United States
Environmental Protection
Agency

Disposition of Comments and Recommendations for Revisions to *"Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization External Review Draft (January 16, 2002)"*

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Because this response document is addressing comments and recommending revisions to the 2002 ERD of the Agency's toxicological review and risk characterization for perchlorate, the history of the assessment is provided to afford historical perspective on how certain issues have evolved.

2002 External Peer Review Draft of the Toxicological Review and Risk Characterization

The 2002 external review draft (ERD) of the Agency's toxicological review and risk characterization that is the subject of this response to comments and recommendations for revision was reviewed at a peer review and public workshop held in Sacramento, CA, on March 5-6, 2002 (U.S. Environmental Protection Agency, 2002a). The workshop was sponsored by the EPA Office of Research and Development and conducted by Eastern Research Group. Comments of the external peer review panel are available as a final report (U.S. Environmental Protection Agency, 2002b) at http://www.epa.gov/ncea. The members of the 2002 external review panel included:

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Internal EPA Reviewers (2002 External Review Draft) of the Toxicological Review and Risk Characterization

In the Fall of 2001, the following EPA scientists contributed their insights on internal Agency review of the draft assessment that was then issued as the 2002 revised ERD of the toxicological review and risk characterization (U.S. Environmental Protection Agency, 2002a):

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The following scientists in the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment were also asked under contract to provide the National Center for Environmental Assessment with comments on the November 15, 2001 internal review draft:

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1998 External Peer Review Draft of the Toxicological Review and Risk Characterization

The Agency's first assessment for perchlorate was issued as the 1998 external peer review draft (ERD) that was reviewed at a public workshop held in San Bernadino, CA on February 10-11, 1999. The workshop was sponsored by the EPA Office of Solid Waste and Emergency Response (OSWER) and the Office of Water (OW), and conducted by Research Triangle Institute. Members of the 1998 external review panel included:

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Preface

The risk assessment of perchlorate proposed by the Agency has undergone two previous external peer review and public comment processes (U.S. Environmental Protection Agency, 1998d; 1999; 2002a, 2002b). In addition, perchlorate has been the focus of several scientific studies and recent risk assessments by several government agencies. These studies have raised concerns among a number of federal agencies regarding how best to characterize the potential risk posed by chemicals that disrupt the function of the thyroid gland. Because there are differences of interpretation regarding the inferences that can be drawn from the available data and with the 2002 ERD released by the Agency, the EPA in conjunction with the Department of Defense, the Department of Energy, and the National Aeronautics and Space Administration requested a review by the National Academy of Sciences (NAS) (Gilman, 2003). The EPA recognized that a review of its 2002 draft risk assessment by the NAS would be beneficial and informative as the Agency moves toward the finalization of the risk assessment.

The purpose of an Agency toxicological review is to provide scientific support and rationale for hazard identification and dose-response assessments based on the emerging data for both human health and ecological effects caused by exposures to perchlorate. It is not intended to be a comprehensive treatise on the chemical or the toxicological nature of perchlorate. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the individual assessments and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

Development of the hazard identifications and dose-response assessments for perchlorate presented in the 2002 ERD followed the general guidelines for risk assessments set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of the 2002 health risk assessment include the Assessment of Thyroid Follicular Cell Tumors (U.S. Environmental Protection Agency, 1998a), Guidelines for Neurotoxicity Risk Assessment (U.S. Environmental Protection Agency, 1998b), 1999 Proposed Guidelines for Carcinogen Risk Assessment (Federal Register, 1999), Guidelines for Reproductive Toxicity Assessment (U.S. Environmental Protection Agency, 1996a), Use of the Benchmark Dose Approach in Health Risk Assessment (Crump et al., 1995), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. Environmental Protection

Agency, 1994), Proposed Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicology Studies (Whalan and Redden, 1994), Guidelines for Developmental Toxicity Risk Assessment (Federal Register, 1991), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. Environmental Protection Agency, 1988), The Risk Assessment Guidelines of 1986 (U.S. Environmental Protection Agency, 1987), and the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

The 2002 risk characterization presented the hazard identification and, where feasible, dose-response assessment for noncancer toxicity for each route of exposure, either the oral reference dose (RfD) or the inhalation reference concentration (RfC). The RfD and RfC are meant to provide information on long-term effects other than carcinogenicity, although more recently, the value of mode-of-action information to inform the potential for a continuum from noncancer toxicity as precursor lesions to carcinogenicity presented as tumors has been recognized (Federal Register, 1996; Wiltse and Dellarco, 1996). Consideration of this continuum is especially pertinent to the evaluation of the potential toxicity of perchlorate. When such a continuum can be characterized, the dichotomous approaches to "noncancer" versus "cancer" toxicity can be harmonized into one route-specific estimate. The objective is to select a prominent toxic effect or key event that is pertinent to the chemical's key mode of action, defined as a chemical's influence on molecular, cellular, and physiological functions (Wiltse and Dellarco, 1996). A harmonized approach to the neurodevelopmental and neoplastic effects of perchlorate is proposed herein.

In a default characterization without mode-of-action information, the RfD typically is based, in part, on the assumption that a threshold exists for certain toxic effects, both for the individual and the population; whereas, a threshold may not exist for other carcinogenic effects. Thus, if the critical toxic effect is prevented, then all toxic effects are prevented. In general, the RfD or RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure or continuous inhalation exposure to the human population (including sensitive subpopulations) that is likely to be without deleterious noncancer effects during a lifetime. The oral RfD is expressed in units of milligrams per kilogram per day. The inhalation RfC considers toxic effects for both the respiratory tract as the portal of entry, as well as for effects remote to the respiratory tract (extra-respiratory or systemic effects). The RfC is expressed in units of milligrams per cubic meter.

The carcinogenicity assessment is meant to provide information on three aspects of the carcinogenic risk assessment for perchlorate: the EPA classification and quantitative estimates of risk from both oral and inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed.

The screening-level ecological risk assessment of environmental contamination by perchlorate follows the Guidelines for Ecological Risk Assessment (U.S. Environmental Protection Agency, 1998c).

Acknowledgments

The authors are indebted to the following individuals who imparted their insights, data, experimental analysis, and expertise to improve specific areas of the ongoing assessment effort and its documentation or to facilitate the process of its development and review.

As noted in the introduction (Chapter 1), this assessment could not have been accomplished without the cooperation of individuals who worked for the governmental entities represented in the Interagency Perchlorate Steering Committee. Each of the subcommittee members contributed to discussions as the process evolved, via stakeholder forums or meetings, and the integrated approach to the overall risk characterization framework began to materialize. Special acknowledgment for oversight of the testing strategy endeavor, notably communication with the contract labs, expediting data delivery, and writing reports goes to Lt. Col. Dan Rogers (U.S. Air Force Materiel Command); Dr. Richard Stotts, Dr. Dave Mattie, and Capt. David Tsui (Air Force Research Laboratory/Human Effectiveness Directorate [AFRL/HEST], Operational Toxicology Branch); and Cornell Long (AFRL/HEST, Human Systems Center).

Special thanks is paid to three scientists who were especially dedicated to development of the physiologically-based pharmacokinetic (PBPK) models for AFRL/HEST: Elaine Merrill, Rebecca Clewell, and Dr. Jeffrey Gearhart. Terri Sterner is particularly appreciated for her attention to reference retrieval and electronic transfer of data.

Other individuals at AFRL/HEST at Wright-Patterson Air Force Base should be noted for their invaluable technical contributions: Dr. William Baker, for all the histopathology analysis and reports that he generated in a short period of time in 1998; Latha Narayanan, for her expert and reliable analyses of thyroid and pituitary hormone data over the years; and Drs. Jeff Fisher and Kyung Yu for their work on iodide and perchlorate kinetics. Deirdre Mahle is also acknowledged for her contributions to the experimental work.

The Perchlorate Study Group (PSG), particularly Michael Girard, is recognized for its aid in sponsoring studies and ensuring timely data delivery in appropriate formats for EPA analyses. Toxicology Excellence for Risk Assessment (TERA) (notably Michael Dourson, Joan Dollarhide, and Jacqueline Patterson), also was very responsive in this regard on behalf of the PSG.

Additional gratitude is expressed to those individuals who submitted unpublished reports and literature summaries for consideration by the EPA review team. Notable contributions were

provided by Dr. Steven Lamm of Consultants in Epidemiology and Occupational Health, Inc., Drs. Gay Goodman and Richard Pleus of Intertox, and Dr. Monte Greer (deceased) of Oregon Health Sciences University.

Drs. George Alapas, William Farland, Paul Gilman, Peter Preuss, and John Vandenberg are paid special thanks for management and sheparding of the various iterations of this assessment and the 2003 response document internally in the Agency and for allocating expertise and funds from across ORD and NCEA to support the analyses. Drs. Amy Mills and Susan Rieth of the IRIS program are appreciated for their dedication to managing the peer review contract and associated logistics. Kate Schalk and staff at Environmental Research Group, Inc. are acknowledged for conducting the peer review of the 2002 ERD. The authors are indebted to Ms. Linda Bailey-Becht, Linda Tuxen and Joseph Corbett for continued support related to technology transfer and communications.

The authors are especially grateful for the dedication and expertise of the following individuals of Computer Sciences Corporation for their roles in the production of the 2003 response and the 1998 and 2002 ERD documents: Carol Seagle, for technical editing; Carolyn Perry and Kelly Quiñones, for word processing; Jessica Long for graphic arts; and John Bennett and Sandra Hughey, for reference retrieval and editing.

Richard Wilson (EPA, NCEA), as always, is applauded for his tireless and cheerful dedication to the task of photocopying the response to comments as well as both drafts of the document and associated supporting materials throughout the years.

1. INTRODUCTION

The risk assessment of perchlorate proposed by the Agency has undergone two previous external peer review and public comment processes (U.S. Environmental Protection Agency, 1998d; 1999; 2002a, 2002b). The members of these two external peer review panels were selected by independent contractors in adherence with Agency policy. The 2002 external review draft (ERD) addressed recommendations made by the 1999 expert peer panel and comments made by public stakeholders. The purpose of this 2003 recommendations for revision document is to provide the Agency's response to the recommendations from the second expert scientific panel provided at the workshop of the 2002 human health and ecological risk assessment ERD document (U.S. Environmental Protection Agency, 2002a) held in Sacramento, CA, on March 5 and 6, 2002. This 2003 revision document also includes response to public comments received as part of the review process. Both public comments made at the workshop as well as those submitted in writing to the Agency have been reviewed and addressed. This document captures summaries of the major comments. A more detailed summary of the expert peer panel comments and public presentations is provided in Appendix H of the peer workshop report (U.S. Environmental Protection Agency, 2002b). The peer panel itself also evaluated public comments provided to them prior to the meeting, presentations made by various stakeholders at the meeting, and public comments sent to the peer panel after the meeting. The Agency also extended the public comment period so that the peer panel could have additional time to consider public comment issues. Based on a project evaluation conducted by Eastern Research Group (ERG, the contractor to EPA on the subject peer review), 12 out of 13 panelists thought that they had adequate or ample time to address the document. Regarding the time given for considering the public comments, one reviewer felt that it was insufficient. Four thought it was limiting. Eight thought it was adequate or ample. The comments submitted by the public and stakeholders in writing have been compiled and are also being made available on an accompanying CD as part of this third and final review process by the National Academy of Sciences (NAS) (U.S. Environmental Protection Agency, 2003a).

This document addresses all recommendations or comments received in the 2002 external peer review process and is based on an update with all data made available to the Agency as of

May 2003. This document describes the evaluations and additional analyses made by Agency scientists as they addressed these peer recommendations and public comments. This 2003 response document provides recommendations for final revision to the 2002 ERD and again proposes its revised reference dose (RfD) to be a harmonized health risk estimate. The EPA defines an RfD as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime." In the case of perchlorate, the Agency has proposed that its harmonized RfD is protective of both noncancer and cancer effects because both are based on the same mode of action.

It should be emphasized that only scientific considerations as dictated by the available health effects data are germane to development of a health risk benchmark such as the RfD that is intended to be protective of public health. It must also be emphasized that the RfD is not a presumptive drinking water standard nor is it a presumptive goal for ground-water or surface-water remediation.

Derivation of the health risk estimate known as the RfD is only one step toward developing a National Primary Drinking Water Regulation (NPDWR) under the Agency's authority provided by the Safe Drinking Water Act (SDWA). The EPA's Office of Drinking Water (OW) has a number of other areas to consider and evaluate (as described in Section 1.4 of this response document) before it will decide whether a drinking water standard is warranted for perchlorate. The OW must apply risk management factors in developing, proposing, and promulgating such a standard.

The same rigor used to evaluate the science and arrive at its proposed RfD will continue to be applied by the Agency as it evaluates the data on exposures, efficacy of treatment technology, and reliability of analytical methods in order to evaluate risk management options for addressing perchlorate contamination. Should the EPA arrive at a determination to regulate perchlorate under authority of the SDWA, these factors, together with costs and benefits, will be thoroughly evaluated to identify various options. This information will be used together with the health risk assessment (i.e., the RfD) to determine whether or not a national drinking water regulation for perchlorate is warranted. These deliberations would once again be vetted in a public process before a regulatory standard would be developed.

1.1 PURPOSE OF REVIEW BY THE NATIONAL ACADEMY OF SCIENCES

Perchlorate has been the focus of several scientific studies and recent risk assessments by several government agencies. These studies have raised concerns among a number of federal agencies regarding how best to characterize the potential risk posed by chemicals that disrupt the function of the thyroid gland. Because there are differences of interpretation regarding the inferences that can be drawn from the available data and with the 2002 ERD released by the Agency, the EPA in conjunction with the Department of Defense, the Department of Energy, and the National Aeronautics and Space Administration requested a review by the NAS (Gilman, 2003).

The EPA recognized that a review of its draft risk assessment by the NAS would be beneficial and informative as the Agency moves toward the finalization of the risk assessment. Another issue specifically posed to the NAS panel is to verify that the principal studies relied upon by the Agency are of the quality, reliability, and relevance that are required to arrive at conclusions about the potential public health implications (including consideration of sensitive subpopulations) of environmental perchlorate contamination. A set of key questions regarding the state of the science or understanding regarding the potential adverse health effects due to disruption of thyroid function were included in the charge that was submitted to the NAS (Gilman, 2003).

The NAS panel is being provided five key references for its review: (1) the 2002 ERD (U.S. Environmental Protection Agency, 2002a), (2) the report from the 2002 expert peer review panel that reviewed that ERD (U.S. Environmental Protection Agency, 2002b) which also includes an Appendix H that is a transcript of stakeholder comments made at the meeting, (3) this response document that discusses the Agency's disposition of comments and recommendations for revisions to the 2002 ERD, (4) a separate CD that has compiled all written public and stakeholder comments received by the Agency during the peer review process (U.S. Environmental Protection Agency, 2003a, and (5) the companion reference CD that contains technical memorandums with details on the new EPA analyses and all reports submitted to the Agency that are not readily available in the public domain (U.S. Environmental Protection Agency, 2003b).

EPA will not disseminate a final revision of its draft risk assessment until the NAS review is completed and the Agency has fully evaluated the recommendations made by the panel. The recommendations in response to the 2002 external expert panel that are represented in this document will be refined or revised in response to the additional comments received from the NAS panel when the Agency finalizes its risk assessment on perchlorate.

1.2 ORGANIZATION OF RESPONSE AND RECOMMENDATIONS FOR REVISION DOCUMENT

Because this is a response document and not the entire revised 2003 ERD, only those sections of chapters that are being revised in direct response to significant comment made by the panel or public will be discussed in any detail. In general, this includes changes to the Executive Summary, Chapter 1, and Chapters 3 through 10 of the 2002 ERD. Comments or recommendations will be summarized and then followed by the EPA response and recommendation, if any, for revision of the 2002 ERD. Recommendations made by the 2002 external peer panel will be noted, but other public comments will not be identified with respect to their originator unless they could not be integrated into those already provided by the expert peer panel. The categories of comments (e.g., citizen stakeholder, defense industry, or public utility) have been provided on the accompanying reference CD (U.S. Environmental Protection Agency, 2003b). Additional revisions that have been recommended by the EPA team that are not in direct response to the peer review panel recommendations or comments will also be provided.

A convention to distinguish comments and recommendations in the text has been adopted in this response document to aid the NAS review. *Comments to the Agency are indicated in single-space italics*. *Significant new text that is recommended for revision of the 2002 ERD is indicated in blue text*.

This document is organized according to the topic areas discussed at the 2002 external peer review held in Sacramento, California, in March 2002. Separate sessions were devoted to these topic areas (A through G) at the peer review, and the resultant report from the panel had a chapter devoted to each (U.S. Environmental Protection Agency, 2002b). The response and recommendation for a revisions document thus also has a separate chapter devoted to each topic area. Public comments were also sorted and placed into these chapters according to topic area.

The charge to the 2002 external peer review panel is provided as Appendix A of this introduction. It should be noted that the charge also included peer review for those contract reports and studies that were not published in journals.

The topic areas discussed at the 2002 external peer review workshop corresponded to the major chapters contained in the 2002 ERD (U.S. Environmental Protection Agency, 2002a). Chapter 2 of the 2002 ERD, Physicochemical Characteristics, was combined with Chapter 3, Toxicokinetics / Toxicodynamics and Mode-of-Action Testing Strategy in the peer review workshop discussion of Topic Area A (Section 2 of the peer panel report) entitled Hazard Characterization and Mode of Action. That topic area is discussed in Chapter 2 of this current response and recommendations document. Topic Area B (Section 3 of the peer panel report), Human Health Effects Data, is discussed in Chapter 3 of this current document and corresponds to evaluation of Chapter 4 with the same name in the 2002 ERD. Comments with respect to the evaluation of the available laboratory animal data were discussed as Topic Area C (Section 4 of the peer panel report). Responses and recommendations for revision regarding the analyses of data from the laboratory animal studies (Topic Area C) are addressed in Chapter 4 of this document and correspond to Chapter 5 on the same subject in the 2002 ERD. Chapters 8 and 9 of the 2002 ERD, Ecological Risk Assessment and Evaluation of Evidence for Indirect Exposures were discussed together under Topic Area D (Section 5 of the peer panel report) at the 2002 peer review and can be found in this document in Chapter 5. Topic Area E (Section 6 of the peer panel report) was devoted to evaluation and discussion on the use of physiologically-based pharmacokinetic (PBPK) modeling to address the mode of action for perchlorate across life stages. The PBPK topic area corresponds to Chapter 6 in both this response document and in the 2002 ERD. The Agency's human health dose-response assessment was discussed and evaluated as Topic Area F (Section 7 of the peer panel report) at the 2002 external peer review. This topic area is addressed in Chapter 7 of the response document and also corresponds to Chapter 7 of the 2002 ERD. Topic Area G (Section 8 of the peer panel report) was devoted to a discussion of the overall risk characterization conclusions and is found in Chapter 8 of this document and in Chapter 10 of the 2002 ERD. Disposition of the summary set of comments presented by the panel during discussion of Topic Area H (Section 9 of the peer panel report; General Comments, Conclusions, and Recommendations) are reflected in responses and

recommendations throughout the chapters of this document. Suggestions for future research made during that session are also recommended in revisions to Chapter 10 of the 2002 ERD

Citations for all references and technical memorandums providing details on the new EPA analyses are provided in Chapter 9 of this document. The EPA memos and all reports submitted to the Agency that are not readily available in the public domain are being provided on the accompanying reference CD (U.S. Environmental Protection Agency, 2003b).

1.3 STATUS OF AGENCY REGULATORY EFFORTS

From the start, the approach by the Agency to addressing the scientific assessment and risk management of the emerging perchlorate situation has been from to pursue a comprehensive characterization through the integration of data and analyses on areas critical to understanding the nature and scope of the environmental contamination. To that end, Chapter 1 of the 2002 ERD described the state of the science in a number of areas critical to developing the perchlorate database for readiness to determine to regulate and gave a historical overview of the evolving health and ecotoxicological risk assessments.

The purpose of this introductory chapter in the final revised assessment will remain to provide background information on the current status of perchlorate (ClO_4^-) contamination in the United States and an historical perspective on how certain issues of concern have evolved to prominence. Changes to the section providing an historical overview of the health and ecotoxicological assessments (Section 1.3 of the 2002 ERD) will not be provided here because these two topics are the subject of the detailed disposition of comments elsewhere in this document under review by the NAS. Thus, the final revision of this section will need to also reflect comments made regarding this NAS review. However, the NAS panel is referred to this section of the 2002 ERD for perspective on the evolution of the various risk assessments efforts to date.

Perchlorate was placed on the Agency's draft chemical contaminant list (CCL) on October 6, 1997, based on broad public input and consultation with the scientific community. Publication of the CCL is required of EPA by the 1996 amendments to the Safe Drinking Water Act (SDWA). The list represents those contaminants that are not currently subject to a National Primary Drinking Water Regulation (NPDWR) and are known or anticipated to occur in public

water systems. The CCL is the source of priority contaminants for research, guidance development, regulatory determinations, and monitoring by the states. As a result of the public comments and the obtainment of additional occurrence information, EPA determined that sufficient information existed to raise concern over perchlorate's potential public health impact and added perchlorate to the final CCL published on March 2, 1998.

Perchlorate was noted to have research needs in various key areas: development of analytical methods to detect the contamination, occurrence surveys and analysis of exposures at different locations with contamination, assessment of adverse human and ecosystem effects, characterization of transport and transformation in the environment, and evaluation of the efficacy of various treatment technology options. The majority of the 2002 ERD and this report are devoted to discussing the risk assessment of the potential human health and ecosystem effects. These additional key topics were not discussed other than as introductory material for the context of the assessment endeavor at the 2002 peer workshop. However, the Agency is continually improving the status of these data in its efforts to arrive at regulatory readiness for perchlorate. The Agency will update this information in its final revised assessment. Consequently, in the following sections a brief description of progress in each area and recommendations for revised text are provided in this response document for review by the NAS panel.

1.3.1 Production Uses and Sources of Perchlorate Contamination

This section provides updates to Section 1.1 of the 2002 ERD. The Agency is not recommending any changes to this section with the exception of one addition and one correction in response to a written comment.

Comment(s): *The Agency received a written comment requesting verification of the sentence that lists other industrial or commercial applications of perchlorate salts on Lines 10 through Line 14 on Page 1-2 (Gullick, 2002). The list of sources was attributed in the 2002 ERD to Siddiqui et al. (1998). This commenter wrote a manuscript recently (Gullick et al., 2001) and could not verify those uses nor could Dr. Siddiqui when the commenter contacted him. The commenter requested deletion of the information if verification of a source not be identified.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency will revise the source of the information to be Schilt (1979).

The Agency is recommending that the following sentences be added to Line 18 on Page 1-2.

The Agency is aware of ongoing research into the possible presence of geological deposits of perchlorate other than the desert salt deposits in Chile's Atacama Desert. Research results from the Phase 1 study of potential perchlorate sources in the High Plains of Texas (Jackson et al., 2003) indicate that "No clear source is apparent from the collected data". Additional research proposed for Phase 2 of this study has not been completed. Other research that could provide insight to perchlorate occurrence in the High Plains Region of Texas have neither been published nor made available to EPA. In every other region of the United States, most notably the State of California where over 6000 public water supply wells have been tested and reported, it is evident that anthropogenic sources can be identified for the vast majority of instances in which perchlorate threatens public water supplies (Mayer, 2003). The U.S. Geological Survey recently published a preliminary report on the detection of perchlorate in a variety of natural materials (Orris et al., 2003). A better understanding of perchlorate occurrence in the environment due to natural and anthropogenic sources, as well as an understanding of perchlorate's transport and transformation in the environment, will require methodical research and more extensive sampling.

