choice of dose metric
  - Subchronic to chronic duration: 3
    - Lack of "womb to tomb" design and *in utero* programming concern — recalibration of feedback system
    - Interdependence with intrahuman factor
  - Database Insufficiencies: 3
    - Concern for immunotoxicity reinforced

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## Operational Derivation

RfD (mg/kg-day) = 0.01 x 0.85 ÷ 300 = 0.00003

Where:

- 0.01 is the point of departure
- 0.85 adjusts to perchlorate anion alone
- 300 is the composite uncertainty factor

## Comparative Risk Derivations

- "What if" calculation based on human data
  - 0.007 mg/kg-day
  - Uncertainty factor of 100 parceled as:
    - Intrahuman variability: 3
    - LOAEL to NOAEL: 3
    - Subchronic to chronic duration: 3
    - Database insufficiency: 3
  - Result is 0.00007 mg/kg-day
- If a larger UF was applied for intrahuman variability then resultant estimate would be essentially equivalent to that proposed

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## Comparative Risk Derivations

- Derivation based on tumor precursor lesions
  - Colloid depletion, hypertrophy and hyperplasia all observed @ > 0.3 mg/kg-day
  - BMDL estimates of 0.9, 0.15 and 0.0004 mg/kg-day
  - HEE estimates of 0.45 and 0.02 for colloid depletion and hypertrophy
  - Uncertainty factor of 100 parceled as:
    - Intrahuman variability: 3
    - LOAEL to NOAEL: 3
    - Subchronic to chronic duration: 3
    - Database insufficiency: 3
  - Result is in range of 0.005 to 0.0002 mg/kg-day
  - A larger UF for intrahuman variability would result in 0.002 to 0.00007 mg/kg-day

## Hypothetical RfD Conversion

- Critical to distinguish the RfD from any guidance value that may result
- Conversion to drinking water equivalent level (DWEL) in ug/L (ppb):
  - Adjustment by 70 kg and 2 l
  - DWEL = 1 ug/l (ppb)
- Derivation of maximum contaminant level goal (MCLG) typically involves the use of a relative source contribution (RSC) factor to account for non-water sources of exposures
  - Range of 0.2 to 0.8
  - Default @ 0.2 when data are inadequate to determine
  - Result would be MCLG between 0.2 to 0.8 ug/l (ppb)

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## Ecotoxicological & Exposure

- Screening-level and not definitive
- Exposure issues:
  - Accumulation in terrestrial and aquatic plants
  - Fate in irrigated soils
  - Potential for dietary toxicity to vertebrate herbivores point to need for lower limits of detection in plant and animal tissues
- Effects need determination:
  - Exposure on aquatic plants and noncrustacean invertebrates
  - Dietary exposures in birds and in herbivorous or litter-feeding invertebrates
  - Dietary and cutaneous exposure for adult amphibians and aquatic reptiles

## Summary: Unique Attributes

- Pro-active partnership to develop data
- Model motivated by mode of action
- Harmonized approach to noncancer and cancer toxicity based on key event
- Human and ecosystem health
- Comprehensive characterization — integrated approach challenging
  - Analytical
  - Occurrence / exposure / transformation & transport
  - Assessment approaches
  - Treatment technology

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## Appendix J

## Post-Meeting Comments Submitted by Peer Reviewers

Note: After the peer review meeting, ERG distributed to the peer reviewers copies of additional public comments received before the submissions deadline (April 5, 2002). The peer reviewers were given the opportunity to prepare post-meeting comments based on the information in these public comments or on any other topics they chose to address. This appendix contains all post-meeting comments that peer reviewers submitted to ERG. ERG modified the format of these comments, but did not alter the content.

**Post-Meeting Comments Submitted by Dr. Thomas Collins:**

Note: During the peer review meeting (see Section 4.2), Dr. Thomas Collins expressed concern about apparent dose-dependent decreases in sperm density and daily sperm production levels observed in a laboratory animal study (Argus 1999). After the meeting, Dr. Collins sent technical questions, through ERG, to the study's authors regarding the sperm analyses. Following are Dr. Collins' questions, responses to these questions from one of the study's authors (Dr. Raymond York), and Dr. Collins' post-meeting comments regarding these responses.