1.3.2 Emerging Occurrence Surveys and Exposure Evaluation

Section 1.2 of the 2002 ERD was entitled, "Evolution of Analytical Detection Methods and Emerging Occurrence Data." The Agency is recommending that these two areas be separated in the final revised document. The title of the new section 1.2 in the revised final assessment will be "Section 1.2 Emerging Occurrence Surveys and Exposure Evaluation".

Because new occurrence data are expected to be continually updated, the maps and tables depicting known contamination or areas with potential release due to manufacturers or users of pechlorate are not provided in this document for the NAS review. Instead, the NAS panel and public are referred to the Agency's Federal Facilities and Restoration Office (FFRO) URL at: http://www.epa.gov/swerffrr/documents/perchlorate.htm *for the most current information. The*

recommended updated text in the new Section 1.2 (Emerging Occurrence Surveys and Exposure Evaluation) is as follows below.

The Region 9 Office of the U.S. Environmental Protection Agency (EPA) first became aware of the potential contamination issues with perchlorate in 1985 when samples measured with a colorimetric method reported contamination in 14 wells ranging from 0.11 to 2.6 ppm (Takata, 1985). The Region 9 office requested assistance from the Centers for Disease Control and Prevention (CDC) to evaluate the potential health effects of these levels of perchlorate (Takata, 1985). In response the CDC recommended validation of the colorimetric measures but could not address the potential for toxicity of the chemical because of toxicity data insufficiencies (Margolis, 1986). The CDC also recommended additional testing to determine potential target tissues and the effects from long-term, low-level exposures. The absence of a valid analytical method to measure low concentrations of perchlorate and the lack of data with which to characterize the risk of toxicity led Region 9 of EPA to focus on chemicals other than perchlorate at these sites. By the early 1990s, however, perchlorate at detectable levels (>1 mg/L) was found in monitoring wells at a California Superfund site, and EPA Region 9 increased its effort to establish a human-health-based reference dose (RfD) in order to help gauge the risk of the contamination that was beginning to be characterized.

In January 1997, the California Department of Health Services' (CA DHS) Division of Drinking Water and Environmental Management requested the Sanitation and Radiation Laboratory Branch (SRLB) test for perchlorate in drinking water wells potentially affected by groundwater migrating from the Aerojet facility near Sacramento. Based on its provisional action level, Region 9 of EPA indicated that a reporting limit of at least 4 ppb would be necessary. Within several months following the March 1997 development of a low-level (4 ppb) ion chromatographic (IC) detection method by CA DHS (see Section 1.3), perchlorate was discovered at various manufacturing sites and in well water and drinking water supplies in California, Nevada, and Utah.

In March 1999, EPA included perchlorate in the Unregulated Contaminant Monitoring Rule (UCMR; Federal Register, 1999). Under the UCMR, all large public water systems and a representative sample of small public water systems were required to monitor for perchlorate beginning in January 2001. The EPA Method 314.0 for the analysis of perchlorate in drinking water using ion chromatographic (IC) methods was published in early 2000 as a direct final rule

(Federal Register, 2000). The EPA Method 314.0 was also approved as a monitoring method for the UCMR (Federal Register, 2000). However, this effort does not extend to investigating potential sources in groundwater and surface water that have not migrated into public water supplies. Additional information about the UCMR is available at the URL: http://www.epa.gov/safewater/ucmr.html.

The CA DHS adopted 18 ppb as its provisional action level in 1997 after perchlorate was discovered in a number of California water supplies. In January, 2002, CA DHS lowered the action level for perchlorate in drinking water to 4 ppb. In 1999 the CA DHS also added perchlorate to the list of unregulated chemicals for which monitoring is required (Title 22, California Code of Regulations §64450). By April 2003, over 5,500 sources of public water supply in California had been sampled by water supply agencies responding to CA DHS requirements. Most of these sources represent water supply wells. Of the sources sampled, 315 (over 5 percent) had perchlorate concentrations greater than 4 ppb in at least two samples. The source of perchlorate contamination in most of these wells is groundwater plumes that have spread as far as nine miles from the site of original release.

At this time, there has not been a systematic national survey of perchlorate occurrence other than EPA's UCMR survey which will be completed in 2004. Several states and EPA regions are taking significant steps to test water supplies for perchlorate, notably the states of Arizona, Utah, New Mexico, and Texas; EPA Regions 6 and 7; and Suffolk County, New York. Figures depicting those states with confirmed perchlorate manufacturers or users and another figure indicating those states in which facilities have, in response to reported releases, directly measured perchlorate in groundwater or surface water are provided on the web site of the Federal Facilities and Reuse Office (FFRO) at the URL: http://www.epa.gov/swerffrr/documents/perchlorate.htm. A table that describes these locations is also provided. Unconfirmed reports have not been included. The table includes a limited number of all releases reported in the December 2002 update of the UCMR survey (Mayer, 2003).

Information on other potential sites across the country is being gathered from the Department of Defense (DoD) and National Aeronautics and Space Administration (NASA) searches, from EPA information requests made to perchlorate manufacturers, and from Congressional inquiries. The EPA has been attempting to obtain these data from the DOD since 1998. The most recent results of these are available from the EPA at the FFRO URL

(http://www.epa.gov/swerffrr/documents/perchlorate.htm). The EPA has also notified state, tribal, and local governments when it obtained evidence of perchlorate manufacture and use in these governmental jurisdictions. The EPA will build on these survey data and other information to discover potential sources and evaluate threats to water resources.

Region 9 officials have collected information concerning detected perchlorate releases throughout the United States and, as of May 2003, have confirmed releases at 95 locations in 25 different states. In California, most areas where perchlorate has been detected are associated with facilities that have manufactured, tested, or disposed of solid rocket fuels and propellants for DoD or NASA. Two facilities that manufactured ammonium perchlorate in Nevada were found to have released perchlorate to groundwater resulting in low levels (4 to 24 ppb) in Lake Mead and the Colorado River. This water is used for drinking, irrigation, and recreation for millions of people in Nevada, California, Arizona, and by Native American tribes.

The concentrations reported in wells and surface water vary widely. At one facility near Henderson, NV, perchlorate in groundwater monitoring wells was measured as high as 0.37% (3.7 million ppb). The highest level of perchlorate reported in any public water supply well was 800 ppb in an inactive well in California. Few active public water supply wells have perchlorate greater than 100 ppb, and very few have been reported at this level outside of California.

Perchlorate was found in a number of water supply wells on Long Island, NY, including several downgradient from a fireworks facility. It has been speculated that the wide distribution pattern of the New York contamination could be a result of low levels of perchlorate contained in fertilizer imported from Chile (TRC Environmental Corporation, 1998; Urbansky, 2000; Suffolk County Department of Health Services, 2001a,b). Agricultural chemicals also have been implicated as a potential source of perchlorate contamination in Nebraska at a shallow well near a speciality fertilizer facility (Williams, 2000) and at several sites in New Mexico and Texas. After state and federal officials in Region 7 added perchlorate analyses in their program testing hundreds of rural wells for fertilizers and agricultural chemicals, their results showed that fertilizer application to farmlands is an unlikely source of perchlorate in Midwestern states. This is the same conclusion reached by a large study of agricultural applications performed by the Agency in partnership with The Fertilizer Institute (U.S. Environmental Protection Agency, 2001a,b).

In addition to discoveries at facilities involved with rocket propellants, explosives, flares, and fireworks, a number of perchlorate detections have been made at current or former military facilities where propellants and explosives were disposed of by detonation and burning. Cooperation from Department of Defense (DoD) and Department of Energy (DoE) officials will continue to be important for examining these types of potential sources.

In the past three years, the increasing interest in investigating the environment has resulted in increasing detections. It is likely that regional positive efforts at detection may largely explain the distribution of known areas of release to the environment when compared to the potential distribution. As the efforts for detection become more uniform nationwide, the occurrence of perchlorate in the environment may more closely resemble the pattern of perchlorate usage.

1.3.3 Analytical Methods Development

The final ERD will be revised extensively to update the current status of analytical methods. A new section entitled, "Section 1.3 Analytical Methods Development" will be separated and updated from the previous Section 1.2 (Evolution of Analytical Detection Methods and Emerging Occurrence Data) in the 2002 ERD. The revised text is provided below to provide the NAS review panel with a sense of the progress in this arena.

Prior to the 1990s, the analytical chemistry methods for the determination of perchlorate anion were based on gravimetric, derivatization/spectrophotometric, or electrochemical methods (Urbansky, 2000; U.S. Army Corps of Engineers, 2001). In the1990s, separation methods such as ion chromatography and capillary electrophoresis began to dominate. Ion chromatography appears to be the most popular separation technique and is amenable to various types of detectors (Hedrick, 2003).

The first ion chromatographic (IC) method using conductivity detection was developed by the California Department of Health Services (CA DHS) in 1997 (CA DHS, 1997). They used an anion exchange column with a mobile phase of NaOH/p-cyanophenol and conductivity suppression. The practical quantifiable limit, also known as the minimum reporting limit (MRL), was determined in a multi-laboratory study in 1998 to be 6 ppb (Eldridge et al., 1999; Tsui et al., 2000; Urbansky, 2000). Better columns for separating large polarizable anions like perchlorate were then developed and in 1999 the EPA published Method 314.0 which used this

next generation of anion exchange resin, electrolytic suppression, and conductivity detection (U.S. Environmental Protection Agency,1999; Jackson et al., 2000).

The minimum detection limit (MDL) of Method 314.0 is 0.53 ppb with a widely achievable minimum reporting limit (MRL) of 4 ppb. Through several simple modifications, however, there are many laboratories achieving lower MDLs and MRLs using Method 314.0. (Yongjan et al., 2002). The modifications being used are (1) large volume injection (2 mL or more), (2) sample preconcentration onto short chromatography columns prior to separation on the analytical column, (3) sample concentration by evaporation and/or, (4) dedication of IC instrumentation to low-level perchlorate analysis.

Generally, the improvements have resulted in MRLs of approximately 1 ppb. It is important to keep in mind that even though these modifications have worked well for relatively clean and well characterized water samples, there are no data to confirm that these modifications work well for a wide range of sample types. Therefore, these modifications should only be made in specialized circumstances and are not appropriate for general monitoring. These considerations for reliability of the analytical methods and limits of detection are especially important when evaluating and interpreting the data on the occurrence of perchlorate in biological samples (plant and animal tissues) for characterizing the potential for indirect exposures.

For samples containing high concentrations of anions (Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}) that elute just prior to perchlorate, further improvements in MDLs and MRLs have been possible using sample clean-up procedures. Removal of highly conducting common anions allows perchlorate to elute on a baseline of low conductivity, further improving detection limits by conductivity. Sample clean-up is typically accomplished with syringe cartridges that contain a resin with ion exchange sites to precipitate or complex the interfering anions. For example, to remove sulfate, a barium-containing resin would be used. Silver ion is used to precipitate Cl^-, and H^+ exchange resin is used to remove CO_3^{2-}. At this time, the main disadvantage to developing methods dependent on these cartridges to achieve desirable MRLs is cost. Depending on the number of cartridges required, the cost per analysis could increase from $8-$24.

Despite the noteworthy advances that have been made in column design for ion chromatography and the advances in sample pre-treatment, there is still the issue of specificity when using conductivity detection. Because perchlorate is identified solely by retention time,

there is the potential for misidentification of contaminant peaks in the same retention time window as perchlorate. In most cases, spiking the suspect sample with perchlorate is sufficient to establish the precise retention time of perchlorate in the matrix and reveals whether or not a suspect peak is indeed perchlorate.

The ideal detector, however, would utilize unique and specific information about the analyte (Hedrick, 2003). The response in mass spectrometric detection is based on very specific information about the analyte, i.e., the mass-to-charge ratio of the ion of interest. In the case of perchlorate ion, the primary mass of interest is 99 based on the 75.77% relative abundance of the chlorine-35 isotope. Mass 101 is a secondary mass of interest based on the 24.23% abundance of chlorine-37. Coupled with chromatographic separation, mass spectrometry (MS) is by far the most promising analytical tool available today for low-level quantitation of perchlorate in drinking water (Handy et al., 2000; Koester et al., 2000, Minteer and Winkler, 2002; Clewell et al., 2000; Urbansky et al., 2000). Typical reagent water method detection limits are 0.03 - 0.07 ppb with minimum reporting limits from 0.1 - 0.25 ppb.

Currently in development at the EPA is a method that uses IC separation of perchlorate ion, as is done in Method 314.0, followed by electrolytic conductivity suppression and electrospray ionization prior to mass spectrometric detection of perchlorate at masses 99 and 101 (Schnute, 2002; Hedrick, 2003). Another approach being pursued by several commercial labs is IC separation of perchlorate followed by electrospray ionization and MS-MS detection. In this approach, perchlorate ion is monitored in its transition from perchlorate, mass 99, to chlorate, mass 83, after the loss of an oxygen atom in a collision cell (General Engineering Labs, 2002). Both of these MS methods are sensitive and specific for perchlorate at sub-ppb levels in drinking water matrices. Additionally, the same sample clean-up and concentration procedures used to improve detection limits of EPA Method 314.0 and to extend its application to more complex matrices can also be used to improve the MS methods.

The Agency continues to work on revising Method 314.0 and on these applications to other matrices. In the event that perchlorate is regulated in drinking water or that a second national occurrence survey is conducted under the Unregulated Contaminant Monitoring Rule (UCMR), this current research will result in inherently more sensitive and specific MS methods that can be readily implemented by most analytical laboratories. The market demand for this capability may determine the commercial availability and expense of this method. Regulatory pressure to

ensure protection or water supplies and to maintain treatment process control is also a factor driving the development of lower reporting limits for perchlorate. Thorough method validation and quality assurance information will be compiled to establish a standard analytical method in the sub-ppb range for various media.

1.3.4 Treatment Technologies

The Agency is recommending that a new section entitled "Section 1.4 Treatment Technologies" should be included in the final revised assessment due to the progress and prominence of this area with respect to regulatory readiness. The recommended text is as follows.

Perchlorate anion is very unreactive toward most reducing agents under typical environmental conditions (e.g., when cold and dilute) and has low reactivity as an oxidant due to kinetic barriers. These same properties made developing treatment technologies difficult, especially at low concentration levels. However, many bench-scale and pilot-scale studies have been completed in the last six years since 1997 and more than fourteen full-scale systems constructed. Perchlorate treatment systems are now operating in the United States, mostly in California.

No one technology or process will likely provide an effective solution for every occurrence of perchlorate contamination in water supplies because there are a large number of independent variables. These include, but are not limited to, perchlorate contamination levels, aquifer types, hydrogeology, and the scale and attributes (e.g., soil or water type) of the site to be remediated; the presence of co-contaminants; Federal, State, and local regulatory constraints; public acceptance; inherent technology or process limitations and side effects; and capital and operating costs. Technology evaluation criteria may also be a function of the intended use of the treated water (e.g., drinking water versus agricultural application).

Treatment technologies for addressing perchlorate-contaminated water may be grouped into three general categories: (1) biological and biochemical reactor treatment systems, (2) conventional chemical reactor treatment systems, and (3) separation and concentration technology. The further development of technology in each of these areas will be essential to provide technically sound, cost-effective treatment options for managing a wide range of perchlorate concentration and water supply situations. Each technology or process either has

technical limitations or has not been demonstrated for treating low-concentration perchlorate-contaminated water. Drinking water authorities may desire, or be compelled, to reduce the perchlorate level in affected supplies below a legislated value. As such, the need for treatment technology for very low concentration levels exists regardless of the findings of the toxicological studies.

The limitations of existing technologies warrant research in development and demonstration of perchlorate treatment process systems. Based on the results of the toxicological studies being conducted, the technology development strategy will be adjusted, if necessary, to produce short-term treatment options unconstrained by capital and operating cost factors, followed by longer term and more cost-effective solutions. It will also be important to establish criteria and ratings for treatment technology effectiveness, e.g., the ratings available from the National Sanitation Equipment Foundation. The following areas of research are recommended for future consideration:

- Biological and biochemical reactor treatment systems,
- Conventional chemical reactor treatment systems,
- Separation and concentration technology, and
- Development of home treatment devices.

Several types of treatment systems designed to reduce perchlorate concentrations are already operative around the United States and are reducing perchlorate to below the 4 ppb quantitation level. Biological treatment and ion (anion) exchange systems are among the technologies that are being used while additional treatment technologies under development.

Biological treatment systems make use of microbes to convert perchlorate to chloride and oxygen. Biological treatment systems used for perchlorate removal require oxygen poor or anoxic conditions and usually the addition of alcohol or some other electron donor to sustain the microbes. Biological systems may be “ex-situ,” passing the contaminated water through a above-ground tank containing a media such as sand or carbon on which the microbes grow, or “in-situ” systems in which the reactions occur in the ground. Biological perchlorate-removal systems are similar to nitrate-removal systems (i.e., denitrification), and systems designed for perchlorate-removal will generally remove perchlorate and nitrate. The advantages of biological technologies for perchlorate removal are that they are effective over a range of conditions, commercially-available, a destructive technology (i.e., no waste brine or waste resin), and likely

to be less expensive than ion exchange. Their main disadvantage applies to systems where the treated water will be used as a source of drinking water. Pilot-scale tests have shown that biological treatment systems can meet all drinking water standards if combined with filtration and other post-bioreactor treatment, but biological treatment methods are not widely used in the United States for drinking water treatment, which may complicate or slow regulatory and community acceptance if the treated water is to be used as a source of drinking water. Full-scale biological treatment systems have been operating in California since at least 1998, reducing perchlorate concentrations from about 2,500 µg/L to less than 4 µg/L.

Ion exchange systems typically remove perchlorate by passing the perchlorate-contaminated water through a tank containing a synthetic resin or other media which removes the perchlorate ion (ClO_4^-) from the water and replaces it with the relatively innocuous chloride ion (Cl^-). Ion exchange systems may also remove other anions in addition to perchlorate, such as nitrate (NO_3^-), sulfate (SO_4^{2-}), and bicarbonate (HCO_3^-). The advantages of using ion exchange technologies for perchlorate removal include: effectiveness over a range of conditions, commercially-available from several vendors, and accepted for use in the production of drinking water. Ion exchange's main disadvantage is that it is a "separation technology" which does not destroy the contaminant. Ion exchange systems either include the capability to regenerate the resin onsite (producing a waste brine), or make use of "one-pass" or disposable resins which are replaced when saturated (producing waste resin). Including onsite regeneration capability increases capital costs, reduces operation and maintenance costs, and results in a waste brine requiring further treatment or disposal. Making use of a disposable resin lowers capital costs, increases operating costs, and simplifies operation and maintenance. Full-scale ion exchange systems have been operating since at least 2000, reliably reducing perchlorate concentrations at a site in California from about 100 µg/L to less than 4 µg/L. The Agency also calls attention to a new resin regeneration technology with complete perchlorate destruction that has been developed by Oak Ridge National Laboratory (ORNL) through funding by the DOD. This new technology allows for the resin to be reused for an extended lifetime with no production of secondary waste (brine). A test system is operative at Edwards Air Force Base.

Many other perchlorate treatment studies have been completed during the last several years. Research is underway on several other perchlorate removal technologies, including reverse osmosis, nanofiltration, phytoremediation, granular activated carbon, chemical reduction,

and electrochemical reduction. The best source of additional perchlorate treatment technology information is the Agency's Technology Information Office web site at the URL: http://www.clu-in.org/perchlorate. This URL has many links to the rapidly expanding information on technology available for the treatment of perchlorate-contaminated water. A May 2001 summary report prepared by the Ground-Water Remediation Technologies Analysis Center (GWARC) available at that site presents results of 65 perchlorate treatment studies. Results of federally-funded perchlorate treatment research managed by the American Water Works Research Foundation (AWWARF) are also becoming available.

1.4 INTERIM GUIDANCE AND REGULATORY AGENDA

As stated in the introduction of this response document, the EPA will not disseminate a final revision of its draft risk assessment until the NAS review is completed and the Agency has fully evaluated the recommendations made by the panel. The recommendations in response to the 2002 external expert panel that are represented in this document will be refined or revised in response to the additional comments received from the NAS panel when the Agency finalizes its risk assessment on perchlorate. When finalized, that assessment will represent ORD's view of the science and potential health risk posed by environmental contamination with perchlorate. It should be emphasized that the final assessment will not be a presumptive drinking water standard nor a presumptive goal for ground-water or surface-water remediation.

Because this response document is expected to have a broad audience outside the NAS panel, the Agency felt it was necessary to include this section to clarify the current status of its interim guidance and to remind the readership of its regulatory agenda. *This section briefly describes pending regulatory activities that this revised evaluation and risk characterization will likely influence and is intended to serve as an update of Section 1.4 (Risk Characterization and Regulatory Agenda) of the 2002 ERD.*

1.4.1 U.S. Environmental Protection Agency Regulatory Plans

The Safe Drinking Water Act (SDWA), enacted by Congress in 1974 and last amended in 1996 (U.S. Code, 1996), provides the basis for safeguarding public drinking water systems from contaminants that pose a threat to public health. Within EPA, the Office of Ground Water and

Drinking Water (OGWDW) develops National Primary Drinking Water Regulations (NPDWR) to control the levels of contaminants that may occur in public drinking water systems. The 1996 SDWA requires EPA to develop a list of priority contaminants every 5 years (the Contaminant Candidate List or CCL), and determine whether or not to regulate at least five contaminants from the CCL every five years. Perchlorate was listed on the CCL in 1998. Contaminants placed on the CCL because they have been identified to occur or have the potential to occur in drinking water and may be a health concern. Once a contaminant is placed on the CCL, EPA collects data and conducts research in a number of areas to determine whether or not a regulation with a NPDWR is necessary. EPA collects information to assess the numbers of people exposed, to evaluate treatment efficacy and cost, to develop analytical methods, and to assess health risk. This information may be useful to develop regulations or guidance under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), or the Resource Conservation and Recovery Act (RCRA).

Because perchlorate is one of the contaminants on the CCL with high concern, EPA is committed to addressing its data gaps quickly and to have the best available data for evaluating its qualifications for issuing an MCL in public drinking water. To promulgate an MCL, the Administrator must make a determination to regulate under the authority of the SDWA that a contaminant has a substantial likelihood of occurring in public water systems with a frequency and at levels of concern to the public health so that its regulation presents a meaningful opportunity for health risk reduction for persons served by public water systems. The EPA may make a determination to regulate perchlorate prior to the next round of CCL regulatory determinations due in 2006. Should the Agency determine that a regulation is necessary, it would be developed in accordance with all legal and regulatory requirements including scientific review of related analyses and opportunity for public comment.

Derivation of the health risk estimate known as the RfD is only one step toward developing a NPDWR under the Agency's authority of the SDWA. If a decision is made to develop a regulation, the EPA's Office of Water (OW) must establish a national public health goal referred to as a Maximum Contaminant Level Goal (MCLG) and the enforceable standard known as the MCL. EPA uses the RfD in developing the MCLG. EPA is required by the statute to specify an enforceable standard as close to the MCLG as feasible based upon an evaluation of best available technology, treatment effectiveness, existing methods for measuring the contaminant

levels, and costs. Section 1412(b)(2) of the SDWA also requires the Agency to complete and publish a national analysis of quantifiable and non-quantifiable health risk reduction benefits associated with a particular treatment level. In addition, the Agency must complete a parallel analysis of quantifiable and non-quantifiable costs that are likely to occur soley as a result of compliance with the MCL.

The EPA issued a memorandum at the beginning of this year (Horinko, 2003) to update information concerning the status of the interim assessment guidance originally transmitted on June 18, 1999 (Noonan, 1999). The new guidance was issued to provide an update as to the status of EPA's scientific review of perchlorate as well as EPA's regulatory process for developing a new standard for drinking water and/or ground-water or surface-water remediation (Horinko, 2003). The interim assessment guidance is a memorandum providing practical information and advice to EPA risk assessors and risk managers at both headquarters and regional offices regarding evaluation of risks for perchlorate, a first step to develop information to assist in making risk management decisions for a substance for which no regulatory standards now exist. This guidance was provided because EPA regional offices and states have sought advice as the number of sites identified as potentially contaminated with perchlorate has increased.

As stated in the 2003 Memorandum, "The 1999 Interim Guidance remains the applicable guidance until supplanted by new guidance based on a finalized risk assessment" (Horinko, 2003). In the absence of a finalized oral health risk benchmark for perchlorate, but in light of ongoing assessment activities by EPA, states and other interested parties, the Agency re-affirmed its standing guidance. Because pregnant women and the fetus *in utero* are the most sensitive populations of concern for perchlorate toxicity in these recent analyses, the standard default body weight and water consumption values of 70 kg for body weight and 2 liters per day of water would be applied in converting a new RfD to provisional clean-up levels in ppb.

This guidance provides direction to EPA Regions, states and tribes involved in environmental cleanups. It also provides guidance to the public and the regulated community on how the Agency intends to assess possible perchlorate contamination. This guidance is not a regulation itself and does not substitute for CERCLA, RCRA, or other EPA regulation. The interim guidance does not impose legally-binding requirements, standards or procedures on EPA, states, tribes, or any other entity, and may not apply to a particular situation based upon the

circumstances. They are intended to inform, but not to prescribe, decisions that must be made in the interim until such time as regulatory standards may be available for perchlorate. This guidance should be considered one of many sources of information that may influence site-specific decisions. EPA and state or tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance.

Additional information on the interim guidance is available at the FFRO URL: http://www.epa.gov/swerffrr/documents/perchlorate.htm.

1.4.2 State Regulatory Plans

The CA DHS and the California EPA Office of Environmental Health Hazard Assessment (CA EPA OEHHA) reviewed the available health effects data and established its action level at 18 ppb based on the provisional RfD values from the EPA Superfund Technical Support Center (Dollarhide, 1992;1995). In January 2002 when new health effects data in the EPA 2002 ERD became available, the CA DHS lowered the action level to 4 ppb. The CA DHS advises water utilities to remove drinking water supplies from service if they exceed the 4-ppb action level. If the contaminated source is not removed from service because of system demands and if drinking water provided by the utility exceeds the action level, the CA DHS advises the utility to notify its customers. On August 1, 1997, the CA DHS informed drinking water utilities of its intention to develop a regulation requiring monitoring of perchlorate as an unregulated chemical.

Legislative action in the 2002 California State Senate to establish a state drinking water standard for perchlorate by January 2003 (California Senate Bill 1822 [HSC 116293]) was signed by the governor after passage by both houses. This legislation required the CA EPA OEHHA to establish a Public Health Goal (PHG) for perchlorate in 2003 and for the CA DHS to issue a maximum contaminant level (MCL) for perchlorate by January 2004. In December 2002, OEHHA released a draft 2- to 6-ppb PHG for public comment that was based on the inhibitory effect of perchlorate on the uptake of iodide by the thyroid gland. Industry sued to require a second peer review and was successful (Howd, 2003). Thus, this draft PHG draft is currently undergoing additional external peer review as mandated by the court decision and though delayed may be finalized later in 2003. The finalization date for the MCL is unknown.

New York, Arizona, and Texas also initially adopted the level of 18 ppb as their advisory level for water supply systems. Texas and Arizona health departments revised their perchlorate

advisory level based on research presented in EPA's December 1998 External Review Draft Toxicity Assessment. In July 1999, Texas arrived at a value of 22 ppb in drinking water by calculating the exposure of a 15 kg child drinking 0.64 liter per day and using the reference dose proposed in the 1998 EPA ERD document. Texas revised this value to 4 ppb in October 2001 based in part on the interim ORD guidance (Noonan, 1999). Arizona derived a 14 ppb level in March 2000 based on a 15 kg child drinking 1 liter per day and using the proposed RfD in the 1998 EPA ERD document. New York State adopted a 5 ppb/ 18 ppb two-tier action level for perchlorate in drinking water with 5 ppb as a planning level.

Massachusetts and Maryland established a 1-ppb advisory level for perchlorate in 2002. Massachusetts' level is a recommendation to a local water district for children and other at-risk populations. Maryland's advice also focused on children and at-risk populations. New Mexico also adopted a 1-ppb interim groundwater screening level for perchlorate in 2002.

The Nevada Division of Environmental Protection (NDEP) has authority under Nevada Water Pollution Control Regulations to address pollutants in soil or groundwater. The state's Corrective Action Regulations direct NDEP to establish action levels for hazardous substances, pollutants, or contaminants using drinking water standards such as a MCL, health advisories, or background or protective levels (determined by IRIS or the equivalent). In August 1997, Nevada determined that the action level of 18 ppb, as established by EPA, would be the recommended action level for cleanup, pending a more current risk assessment.

1.5 SUMMARY

The summary in Chapter 1 of the 2002 ERD will be revised to reflect the final disposition of the Agency's risk assessment efforts. The current summary for the purposes of this response document to the NAS panel is as follows.

Perchlorate contamination is a concern for several reasons. First, there are uncertainties in the toxicological database that is used to address the potential of perchlorate to produce human health effects at ambient contamination levels. Additionally, the actual extent of perchlorate occurrence in ground and surface waters and other media (soils or plant and animal tissues) is unknown — a problem compounded by limits of the analytical detection method applied to other matrices — so that the relative contribution of direct and indirect exposures to human body

burden has not been established. The nature and extent of ecological effects and details about transport and transformation phenomenon in various environmental media have only begun to be studied rigorously. Finally, the efficacy of different treatment technologies for various water uses, including drinking and irrigation, needs to be better established with full-scale systems. EPA continues to comprehensively characterize the risks to human and ecological health from perchlorate contamination through the integrative approach presented in Figure 1-1. *NOTE: Figure 1-1 in this response document provides recommended revisions to Figure 1-5 in the 2002 ERD. Figure 1-5 has been revised to explicitly identify direct versus indirect exposure pathways and to better identify operations at the source that result in contamination.*

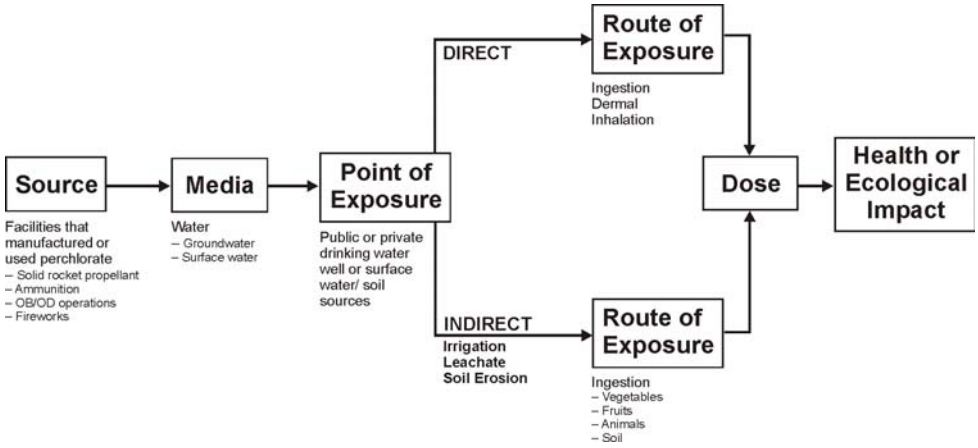

Figure 1-1. Recommended revisions of Figure 1-5. Considerations for comprehensive characterization of perchlorate contamination.

Modified from Underwood (1998) and U.S. Environmental Protection Agency (2002a).

Thus, the Agency is addressing a number of key pieces of information; and scientific advances are essential to adequately characterize the risks of perchlorate contamination and to develop scientifically based management strategies that effectively mitigate the potential risks posed by perchlorate contamination. Accurate characterization of exposures requires reliable analytical detection methods. The exposure estimates cannot be gauged with respect to their risk

unless a robust health risk estimate is available. Treatment technologies should be targeted to levels of concern and tailored to the intended water use. Technology transfer is necessary so that all affected parties and concerned citizens are appraised of accurate and reliable information that is up-to-date with the evolving state-of-the-science. The purpose of the revised risk characterizations presented in this document is to serve in this integrative approach by providing more robust risk estimates than those that currently exist provisionally in order to better gauge the potential human health and ecological impacts. As with any risk assessment, incorporation of new data is an iterative process.

It should be noted that the Agency's assessment effort was accomplished in a relatively expedited time frame through the partnership and cooperation of a number of governmental entities. In 1998, an Interagency Perchlorate Steering Committee (IPSC) was established by EPA under a Congressional directive. Membership of the IPSC included personnel from the EPA, the National Institute for Environmental Health Sciences (NIEHS), the Department of Defense (DoD), and other federal agencies as well as affected state, tribal, and local governments. The purpose of the IPSC was to facilitate and coordinate accurate accounts of related technological issues (e.g., occurrence, health / toxicological effects, treatability and waste stream handling, analytical detection, ecological impacts, and environmental transport and transformation) and to create information-transfer links for interagency and intergovernmental activities regarding these areas of concern. A collaborative and innovative approach that devoted subcommittee work to focus on these key areas allowed the IPSC to serve as an information clearinghouse for summaries of the state of the science in the areas reflected in Section 1.4 of this document as well as the ensuing chapters on health risk, ecological risk, and indirect exposures. The IPSC sponsored a number of stakeholder workshops that were very useful to problem formulation of the perchlorate risk characterization endeavors. *NOTE: Because the IPSC is no longer active and many of the membership are no longer at the same institutions or in the same roles, the Agency is recommending that Figure 1-6 of the 2002 ERD be deleted from the final document.*

The IPSC collaborated in 1998 with EPA on a draft report to a Congressional committee that assessed the state-of-the-science concerning the health effects of perchlorate on humans and the environment and the extent of perchlorate contamination. The draft report also contained recommendations for future research to address emerging issues (U.S. Environmental Protection

Agency, 1998e). Updates to these efforts were largely reflected in the 2002 ERD and provided as updates on Agency web sites. Although the IPSC is no longer active, it is hoped that the newly formed Interagency Working Group (IWG) on perchlorate will continue the degree of collaboration, resource leveraging, and information sharing that was the hallmark of the IPSC. The IWG consists of a group of interested federal agencies convened under the auspices of the White House Office of Science and Technology (OSTP). It is expected that the draft Congressional report will be revised once the Agency's health and ecological risk characterization is finalized after consideration of recommendations from this NAS review.

Updates on EPA activities pertaining to perchlorate can be found via links at the following web sites maintained by the Agency: on the National Center for Environmental Assessment (NCEA) home page: http://www.epa.gov/ncea; on the EPA Office of Water (OW) web site: http://www.epa.gov./ogwdw/ccl/perchlor/perchlo.html; on the Federal Facilities Restoration and Reuse Office (FFRO) web site: http://www.epa.gov/swerffrr/documents/perchlorate.htm.; and on the Technology Information Office URL web site: http://www.epa.gov.

2. HAZARD CHARACTERIZATION AND MODE-OF-ACTION

This chapter addresses recommendations by the panel or comments received regarding the Agency's hazard identification of potential adverse health effects of perchlorate exposure and the proposed conceptual model of its mode of action. Additional discussion of how the mode of action underpins the integration of diverse types of data available leading to a point of departure can be found in Chapter 7 of this document.

These responses and recommendations are suggested primarily for revision of Chapter 3 (Toxicokinetics/Toxicodynamics and Mode-of-Action Testing Strategy) in the 2002 ERD. Some recommendations will also be reflected in refinements to sections in Chapter 5 (Laboratory Animal Studies) and Chapter 7 (Dose-Response Assessments for Human Health).

Figure 2-1 shows a revised schematic of the EPA's proposed conceptual model used to harmonize the assessment of noncancer and cancer outcomes based on the mode of action. The mode of action is proposed as a continuum of pathogenesis linking key events from exposure to adverse biological effects. The mode of action for perchlorate is proposed to be inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS), causing perturbation of thyroid hormone synthesis and regulation, and leading to adverse neurodevelopmental and neoplastic sequelae.

This revised figure will replace Figures 3-12, 6-1 and 7-1 in revision of the 2002 ERD. The revision to the schematic is based on discussions by the panel regarding the nature of perchlorate interaction with the sodium (Na^+)- iodide (I^-) symporter (NIS) in the thyroid gland and recognition that the presence of NIS in other tissues such as the gastrointestinal (GI) tract, placenta, and mammary gland has important implications for different perchlorate dosimetry during various life stages. The revisions were made in response to comment(s) that will be discussed in greater detail in the following sections.

Based on the mode of action for the toxicity of perchlorate, the Agency has proposed a single oral risk health benchmark based on key events of this mode of action to be protective of both neurodevelopmental (noncancer) and thyroid neoplasia (cancer) effects.

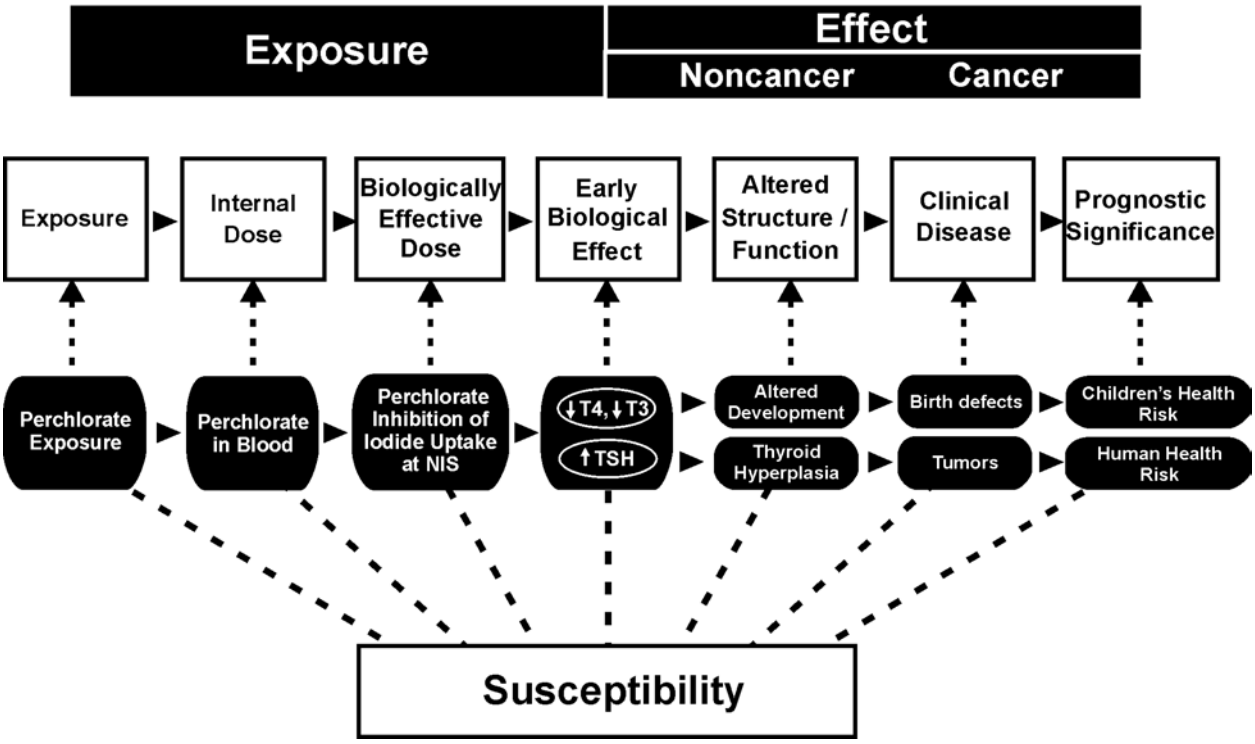

Figure 2-1. Recommended Revision to Figures 3-12, 6-1 and 7-1. Mode-of-action model for perchlorate toxicity proposed by the U.S. EPA (U.S. Environmental Protection Agency, 1998, 2002a). Perchlorate interferes with iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS) present in various tissues such as the thyroid, gastrointestinal (GI) tract, skin, placenta, and mammary gland. Schematic shows the exposure-dose response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (U.S. Environmental Protection Agency, 1994a; Schulte, 1989). The model maps the toxicity of perchlorate on this basis by establishing causal linkage or prognostic correlations of precursor lesions.

2.1 COMMENTS ON THE INTERACTION OF PERCHLORATE WITH THE SODIUM (Na^+)-IODIDE (I^-) SYMPORTER (NIS)

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question A.1 — Have all the data on toxicokinetics and toxicodynamics been identified and appropriately utilized and have the similarities and differences in the toxicity profile across species been adequately addressed?

The peer reviewers offered many comments when responding to this charge question. Most of the discussion focuses on the nature of the interaction between perchlorate and the NIS. Most of the comments suggested that the revised document should present more detailed information on toxicokinetics and toxicodynamics. Some specific key comments and responses follow.

2.1.1 Perchlorate Translocation into Thyroid Cells

Comment(s): *There was considerable discussion by the panel on details of NIS inhibition by perchlorate, including a debate regarding whether perchlorate is a competitive inhibitor of iodide uptake and can be translocated into follicular cells or whether the inhibition is a conformational change of the NIS and irreversible. It was noted that iodide uptake is an electrogenic process and not driven by adenosine triphosphate (ATP). One reviewer stated that perchlorate is not translocated into thyroid cells as the 2002 ERD states. This reviewer first critiqued a study cited in the 2002 ERD (Anbar et al., 1959). In this 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. The reviewer argued that using this study design one could not discern whether perchlorate translocated into thyroid cells or simply bound to them. This reviewer then noted that the recent cloning of the NIS has enabled laboratories to conduct more rigorous research on iodide uptake inhibition. 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 that it does not translocate into thyroid cells. This reviewer questioned the validity of EPA's assumption that NIS translocates perchlorate into thyroid cells and noted that none of the references cited in the 2002 ERD provide compelling evidence that active translocation of perchlorate occurs. The reviewer indicated that even the most recent studies reporting concentrations of perchlorate in the thyroid (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.*

Given that perchlorate does not translocate into thyroid cells, the reviewers briefly discussed what terminology most accurately describes perchlorate interactions with the NIS. One reviewer noted that a recent study (Eskandari et al., 1997) and others suggest that perchlorate is a "blocker" rather than a "competitive inhibitor". Another reviewer noted that both terms characterize perchlorate interaction with the NIS. Regardless of the terminology EPA eventually adopts, both reviewers recommended that the 2002 ERD should be updated to reflect current findings on whether perchlorate is translocated into thyroid cells. Subsequent submissions by stakeholders also addressed this issue.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that a more detailed discussion of perchlorate translocation studies would benefit the interpretation of potential risks and thus will update appropriate sections in Chapter 3 of the 2002 ERD.

Figure 2-2 will be added to the revised document in that chapter to show a more detailed view of the current mechanistic understanding of the NIS and its transport process.

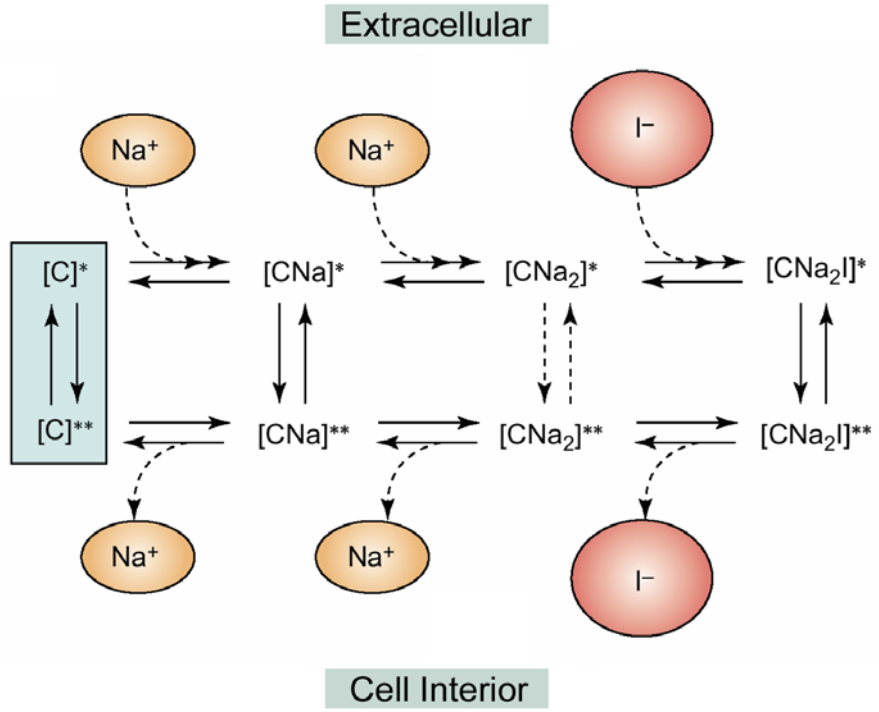

Figure 2-2. New Figure 3-3. Electrophysiological characterization of the sodium (Na^+)-iodide (I^-) symporter (NIS). First one sodium (Na^+) binds to the NIS which in the absence of substrate is able to cross the membrane via the NIS carrier (C) in a Na^+ uniport mode (CNa* → CNa) by the return of the empty binding site. The kinetic data suggest that Na^+ binds to NIS before iodide (I^-). In the presence of iodide (I^-), the carrier complex CNa_2I^* is formed which undergoes a conformational change to expose the bound iodide (I^-) and two sodium (Na^+) to the interior of the cell ($CNa_2I^* \rightarrow CNa_2I^{**}$). Both sodium ($Na^+$) and iodide ($I^-$) are released into the cytoplasmic compartment and the empty carrier undergoes another conformational change to expose the binding sites to the external solution again. Charge movement data suggest that the sodium (Na^+) binding dissociation does not contribute greatly to the total observed charge. Eskandari et al. (1997) have proposed that NIS charge movements arise primarily from conformational changes of the empty carrier (C' → C").**

Source: Redel et al. (2003).

The NIS is an integral plasma membrane glycoprotein that mediates the translocation of iodide into the thyroid and other tissues including the gastric mucosa, lactating mammary gland and placenta (Dohan et al., 2003). Eskandari et al. (1997) have examined the mechanism, stoichiometry, and specificity of NIS by means of electrophysiological, tracer uptake, and electron microscopic methods in Xenopus laevis oocytes expressing NIS.

Figure 2-2 shows a schematic representation of a model for sodium and iodide cotransport developed in *Xenopus laevis* oocytes via cDNA clone encoding of the NIS. The kinetics of transport as a function of external sodium cation and substrate concentration on this system suggest an ordered binding of sodium and substrate to the transporter in which binding of sodium occur first. Charge movement data suggest that sodium binding/dissociation does not contribute greatly to the total observed charge so that it is proposed that NIS charge movements arise primarily from conformational changes of the empty carrier (shaded region on Figure 2-2).

Using a two-microelectrode technique voltage-clamp technique, they have demonstrated an inward steady-state current (i.e., net influx of positive charge) is generated in NIS-expressing oocytes upon addition of iodide to the bathing medium leading to depolarization of the membrane. Because the recorded current is attributable to NIS activity, this observation confirms that NIS activity is electrogenic. Simultaneous measurements of tracer fluxes and currents revealed that two sodium cations are transported with one anion, demonstrating unequivocally a 2:1 Na^+/I^- stoichiometry. Therefore the observed inward steady-state current is due to a net influx of sodium cation.