**Question #1 (regarding sperm evaluation):**

*Dr. Collins' question:*

"Does the percent motility presented in the report refer to 'progressive motility' or to 'motility'? If this value represents 'progressive motility,' how was 'progressive motility' defined using the Hamilton-Thorne Sperm Analysis System?"

*Dr. York's written response:*

"The value represents motile sperm versus nonmotile sperm. We have never been able to validate 'progressive motility' for GLP studies. We have been able to validate curve linear velocity, average path velocity but not smooth line (straight line) velocity or beat-cross frequency which are needed in the calculations."

*Dr. Collins' comment on the response:*

"Acceptable."

**Question #2 (regarding tissue evaluation):**

*Dr. Collins' question:*

"What embedding material was utilized for the testicular tissue? Paraffin? Methacrylate? How were the samples stained? PAS? H and E?"

*Dr. York's written response:*

"The protocol-specified tissues were routinely processed, embedded in paraffin, sectioned and stained with hematoxylin and eosin for microscopic evaluation. The copy of page 817 from the final report is attached." (See page 817 of Argus 1999 for attached material.)

*Dr. Collins' comment on the response:*

"Argus laboratories indicated that testicular tissues were routinely processed, embedded in paraffin and stained with hematoxylin and eosin (H and E). Testicular tissues have traditionally been immersion-fixed with formalin and embedded in paraffin for

histological observation, however it is generally accepted that the use of this fixative induces many artifacts and that subtle changes in testicular histology may be missed or not observed. Many testing guidelines (FDA, EPA, OECD, and ICH) suggest using Bouin's fixative or another suitable fixative to preserve testicular tissues. It is surprising that Argus laboratories continued to use formaldehyde as the fixative of choice for the fixation of testicular tissues. Because the testicular tissues were not fixed utilizing appropriate fixatives, histopathological data should be considered questionable with respect to more subtle changes which may have occurred in the seminiferous epithelium but not with respect to more gross histopathological changes."

**Question #3 (regarding sperm density):**

*Dr. Collins' question:*

"To what do the authors attribute this reduction in cauda epididymal sperm density in 30-mg/kg/day-dose group of the F1 generation?"

*Dr. York's written response:*

"Cauda epididymal sperm density was 1543.6 ± 520.8 for the control male rats in the F1 generation and 1372.6 ± 444.6 for the male rats in the 30 mg/kg/day exposure group. The authors attribute the reduction in cauda epididymal sperm density in 30 mg/kg/day dose group to chance. The data for this parameter had overlapping standard deviations indicating a common dispersion of data points and there was no statistical difference when an ANOVA, the workhorse of toxicology, was applied. The ANOVA is very robust for moderate departures from equality of variances when the sample sizes are approximately equal."

*Dr. Collins' comment on the response:*

"Acceptable."

**Question #4 (regarding sperm density):**

*Dr. Collins' question:*

"Why is the sperm density for the P and F1 controls so dramatically different?"

*Dr. York's written response:*

"The raw data for this study was re-reviewed. The age, body weight and caudal weight for the male rats of both generations were approximately the same. The settings on the Hamilton-Thorne Sperm Analysis System were the same for both generations. We believe the difference is a matter of a slight difference in technique between two technicians. Technician #1 analyzed all 119 samples of the P generation epididymal sperm concentration by himself while Technician #2 ran most of the F1 generation (98 samples), with Technician #1 running some samples (18 samples). Technician #1's counts

were lower than Technician #2, on average, which we discovered and discussed at the time. What we determined then was that Technician #1 handled the homogenates differently than Technician #2 did when he made the stained sample from the homogenate. Technician #2 would invert several times and then vortex, pipetting the sample of while the homogenate was still spinning from vortexing, keeping the cells in suspension and, hopefully, evenly distributed. Technician #1 would vortex, then when the sample stopped spinning he would invert several times, and draw the sample. When Technician #1 used Technician #2's ordering of steps, he also achieved higher counts. It may seem odd, but sometimes a small difference in ordering of steps will produce a significant difference in the results. Technician #1's range of values overlapped Technician #2's range, but Technician #2 was higher overall than Technician #2, so Technician #2 would fall on the high end of the bell curve and Technician #1 would fall on the low end. This did not show up on the spermatid counts for this study because Technician #1 and Technician #2 split the analyses more evenly, each doing about half of the samples."

"I have also attached the Testing Facility's historical control for the Hamilton-Thorne Sperm Analysis System. It includes the type of study, date report finalized and covers 1314 rats from 51 studies conducted 1998 through 2002. The Sponsor's protocol number has been removed except for this study. The average sperm density is 1099.9 with a range of 730.7 to 1563.9 so the sperm density values for both generations for this study fall within the historical control range but towards the two tails. By reviewing the historical control file, it can be noted that 5 multigenerational studies have been completed since the two-generation ammonium perchlorate study (Code 353A and B; protocol 1416-001). The codes and sperm density values for the P and F1 generation control rats for these studies are 489A (1255.0) and B (940.2); 513A (1046.8) and B (1010.8); 522A (1044.8) and B (1061.0;) and 570A (962.7) and B (1194.3)."

*Dr. Collins' comment on the response:*

"The authors indicated that the differences between the sperm density counts in the P and F1 generation were attributed to technicians using different techniques to prepare samples for analysis with the Hamilton-Thorne Sperm Analysis System. This would suggest that one or both of the technicians did not follow the Standard Operating Procedures for the preparation of sperm samples for analysis by CASA. This could compromise the results because not all samples were handled in a similar manner. Although this explains the different counts obtained between the two generations, it still does not explain the non-significant dose-related decrease in sperm density and daily sperm production observed in the F1 generation. It seems unlikely that a dose related decrease in sperm density would occur if two different technicians using different techniques for sample preparation randomly assayed the samples. Although an examination of the historical control data indicated that the counts obtained from the animals in the 30-mg/kg/day-dose group were within the range of historical control values, the dose related decrease in sperm numbers observed in the F1 generation is still puzzling. Because two different methods were utilized for the determination of testicular spermatid densities and because of the

unexplained dose-related decrease in spermatid density in the F1 generation, spermatid density data should be considered questionable but should not be discounted with respect to the accuracy of the spermatid counts."

**Post-Meeting Comments Submitted by Dr. David Hoel:**

*Point of departure based on human data:*

The Greer et al. 2002 study provides the best data for NOAEL estimation of iodine uptake. If the data for the original study are combined with the data from the subsequent 0.007 mg/kg dose group one finds a good fit using percentage uptake at 24 hours and 14 days versus the log of administered dose. One individual is a clear outlier (mj in the 0.007 mg/kg) and is eliminated from the analysis. The regression yields Percentage uptake = 0.230 –0.88*log(dose). The data is well described by this expression which predicts a no-effect level (as opposed to a NOAEL) of 0.005 mg/kg. Using the lower limit of the 95% confidence interval the no-effect level becomes 0.0025 mg/kg. If one includes a covariate for the background uptake level one obtains the expression: Percentage uptake = 0.401 – 0.18 background – 0.85 log (dose). For a background level of 0.1 mg/kg the no-effect level value is 0.008 mg/kg and 0.0045 mg/kg for a background level of 0.2 mg/kg. Since the concern is with those with a lower background level using a value of 0.005 is conservative. Comparing this data with that of the other clinical study by Lawrence one finds that the Lawrence study estimates a somewhat higher value for the no-effect level. We therefore conclude that a very conservative human point of departure is 0.0025 mg/kg/day. Considering the large amount of human data which is generally negative it is reasonable therefore to use an uncertainty factor of 3 for intra-human variability. This results in a reference dose of 0.0008 mg/kg. If a more conservative uncertainty factor of 10 is desired then one has a reference dose of 0.00025 mg/kg. I would, however, use the value 0.0008 mg/kg/day as an RfD since conservatism has already been incorporated into the calculation prior to applying the 3x uncertainty factor. Further it is not clear that a small reduction in iodine uptake indicates any adverse health effect.