Measurement of charge movements associated with the NIS conformational changes and substrate-uncoupled sodium-dependent leak currents have provided insights into the nature of the NIS cotransport. These researchers have reported that whereas iodide and a wide variety of other anions including ClO_3^-, SCN^-, $SeCN^-$, NO_3 Br^- , BF_4^-, IO_4^- and BrO_3^- generated steady-state inward electrical currents in the system that indicate these anions are transported, perchlorate (ClO_4^-) did not. This strongly suggested perchlorate is not transported (de la Vieja et al., 2000). Figure 2-3 shows the relative substrate selectivity of NIS. In their experiment, current was monitored as anions were added (500 μM) to the perfusion solution. Perchlorate completely blocked the inward current induced by 50 μM iodide. The relative apparent affinity of the NIS for anions were I^- (1.00) ≥SeCN (0.87) > SCN^- (0.34) > $Clo3^-$ (0.12) > $NO3^-$ (0.04). Any

comparative assessments of anions would need to account for these dramatic differences in potency to inhibit the NIS.

Eskandari et al. (1997) suggested that perchlorate was not translocated, although an electroneutral transport was not excluded by these authors. As discussed at the peer review meeting, earlier experiments showing that ^{36}Cl-perchlorate enters the cell were probably misinterpreted. These authors suggested that chlorate ($^{36}ClO_3^-$) rather than $^{36}ClO_4^-$ enters the cells and can be misinterpreted. These authors cite the work of Yoshida and colleagues in support of their hypothesis. Yoshida et al. (1984, 1997) reported that perchlorate did not induce an inward current in FRTL-5 cells or in Chinese hamster ovary cells stably expressing NIS.
Eskandari et al. (1997) suggested that perchlorate was not translocated, although an electroneutral transport was not excluded by these authors. As discussed at the peer review meeting, earlier experiments showing that ^{36}Cl-perchlorate enters the cell were probably misinterpreted. These authors suggested that chlorate ($^{36}ClO_3^-$) rather than $^{36}ClO_4^-$ enters the cells and can be misinterpreted. These authors cite the work of Yoshida and colleagues in support of their hypothesis. Yoshida et al. (1984, 1997) reported that perchlorate did not induce an inward current in FRTL-5 cells or in Chinese hamster ovary cells stably expressing NIS.

There was no debate about the ability of perchlorate to inhibit the NIS at the 2002 peer review. There was considerable discussion, however, as to whether the inhibition was competitive or if instead perchlorate was irreversibly bound to the NIS. Further discussion has ensued in some publications (Soldin, 2002, Clewell and Gearhart, 2002a,b). Van Sande et al. (2003) report a different selectivity of the NIS. Clewell et al. (in preparation) assert the principal of parsimony and state that for kinetic modeling that perchlorate can be described as a competitive inhibitor, citing some of the literature questioned above.

To clarify terminology, irreversible "blocking" referred to elsewhere in comments by the peer panel would be classified as non-competitive inhibition. In the latter case, perchlorate would still inhibit the translocation of iodide but would not itself cross into the cell. Table 2-1 shows the numerator and denominator for an equation to address inhibition. Non-competitive or "blocking" as stated in some of the peer review comments would imply that the maximum velocity of the Michaelis-Menten equation (Vmax) for iodide is reduced in the presence of perchlorate. Competitive inhibition would imply that the Michaelis-Menten affinity (Km) for iodide transport is increased by the presence of perchlorate. Quantitative differences in iodide

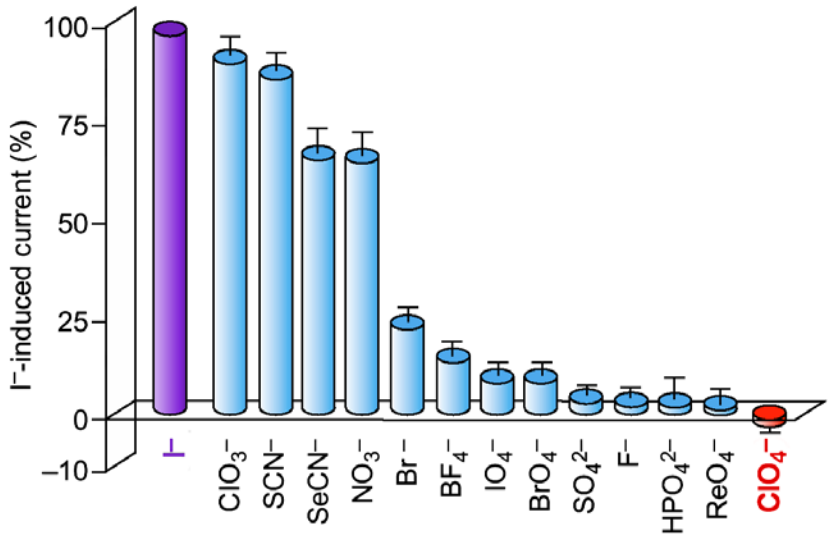

Figure 2-3. New Figure 3-4. Schematic of substrate selectivity for the Sodium (Na^+)-Iodide($^-$) Symporter (NIS). Currents for each anion shown were normalized to the current generated by iodide (I-). Data shown are reported as mean ± S.E. (N = 3).

Source: Eskandri et al. (1997).

Table 2-1. Comparison of Equations to Represent Various Types of Inhibition by Perchlorate at the NIS

Inhibition	Numerator	Denominator
No inhibition	Vmax*Ci	Ci+Km
Competitive inhibition	Vmax*Ci	Ci+Km (1+Cp/Kp)
Non-competitive inhibition	Vmax/(1+Cp/Kp) *Ci	Ci+Km

Vmax = Michaelis-Menten maximum velocity
Km = Michaelis-Menten affinity constant for iodide
Ci = Concentration of iodine
Cp = Concentration of perchlorate
Kp = Michaelis-Menten affinity constant for perchlorate

uptake between these two descriptions (calculated as the numerator over the denominator) would depend on the various parameters involved.

Resolution of this controversy will likely take additional experiments to discern differences in selectivity of the NIS and to characterize the precise mechanism of action for perchlorate. As discussed in Chapters 6 and 7, the dose metric that is currently chosen for interspecies extrapolation and based on available physiologically-based models is the area under the curve of perchlorate in blood (AUCB). Given the small volume that the thyroid represents in these model structures, definitive resolution of this debate will not impact the predicted human equivalent estimates to a significant degree. Accurate characterization will be required, however, to refine the modeling to more mechanistic descriptions, not only in the thyroid but especially in other tissues important for the toxicity of perchlorate different lifestages (e.g., placenta and lactating mammary tissue).

2.1.2 Metabolism

Comment(s): *When discussing the implications of iodide inhibition at NIS, one reviewer asked the others if this inhibition is reversible. One reviewer noted in response 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. The reviewer recommended that the EPA reconsider its assumption that perchlorate is not metabolized and is "excreted virtually unchanged". This assumption was 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 suggests that 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 and that these hypotheses are the subject of ongoing studies.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that its presentation of the nature of perchlorate interaction with the NIS needs to be expanded to discuss current concepts regarding the processes of non-competitive blocking by perchlorate or 1:1 exchange with sodium.

2.1.3 NIS in Other Tissues

Comment(s): *One reviewer noted that the 2002 ERD provides limited information on other tissues known to contain NIS (e.g., lactating mammary gland, placenta, stomach, salivary gland,*

choroid plexus) and whether perchlorate exposure can lead to adverse effects through iodide uptake inhibition in these tissues. This reviewer 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 recommended that the revised document should provide much more information on the potential for perchlorate to inhibit NIS in other tissues.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that the 2002 ERD did not discuss in sufficient detail that the inhibition caused by perchlorate exposure would also inhibit important iodide transfer by the placenta to the fetus and by mammary tissue to lactating neonates. These features were described in Chapter 6 of the 2002 ERD regarding the necessary processes and compartments for development of the PBPK models to describe perchlorate disposition, but they were not adequately discussed in Chapter 3 on the mode of action. The differences in perchlorate dosimetry during these various lifestages contributes to their increased sensitivity. An important aspect to emphasize, as noted above, is that the other tissues that contain NIS do not respond to regulation by the HPT axis. Neonatal thyroid glands have limited stores of iodine and thyroid hormones (van den Hove et al., 1999; Savin et al., 2003) so inhibition of the NIS in mammary tissue could have significant consequences. The importance of thyroid hormone to brain development of the fetus and thus the importance of inhibition of NIS to impair placental transfer is noted.

Consequently, the Agency has modified the mode-of-action model in Figure 2-1 to show that the biologically effective dose is at the NIS and not only in the thyroid. The legend explicitly lists some of the other key tissues in which NIS is located and discusses that inhibition of the NIS in these tissues is critical to different life stages. The Agency is also including Figure 2-4 to schematically illustrate perchlorate disposition and its effects iodide uptake in the adult / pregnant woman, fetus, and neonate. The affinity of the NIS for perchlorate was addressed in response to the comment above.

The Agency will include a contemporary list of tissues that have had their NIS expression and activity characterized (Dohan et al., 2003). An important note will also be made that while the NIS transport systems in these extrathyroidal tissues exhibit functional similarity with the thyroid such as a susceptibility to perchlorate and similar concentration gradients, they also display some important differences that are important to consider in evaluating dosimetry and

risk to different life stages: (1) these nonthyroid tissues do not have the ability to organify accumulated iodide (with the possible exception of the lactating mammary gland), the HPT axis exerts no regulatory control via TSH, and certain tissues (salivary gland and gastric mucosa) have been shown to concentrate thiocyanate which by contrast is metabolized after uptake in the thyroid. Despite these differences, several reports of patients suffering the simultaneous genetic absence of iodide transport in the thyroid, the salivary glands, and the gastric mucosa strongly hinted at a genetic link among these iodide transport systems and suggesting that extrathyroidal transport is catalyzed by plasma membrane proteins that are very similar if not identical to thyroid. This is an important consideration for the risk assessment at hand because inhibition by perchlorate in the placenta and mammary tissue would prevent proper iodide transfer to critical life stages. The Agency intends to include Figure 2-4 to schematically illustrate perchlorate disposition and its effects iodide uptake in the adult / pregnant woman, fetus, and neonate. The affinity of the NIS for perchlorate was addressed in response to the comment above.

2.1.4 NIS Inhibition and Upregulation

Comment(s): *The peer reviewers identified additional areas where more detailed toxicokinetics is warranted. One reviewer recommended that the revised document more prominently acknowledge that the 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 (Km) for iodide transport via the NIS to published values for the inhibition constant for perchlorate. Further, this peer reviewer recommended that the revised document note that the NIS upregulation mechanism triggered by the HPT axis regulates expression of NIS only in the thyroid and not in the other tissues mentioned in Section 2.1.3.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that the inclusion of Figure 2-3 and the discussion of NIS selectivity is consistent with this comment.

2.1.5 Toxicodynamics

Comment(s): *Peer reviewers offered several comments regarding toxicodynamics. Most of these identified specific topics in the 2002 ERD that should include more detailed information. One reviewer noted that the 2002 ERD lacks detail both on exactly how increases in TSH lead to cancer and on the similarity between the specific sequence of events in rodents and humans. Elaborating on this topic, another reviewer suggested that the revised document could improve the discussion of carcinogenesis by describing more steps in the sequence of events such as how perchlorate exposure leads to compensatory proliferation or excess mitoses per unit time. This*

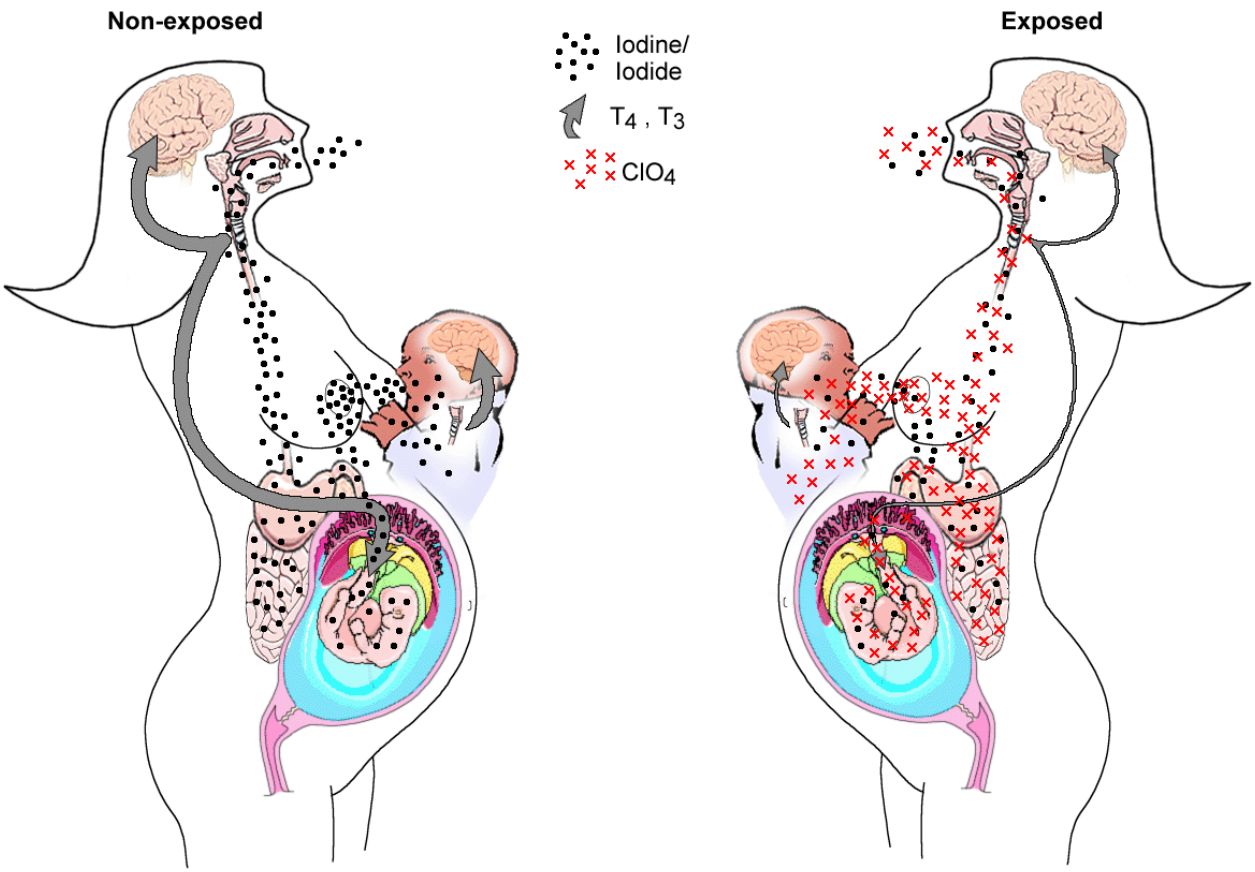

Figure 2-4. Recommended new Figure 3-5. Dosimetry of ingested perchlorate and effects of its impact on the sodium (Na^+)-iodide (I^-) symporter (NIS) in various tissues at different life stages. Perchlorate inhibits iodide uptake at NIS present in the thyroid, GI tract, placenta, skin and mammary gland. This decreases the synthesis of circulating thyroid hormones in the adult and their placental transfer to the fetus in pregnant women. Interaction of perchlorate at the NIS can also inhibit placental iodide transfer to the fetus or lactating neonate and thereby additionally decrease thyroid hormone synthesis at these life stages.

reviewer acknowledged that the background discussions in the 2002 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 reviewer 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 2002 ERD on carcinogenesis is based on observations of precursor lesions which do not necessarily result in cancer. On a similar note, another reviewer suggested that the EPA apply the term "precursor" carefully. The term conventionally refers to an event that lies on the causal pathway of an adverse effect.

This reviewer recommended that EPA list all events that are considered precursors and the specific adverse effects that ensue.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that the mode of action model addresses the continuum of pathogenesis from precursor lesion to tumor with Figure 2-1 and descriptions of the key event in the text of Chapter 3. The data evaluated to determine whether perchlorate was genotoxic are presented in Chapter 5 and an overall synthesis of the data to arrive at a conclusion regarding the database supporting a non-linear approach to its anti-thyroid effects in Chapter 7.

The Agency appreciates that panel advocated for more mechanistic descriptions of mechanistic linkages for both neurodevelopmental sequelae as well as for thyroid tumors. The EPA anticipates that systems biology approaches will aid the understanding of the pathogenesis for these endpoints and expects that the revised assessment will include additional reviews and insights at that time.

2.1.6 Other Comments

Comment(s): *When summarizing the pre-meeting comments, the discussion leader identified other sections of the 2002 ERD that EPA could clarify but these comments were not discussed in detail. First, the discussion leader suggested that EPA provide additional detail on the re-analyses of the radioimmunoassay (RIA) data. The revised document should document how the analyses were conducted, what standard curves were used, and whether data points were interpolated from the standard curve to levels below the lowest available standard. Second, he noted that the 2002 ERD lacks detail on the levels of iodide in maternal serum, how perchlorate may affect these levels, and the potential consequences to fetuses and neonated. Finally, the potential for re-programming the HPT axis was noted, and the reviewer suggested that current research on the HPT axis might offer perspective on the issue.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency notes that some of the information requested by the reviewer is not readily available but intends to update descriptions of the studies with these details where possible. A description of the concern for the use of human RIA kits to assay rodent samples will be provided in the introductory section of Chapter 5 of the 2002 ERD that discusses the interlaboratory variability study performed on serum hormone measurements across three laboratories by the AFRL (Narayanan, 2000). However, the reviewer's concern is the measurement of hormone levels (especially for control

animals) that in some cases may fall below the lowest standard used to generate the calibration curve. This will be noted as a consideration for interpretation of hormone data.

Data on the levels of maternal, fetal, and neonatal iodide and thyroid hormone are beginning to be quantified in a systematic fashion to better understand the maternal-fetal thyroid hormone relationships before the onset of fetal thyroid secretion. These may be informative to efforts to detect and prevent maternal hypothyroxinemia in early pregnancy. The Agency agrees with the comment and will include discussion of maternal-fetal nutrient transfer in revisions to Chapter 3 of the 2002 ERD. For example, a recent article has raised some new questions about whether the thyroid hormone concentrations found inside the gestational sac could be biologically relevant for the occupation of nuclear TR in the fetal brain (Calvo et al., 2002). A decrease in the availability of free T4, a major precursor of intracellular nuclear receptor-bound T3, may result in adverse effects on the timely sequence of developmental events.

The Agency agrees that *in utero* programming is a critical consideration for effects on thyroid hormone economy and will update any inferences with new references as they are published.

2.2 COMMENTS ON CONCEPTUAL MODEL

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question 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 on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

Comment(s): *The panel recommended that the revised document should discuss in greater detail the mechanisms by which inhibition of iodide uptake and subsequent decrements in thyroid hormones and increases in TSH alter neurodevelopmental and neoplastic processes. It was noted that the 2002 ERD lacked detail on the levels of iodide in maternal serum, how perchlorate may effect these levels, and the potential consequences to fetuses and neonates. The potential for re-programming of the HPT axis should be discussed. It was also suggested to discuss the role of different thyroid hormones (i.e., T3 and T4) on other biological processes.*

EPA Response(s) and Recommendation(s) for Revision(s): As will be discussed in Chapter 4, there is increasing evidence from epidemiological and experimental data that first trimester

maternal thyroid status is pivotal for the outcome of pregnancy and for the neuropsychomotor development of the child . The importance of thyroid hormone in brain development and the irreversibility of thyroid hormone insufficiency is emphasized in this risk assessment. The dose-response and precise mechanistic steps of hormone action on brain receptors that affect neurodevelopment are an area of active research. The Agency agrees that expanded discussion of potential mechanisms that link iodide uptake and hormone level alterations and associated neurodevelopmental and neoplastic outcomes would be beneficial to the document. These will be incorporated in Chapter 3 of the revised assessment.

2.3 COMMENTS ON THE CARCINOGENIC POTENTIAL OF PERCHLORATE AND IMPLICATIONS FOR PROCEDURES OF LOW-DOSE EXTRAPOLATION

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding 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: (1) the use of thresholds in low-dose extrapolations and (2) the choice of dose metric. The two general topics focused on by the panel in this response are addressed in separate sections below. This section also addresses the summary comment on Page 9-3 of the peer review report (US EPA, 2002b) regarding the genetic toxicology of perchlorate.

2.3.1 Use of Nonlinear Low-Dose Extrapolation

Comment(s): *The panelists that commented on this topic felt that the 2002 ERD provided compelling evidence that perchlorate is not genotoxic and that the use of non-linear dose extrapolations is appropriate and adequately defended in the document. Clear and convincing mechanistic arguments were presented to support that the toxicity of perchlorate being a nonlinear process. It was suggested by one reviewer that iodide deficiency would not result from a single, low perchlorate exposure but rather sustained elevated exposures. Another reviewer asked for toxicodynamic modeling to support the non-linear approach.*

Dr. David Jacobson-Kram was the peer reviewer assigned to evaluate the genotoxicity data and he indicated that no new studies have been published since the 1999 peer review. He agree with the findings of the previous peer review panel (1999) 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.

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees with both the 1999 and 2002 peer review panels that a sufficient database has been developed to arrive at a weight of evidence conclusion that the carcinogenicity of perchlorate is due to its anti-thyroid effects and not direct genotoxicity per existing EPA guidance (US EPA, 1998a).

Section 5.1.1 of the 2002 ERD described the historical cancer studies and Section 5.1.2 summarized the battery of genotoxicity studies performed to evaluate the potential mutagenicity of perchlorate as part of the testing strategy. As described in Section 7.1.5.2.1, the 2002 ERD presented an evaluation of the evidence to support a non-linear dose response approach to the assessment of the risk of thyroid neoplasia from perchlorate based on its mode of action. Because the derivation is based on the mode of action model, the proposed harmonized RfD is viewed by the EPA to be protective of both neurodevelopmental and neoplastic toxicities.
To address these requests, the EPA will include a new summary section in the revised document, Section 5.1.3 (Summary and Cancer Hazard Assessment). The Agency will also include a new Section 7.2 (Cancer Hazard Characterization) in the revised assessment. The new text for Section 5.1.3 is provided in this chapter and the text for Chapter 7 in Section 7.6.1 herein.

While the EPA agrees with the panel that the use of a non-linear approach to evaluate thyroid tumors was adequately presented, the Agency has included text in various inserts in both Chapter 4 and Chapter 7 to highlight comments made by the peer review panel that indicated that the shape of the dose-response is not known for neurodevelopmental effects nor has a threshold for these effects been established. While a reference dose approach would still be considered appropriate for neurodevelopmental toxicity, the lack of understanding of the shape of the dose-response and existence of a threshold needs to be considered carefully when evaluating the point of departure for such a derivation. Likewise these considerations should be weighed when assigning the magnitude of uncertainty factors.

The Agency agrees that biologically based dose response modeling, including components to address both pharmacokinetics and pharmacodynamics would be worthwhile. However, as will be discussed in subsequent chapters, the development and validation of BBDR models is viewed as an effort that will take considerable time to achieve success due to the need for

significant amounts of additional experimental studies. Development and validation of BBDR models for different life stages will be particularly challenging.

2.3.1.1 New Section 5.1.3 (Summary and Cancer Hazard Assessment)

As described in Section 5.1.1, perchlorate has been demonstrated to produce thyroid follicular tumors (adenomas or carcinomas) in laboratory animals (male Wistar rats and female BALB/c mice) at high doses. These studies were single high-dose drinking water studies that precluded determination of the dose-response relationship for tumor formation. There are no other chronic data on perchlorate with which to evaluate the carcinogenic risk.

As will be described in Section 5.5.2.2 (of the 2002 ERD and revised document), thyroid follicular tumors (adenomas) were also observed in the F1 generation at 19 weeks during a two-generation study in Sprague-Dawley rats. This study had been designed to evaluate the ability of perchlorate to produce transgenerational effects. The thyroid tumors occurred with a statistically significant increase in incidence and decrease in latency when compared to the incidence of all other studies showing this type of tumor in this strain and sex at terminal sacrifice of 2-year bioassays in the NTP archives (Dunson, 2001b). While all of these studies suggest the potential for thyroid carcinogenicity associated with disruption of thyroid physiology, the early onset of tumorigenesis in the offspring of treated animals is particularly noteworthy, suggesting the potential for fetal impacts.