*Human Clinical Studies:*

Concerns are raised about the fact that the clinical studies were carried out on healthy adults. This concern, if valid, should also then be expressed with the toxicological studies since the rodents are presumably also healthy and genetically even more homogeneous than the humans.

*Peer Review:*

One minor comment is on the idea that EPA considers a thesis as a peer reviewed paper. The problems with this view are as follows:

1) Often only the student and the advisor are expert in the topic and not the other committee members.

2) Often little attention is given by other committee members to the research work especially for a MS thesis which is not necessarily required to be publishable.

3) In journal reviews the editor seeks out the most knowledgeable and anonymous reviewers which one does not have with a graduate advisory committee.

4) After publication problems and errors with a paper can be brought to everyone's attention through "letters to the editor."

5) I know of theses whose results have been rejected for publication in the scientific literature.

**Post-Meeting Comments Submitted by Dr. Merle Paule:**

In the absence of convincing changes in brain morphometry, the point of departure using only changes in thyroid hormone, TSH and thyroid histology becomes hard to defend. In the absence of another study to replicate, yet again, the morphometric findings, it should be more expeditious to have the morphometrics analyzed again by a blinded expert. While several peer review members indicated that they were comfortable with the interpretation of the morphometric data proffered by the EPA staff, it was clear that several others felt they would never be able to trust the data from the Argus 2001 study.

It is also critical, that in any attempts at replicating rodent studies (and likely those of other species), time of year (season) needs to be taken into consideration and controlled. The effects of chemicals in animals can vary tremendously from winter to summer, even when such animals are housed inside, with no known exposure to natural light, etc. It is my opinion, that the difficulty in (or inability to) replicate rodent studies may in some cases relate to seasonal confounds.

In general discussion with other reviewers who also were asked to assess the Bekkedal motor activity study; the consensus was that the data did in fact indicate a signal (effect) of perchlorate exposure: Specifically, the 18 day old males and the 22 day old females showed perchlorate effects when the Bayesian analysis was employed. Similarly, Bayesian analysis of the behavioral data from an earlier Argus study also showed effects, albeit in 14 day old subjects. The absence of an effect in the 14-day-old animals in the Bekkedal study was not considered problematic because of issues surrounding the ability to replicate findings in rodents.

If follow-on studies are undertaken, it is clear that much more sophisticated behavioral analyses need to be employed to explore the potential of perchlorate-induced functional deficits. These could include but not be limited to: studies on classical conditioning (thought to depend, at least in part, on cerebellar function); learning tasks (hippocampal function); and attention tasks (frontal cortical function).

Several observers at the Workshop brought up the issue of potentially confounding effects of diet: soy-based products or others may contain compounds with the ability to interact with and influence thyroid hormones or TSH. This possibility needs to be explored and it needs to be determined whether the diets used in the earlier studies might have affected the outcome of those studies. If so, studies with other diets that do not have the ability to influence the system would need to be conducted.

In general, this reviewer feels that the Agency did an exceptional job of providing reasonable analyses and interpretations of the data and in defending the Uncertainty Factors proposed. After discussion at the workshop, the consensus of the panel was clearly leaning toward eliminating the UF of 3 for database insufficiency, based on the lack of concern expressed by the immunologist on the panel for the likelihood that perchlorate may have adverse effects on immune system function. Those arguments were persuasive, but, of course, if one were to poll

other immunologists, one might get different perspectives. I would not, however, feel uncomfortable about dropping the UF of 3 for database insufficiency.

The issue of the inverted U-shaped dose-response curve for endpoints used to support the point of departure need to be clearly telegraphed to the non-science stakeholders. This is a confusing issue, but such non-linear relationships are not uncommon. Thus, while I am not skeptical of the existence of such a relationship in the perchlorate matter, a clear explanation of such will be necessary.

**Post-Meeting Comments Submitted by Dr. Thomas Zoeller:**

**1. Borak, J. and Russi, M. Comments on: Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (External Review Draft January 16, 2002)**

The executive summary identifies 3 "value judgements" made by the EPA in its document which these authors find problematic:

1. Exclusion of the clinical studies as "principal studies" for use in the risk assessment.
2. Use of the animal studies despite the almost certain confounding variable of the diet containing soy protein.
3. Characterization of the Schwartz epidemiological study by the EPA as a "strong" study.

CRITIQUE:

1. Neither the EPA nor a regulated industry should define the ethics of identifying a LOAEL/NOAEL for compounds in humans. This is an issue that is reasonably considered by a national body such as the NAS, which the EPA has contacted.

2. The authors are unreasonably secure in their argument that the ARGUS studies were confounded by isoflavones (e.g., genestein) in the diet. Although this is an important hypothesis that should be formally evaluated, the diet used by ARGUS is a very common one in rodent studies. Therefore, if there were the kind of confound the authors propose, and as described by Ikeda *et al.* (1), it would be very obvious in the literature considering the values for TSH reported in the *Carcinogenesis* paper (1). This report indicates that animals on the soy-enriched diet exhibited TSH levels of about 125 ng/ml. This is greater than 10-fold higher than what is considered to be elevated TSH levels in rats, produced by the well-known goitrogenic agents methimazole (MMI) and propylthiouracil. In fact, I have recently completed a study in my lab combining MMI and 0.5% perchlorate and still observed TSH levels in the 10-20 ng/ml range, unlike the astronomical values of Ikeda *et al.* Thus, although it is important to consider this kind of potential interaction, it is highly unlikely that it has any bearing on the ARGUS studies. The TSH levels reported in the ARGUS studies were not outside those observed in the thyroid literature, and certainly were not in any way similar to that reported by Ikeda *et al.*

3. Characterization of the Schwartz Thesis. These are potentially valid comments, though I don't believe I have an original copy of the Schwartz thesis and therefore cannot comment. See the public comments by Schwartz.

**2. Bruce, G. and Pleus, R.C. Summary of the Expert Review of the ARGUS, 2001 ("Effects Study") Evaluation of Perchlorate Effects on Brain Morphometry in Neonatal Rats.**

Conclusions regarding the adequacy of the methodology in the ARGUS 2001 Effects study.

a. *Use of coronal sections.* There is no doubt that coronal sections were not optimal for all the brain areas measured. However, for others, it was. Finally, linear measures were not the best choice of endpoint – though there are no valid and validated endpoints in the developing brain for thyroid toxicity.

b. *Use of single width measures.* The corpus callosum measurements are clearly not reliable in coronal plane.

c. *Lack of evaluation of post-puberty animals.* In both humans and animals, perinatal thyroid insufficiency produces a lag in myelination that "catches up" later in development. However, both humans impacted by postnatal hypothyroidism and rats experimentally manipulated to model this deficiency, exhibit permanent neurological deficits despite the "catch up" in certain measures. Thus, the requirement for persistence of an anatomical anomaly is not valid.

d. *Lack of demonstrated association between changes in linear dimensions of brain structures and the presumed mode of action of perchlorate (hypothyroidism).* It is true that there is no information linking incremental deficits in thyroid hormone levels with linear brain measurement. In fact, there is no information linking incremental deficits in thyroid hormone levels with anything. Did the experts propose a series of endpoints that have been validated for toxicological studies such as ARGUS 2001?

e. *Lack of a positive control.* This is simply wrong. We know enough about perchlorate mechanism of action that using a drug like methimazole or PTU which acts in a fundamentally different way with different pharmacokinetics compared to perchlorate is a weak and illogical design. Rather, using $T_4$ to ameliorate the effects of perchlorate, restoring levels to those within a physiological range, would be better.

f. *A clear dose-response relationship is not apparent.* It is not logical to assume, *a priori*, that linear measures of brain structures will exhibit a clear dose-response with perchlorate. The control of size of brain structures is not well understood. The role of thyroid hormone in this process is not well understood. The relationship between thyroid hormone activation of receptors and down-stream events that may play a role in controlling the size of specific brain structures is also not well understood. What possible basis would experts have to conclude that this should be a requirement for a clear dose-response relationship between perchlorate and size of individual brain structures as measured by a single linear measurement?