The battery of *in vitro* and *in vivo* genotoxicity studies described in Section 5.1.2 established that ammonium perchlorate does not have significant mutagenic or clastogenic activity. Additional evidence for an anti-thyroid effect of perchlorate is presented in Section 7.1.5.2.1.

Based on these data, it is concluded that perchlorate is carcinogenic in laboratory animals. The mode of action for perchlorate is established to be operative in laboratory animals, and is considered relevant to humans. While the laboratory animal data may support a concern for a thyroid cancer hazard from perchlorate exposures, human cancer risk will be dependent on the level of exposure and whether thyroid function has been disrupted. A hazard characterization is presented in Section 7.6.1.

2.3.2 Selection of Area-Under-the-Curve in Blood (AUCB) as the Dose Metric

Although this set of comments may be more appropriately placed in Chapter 6 (PBPK Models to Address Perchlorate's Mode of Action), this response document is adhering to the report of the peer review (US EPA, 2002b). Additional discussion on this topic is found in Chapter 3 (Human Health Effects Data), Chapter 4 (Toxicological Effects in Laboratory Animal Studies) and Chapter 7 (Human Health Risk Assessment).

Comment(s): *Clarification occurred between the two panel members responsible for review of the PBPK models that the choice of dose metric by the EPA was area under the curve in blood (AUCB) and not the area under the curve in tissue (AUCT). This choice is appropriate if perchlorate is not translocated into thyroid cells. One reviewer questioned if the selected dose metric was the best predictor of toxic effects and wondered if intermediate measurements (e.g., compensating hyperplasia or excess mitoses per unit time) may be better indicators, notably of neoplasia. Another reviewer disagreed emphasizing the limitations of studies of short durations (e.g., 2 weeks or less in humans) and that perchlorate exposure can clearly cause changes in thyroid hormones and TSH levels in humans even if not observed within this time period.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that its choice of the area under the curve for perchlorate in blood (AUCB) is the most appropriate at this time. The rationale for this choice is provided in Section 7.2.1 As will be described in Section 4.4, the Agency considers the three indices of histopathology in the gland (colloid depletion, hypertrophy, and hyperplasia) to be mutually representative of a change in the thyroid hormone economy of the gland. Mitoses per unit time or labeling index studies were not performed and would not be particularly useful for the assessment of neurodevelopmental effects. The issues of exposure duration for specific studies are discussed in greater detail in Chapters 3, 4, and 7.

2.4 COMMENTS ON HARMONIZED APPROACH

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding 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.

Comment(s): *As summarized in the pre-meeting comments, the peer panel noted that they generally supported the proposed approach to harmonize the risk assessment by focusing on a key event (iodide uptake inhibition) that precedes neoplasia, neurodevelopmental effects, and other noncancer effects.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has no additional recommendations for revisions to address this topic area other than those suggested in the previous sections.

3. HUMAN HEALTH EFFECTS DATA

This chapter addresses recommendations by the panel or comments received from the public and interested stakeholders regarding the Agency's analyses of the human health effects data on perchlorate. This chapter is restricted to the analysis of the data. The discussion of their potential use in identifying the point of departure is provided in Chapter 7 of this document. Chapter 7 also addresses limitations of experimental design, attributes of the outcome measures or models used in the human studies, and how these impact the utility of the data for drawing inferences or for bounding the dose-response assessment generalized to protect public health.

These responses and recommendations are suggested primarily for revision of Chapter 4 (Human Health Effects Data) in the 2002 ERD and in the Introduction and Section 7.1.5.1 (Comparison with Derivation Considering Human Data) of Chapter 7 (Dose-Response Assessments for Human Health). Refinements regarding the discussion of human data are also recommended by the Agency for Chapter 10 of the 2002 ERD (Major Risk Characterization Conclusions) and are also presented in Chapter 8 of this response document.

The available human studies on perchlorate include two types of studies, ecological epidemiological studies and controlled human dosing or clinical studies. The comments and response / recommendations will be discussed separately for each type of data.

3.1 STATUS OF EPA POLICY ON THE USE OF THIRD-PARTY HUMAN DATA

This topic was not posed to the 2002 expert peer review panel for review, nor is it posed to this NAS panel. However, because there has been considerable confusion caused by conflicting reports regarding whether the available human studies on perchlorate were considered in the EPA health risk assessment, the EPA is recommending revisions to the discussion of this topic in the new ERD. The use of the human data was discussed in the 2002 ERD in the Executive Summary, in the introduction to Chapter 4 (Human Health Effects), in the introduction to Chapter 6 (Construction of PBPK Models to Address Perchlorate's Mode of Action), and in two

different sections of Chapter 7 (Dose-Response Assessments for Human Health) — its introduction and Section 7.1 (Comparison with Derivation Considering Human Data).

The EPA is now recommending a separate section in the introduction of each of these chapters (e.g., 4.1., 6.1 and 7.1, entitled, *"Status of EPA Policy on the Use of Third-Party Human Data"*) to reinforce the message that the Agency considered all of the human data in depth to ensure that they were obtained ethically and to address necessary safeguards for such studies, as well as to assess them for their utility in arriving at a dose-response assessment of perchlorate for the general public. As before, Section 7.1.5.1 (Comparison with Derivation Based on Human Data) will address issues of using these data as the basis for an operational derivation of the health risk estimate. Further, the utility of human data in arriving at the point of departure will be emphasized in Sections 7.1.1 (Key Events and Weight of the Evidence) and 7.1.3 (Point-of-Departure Analysis).

For clarification, the Agency analyzed the raw data on iodide uptake inhibition from the Greer et al. (2002) study that were available from the Quality Assurance and Quality Control (QA/QC) review performed by the Air Force Research Laboratory (AFRL) (Merrill, 2001a). These data were used by the AFRL to develop a physiologically-based pharmacokinetic (PBPK) model that describes the disposition of ingested perchlorate in humans (Merrill, 2001c). The EPA then relied upon this model to inform interspecies extrapolation. What was not available to the Agency until the March 2002 peer review meeting was a draft of a manuscript that had been written regarding the study. The draft of the accepted manuscript was made available to the panel at the peer review and it has subsequently been published (Greer et al., 2002). The current Agency analysis of Greer et al. (2002) and its utility in dose-response assessment is discussed in Section 3.3.

The following is the introductory section to be inserted at the beginning of Chapters 4, 6, and 7 to augment the discussion already found on this topic in the 2002 ERD.

On December 14, 2001, after an internal review of the 2002 ERD had already occurred and the document was in final production for its January 2002 release, the Agency articulated its interim policy on the use of third-party human studies submitted by regulated entities (U.S.

Environmental Protection Agency, 2001c).[1] EPA considers "third party studies" to be studies that have not been conducted or funded by a federal agency.

Some of the clinical studies contained in the perchlorate database fall in this category. The scientific and technical strengths and weaknesses of these perchlorate studies were described before this Agency policy was articulated. It is noted that the motivation for including controlled human studies in the original perchlorate testing strategy (as discussed in Chapter 3) was to provide data to support the development of physiologically based pharmacokinetic (PBPK) models for use in interspecies extrapolation. The data from the clinical studies that passed Quality Assurance and Quality Control (QA/QC) review were used by the Air Force Research Laboratory (AFRL) to develop the PBPK model which describes the disposition of ingested perchlorate in humans (Merrill, 2001c). The EPA relied upon this model to inform interspecies extrapolation. The two principal intentional human dosing studies (Greer et al., 2002, Lawrence et al., 2000, 2001) used in the PBPK modeling and validation were performed at institutions currently holding Federal Wide Assurances; underwent institutional review board approval; and report funding sources to include the Perchlorate Study Group (an industry consortium) and NIH grants.

The human data were not used to derive NOAEL estimates for thyroid effects in the general human population not because of the EPA interim policy, but rather because the EPA feels that both the observational (ecological) epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the sole basis for a quantitative dose-response assessment protective of the general public health. The observational studies suffer from a lack of statistical power, of control for confounding, of surveillance of disease outcome measures, and of exposure characterization. The controlled studies are very limited with respect to the type of subjects used (e.g., thyroid status, age, not representative of susceptible subpopulations) and experimental design (e.g., sample size, time points, duration, outcome measures). The independent analyses that led the Agency to these conclusions were provided in the 2002 ERD and additional considerations are included in subsequent chapters of this response document.

A federal court has vacated the December 14, 2001, Interim Policy. CropLife America v. EPA, D.C. Cir., No 02-1057 (June 3, 2003).

Therefore, while these data were used to inform the weight of evidence and database analysis to arrive at a point of departure, these data were considered less reliable than the laboratory animal data for the operational RfD derivation and are not used as the sole "principal study."

3.2 COMMENTS ON THE EPA SUMMARY OF THE ECOLOGICAL EPIDEMIOLOGICAL DATA

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding the ecological epidemiological data for perchlorate that can be found in the Executive Summary, Chapter 3 (Human Health Effects Data), and Chapter 9 (General Comments, Conclusions and Recommendations) of the 2002 peer review report (U.S. Environmental Protection Agency, 2002b). Specific pre- and post-meeting comments by those panel members assigned to this topic (Drs. Carrasco, Cox, Hoel, Razzaghi, and Zoeller) have also been considered. Comments received from the public and other stakeholders are also included in this evaluation. Discussion of the use of the human data as the basis of the point of departure for the RfD derivation is found in Chapter 7 (Dose-Response Assessment for Human Health) of this response document.

***Comment(s)**: A number of the reviewers commented on the way in which the 2002 ERD presented the human health effects data, noting in general a careful and systematic analysis with a fair and balanced discussion. One reviewer noted that a severe weakness in all of these studies is that measures of exposure are not adequate to identify specific dose-effect relationships. Another reviewer noted that none of these studies had overcome the basic limitations due to the lack of good exposure data, of well-controlled confounding, of adequate power, etc. Another reviewer encouraged caution in interpreting the causality and quantitative results of the ecological studies, noting that one of the largest ecological studies on radon 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. Agreeing that such studies have potential limitations, another reviewer suggested that EPA present a balanced overview for all ecological studies, including those with positive findings and specifically referred to the Schwartz (2001) study. Two reviewers highlighted specific concerns regarding the utility of the ecological studies including that the most sensitive health endpoints (e.g., neurodevelopmental effects) may not have been considered and that no single metric of human thyroid function identifies potentially significant thyroid impairment and toxic effects. These two reviewers also noted that researchers have found associations between intellectual deficits in children whose mothers had decreased T4 levels during pregnancy but not increased TSH levels. One of these reviewers also 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 of TSH. These reviewers said that these findings show that adverse effects may result from impaired thyroid function even if evidence of upregulation is not observed.

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees with the general conclusions of the 2002 peer panel regarding the utility of these observational epidemiological data for quantitative dose-response analysis. No substantive recommendations for revision of the discussion regarding these studies in the 2002 ERD are proposed with the exception of the responses provided to revise discussion of the specific studies noted herein, e.g., specific comments on the Schwartz (2001) study below. The Agency further notes that these same concerns apply to the occupational epidemiological studies. As discussed in the 2002 ERD, those occupational studies are by inhalation exposures, and adequate adjustment for particle size distribution and deposition in different aspects of the respiratory tract were not addressed. However, as discussed in Section 3.4 below, text regarding the use of the human data to evaluate the point of departure and to evaluate the proposed dose-response estimate with a bounding exercise will be included in revisions to Chapter 7.

Comment(s): On Soldin et al. (2001). *One peer reviewer requested that the Soldin et al. (2001) review of clinical data be discussed in more detail in order to gain possible insight concerning the comparison of human data with the laboratory animal data. Another expressed concern about the analyses of the serum perchlorate levels presented in the Soldin et al. (2001) review. The review suggests that humans exposed to 10 mg of perchlorate a day had serum concentrations of 0.6 ug/ml while perchlorate was not detected in the serum of humans exposed to 3 mg of perchlorate per day. This reviewer could not understand the non-detects in this latter group because she expected that they would have serum concentrations of approximately 0.2 ug/ml – a level about 50 times higher than minimum detection limits reported for ion chromatography. The other reviewers could not explain this discrepancy.*

EPA Response(s) and Recommendation(s) for Revision(s): As discussed on Page 4-30 of the 2002 ERD, the Agency notes that Soldin et al. (2001) is a review article which was co-authored by two major participants in industry-funded perchlorate research. Each of the studies in the review was addressed individually by the EPA in its ERD and the difference in opinion stated on Page 4-30. The Agency also stands by its summary in Table 4-5 (now Table 4-15 in the revised final document) that indicates a number of critical issues not considered in the review. Further,

one peer panel member in post-meeting comments offered that the Soldin et al. (2001) review is based on the assumption that neonatal T4 is a valid biomarker of prenatal thyroid status and that this was clearly false given the 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. The EPA has no comment on the discrepancy noted above by the other peer panel member with respect to the non-detects given current analytical methods. In conclusion, the Agency is not revising its treatment of the Soldin et al. (2001) review in the final document.

Comment(s): On Crump et al. (2000). *The peer reviewers had various comments on EPA's interpretation of the Crump et al. (2000) study. First, based on comments submitted by an author of the study, one reviewer recommended that EPA rewrite its review but did not specify the nature of the necessary revisions. This reviewer questioned why the negative findings of this study were dismissed when studies of cohorts in Chile have been used to develop dose-response data for exposures to nitrate and nitrites in drinking water and its associated effects. This reviewer also noted that Crump et al. (2000) suggests that humans can experience perchlorate exposures much higher than the proposed point of departure without having impaired thyroid function. Another reviewer noted paradoxical findings in this study, noting the lower TSH levels observed among the more highly exposed individuals. Another reviewer noted 30% of the reference population exhibited goiter in 30%, which makes it difficult to defend or make conclusions.*

EPA Response(s) and Recommendation(s) for Revision(s): In response to these comments and those submitted by stakeholders suggesting that the Crump et al. (2000) study provides characterization of a dose-response for exposure of children to perchlorate, the EPA has performed additional analysis (Marcus, 2003b) of Crump et al. (2000). These new analyses combined with consideration of previously noted problems with this study have reinforced the EPA's opinion expressed in the 2002 ERD that this study has significant design flaws which limit its utility for informing the dose-response assessment of perchlorate. A full discussion of these new analyses and interpretation of the results is provided in Section 3.2.1 below. This section will be used to revise text in the 2002 ERD found on Pages 4-7 through 4-8. A discussion of the relative potency of nitrate versus perchlorate with respect to inhibition of iodide uptake at the NIS was provided in Chapter 2 of this response document.

Comment(s): On Schwartz (2001)*. The peer reviewers offered various opinions about the findings in this study and about EPA's interpretation. One recommendation was to eventually consider any publication that may result. Another reviewer, reiterating concerns about the*

inferences that can be drawn from ecological studies, cautioned against drawing conclusions from the Schwartz (2001) study. A third reviewer recommended that EPA interpret the significance of the transient changes in thyroxine (T4) levels observed in the newborns. Another reviewer wanted clarification on why the T4 declined at four of the perchlorate exposure levels with age until about 18 hours and then increased over the next 30 hours. One reviewer noted that although the study appeared to be more sophisticated in its estimate of exposure, it still lacked the power of individual measurements of perchlorate and their relationship to thyroid function.

EPA Response(s) and Recommendation(s) for Revision(s): With respect to the newborn T4 levels, a review of Table 2 in Schwartz (2001) reveals only minor deviations in the early postnatal T4 values. Because thyroid hormone values in the first few days after birth are highly variable due to relatively large fluctuations just prior to parturition, EPA does not find any significant interpretation with respect to this observation.

The EPA is not recommending any significant new changes to the discussion of the Schwartz (2001) study. At this time, the thesis is not yet published. The EPA stands by its analysis of the results and comments that the Schwartz (2001) study remains the most convincing of the neonatal ecological studies given its elaborate exposure assignment and its detailed collection of covariate information pertaining to neonatal thyroid function. The implication of the study is that a statistically significant trend in decreases of T4 at all exposure levels (categorized as low, 1-2 ppb; medium 3-12 ppb; and high ≥ 13 ppb) suggests that ambient levels of perchlorate may affect thyroid hormone economy in newborns.

However, the Agency points out that this study was not relied upon to any greater degree than any of the other observational (ecological) studies due to the concerns noted above about this type of study design. Further, the Agency agrees with the reviewer that suggested both negative and positive findings be used in any evaluation of bounding the laboratory animal data with human studies. Given that this study had more robust estimates of exposure than any of the other observational studies and that it did suggest a positive finding in neonates, this study should also be considered in any bounding exercise (see Chapter 7) using these types of human data.

The Agency is aware that a new ecological study will also offer the advantage of better defined exposure estimates for an evaluation of neonatal serum hormone outcomes. The Environmental Health Investigations Branch of the California Department of Health Services is conducting an ecological, cross-sectional study of *in utero* perchlorate exposure and newborn

thyroid function in a population of Rancho Cordova, California, neonates who were born during the years that this city's water supply was contaminated with perchlorate. Perchlorate exposure will be assigned based on hydrogeological modeling and water distribution system simulations conducted by the University of California at Davis. Data on outcome measures were collected by the Newborn Screening Program of the California Department of Health Services. The "Perchlorate Exposure in Drinking Water and Neonatal Thyroid Hormone Levels" health study is funded by the Agency for Toxic Substances and Disease Registry through a Centers for Disease Control and Prevention Grant Award #U50/ATU990079.

At the 2002 peer review meeting, Ms. Schwartz provided a correction to a typographical error regarding the T4 differences; these changes will be made to the text on Page 4-12, lines 18 to 20 and the summary Table 4-5 in the 2002 ERD (now Table 4-15 in the revised document). The lines on Page 4-12 will be changed to read as: *Controlling for age at screening (6-hour increments up to 48 hours), gender, single versus multiple birth, birth weight (in 5 levels), and ethnicity (20 categories), a highly statistically significant declining trend was observed for T4 with the four perchlorate exposure levels (0, -0.97, -1.12, -1.82 μg/dL)*. The entries in Table 4-5 of the 2002 ERD (now Table 4-15 in the revised document) will be changed as follows: in the "outcomes studied" column: T4: 16.6 μg/dL; in the "findings column": *ANCOVA model with extensive control of most confounders finds highly significant decrease in T4 (mean 16.6 μg/dL) with P level (0, -0.97, -1.12, -1.82) and large effects for birthweight (-7.2 for birthweight 1500-2500), age (-5.0 for hours 7-18) and ethnic groups (-1.0 to -3.0)*. Clarifications to entries in Table 4-5 (now Table 4-15) in the "Problems / Comments" column include: *"Definition of presumptive positive": For each laboratory tray: all infants with T4 levels below 9 μg/dL plus the lowest 5% of those remaining infants immediately above 9 μg/dL*; and *"Age at screen was included in the logistic model, but was not significantly associated with congenital hypothyroidism (and was therefore removed from the model)."* One other correction to the 2002 ERD description should read as follows on Page 4-39, lines 17-18: *The study with the strongest findings, Schwartz (2001), included all newborns, not just those whose blood samples were collected during the first two days after birth although 88.1% of the study sample did have their blood sampled during the first two days after birth.*

3.2.1 Revised EPA Analysis of Crump et al. (2000)

This section will be used to augment text in the 2002 ERD found on Pages 4-7 through 4-8 in the 2002 ERD that discusses the findings of Crump et al. (2000). This new text reflects additional evaluation of the original study design and methods employed by Crump et al. (2000) as well as additional analyses performed by the Agency to explore the potential for confounders in response to recommendations made by the 2002 peer review panel. The existing text in Section 4.1.1 of the 2002 ERD (Ecological Studies) will be separated into subsections devoted to general health mortality and health assessments (Section 4.1.1.1), general population studies (Section 4.1.1.2), and screening studies of children or newborns (Section 4.1.1.3). Individual studies will then be described separately so that the discussion of the Crump study becomes Section 4.1.1.3.1. The EPA specifically aimed to determine the usefulness of this study for a dose-response derivation for perchlorate applicable to children or the general public. Details discussed in this summary section are found in Marcus (2003a).

Crump et al. (2000) describes an ecological epidemiologic study of neonates and school-age children in three communities in Chile with different degrees of contamination by perchlorate in their drinking water. The 2002 ERD concluded that the questions left unresolved by Crump et al. (2000) seriously limited the ability of the Agency to use the study to inform a dose-response relationship applicable to the general U.S. population or to susceptible sub-populations of infants and children. As part of the review process, the Agency received several stakeholder comments which suggested that the study was informative to consideration of dose-response of perchlorate effects in children. Based on suggestions presented at the peer review, the panel recommended that EPA re-evaluate Crump et al. (2000). The purpose of this subsequent review is to further evaluate the authors original analyses and to explore issues of potential confounding that were recommended by the EPA peer review panel report in 2002 (U.S. Environmental Protection Agency, 2002b).

As described above, Crump et al. (2000) describes two different epidemiologic samples: (1) 163 school-aged children (ages 7 to 8 years) with responses to a parental questionnaire regarding town of birth, residence, maternal and neonatal health history and individual measurements of clinical chemistry, thyroid hormone (triiodothyronine, T3; thyroxine, T4; and free thyroxine, fT4), and thyroid stimulating hormone (TSH) and (2) 11,967 neonates whose

TSH levels were measured as part of the Chilean neonatal thyroid screening program, about 75 percent of them at neonatal age 3 days.

The 2003 EPA reanalyses of the methods and results generally confirmed the Crump et al. (2000) findings as reported. However, the 2003 EPA reanalyses produced additional findings and raised additional questions regarding the appropriateness of the limited study population, the experimental design, and statistical analyses. These concerns can be grouped according to issues regarding the data and analyses as follows:

(1) that the high background incidence of goiter and family history of thyroid disease in the three cities (both schoolchildren and neonatal infants) may confound a study of the effects of perchlorate;
(2) the adequacy of the exposure characterization;
(3) the comparability of personal demographic and general health / nutritional characteristics; and
(4) the sample size and variability of serum hormone analyses.

Each of these issues is summarized in the following sections, and detailed discussion can be found in the accompanying technical memorandum, Marcus (2003a).

3.2.1.1 New Section 4.1.1.3.1.1: Concerns Regarding Thyroid Disease Status in the Study Population

The biggest concern for confounding and thus the appropriateness of this population for any inference regarding dose-response to historical perchlorate exposures is the high prevalence of goiter and family history of thyroid disease in the control population as well as in the cities used to represent exposed populations. This concern was raised by the 2002 external peer review panel (US Environmental Protection Agency, 2002a). Figure 3-1 shows the prevalence of goiter and family history of thyroid disease reported in Crump et al. (2002) for all school children and for school children with lifelong residence in each city.

The prevalence of goiter in the population in the city used as the control (22.2%) as well as in those cities used to represent the exposed population (19.5 and 26% in Chañaral and Taltal, respectively) is much greater than estimates of goiter rates in U.S. children which range from 1 to 8% (Xu et al., 1999; Hollingsworth et al., 1977; Trowbridge et al., 1975). The prevalence in each is also greater than both the 4% reported for the thyroid-disease-free general U.S.

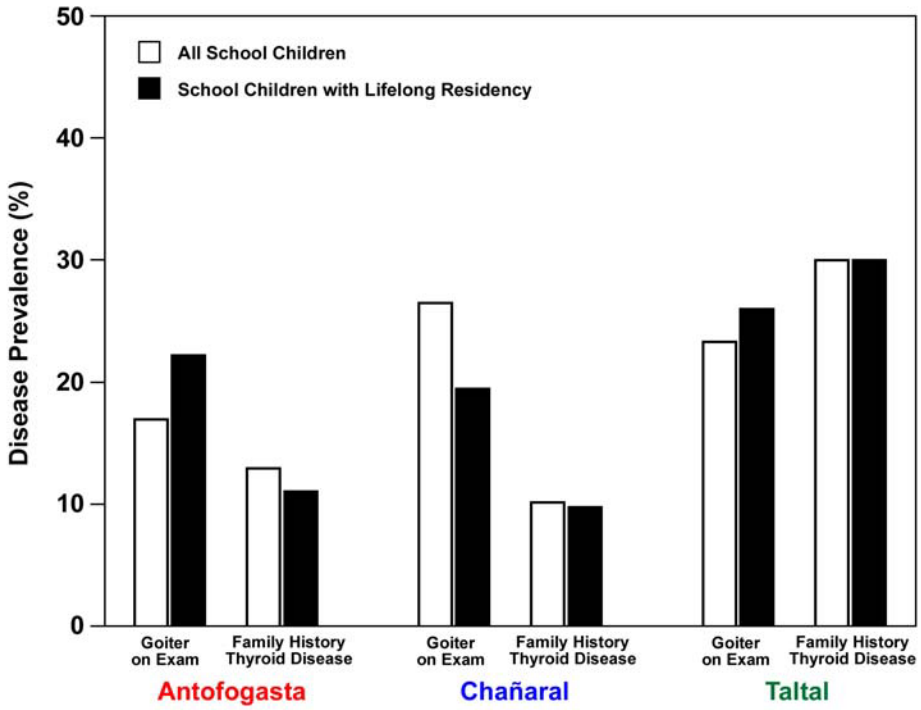

Figure 3-1. New Figure 4-2. Prevalence of thyroid disease in schoolchildren of study population in Crump et al. (2000). Samples were stratified as either "all" of the schoolchildren or those with a "lifelong residence in their respective city." Goiter was classified with exam based on standardized World Health Organization criteria (DeMaeyer et al., 1979). Family history of thyroid disease was determined by take-home questionnaire completed by the parents and based on report of direct relatives with history of goiter, hypothyroidism or subtotal thyroidectomy.

population or the 15% reported for the U.S. population self-reporting thyroid disease (including goiter) for the years 1988 to 1994 (Hollowell et al., 2002). These levels are indicative of an abnormal thyroid status in the study population used by Crump et al. (2000). Indeed, if an area has a goiter rate > 5% in children aged 6 to 12 years, it is classified as endemic with respect to goiter (World Health Organization - United Nations International Childrens Education Foundation - International Council for the Control of Iodine Deficiency Disorder, 1994). The high prevalence of goiter in the population is particularly puzzling and of concern given that the region has been established to be in iodine excess (Iodine Deficiency Disorder Prevalence and Control Program Data, 2002).

The concern that the high prevalence of goiter is indicative of some degree of abnormal thyroid status is exacerbated by the high levels of TSH in the population when compared to levels reported for the US population in 1988 to 1994 (Hollowell et al., 2002), whereas the total T4 in the population is more comparable. The mean ± SD for the TSH level in the control city of Antofagasta (3.1 ± 1.2 μU/mL) is more than twice as high than that reported (mean ± SE) by Hollowell et al. (2002) for the general thyroid-disease free US population (1.4 ± 0.02 μU/mL) or US population with risk factors (1.71 ± 0.05 μU/mL) in the years 1988 to 1994.

There is also a high degree of variability in the TSH measurements. Table 3-1 (new Table 4-2 in the revised final document) shows a coefficient of variation (CV, calculated as mean divided by the standard deviation) of 39 to 55% for this hormone analysis. This variability is particularly troubling in light of the lab error that resulted in a number of TSH measurements over a 7-month period being excluded (Crump et al., 2000). The 2002 expert peer review panel raised a concern over the need to provide for normalization of the radioimmunoassay (RIA) kits against a calibration curve. This concern is compounded by the fact that the data in this study represent a single blood draw for hormone analyses in each individual for each population. No QA/QC information or record of the sampling is provided for the hormone or for any other clinical chemistry parameter. The variability due to timing of the hormone sample is discussed in greater detail in Marcus (2003a) in the section on the 2003 secondary analyses performed by EPA. These analyses show there were statistically significant differences in the relationship of TSH to city, sex, and postnatal age in the neonatal sample and suggests that a statistical model with a city-sex-age interactions model may be needed in addition to the main effects model presented in Crump et al. (2000) that used only city, sex and neonatal age as covariates. This is discussed in greater detail in the section below on new EPA analyses.

Thus, given that Crump et al. (2000) represents an evaluation of thyroid hormone economy in a population exposed historically for an undetermined period of time and whose exposure is characterized by only one set of contemporary water supply samples, these findings call in to question the reliability of any inference drawn from these data regarding dose-response in the individuals of these populations. The elevations in goiter and TSH in this population suggests that compensatory mechanisms to thyroid stressors in the environment may have long been operative. Further, an area with unexpectedly high urinary iodine is not a good location in which to study the effects of perchlorate because its anti-thyroid mode of action is to block iodide

Table 3-1. New Table 4-2. Coefficients of Variation[1] (%) For School Children Samples in Each City from Crump et al. (2000)

Measurement	Sample of Children Ages 6-8 Years	Coefficient of Variation (%)		
		Antofagasta	Chañaral	Taltal
TSH	All in sample	54	45	47
	Lifelong residents	39	45	43
T4	All in sample	14	14	13
	Lifelong residents	16	13	12
Free T4	All in sample	8	8	14
	Lifelong residents	8	8	7
T3	All in sample	14	10	14
	Lifelong residents	15	10	10
Urine Iodine	All in sample	53	58	62
	Lifelong residents	50	56	64
Urine Iodine per Unit Creatinine	All in sample	36	43	38
	Lifelong residents	37	44	38

[1]Calculated as: mean / standard deviation.

uptake. A high background iodine status indicated by the elevated urinary iodine concentration in this population may mask any effects of perchlorate. Additional support that the Crump et al. (2000) study population had abnormally high urinary iodine values comes from a recent reanalysis of the third National Health and Nutrition Examination Survey (NHANES III) data (Soldin et al., 2003). Soldin et al. (2003) estimated geometric mean values ± standard deviation (95% confidence intervals) of urinary iodine (µg/dL) UI per creatinine ratio (UI/Cr) for 6 to 9 year-old males and females to be 25.20 ± 0.56 µg/dL (24.10 - 26.34) and 20 ± 0.52 µg/dL (19.30-21.39) µg/dL, respectively. The mean values for this same parameter in each of the cities studied by Crump et al. (2000) are at least more than 30-fold higher than those reported by Soldin et al. (2003).

3.2.1.2 New Section 4.1.1.3.1.2: Concerns Regarding Adequacy of Exposure Characterization

Crump et al. (2000) describes an epidemiological assessment of neonates and school-age children in three communities in Chile with different degrees of contamination by perchlorate levels in their drinking water:

(1) Antofagasta, a large city (population about 150,000) with non-detectable levels of perchlorate;

(2) Chañaral, a much smaller city (population about 20,.000) with concentrations 5 to 7 µg/L; and

(3) Taltal, a much smaller city (population about 15,000) with concentrations 100 to 120 µg/L.

Larger cities would be expected to generate more precise estimates of differences among cities in general unless the variability is increased in larger cities due to greater heterogeneity in demographics and exposure. However, as shown in Table 3-2 (Table 4-3 in the revised final document), the largest city (Antofagasta) with all sample values for perchlorate below detection limit (BDL), had the greatest CV in infant TSH. This suggests other sources of variability need to be considered. An expanded discussion of potential determinants of diversity is provided in Marcus (2003a). These issues are compounded by concerns regarding the adequacy of the limited number of samples and the arbitrary assignment of a constant value to samples below the analytical detection limit. A summary of these concerns are listed in the following sections, and a more detailed discussion can be found in Marcus (2003a).

3.2.1.2.1 New Section 4.1.1.3.1.2.1: Limited Exposure Sampling and Use of Indicator Variable

Individual exposure values were not measured in Crump et al. (2000). Community-wide mean concentrations were estimated from 25 separate samples of sources in each city and were the *de facto* units of exposure for analysis to ascertain any dose-response relationships among parameters assayed. Only the city indicator was used in the dose-response analysis, without further refinement of quantification through the use of separate sample measurements for each city. There was no indication in the paper that replicates of each sample were performed.

The use of indicator variables is based on a hypothesis that "city" is a satisfactory surrogate for between-city differences in perchlorate exposure alone and that no other factors affecting

Table 3-2. New Table 4-3. Coefficients of Variation[1] (%) for TSH in Neonatal and School Children Samples in Each City of Crump et al. (2000)

		Coefficient of Variation (%)		
Group	**Sub-Group**	**Antofagasta**	**Chañaral**	**Taltal**
School Children	All in sample	55	45	47
	Lifelong residents	39	45	43
Neonates by sex	Female	231	85	79
	Male	169	79	84
	Both	229	83	77
Neonates by postnatal age	Age 1-2 days	59	109	29
	Age 3 days	189	71	73
	Age 4 days	133*	84	86
	Age 5 days	70	90	77
	Age 6 days	79	81	77
	Age $\geq$ 7 days	83	67	81

[1]Calculated as: mean / standard deviation.
* Assuming the value of 14.4 in Table 8 of Crump et al. (2000) is correct and not an outlier or misprint of 4.4, the coefficient of variation in this cell could be 14.4 / 3.3 = 436%.

thyroid status differ among cities. If this assumption is not correct, then presumed perchlorate effects (or lack of effects) may be attributable to those other factors, and the analysis of perchlorate effects may be confounded. Concerns about confounding are summarized in Section 3.2.1.3 (New Section 4.1.1.3.1.3 in the ERD) which addresses the comparability of demographic and general health characteristics. A more detailed discussion can be found in Marcus (2003a) in the section on additional new 2003 EPA analyses.

3.2.1.2.2 New Section 4.1.1.3.1.2.2: Arbitrary Assignment of Values for Concentrations Below Detection Limits

The arbitrary assignment of a constant value to non-detects (Below Detection Limit or BDL) in Chañaral by Crump et al. (2000) potentially biases the estimated mean and standard deviation of the drinking water concentrations. Superior statistical methods for dealing with non-detects are widely available in the literature on water quality assessment (e.g., Gibbons

et al., 1994; Gilbert, 1987; Lambert et al., 1991; U.S. Environmental Protection Agency, 2000), but were not incorporated in Crump et al. (2000). These methods typically combine the fraction of BDL or "left-censored" data with the above-BDL data to estimate the probability distribution of all the data, log-normal or Weibull for example.

To illustrate the potential bias, suppose all four BDL (16% of the sample) are 2 μg/L as the authors assume. Then the mean of the other 21 non-BDL concentrations is $(25 \times 5.5 - 4 \times 2)/21 = 6.17$ μg/L. If the true BDL values are 0 instead of 2 μg/L, the true mean (averaging the above-BDL and below-BDL values) is $(21 \times 6.17 + 4 \times 0)/25 = 5.18$ μg/L. The treatment of 100% BDL in Antofagasta (all below 4 μg/L) poses another challenge. One alternative is to assume that all BDL values in Antofagasta are derived from a distribution of perchlorate concentration with almost no probability of being greater than 4 μg/L. On the other hand, if the true BDL values were closer to 4 μg/L then the difference between Antofagasta and Chañaral becomes less as does the power to detect an effect due to perchlorate.

It might be reasonable to assume that the distribution of perchlorate concentrations in groundwater at Chañaral and Taltal is log-normal with the geometric mean (GM) and geometric standard deviation (GSD) estimated from the complete left-censored data set. If the geometric standard deviations of Antofagasta and Chañaral are not significantly different, one might assume that perchlorate exposures at Antofagasta were also log-normally distributed with a composite GSD. The GM could be estimated assuming that the largest value corresponds to the 96th (= 24/25), 96.15^{th} (25/26), or 98th (= 24.5/25) etc., percentile of the distribution at Antofagasta. Thus the missing GM and mean at Antofagasta could be estimated and a quantitative concentration or concentration estimate could be used in a quantitative dose-response model in Crump et al. (2000). Consequently, it would then not be necessary to use "City" as a categorical exposure surrogate in Tables 4 through 7 of Crump et al. (2000). While these uncertainties in the calculation of the exposure estimates exist, no attempt was made to account for these uncertainties in the subsequent analyses. No quantitative use of the exposure data was made, and no ranking of cities by estimated exposure status was used in any of the regressions performed by Crump et al. (2000).

3.2.1.2.3 New Section 4.1.1.3.1.2.3: Assumption of Mean Concentration as Accurate Exposure Metric

The assumption that the mean concentration of perchlorate accurately characterizes the exposure in any given community has not been examined. Crump et al. (2000) have apparently accepted the hypothesis that there is no relevant spatial and sociodemographic variability in exposure. Even if the groundwater concentrations and the potable water concentrations were homogeneous in space and time, human exposure may be affected by a number of economic and demographic factors that are heterogeneous in space and time. The heterogeneity not only includes water sources, but may also include consumption of food products grown locally or reconstituted using local water supplies. While the authors could have provided data from which quantitative dose-response relationships for perchlorate exposure might have been calculated, only the city identifier was used as a surrogate variable for perchlorate exposure. Analyses performed by EPA in response to recommendations to evaluate the potential for covariates described in the next section cast considerable doubt on the validity of this assumption regarding the mean as well as the use of city as a categorical dose indicator.

3.2.1.3 New Section 4.1.1.3.1.3: Concerns Regarding the Comparability of Personal Demographic and General Health / Nutritional Characteristics

As discussed by the 2002 peer review panel, a basic concern in semi-ecological epidemiological studies is the possibility that unobserved personal covariates (i.e., potential confounders) could be responsible for the observed effects based on grouped exposure data.

The sample of schoolchildren in Crump et al. (2000) had a number of similarities across all three cities:

- parents mogeneity of variance across dose groups ("homoscedasticity") predominantly of Hispanic ethnicity,
- similar socioeconomic characteristics (not specified), and
- age 6 to 8 years.

Potential confounders include nutrition and diet, access to medical care, behaviors that affect the daily intake of drinking water, breast-feeding or lactation for neonates, and ethnicity. Other covariates available in the survey were child's contact information, date and town of birth, residence history, mother's residence for the year preceding the child's birth, history of breast feeding, nutrition during the first 6 months of life, current child medications, parents'

occupations, and family history of thyroid disease. However, only residence history, age, sex, and urine iodine were used as covariates in the analyses by Crump et al. (2000) as presented in Tables 4, 5, 6 and 7, to adjust the estimated effects on hormone levels. Notably absent from these analyses were the use of the measured perchlorate levels or lactation information.

The detailed discussion in Marcus (2003a) emphasizes that considerable heterogeneity and unexpected relationships exist across cities for the various clinical chemistry parameters and key indicators of iodine status suggesting that effects on the endocrine system of perchlorate in groundwater may be confounded with other factors affecting these indicators. The Agency is concerned with the lack of logical consistency in related indicators that would serve to reinforce or verify relationships attributed to perchlorate exposure. In many cases, the rank ordering of the cities is different from what have been expected from the rank ordering of mean perchlorate concentrations in groundwater, either Antofagasta < Chañaral < Taltal or Antofagasta > Chañaral > Taltal, depending on the indicator. As discussed above, the selection of Antofagasta as a reference city and the use of a city as a dose indicator based on a mean value may need to be reconsidered.

Close scrutiny of Crump et al. (2000) reveals that there are also significant differences in demographic characteristics, as in some cases the relationship between goiter and a history of family thyroid disease and TSH, free T4. Additionally, urine iodine elimination appears to differ in the sample of all school children (N = 163) versus the subsample of lifelong resident children.

To address the issue of heterogeneity and the potential for confounding, the Agency undertook secondary analyses of the schoolchildren data with twelve variables selected in advance of carrying out the reanalyses: (a) four response variables that indicate thyroid status: T3, T4, fT4, and TSH; (b) two demographic variables: age and weight; (c) four blood chemistry variables: aspartate aminotransferase, lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and glucose; and (d) two indicators of urinary iodine elimination: urine iodine concentration and urine iodine excretion normalized to creatinine clearance.

Many statistically-significant differences between cities were found using parametric t-tests and ANOVA with the different cities used as main effect surrogate for exposure. The issue of potential confounding may be even more serious for the neonates than for the school children. Apart from gender and postnatal age, the analyses performed by Crump et al. (2000) did not

attempt to address other factors that may affect TSH levels such as differences in fetal and postnatal exposure (e.g., lactation in addition to drinking water).

Age-wise ANOVA tests performed by the EPA on the neonatal data showed no significant differences in mean TSH in infants. However, unadjusted pairwise t-tests show that at an age of 3 days, there is a highly significant difference between mean TSH in Antofagasta and Chañaral and a less significant difference between mean TSH in Antofagasta and Taltal. At an age of 4 days, unadjusted pairwise t-tests show that there is a highly significant difference between mean TSH in Antofagasta and Taltal and a less significant difference between mean TSH in Chañaral and Taltal. There is no difference between any of the cities at an age of 7+ days. If it is decided that other experimental design deficiencies do not prevent further evaluation of the data, it might be worth reanalyzing the TSH data for neonates and infants with interactions between city, age, and sex.

3.2.1.4 New Section 4.1.1.3.1.4: Concerns Regarding the Sample Size and Variability of Serum Hormone Analyses

The question of sample size is always a concern in observational epidemiological studies. Figure 2 in Crump et al. (2000) shows that neonatal TSH in Antofagasta is much more variable than in the two smaller cities. A number of temporal and spatial factors discussed in Marcus (2003a) may be responsible for this difference. Potential confounding with other variables in the three cities therefore seems possible and is indeed likely. With respect to the hormone analyses, Crump et al. (2000) discuss statistical power on Page 607, but leave open a number of questions.

(1) Was the power analysis done before the study or afterward?

(2) If done prospectively, as it should have been, how was the median effect size selected for a prospective power analysis?

(3) Was this based on a pilot study?

The median TSH concentration for euthyroid adults with low perchlorate dose reported in Greer et al. (2002) was about 2 μU/mL with a standard deviation of about 1 μU/mL. The median value for the group with the highest perchlorate dose in Greer et al. (2002), whose radioactive iodine uptake was inhibited to about 40% of the baseline value, had about the same median and standard deviation for TSH as the low-dose group. The design value for power

calculations on Page 607 in Crump et al. (2000) was an effect size of 1 μU/mL, larger than the largest inter-city difference observed post-study (0.3 to 0.4 μU/mL).

From a clinical perspective a difference of 1 μU/mL may seem to be small for an individual given the inherent variability in TSH measurement at any point in time. However, if this difference in the mean values represents a shift in population distribution curves, then there may be an important shift in the number of individuals in the tails of the distribution. This may result in many more children above or below levels that would be expected in unexposed populations. In general, population-based reference ranges are of limited value for the interpretation of measurements in the individual if variation within individuals is small compared with variation between individuals. Accordingly, a test result within laboratory reference limits is not necessarily normal for an individual. Andersen et al. (2002) found that the individual reference ranges for serum T4 and T3 to be about half of the width of population-based reference ranges. Evidenced for this concern is confirmed by careful analysis of the Greer et al. (2002) data — those individuals with high TSH or low T4 with respect to the other subjects tended to remain at the ends of distribution across the 14-day study (Marcus, 2003c). Without more information on the reliability of the RIA kits and laboratory performance standards it is difficult to accurately determine potential consequences. Thus, another question raised is:

(4) Why did the authors select this larger TSH difference and use an excessively small sample for evaluation?

Likewise, the median fT4 concentration for euthyroid adults with low perchlorate dose reported in Greer et al. (2002) was about 1.2 ng/mL with a standard deviation of about 0.15 ng/mL. The effect size evaluated by the authors was 0.5 ng/dL. The median value for the group with the highest perchlorate dose in Greer et al. (2002), whose radio-active iodine uptake was inhibited to about 40% of the baseline value, had about the same median and standard deviation as the low-dose group. These considerations raise the following additional questions:

(5) Why did the authors select the larger fT4 difference for evaluation? Perhaps the largest effects of perchlorate occur during prenatal and early postnatal periods and are not easily detected by TSH levels in older humans or even in schoolchildren.

(6) If the authors were looking for differences for effects in human children, should they have determined whether a much smaller effect could have been detected with a much larger sample size?

The sample size for the school children samples may be too small to permit detection of real differences of small magnitude given that the ages only ranged from 6 to 8 years; whereas for neonates, much larger differences in sample size and city effect allow identification of differences in TSH at postnatal ages 3 and 4 days. As above, the EPA analyses suggest that more complicated higher-order models may be needed to adequately characterize multivariate relationships among outcomes and covariates for neonates as well as for school children.

Based on the data presented in Table 8 of Crump et al. (2000), the numbers of neonates in separate age groups in Chañaral and Taltal are very small for comparisons among the three cities except in age groups 3, 4, and 7+ days. Assuming that the age groups are distributed in the same proportion across each city, given the total number in the left column of Table 8, the only ages with at least 40 children in each city are ages 3 days (estimated n = 6367 in Antofagasta, estimated n = 335 in Chañaral, and estimated n = 307 in Taltal), age 4 days ((estimated n = 1157 in Antofagasta, estimated n = 61 in Chañaral, and estimated n = 56 in Taltal), and age 7+ days (estimated n = 848 in Antofagasta, estimated n = 45 in Chañaral, and estimated n = 41 in Taltal). All other age groups in the two smaller cities have fewer than 15 subjects.

The imputation scheme used here is the most favorable for providing a *post-hoc* design that minimizes the potential for age-gender-city confounding. Other sample sizes may introduce biases into the analyses. For example, suppose there are only n = 5 observations in Chañaral and Taltal at age 3 days, and that the n = 6914 in Antofagasta: Antofagasta would dominate the age 3- days estimates, but the uncertainty in the estimated means for the smaller cities would be larger. Conversely, suppose that Chañaral and Taltal each have n = 30 subjects on days 1-2, and Antofagasta only has 3: the smaller cities would dominate the results in the youngest infants. Additional justification is needed for the statement on Page 609 of Crump et al. (2000) that *"The small amount of confounding identified in these analyses was almost entirely owing to [neonatal] age at time of screening. Sex and age were both significant predictors of [neonatal] TSH."* At the very least, a more complex statistical model as suggested below is required to describe the relationship.

3.2.1.5 New Section 4.1.1.3.1.5: EPA Conclusions Regarding Reanalysis of Crump et al. (2000)

The Agency undertook a second and more thorough evaluation of the original analyses in Crump et al. (2000) and then performed new analyses in response to recommendations of the

2002 external peer review panel to explore issues of potential confounding (US EPA, 2002b). The EPA evaluation generally confirmed the original Crump et al. (2000) findings as reported. However, the new EPA reanalyses performed in 2003 using the published data (secondary analyses) produced additional findings and raised additional questions regarding the appropriateness of the limited study population, the experimental design, and statistical analyses. Findings of the 2003 EPA analyses suggest the following:

(1) The high background incidence of goiter and thyroid disease and elevated urinary iodine excretion levels in the three cities suggests that the entire study population and the location are inappropriate to study the effects of perchlorate.

(2) Exposure to perchlorate in these cities may not be adequately characterized by the mean of the limited number of samples for groundwater concentration of perchlorate; and the arbitrary assignment of a constant value to concentrations below the analytical detection limit may have introduced bias.

(3) There may exist potential confounding between outcome variables and uncontrolled covariates (e.g., demographics, nutrition) due to the use of "City" as a surrogate for perchlorate exposure.

(4) The sample size for the school children samples (36 to 60 per city) may be too small to allow for detection of real TSH differences of small magnitude, especially in view of the moderately large intrinsic heterogeneity of the TSH concentrations among children.