g. *Hypothyroidism was not induced.* (see discussion of #7)

**3. Goodman-1. Letter to EHP editor.**

No comment

**4. The DoD Perchlorate Working Group. "Comments on the U.S. Environmental Protection Agency's Draft Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (NCEA-1-0503, 16 January 2002)**

Several points are made and expanded in the text:

1. Issue of using clinical studies (see above)
2. Argue that the use of brain morphometry and thyroid hormone levels are problematic because of the inconsistencies. These inconsistencies were well-recognized by the peer-review group and it is clear that there are problems. However, these data are endpoints with the most important implications and need to be emphasized in the EPA analysis.
3. Argues that "The screening benchmark for herbivores should be derived using toxicity values associated with endpoints known to be ecologically relevant (development, reproduction)." I fully disagree with this statement because, in my opinion, it is based more on the common practice of using reproductive endpoints in toxicity studies because those endpoints are better developed, not because they are more important or valid. Because reproduction can occur even in cretins – the most severe form of developmental defect due to thyroid hormone insufficiency – I believe it is not wise to use reproductive indices alone in evaluating the toxicity of a compound like perchlorate which affects the thyroid.

**5. Schwartz, H. public comments, "<u>Thyroid Hormone Effects on the Developing Brain: Critical Review...</u>".**

The Executive summary concludes that, "For all these reasons, we must conclude that the reported results are artifacts of methodology and are of no value in evaluating the possible effects of perchlorate in the rat. Dr. Schwartz presents a clear set of arguments to support this conclusion. However, many of these arguments are weak, if not incorrect, and this reduces the strength of the conclusion. These arguments are as follows.

A. The concept of a "critical period" of thyroid hormone action on the rat brain, as defined by Dr. Schwartz, is incorrect. Dr. Schwartz defines the critical period of thyroid hormone action in rodents (rat) as the early postnatal period, and that this period is absolute. Recent studies show that maternal thyroid status of the dam can influence gene expression (2-4), behavior (5), hearing (6), and migration of cortical neuroblasts (7). The Dowling studies also show clearly that changes in maternal $T_4$ *within the physiological range*, can affect the expression of known thyroid hormone-responsive genes in the fetal brain <u>before the onset of fetal thyroid function.</u> Thus, it is incorrect to assert that thyroid hormone of maternal origin does not play an important role in fetal brain development, even before the onset of fetal thyroid function. The important conclusion from these studies is that it is more appropriate to think of "critical" periods of thyroid hormone action in specific developmental contexts. For example, Schwartz

demonstrated that manipulating maternal thyroid status late in gestation did not influence MBP gene expression in the late-gestation fetus (8). This is undoubtedly a valid, reliable, and important observation as it relates to the control of myelination in the cerebellum. However, it is inappropriate to consider MBP regulation by thyroid hormone to be a surrogate marker of thyroid hormone effects on the entire brain at all times.

B. Dr. Schwartz describes the effects of hypothyroidism on rat development as it has been amply shown throughout many years and studies, and correctly observes the lack of these effects in the Argus studies. Specifically, lowered growth rate of the dams, her fetuses and her pups. However, it cannot be overemphasized that the thyroid literature is characterized by the use of high doses of potent goitrogens and the production of severe hypothyroidism. In no case were animals exposed to multiple doses of these goitrogens with the goal of identifying a NOAEL/LOAEL of thyroid hormone. Although pharmacodynamic studies exist for some goitrogens (9), they have not been performed to look at effects on the brain. Therefore, it is not valid to directly compare the dose-response studies of perchlorate in the Argus studies with this body of literature. Having said that, Dr. Schwartz is absolutely correct in stating that the Argus studies used endpoints of perchlorate toxicity that were completely devoid of any known relationship to thyroid hormone. Moreover, this point was made clearly in the first Peer Review meeting and this fact alone weakens the interpretation of the Argus studies. However, it must also be recognized that even known markers of thyroid hormone action on the developing rat brain have not been validated for the kind of toxicological screen used in the Argus studies.

C. The concept that we can surmise that changes in circulating levels of thyroid hormone did not produce adverse effects in the brain would obviate all empirical measures of neurotoxicity of chemicals that reduce circulating levels of thyroid hormone. For example, we could simply surmise that a threshold of thyroid hormone levels exist, above which no adverse effects can be assumed and below which adverse effects can be assumed. The fact is that no experimental studies to date have attempted to identify a NOEL for thyroid hormone deficits on specific measures of thyroid hormone action in the developing brain that control important developmental events. Therefore, the conclusion of Dr. Schwartz is unwarranted. Moreover, the assumption that we can predict or calculate the thyroid hormone decrement in maternal serum required to observe an adverse effect on the brain is based on estimates of receptor occupancy taken from whole brains and pooled. This is valid if and only if the brain is a homogeneous aggregation of cells with respect to thyroid hormone, which is clearly not the case.

D. The effect of perchlorate on linear measures of the brain are not validated measures of thyrotoxic endpoints, as Dr. Schwartz points out. However, the use of a goitrogen as a positive control would be less valuable than demonstrating that the effect(s) of perchlorate on brain development could be ameliorated with exogenous $T_4$. Clearly, with all the research focused on perchlorate, there is little doubt about its mechanism of action.

**6. Wahlsten, D. Summary and Re-Analysis of Data: Brain morphometry results from a perchlorate toxicity study (Primedica 2001).**

Dr. Wahlsten reanalyzed the data from the Argus 2001 "Effects" study and found that there was a small but statistically significant increase in the thickness of three brain regions, including the frontal cortex, the parietal area and the striatal area. This is a reasonable analysis of the data acquired in the ARGUS study. The critical element of Dr. Wahlsten's comment is that the analysis should consider the size of the effect as well as their statistical significance. Dr. Wahlsten presents a very strong argument that there are in fact treatment effects. However, he argues that these effects are not biologically significant because they are not large. Considering that the measures under consideration are simple linear measurements of brain regions, it is impressive that any effects were observed. Moreover, it is important to recognize that any observed effects must necessarily be related to reductions in thyroid hormone. If there is an effect on the size of specific brain regions, then it is not unreasonable to infer that there are potentially a number of effects that do not contribute to size, such as neurochemical effects.

**7. Bruce, G., Peterson, M., Lincoln, D.R., and Pleus, R.C. Review and assessment of TSH and thyroid hormones during pregnancy in the rat and human and comparison to hormone values in the 2001 Effects Study.**

Bruce *et al.* propose that gestational TSH and thyroid hormone levels in normal (control) rats can be compiled across many publications to generate a "reference" range to which the values in the Argus 2001 "Effects Study" can be compared. Bruce *et al.* state that, "Reported mean TSH concentrations for pregnant control rats late in gestation (gestational day [GD] 19 to 22), range from 0.43 to 469 ng/mL." Using this reference range, it is found that the TSH levels are not outside this "normal" range following perchlorate exposure in the ARGUS 2001 study. It is my understanding that the authors are suggesting that this range should reflect a "reference" for pregnant rats. There are several reasons this is not possible. First, the laboratories from which the data were obtained to determine this reference range were not using pools to calibrate their assays across laboratories. It is likely that they were not all using the same reference preparation – they may not have even been using purified TSH for the reference standard and for labeling from the same source. Clearly, a TSH level of 469 ng/mL is not believable, so the authors do not seem to be using critical judgement in evaluating the literature. For these reasons, it is absolutely essential that studies such as ARGUS 2001 include control groups and that the control groups are used as the reference to which all other measures are compared. This kind of comparison will also take into consideration the potential confounding variables that the authors suggest are problematic. Using appropriate controls, and avoiding systematic errors such as sampling different groups at different times of day or on different days, is simply basic scientific methodology. The same logic holds for all the other hormones measured in ARGUS 2001.

The authors of this comment appear to misunderstand the concept of hypothyroidism. Clinically, a diagnosis of hypothyroidism requires low $T_4$ and high TSH (outside the reference range) and the simultaneous presentation of some of the symptoms of hypothyroidism.