(5) The neonatal samples (ages 1 to 7+ days) were much larger than in the school children study, with 428 neonates in Taltal, 468 in Chañaral, and 8888 in Antofagasta. Even though the TSH levels are very heterogeneous, the relatively large sample sizes allowed identification of differences in mean TSH among the cities at postnatal ages of 3 and 4 days. The EPA analyses suggest that if the population were deemed appropriate for the study of perchlorate effects, more complicated higher-order models may be needed to adequately characterize multivariate relationships (confounding) among TSH, gender, and postnatal age for neonates.

Based on these concerns, the EPA once again concludes that the findings of the observational (ecological) epidemiological study of Crump et al. (2000) are of limited use to informing the dose-response function of perchlorate effects for Chilean children and much less so for generalization to the U.S. public. Critical design deficiencies include the disease status of the study population, the adequacy of the exposure characterization, the lack of control for potential covariates (notably of neonatal age), and a small sample size used for evaluation of effects on serum hormones. Further, the study did not evaluate any neurodevelopmental outcome measures which are the fundamental concern. It is especially notable that recent studies have shown that subtle changes in the serum thyroid hormone T4 alone (hypothyroxinemia) are associated with neurodevelopmental deficits (Haddow et al., 1999; Pop et al., 1999; Morreale de Escobar, 2000; Lavado-Autric et al., 2003).

One of the documents submitted by to the PSG was a protocol for a study in pregnant women from this Chilean population that is apparently underway (Tellez, n.d.). Based on a preliminary review of the protocol, it is evident that a number of the same concerns enumerated in the preceding section would have to be addressed, most notably the apparent thyroid disease status and elevated iodine status, as well as provisions made for determination of neurodevelopmental indices in order for this new study to be particularly informative.

3.3 COMMENTS ON THE EPA REVIEW OF THE HUMAN DOSING AND CLINICAL DATA

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding the use of human dosing and clinical data that can be found in the Executive Summary, Chapter 3 (Human Health Effects Data), and Chapter 9 (General Comments, Conclusions and Recommendations) of the 2002 peer review report (US EPA, 2002b). Specific pre- and post-meeting comments by those panel members assigned to this topic (Drs. Carrasco, Cox, Hoel, Razzaghi and Zoeller) have also been considered. Comments received from the public and other stakeholders are also included in this evaluation. Discussion of the use of the human data as the basis of the point of departure for the RfD derivation is found in Chapter 7 (Dose-Response Assessment for Human Health) of this response document.

***Comment(s)**: The panel provided a mixed review of the controlled human studies. One reviewer stated that the EPA treatment of the studies by Lawrence et al. (2000, 2001), Greer et al. (2000) and Merrill (2001a) appeared thorough and reasonable and that the interpretations were clear and the conclusions warranted. Another reviewer agreed that the EPA provided a good summary of the available studies, but stated that the studies were not sufficiently informative to warrant reliable conclusions. The third reviewer felt that Greer et al. (2002) was the most relevant published since 1999 and encouraged revision of the EPA document now that the detail of the published manuscript was made available. Another reviewer noted that most human studies with exposure durations of 2 weeks or less (e.g., Greer et al., 2000, 2002; Lawrence et al., 2000, 2001) would not likely identify toxic effects that may occur due to long exposure durations because most humans can continue to produce thyroid hormones for weeks following iodide uptake inhibition. Additional specific comments on the individual studies are provided below.*

EPA Response(s) and Recommendation(s) for Revision(s): No additional response is necessary to these comments that is not already provided in the sections that address specific issues below.

***Comment(s): On Lawrence et al. (2000, 2001)**. The "Lawrence study" is a clinical (human dosing) study documented in an article (Lawrence et al., 2000) and a letter to the editor that describes a different dose tested later (Lawrence et al., 2001). Like Greer et al. (2002), perchlorate was administered in drinking water for 14 days to euthryoid adults. The study considered nine subjects and reported radioactive iodide uptake (RAIU), circulating thyroid hormone levels, urine and serum perchlorate levels, and other parameters at selected days during and after exposure. The discussion leader noted that the results were generally consistent with those of Greer et al. (2002). Like Greer et al. (2002), the Lawrence et al. (2000, 2001) studies suffer 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 et al. (2000, 2001) studies during their deliberations. Regarding Lawrence et al. (2001), one reviewer noted that no neurodevelopmental or neoplastic effects were evaluated. This same reviewer felt that the dose was arbitrarily chosen and that the number of subjects was probably insufficient to detect an effect although no analysis of the power was provided.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees with these comments on the limitations of the Lawrence et al. (2000, 2001) studies. No revision to the EPA analysis of the Lawrence (2000, 2001) provided on Pages 4-20 To 4-23 of the 2002 ERD is recommended. The EPA reiterates its concern about the reliability of these data due to their failure to pass the QA/QC performed by the Air Force Research Laboratory (AFRL) (Merrill, 2001a,b). Further, Lawrence et al. (2001) was not published except as a letter to the editor.

Thus, the EPA will consider these data as ancillary or supporting to the findings of the Greer et al. (2002) study, but not suitable to be relied upon.

Comment(s): On Greer et al. (2000, 2002)*. The discussion leader noted that the Greer study (Greer et al., 2000, 2002 and data in Merrill, 2001a) was generally well conducted and informative, yet the EPA 2002 ERD gave disproportionately greater discussion to the Lawrence et al. (2000, 2001) studies. The discussion leader noted that the Greer study, like the Lawrence studies, suffers from several limitations, including lack of control for potential confounders (most notably dietary intake), small sample size, consideration of only healthy adults, and use of a 2-week exposure duration when human thyroid reserves can generate thyroid hormones for weeks after iodide uptake is inhibited. The other peer panelists expanded on these concerns. One reviewer noted in both oral and written comments that there was insufficient information in this study to assess the reliability of the results. Regarding the design of the Greer study, no information is provided about the subject selection other than gender and having a normal thyroid. The distribution of age and gender in each dosage was not provided. Other missing information included health status, smoking status, weight, ethnicity, etc. No information regarding the choice of dosage was provided. This reviewer questioned whether a randomized block design to help control for confounding factors (such as age, health status, etc.) would have been more appropriate and noted that a regression model on log-dose with three doses in a one-way layout is not an adequate approach to analyze the data. This reviewer also wondered how goodness of fit was evaluated for the model used in the Greer et al. (2002). This reviewer was uncomfortable with the conclusion noting that the design deficits and analysis make a generalization of the findings to the public unreliable. Two other reviewers voiced strong concerns and disagreement regarding the conclusion made by Greer et al. (2002). They questioned whether similar findings would have resulted if more subjects had been studied, if the duration were longer, or if potentially susceptible populations were studied. Concern for variability observed in the baseline levels also raised concern that the study truly supports a no-effect level.*

EPA Response(s) and Recommendation(s) for Revision(s): The 2002 ERD gave more attention to the Lawrence et al. (2000, 2001) studies than to Greer et al. (2002) because the draft manuscript of the latter was not made available until at the peer review workshop. The EPA had done some analyses of the Greer et al. (2000) data that were available in the QA/QC report from the Air Force Research Laboratory (AFRL) (Merrill, 2001a). In the 2002 ERD, a post-hoc power analysis of the usual t-test for the 14-day exposure data was performed using the log transform of the ratio of individual values to the baseline values based on the non-central t-distribution. That analysis showed that the number of subjects tested at the lowest dose (0.007 mg/kg-day) resulted in low power (0.1) with which to detect a difference of the same effect size when compared to the other doses (0.95, 0.998 and 0.999 at the 0.02, 0.1 and 0.5 mg/kg-day

levels). The peer panel was clear in its reservations regarding the use of results from this one human study to generalize to the population at large or to susceptible populations. Nevertheless, the Agency was encouraged to more fully evaluate the published Greer manuscript.

The EPA fully evaluated the model employed by Greer et al. (2002). The EPA also undertook analyses to further evaluate specific concerns (e.g., duration of the study and statistical model structure) to ascertain if any additional insights could be gained. The EPA used alternative statistical models to analyze these data using a "benchmark dose" approach that allowed calculation of true confidence limits on the model parameters, test of the model fit to the data, and which is a more acceptable approach to dose-response analysis in the regulatory arena. All models were evaluated for goodness of fit to the data. The new analyses and conclusions are described in the text provided as Section 3.3.1. This text will replace the discussion in the 2002 ERD found on Pages 4-23 to 4-24. As in the section on ecological studies, the Section 4.2 (Studies in Healthy Human Subjects) will be separated into separate subsections describing each study. Thus, this revised description for the Greer et al. (2000, 2002) study becomes Section 4.2.1.3 following the existing description in the 2002 ERD of Burgi et al. (1974) which becomes Section 4.2.1.1 and Lawrence et al. (2000, 2001) which becomes Section 4.2.1.2.

3.3.1 New Section 4.2.1.3: Revised EPA Analysis of the Greer et al. (2000, 2002) Study

A third study of RAIU in healthy euthyroid subjects was reported by Greer et al.(2000, 2002). The data from this study underwent QA/QC by the Air Force Research Laboratory (AFRL) and were used to help develop the PBPK model that describes the disposition of ingested perchlorate in humans (Merrill, 2001a,c).

The data presented in the QA/QC report and in an abstract by Greer et al. (2000) were analyzed by the EPA in its 2002 ERD. In the 2002 ERD, a post-hoc power analysis of the usual t-test for the 14-day exposure data was performed using the log transform of the ratio of individual values to the baseline values based on the non-central t-distribution. That analysis showed that the number of subjects tested at the lowest dose (0.007 mg/kg-day) resulted in low power (0.1) with which to detect a difference of the same effect size when compared to the other doses (0.95, 0.998 and 0.999 at the 0.02, 0.1 and 0.5 mg/kg-day levels). This section provides an evaluation of Greer et al. (2002) and a description of the new analyses that the EPA has

performed in order to address the peer panel recommendations and other comments made in the 2002 peer review process.

The design and performance of the study will first be described; an evaluation of the linear-log regression model used in Greer et al. (2002) will then be discussed. Finally, additional analyses performed by the EPA with different models to address peer-panel recommendations will then be presented. The specific recommendations by the 2002 peer review panel addressed in the new EPA analyses include (1) exploration of the influence of any apparent outlier(s); (2) use of a more sophisticated and alternative model structure that allows for evaluation of the goodness of fit of the model to the data; (3) calculation of confidence intervals to attempt to address variability in the data; (4) evaluation of the influence of age and gender with a randomized block design; and (5) evaluation of the influence of duration of the study exposure on resultant estimates of doses that effect RAIU. An additional objective of the new EPA analyses was to verify the results reported by Greer et al. (2002). These analyses are described in greater detail in Marcus (2003b).

3.3.1.1 New Section 4.2.1.3.1: Greer et al. (2002) Study Design

Greer et al. (2002) performed a study in healthy human subjects at the Oregon Health and Science University. A consent form, approved by the university's institutional review board (IRB) informed the volunteers of the study's sponsor and explained the purposes, risks, discomforts, benefits, and alternatives. The consent form was signed at the screening visit and witnessed by the principal investigator and another witness. Volunteers were compensated for their participation.

The screening consisted of a history, a physical examination, blood sampling (for complete blood count [CBC], routine serum chemistry, and serum thyroid function tests) and urine sampling (for routine urinalysis, screening for drug abuse, and pregnancy testing for eligible women). Candidates were excluded based on a history of thyroid disease, recent ingestion of an iodine-containing pharmaceutical, or significant thyroid enlargement. The first twenty-four subjects were tested according to a "main study" protocol at doses of 0.02, 0.1 or 0.5 mg/kg-day. An additional set of thirteen (n = 13) subjects were later exposed in a more limited "uptake study". Six women and one man were tested in this "uptake" study at a new dose (0.007 mg/kg-day), and two additional subjects each were tested at the previous doses. A total of 37 subjects

(16 males and 21 females) were tested in the entire study (both "main study" and "uptake study" combined). The age of the subjects ranged from 18 to 57 years with a mean of 38 (SD ± 12) years. Study size and dosing was based on Lawrence et al. (2000, 2001).

Pharmaceutical-grade potassium perchlorate was dissolved in 0.5 to 1.0 ml of lemon juice to make a slurry that was mixed with spring water to make a stock solution of 50 mg potassium perchlorate per 100 ml. Dosing solutions were prepared by appropriate dilution of the stock solution with spring water to a volume of 400 ml. Subjects were given cups with the 100 ml level marked and instructed to drink 100 ml at each of four time points throughout each day of exposure: 0800, 1200, 1600 and 2000 hours.

An outline of the "main study" protocol used for doses of 0.02, 0.1 or 0.5 mg/kg-day was as follows (exposure is denoted with an "E" and post-exposure recovery period with a "P"):

- Ingestion of ^{123}I at 0900 hours on (1) the baseline visit (BV) one day prior to the experiment; (2) on the second day of exposure (E2), i.e., after one day of perchlorate exposure; (3) on the fourteenth day of exposure (E14), i.e., after 13 days of perchlorate exposure; and (4) on post-exposure day 15 (P15), i.e., 14 days after ceasing perchlorate ingestion.
- RAIU measurement at 1700 hours on the days of ^{123}I ingestion and 0900 hours the following morning.
- A blood draw at the screening visit and a total of 22 blood draws on 11 days throughout the study period of 35 days.
- Collection of 24-hour urine in five pooled collections on 5 days: (1) the day before the BV, and days E1, E2, E8, and P1. Collection of 24-hour pooled urine on three days (E14, P2 and P14).

An outline of the "uptake study" protocol used for the lowest dose (0.007 mg/kg-day) and for two additional subjects at each of the previous doses was as follows:

- Ingestion of ^{123}I at 0900 hours on three days: BV, E14, and P15.
- RAIU measurement at 1700 hours on the days of ^{123}I ingestion and 0900 hours of the following morning for BV and day E14 and at 0900 hours only on the day following ^{123}I ingestion on day P15.
- Blood draw at screening and on days E8 and E14.
- Collection of 24-hour pooled urine on the day before the BV and on day E14.

Assays for serum levels of total T4, free thyroxine (fT4), total T3, and TSH were performed by a contract lab. In the "main study," these assays were performed on blood drawn on 16 occasions: screening visit (SV), BV (0800 hr); E1 (1200 and 1600 hr); E2 (0800, 1200, and 1700 hr); E3 (0900 hr); E4 (0800 and 1200 hr); E8 (0900 hr); E14 (0800, 1200, and 1700 hr); P1 (0900 hr) and P15 (0900 hr). In the "uptake study," these hormones were analyzed only in blood drawn at the SV and on E14 (0800 hr). Serum anti-TPO levels were measured in blood drawn at the SV and on day P15.

A serum chemistry panel (sodium, potassium, calcium chloride, total CO_2, glucose, urea nitrogen, total bilirubin, albumin, TBG, total protein, creatinine, aspartate transaminase, and alkaline phosphatase) and CBC (including differential) was performed on blood samples drawn at the SV and on days E2, E14 and P15. In the "uptake study" these were performed only at the SV. No significant change in any of these parameters was reported.

3.3.1.2 New Section 4.2.1.3.1.1: Greer et al. (2002) Statistical Analyses and Results

This section describes the analyses and results as reported in the Greer et al. (2002) manuscript.

3.3.1.2.1 New Section 4.2.1.3.1.1.1: RAIU Inhibition

Greer et al. (2002) used two major statistical approaches to analyze the results of the RAIU component of the study. For pairwise comparisons with baseline values, a two-tailed t-test for dependent samples and the nonparametric Wilcoxon matched pairs test was used. Table 3-3 presents the summary statistics of the pairwise comparison provided in Greer et al. (2002). Using such a pairwise comparison with baseline, the lowest dose producing no statistically significant inhibition of RAIU was 0.007 mg/kg-day. However, the points made by EPA in the 2002 ERD regarding the limited power of the sample size at this dosage to detect an effect still hold.

Greer et al. (2002) also extrapolated the results from a linear regression model of the relative uptake (time point compared to baseline value) against the logarithm of perchlorate dose to estimate a "true" no-effect level. The regression model used to evaluate the dose-response of RAIU (Y) on perchlorate dose was of the general form

Table 3-3. New Table 4-7. Descriptive Statistics Provided by Greer et al. (2002) for the Inhibition of Radioactive Iodide Uptake (RAIU) at Various Sample Times and Different Days of Exposure

	8-hr uptake (mean ± SE)			24-hr uptake (mean ± SE)		
Dose	**No.**	**Raw (% ^{123}I)**	**Percent of baseline**	**No.**	**Raw (% ^{123}I)**	**Percent of baseline**
0.5 mg/kg-day						
Baseline visit	10	14.1 ± 1.4	–	10	21.6 ± 2.0	–
E2	8	4.4 ± 0.4	31.6 ± 2.9**	8	6.5 ± 0.6	30.6 ± 2.6**
E14	10	4.5 ± 0.5	32.6 ± 3.3**	10	6.9 ± 0.9	32.9 ± 3.8**
P15	8	14.7 ± 1.4	107.7 ± 11.3	10	21.7 ± 2.0	104.6 ± 9.4
0.1 mg/kg-day						
Baseline visit	10	12.8 ± 1.5	–	10	19.9 ± 2.1	–
E2	8	7.7 ± 1.1	59.4 ± 2.0**	8	11.8 ± 1.7	59.2 ± 3.5**
E14	9	7.4 ± 1.3	56.7 ± 5.2**	10	11.0 ± 1.6	55.6 ± 3.9**
P15	8	12.9 ± 1.8	103.5 ± 10.4	10	20.8 ± 2.2	106.6 ± 9.1
0.02 mg/kg-day						
Baseline visit	10	11.8 ± 1.0	–	10	18.4 ± 1.2	–
E2	8	10.2 ± 1.0	83.8 ± 6.3*	8	15.7 ± 1.4	82.8 ± 5.6*
E14	10	9.4 ± 0.7	81.8 ± 4.2**	10	15.2 ± 1.1	83.6 ± 4.1**
P15	8	13.5 ± 1.2	111.7 ± 8.1	10	19.1 ± 1.3	105.3 ± 5.5
0.007 mg/kg-day						
Baseline visit	7	12.6 ± 2.5	–	7	18.1 ± 3.1	–
E14	7	10.6 ± 1.1	93.8 ± 9.0	7	16.5 ± 1.6	98.2 ± 8.3
P15	–	–	–	7	17.3 ± 2.5	100.3 ± 8.4

* $p < 0.05$; ** $p < 0.005$ (pairwise comparison to baseline).
E2 = Exposure Day 2; E14 = Exposure Day 14; P15 = Post-exposure Day 15.

$$Y = \beta_0 + f(DOSE) + \varepsilon \qquad \text{(Equation 3-1)}$$

where ε is a random error term and β_0 is an intercept parameter which is estimated from the data. The amount of information the data provide for fitting the function is limited as there are only 4 doses (0.007, 0.020, 0.100, and 0.500 mg/kg-d). Consequently, only models with $\leq$ 3 parameters can be fitted and tested for goodness of fit.

The regression analysis performed by Greer on the logarithm of dose (D) resulted in the following equations for the 8-hr and 24-hr sample points on exposure day 14 (E14), where RU is the relative uptake of RAIU compared to the corresponding individual baseline value.

$$(RU_8)E_{14} = (-0.337 \pm 0.037)\log_{10}D + (0.229 \pm 0.052) \qquad \text{(Equation 3-2)}$$

$$(RU_{24})E_{14} = (-0.359 \pm 0.034)\log_{10}D + (0.213 \pm 0.048). \qquad \text{(Equation 3-3)}$$

To predict a "no-effect" level, Greer et al. (2002) set the response (RU) in these regression model Equations (3-2) and (3-3) to 1.00 (equivalent to 0 % inhibition of uptake). Using the regression model in this way for the RAIU data at the 8-hr (n = 36) and 24-hr (n = 37) time points resulted in predicted doses of 0.0052 and 0.0064 mg/kg-day. Converting this range with the standard adult default body weight of 70 kg and water consumption of 2 L/day resulted in the range of 180 to 220 ppb (average 200 ppb) for a predicted "no-effect" level.

Greer et al. (2002) used these regression equations to extrapolate below the lower end of the experimental data by setting the linear-log regression to 1.00 (0% inhibition) and assuming linearity. This was an extrapolation outside of the observed range so that the estimate becomes uncertain as does any estimation of uncertainty in the extrapolation. There is no *a priori* justification to assume linearity in the dose-response function as was done for the extrapolation. In addition, Greer et al. (2002) did not calculate true confidence limits. Instead, Greer et al. (2002) set the relative uptake (RU) to various values (e.g., 5%, 10%, 20%) to predict dose estimates. These dose estimates were then inserted into the equations and the resultant values were called "upper confidence limits." This procedure does not properly propagate the uncertainty of using the equation with the given data in arriving at these estimates. Consequently the values estimated are mathematical exercises and not formal statistical

procedures so that they can not be considered true or credible "upper confidence limits". It is also noteworthy that Greer et al. (2002) only calculated upper confidence limits whereas risk assessment is interested in lower limits on the dose estimate. The EPA benchmark approach described in Section 3.3.1.3.1 is considered by the Agency to be a more transparent procedure that explicitly incorporates uncertainty and is designed to calculate confidence intervals.

3.3.1.2.2 New Section 4.2.1.3.1.1.2: Serum Hormones

Analysis of the hormone data (T4, fT4, T3 and TSH) by Greer et al. (2002) was restricted to the "main study" subjects (n = 24) because limited data were available for the "uptake study" subjects. A categorical time of exposure variable (before, during and after perchlorate) and a categorical dose variable (high, middle and low) were used in a two-way ANOVA. This analysis revealed no significant dependence of any serum hormone on the categorical dose variable. A mild decrease, the opposite of what is expected in TSH, was observed during the 2-week exposure at the high (0.5 mg/kg-day) dose.

Analysis of the effect of the time of blood draw on serum hormones was performed with ANOVA separately within each dose group for each time-of-day category. Serum T3 was found to be significantly affected ($p < 0.03$) in subjects of the 0.1 mg/kg-day group, but this was attributed by Greer et al. (2002) to low initial values at the time of the screening visit.

3.3.1.3 New Section 4.2.1.3.2: Revised EPA Analysis of RAIU Data

As discussed above, several concerns were raised at the 2002 external peer review regarding the statistical model used by Greer et al. (2002). In response to these comments, EPA scientists conducted new analyses to arrive at a dose-response estimate for the RAIU inhibition in these subjects. These analyses are described in greater detail in Marcus (2003b).

3.3.1.3.1 New Section 4.2.1.3.2.1: Alternative Model Structure and Benchmark Dose Analyses

Because of the transformation of perchlorate dose by the logarithm in the regression model used by Greer et al. (2002), its biological plausibility is questionable except possibly within a limited range. It can not be used to extrapolate the effect at low doses because the form of the regression equation dictates that uptake becomes infinitely large (inhibition eventually becomes negative) as the perchlorate dose approaches zero. The regression equations in Greer et al.

(2002) imply the opposite behavior for extrapolation of the effect at very high doses, with negative uptake and infinitely large inhibition. The suggestion by the authors that the linear-log regression equation can be linearly extrapolated outside the observed range can not be tested using these data. Thus, while reasonable for interpolation, extrapolation outside the limited range between the observed doses as used to predict the "true no-effect levels" is not recommended. Further, no statistical evaluation of the goodness of fit of the model was performed. Perhaps most importantly, true confidence limits were not calculated.

An alternative model chosen by the EPA for evaluation was the Hill function. The Hill function is a continuous dose-response model available in EPA's widely used Benchmark Dose (BMDS) software (U.S. Environmental Protection Agency, 2002c). This Hill model is the one most often used for evaluation of continuous outcome measures such as RAIU. The benchmark approach has been recommended for dose-response analysis in regulatory risk assessment (Barnes et al., 1995; U.S. Environmental Protection Agency, 1995) because it offers the advantages of using the entire data set to inform the model fit and because it can account for variability in the sample when confidence limits are calculated. The BMDL is routinely used in operational derivation of an RfD or RfC for these reasons.