Translating this definition to rats is somewhat difficult because there is no such reference range for thyroid hormones (see above) and because usually clinical observations are not sophisticated as it applies to rats. Accordingly, the term hypothyroidism applied to rats usually refers to the most severe situation where rats are treated with a potent goitrogen (methimazole or propylthiouracil), exhibit undetectable $T_4$ and TSH levels above 10 ng/mL. Clearly, perchlorate did not induce hypothyroidism (as the term is used in the literature) in rats in the ARGUS 2001 Effects Study. However, in rats as in humans, subtle hypothyroxinemia can produce adverse effects especially if it occurs during development. Thus, the issue is not whether the animals exhibited hypothyroidism, it is whether the hypothyroxinemia produced by perchlorate produced adverse effects.

*Factors contributing to variability in measured TSH and thyroid hormone levels.* Hormone levels within the hypothalamic-pituitary-thyroid axis all vary significantly over the 24 hour day. This is not technically a circadian rhythm (which would imply that it persists in the absence of light/dark cycles. However, ensuring that blood is collected at the same time of day, staggered across treatments, is clearly the best way to perform the experiment. It is highly unlikely that temperature changes would have in any way affected the ARGUS studies. I studied cold exposure in rats for a number of years (10-16). Small changes in temperature for short duration do not affect hormone levels. Moreover, cold exposure produces effects in the afternoon, not in the morning (13). Considering this, temperature changes associated with routine maintenance of the animals is not likely to affect the results in any way.

**8. Soldin, O.P., Nandedkar, K.N., Japal, K.M., Stein, M., Mosee, S., Magrab, P., Lai, S., Lamm, S.H. Newborn thyroxine levels and childhood ADHD.**

This paper is based on the assumption that neonatal $T_4$ is a valid biomarker of pre-natal thyroid status. This is clearly false. There is a great deal of evidence demonstrating that prenatal thyroid status can be variable without being indicated by the point-estimate of thyroid status at the time of birth.

**9. Lockheed Martin Corp. Comments on U.S. EPA's perchlorate environmental contamination: toxicological review and risk characterization.**

1. EPA's External Peer Review lacked a single medical expert on the subject of the thyroid. The authors argue that clinical endocrinology is central to the issue under debate, and therefore, the peer review committee should have included clinical endocrinologists specializing in thyroid endocrinology. Drs. Greer and Braverman were highlighted for their expertise. Clearly, Drs. Greer and Braverman are truly distinguished physician scientists. However, neither of these clinicians studied the role of thyroid hormone in development. Thus, it would have been a valid argument to include clinicians experienced in clinical thyroidology who study the effects of maternal or postnatal hypothyroxinemia on neurological function.

2. EPA's External Peer Review panel included interested participants. The authors argue that Dr. Zoeller's inclusion on the peer review raises at least the appearance of partiality because he was a returning member of the '99 peer review panel and because he was cited by the Environmental Working Group in their "Report" on perchlorate. First, I would like to point out that my criticisms of the animal studies provided to the '99 peer review were very similar to those being articulated by the authors reviewed above in the present document. Specifically, the use of linear measures in brain morphometry was poorly supported. Why use the statistical approach being used? Etc. However, I was critical because I thought that these criticisms would lead to a change in the approach that could have prevented some of the problems associated with those studies as has been amply debated. Thus, it seems disingenuous to argue that my comments were biased in the '99 peer review meeting but other's are not biased now.

Second, it is reasonable to wonder about the integrity of a scientist cited in the EWG report, which is accurately portrayed as "long on hyperbole and short on science". However, I have made a great number of public comments before and since that time that can and should be held up to scrutiny as to my propriety, bias, and partiality.

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# Appendix K

## Index of Written Materials Submitted by Observers at the Peer Review Meeting

During the observer comment period, several observers distributed written materials to the peer reviewers for their consideration. Some of these materials were copies of the comments spoken at the meeting (i.e., those documented in Appendix H), but others were supplemental information not covered during the observer comments. Following is an index of the written materials that observers submitted during the observer comment period:

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