The general form of the Hill model for a response (RAIU inhibition) is

$$X = \beta_0 - v \times DOSE^n / (k^n + DOSE^n) + \varepsilon \qquad \text{(Equation 3-4)}$$

where: β_0 is the intercept, expected to be 1 as X is a ratio relative to base;
v is the "velocity," with $v > 0$ and $\beta_0 \geq v$ expected;
k is the scale parameter; and
n is the exponent with $n \geq 1$ expected.

It was assumed that the random variability or measurement error, ε, has constant variance. This assumption can be relaxed, but the hypothesis tests in BMDS suggested little reason to do so. BMDS uses likelihood profile methods to estimate the model parameters, the BMD for any specified fractional reduction in the expected response, E{X}, expressed as a percentage, p.

Estimates of the benchmark dose (BMD) and the lower 95% confidence limit on that dose (BMDL) were calculated at a benchmark response level (BMR) of 5% using both absolute and

extra risk forms of the function. This is the conventional BMR for continuous endpoints (U.S. Environmental Protection Agency, 1995, 2002c). The analyses of developmental endpoints by Allen et al. (1994 a,b) and Kavlock et al. (1995) also support 5% as the appropriate response level for these endpoints. This response level is above that associated with the lowest dose (0.007 mg/kg-day) in the Greer et al. (2002) study and less than the approximately 20% seen at the 0.02 mg/kg-day dosage where a statistically significant difference from baseline was evident by pairwise comparisons.

Benchmark dose estimates were calculated for each of the four experimental sample-time conditions:

- Exposure day E2 at (1) 8 hr (denoted sample set 8B2) and (2) 24 hr (denoted sample set 24B2) and
- Exposure day E14 at (3) 8 hr (denoted sample set 8B14) and (4) 24 hr (denoted sample set 24B14).

Tables 3-4 and 3-5 show the results from the BMDS software for absolute and extra risk models, respectively. A discussion of the full data sets versus restriction of outliers is found in the next section. Constraints on the exponent were also explored. Estimates for BMR levels of 1% and 5% can be found in Marcus (2003b).

The more stable estimates of benchmark dose were obtained with the exponent n constrained to be ≥ 1 or to be $\equiv 1$. BMDL estimates ranged from 0.00130 for the 24-hr sample on exposure day 14 (full data set) to 0.01045 (without outliers) for the 8-hr sample on exposure day 2. The lower estimates were consistently shown to be associated with the second sample (24 hr) and longer duration (exposure day 14). A more detailed evaluation of the sample-time and dose-duration dependence is provided in Section 3.3.1.3.3.

3.3.1.3.2 New Section 4.2.1.3.2.2: Evaluation of Outliers and Tests of Goodness of Fit

Members of the 2002 external peer review panel noted that at least one of the observations (Case 47, 24-hr sample on exposure day 14, x =1.61) appeared to be an outlier. The EPA was encouraged to consider this outlier issue when re-evaluating the study. The issue was considered in detail in Marcus (2003b) because the removal of suspected outliers from an analysis is always a matter of concern. Because the BMDS software produces chi-square residuals regarding

Table 3-4. New Table 4-8. Estimates of BMD and BMDL at BMR = 0.05 Absolute Risk for All Four Sample Sets on Thyroid Radioactive Iodide Uptake in Greer et al. (2000, 2002) and Merrill (2001a)

Sample set	BMDL*	n unconstrained		n ≥ 1		n ≡ 1	
		Full data	w/o out.	Full data	w/o out.	Full data	w/o out.
8B2	BMD	0.0073	0.03249	0.0082	0.02789	0.0082	0.01909
	BMDL	0+	NE **	0.0029	0.01045	0.0029	0.01045
24B2	BMD	0.0065	0.02388	0.0059	0.0089	0.0059	0.0087
	BMDL	0+	0+	0.0015	0.0031	0.0015	0.0031
8B14	BMD	0.001	0.01118	0.0054	0.01118	0.0054	0.0072
	BMDL	0+	0+	0.0019	0+	0.0019	0.0027
24B14	BMD	0.01276	0.0079	0.01276	0.0079	0.0045	0.0063
	BMDL	0+	0+	0+	0+	0.0013	0.0024

*mg/kg-day
**Not estimable

Table 3-5. New Table 4-9. Estimates of BMD and BMDL at BMR = 0.05 Extra Risk for All Four Sample Sets on Thyroid Iodide Uptake in Greer et al. (2000, 2002) and Merrill (2001a)

Sample set	BMDL*	n unconstrained		n ≥ 1		n ≡ 1	
		Full data	w/o out.	Full data	w/o out.	Full data	w/o out.
8B2	BMD	0.0058	0.02079	0.0064	0.01811	0.0064	0.0137
	BMDL	0+	0	0.0024	0.0068	0.0024	0.0068
24B2	BMD	0.0055	0.01724	0.0051	0.007	0.0051	0.007
	BMDL	0+	0+	0.0016	0.0026	0.0016	0.0026
8B14	BMD	0.001	0.0077	0.0041	0.0077	0.0041	0.0051
	BMDL	0+	0+	0.0015	0.002	0.0015	0.002
24B14	BMD	0.0097	0.0053	0.0097	0.0053	0.0036	0.0043
	BMDL	0.0012	0+	0.0012	0.0017	0.0012	0.0017

*mg/kg-day

model fit for each observation, these statistics can be used to aid the identification of potential outliers.

The following general criteria were used:

(a) chi-squared residuals > 2.25 in absolute value;

(b) greater emphasis on removing high outliers (X > 1) at low doses as these were judged to be the least plausible and most influential observations;

(c) little emphasis on removing negative outliers at high doses even when the chi-squared residual was less than -2.25; and

(d) homogeneity of variance across dose groups ("homoscedasticity").

To conduct a sensitivity analysis of the Hill model, Equation 3-4 was exercised using the BMDS software and then with different software. To evaluate goodness of fit and the sensitivity of the BMDL estimate to model structure, a completely different and third model, the exponential model that is used routinely to describe biological systems, was also used. These two models were also compared to the linear-log model (Equation 3-1) used by Greer et al. (2002). The SYSTAT statistical package non-linear regression procedure (SYSTAT 10, 2002) using a least squares Gauss-Newton method was used to exercise both the Hill model and the exponential model.

The form of exponential model is

$$X = \beta_0 + \beta_1 \times \exp(-DOSE / \beta_2) + \varepsilon \qquad \text{(Equation 3-5)}$$

with all three unknown parameters (β_0, β_1, β_2) assumed to be positive. Equation 3-5 then has the following useful properties:

(1) The intercept = $\beta_0 + \beta_1$ is positive when DOSE = 0;

(2) The inhibition component = $1 - \exp(-DOSE / \beta_2)$ is 0 when DOSE = 0 and steadily increases with increasing dose;

(3) The estimated thyroid iodine uptake is always finite and positive, unlike the Greer model; and

(4) The model can be easily interpreted as the sum of dose-dependent and dose-independent components as:

$$\underbrace{X = \beta_0 + \beta_1}_{\text{Uptake Dose–independent}} + \underbrace{\beta_1 \times (1 - \exp(-\text{DOSE} / \beta_2))}_{\text{Dose–dependent by perchlorate ingestion}}. \qquad \text{(Equation 3-6)}$$

It is likely that studies with longer duration as well as more numerous dose levels would find that part of the "dose-independent" component found in this study is also dose-dependent, and that a more complicated function than Equations 3-5 or 3-6 must be estimated from the data. Equations 3-5 and 3-6 allow easy estimation of secondary parameters, such as the intercept for no dose (0 mg/kg-day).

The full data models including possible outliers were run first so as to provide some quantitative identification of outliers. Results for outliers are shown in Table 3-6. The most difficult decision was whether to remove the data for Case 41 from sample set 24B14. The EPA decided to remove this observation from the 24B14 analyses for several reasons. Even though the value of X = 1.39 was larger than all but one other ratio in the whole study, excluding both observations 41 and 47 yielded estimates of n = 1.080 which was consistent with most other analyses and a value of β_0 = 0.917 which was consistent with almost all other analyses with outliers removed. Removing only Case 47 but retaining Case 41 yielded the highly unusual estimates of n = 0.495 and β_0 = 1.319, suggesting that Case 41 was also highly influential. Restricting the analyses to n ≥ 1 when Case 47 was removed and Case 41 included produced much more reasonable estimates of β_0= 1.014, BMD = 0.00858 and BMDL = 0.00234 mg/kg-day for absolute risk and BMD = 0.00632 and BMDL = 0.00203 mg/kg-day for extra risk at BMR = 0.05. These estimates for sample set 24B14 are concordant with those in the outlier columns of the other three data sets in Tables 3-3 and 3-4 when n ≥ 1.

Table 3-7 shows that the BMDS goodness-of-fit P value for sample set 24B14 is adequate (P > 0.05) with or without outliers for all of the models shown. The determining factor justifying removal of both Case 47 and Case 41 was the test of the equality of variances of the error term, called "Test 2" in the BMDS output. When all of the data for the 24B14 sample set are included, the hypothesis of equal variances would likely be rejected by most investigators because P = 0.0214 and this is less than 0.05 (see Table 3-7). This result suggests that in sample set 24B14 some of the dose groups are much more highly variable than others. Removing both Cases 47 and 41 produces evidence of homogeneous variances in sample set 24B14 across dose

Table 3-6. New Table 4-10. Suspected Outliers in the Four Sample Sets on Thyroid Iodide Uptake in Greer et al. (2000, 2002) and Merrill (2001a)

Sample set	Case ID	Dose *	X	Chi-squared residual
8B2	8	0.02	1.15	2.83
	10	0.02	1.09	2.28
24B2	47	0.02	1.25	2.71
8B14	3	0.007	1.28	2.3
24B14	41	0.007	1.39	2.21 **
	47	0.02	1.61	3.75

*mg/kg-day
**Determined to be an influential observation and retained as an outlier

Table 3-7. New Table 4-11. Goodness-of-Fit P values (Test 3) for the Hill Model with Sample Sets 8B14 and 24B14 and a Test of the Equality of the Variances Among the Four Dose Groups (Test 2)

	Sample set 8B14		Sample set 24B14	
Model	**Full data**	**w/o outliers**	**Full data**	**w/o outliers**
any β_0, any n	NA	NA	NA	NA
any β_0, $n \geq 1$	NA	NA	NA	NA
any β_0, n = 1	0.6851	0.8642	0.6428	0.9293
any n, $\beta_0 = 1$	0.7333	0.6568	0.8271	0.7123
$n \geq 1$, $\beta_0 = 1$	0.6684	0.3315	0.8271	0.3449
n = 1, $\beta_0 = 1$	0.9123	0.6239	0.8195	0.6402
Test 2 for equal variances among all dose groups (same in all models)	0.1143	0.3505	0.0214	0.8961

groups with P = 0.8961. However, these outliers suggest that other sources of variability or heterogeneity were present.

A visual inspection of the results is indispensable for judging whether any of the observations are outliers with respect to a fitted model. Figure 3-2 shows the fit of the Hill model with and without the two outliers for the 24-hr sample set on exposure day E14.

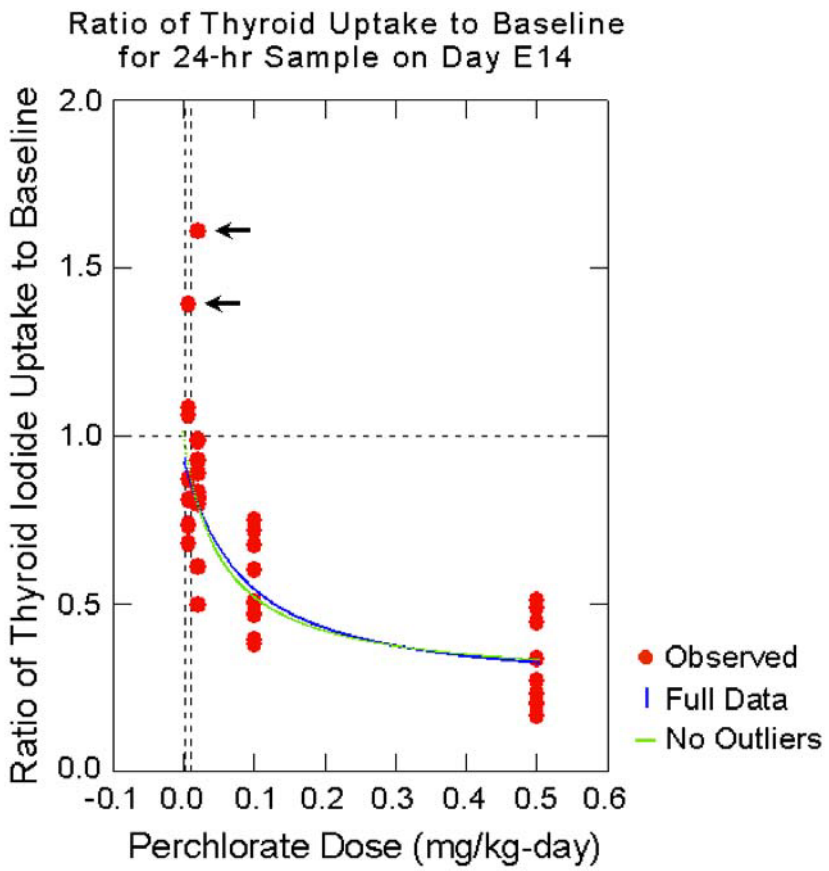

Figure 3-2. New Figure 4-2. Comparison of Hill model fit to the dose-response data for the effect of ingested perchlorate on thyroid radioactive iodide uptake (RAIU) relative to pre-exposure baseline for individuals in the 24-hour sample on exposure day 14. Model fit for both the full sample set or with excluding two outliers (shown by arrows) is illustrated.

The estimates of BMDL for the four data sets using each of the three models (the Hill model exercised with two different software packages and the exponential model) are shown in Figure 3-3. All three models gave reasonably similar results. The Hill function models (using either BMDS or SYSTAT software) were numerically unstable when three or four parameters were estimated for the three or four available dose groups. Numerically stable estimates using BMDS or the nonlinear-regression (SYSTAT) Hill function models were obtained by allowing the two parameters v and k to be estimated from the data and specifying that the power (n) and intercept (β_0) parameters were equal to 1. These were reasonable assumptions in this case

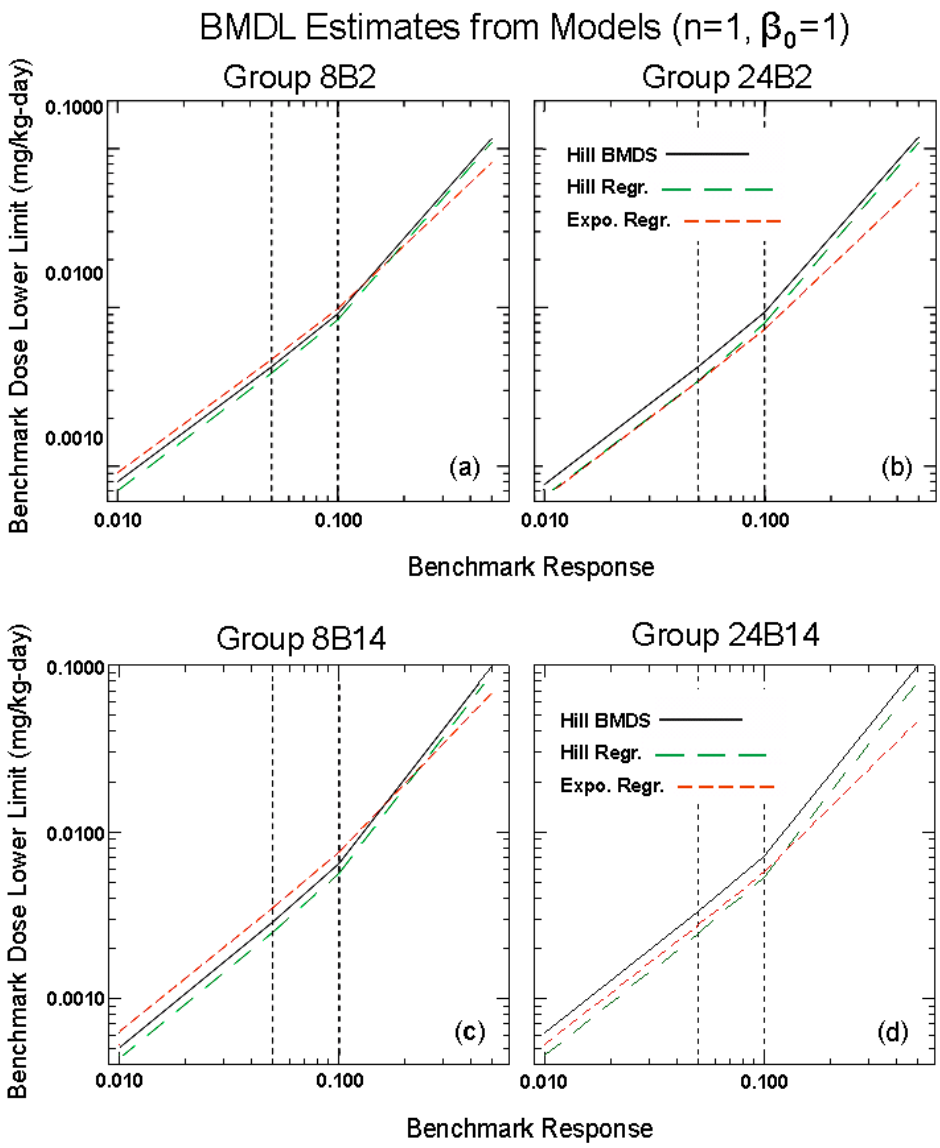

Figure 3-3. New Figure 4-3. Comparison of three methods for estimating the benchmark dose lower limit (BMDL) for a 5 percent inhibition of radioactive iodide uptake (RAIU) in the thyroid relative to pre-exposure baseline value. Data are for full data sets taken after 8 hours (Panel a) or 24 hours (Panel b) on exposure day E2 (sample sets 8B2 and 24B2) and after 8 hours (Panel c) or 24 hours (Panel d) on exposure day E14 (sample sets 8B14 and 24B14). The three methods are (1) Hill function fit by Benchmark Dose (BMDS) software (black solid line), (2) Hill function fit by a non-linear regression program (green long dashed line), and (3) exponential function fitted by nonlinear regression program (red short dashed line).

because the confidence interval covers 1 for both of these parameters when using the full data set.

For the data from sample sets 8B14 and 24B14 in which there were more dose groups (4) than the number of parameters to be estimated from the data (2 or 3), it was possible to quantify the goodness of fit of different models, including non-nested models, by the use of the Akaike Information Criteria (AIC) as discussed below.

Goodness of fit P values are presented in Table 3-7 for various combination of parameter values in the model for the two different sample sets on exposure day 14. The bottom row shows the goodness-of-fit P values for testing the equality of the within-dose-group variances. If outliers are present in one or more dose groups, one should expect a much larger variance than for within-dose-group variances with no outliers. The P value for the full data models with no suspected outliers removed is not large for sample set 8B14 but is statistically significant for sample set 24B14. Removal of suspected outliers improves the goodness of fit substantially for both data sets, but the evidence for deleting Case 47 in sample set 8B14 is not compelling. In most of the analyses discussed below, we will therefore use the full data sets for samples 8B2, 24B2, and 8B14, but will use 24B14 with Cases 41 and 47 removed.

The EPA also then compared the goodness of fit of the Hill models (both using BMDS and SYSTAT) and exponential model with the linear-log model (Marcus, 2003b). Several different criteria are available to compare the goodness of fit of statistical models to data, even when the models are not nested. Classical goodness-of-fit indicators such as the coefficient of determination (R^2) can be used to evaluate nested models in which the form of the model remains the same, but the number of parameters is allowed to vary, as in selecting a parsimonious set of covariates in a linear regression model. Models with more parameters (more covariates) will have a larger R^2 than models with fewer covariates among the same set of covariates. The model selection is regarded as complete when additional covariates produce little if any increase in the R^2 statistic.

A formal statistical test of goodness of fit is particularly easy when the covariates are clustered in identical groups, for example by "dose" as in the present problem. The classical coefficient of determination or multiple R-squared (R^2) has long been used to compare linear models in which fewer terms are embedded or nested within larger models. Developments in statistical theory since the 1970s allow evaluation of goodness-of-fit for non-nested models

using information-theoretic criteria to assess the distance between the unknown true distribution and an approximation based on data. These criteria include

- Akaike Information Criterion (AIC),
- Bayes (Schwarz) Information Criterion (BIC), and
- Consistent Akaike Information Criterion (CAIC).

These criteria were used to compare the fitted Hill model, the exponential model, and the Greer linear-log dose model (Marcus, 2003b). The number of estimated parameters was restricted to 2 or 3 as there only 3 or 4 different dose groups from which a dose-response model can be estimated.

The smallest value (i.e., the largest negative value) indicates the preferred model among the set of models being compared. In general, the AIC retains more parameters than are necessary (overfitting) even in very large samples. The AIC adjusts the deviance (in the case of normally distributed data, essentially the sum of squared deviations of the observed minus the fitted values) for the number of degrees of freedom used to estimate the parameters from the data (Akaike, 1973). The AIC differences have an asymptotic chi-squared distribution. The BIC and CAIC retain about the correct number of parameters that are necessary even in very large samples. The AIC is the usual criterion and is used in BMDS.

Results for the AIC are presented in Table 3-8. The results for the other tests are presented in Appendix D of Marcus (2003b). The two-parameter models evaluated included the Hill model (run in neither BMDS or in SYSTAT as a non-linear regression) with n =1 an intercept = 1; the exponential model with intercept = 1; and the linear-log model. The three-parameter models included the Hill model (both versions) with n = 1 and the exponential model with no parameters specified. The linear-log model has only two parameters.

The AIC and other criteria for goodness of fit of the Hill, exponential, or linear-log models depend on the data because the estimated residual variance is penalized by the number of parameters used to fit the model. These criteria are easily calculated from the output of statistical programs for linear and nonlinear regression models. The AIC in Table 3-8 can be compared by the number of degrees of freedom (nested by rows within the model type) across the different model structures (columns) or both.

Table 3-8. New Table 4-12. Comparison of AIC in Four Different Models for RAIU Inhibition by Perchlorate

Sample Set	Number of Parameters	N	Hill Function		Exponential Function	Linear-Log Function
			BMDS	Regression		
8B14	2	36	-95.2041	-97.2041	-93.5346	-97.157
	3	36	-93.2231	-95.2232	-94.9099	NA[1]
24B14 no outliers	2	35	-97.2116	-101.2029	-98.8618	-101.2292
	3	35	-96.0958	-100.1131	-100.0906	NA
8B2	2	24	-75.6363	-77.6364	-76.6478	-77.8484
	3	24	-73.9723	-75.9723	-75.9723	NA
24B2	2	24	-66.0989	-68.1987	-68.1987	-68.1983
	3	24	-64.202	-66.2021	-66.2021	NA

[1]NA: Not available for linear-log model, which has only two parameters.

As shown in Table 3-8, all of the two-parameter models fit the RAIU data from Greer et al. (2002) almost equally well for all sample sets and criteria. In general, the AIC values must be on the order of 4 units or greater to be considered different. It is likely that the two-parameter models are almost indistinguishable by the above criteria as there are only 3 or 4 dose groups, and the dose groups at 0.007 and 0.020 mg/kg-day are too closely spaced to provide much information.

The three-parameter Hill and exponential models fit the observed data almost equally well as the two-parameter models. However, because the information-theoretic criteria penalize models with a larger number of degrees of freedom, the better fit of the larger models as measured by smaller estimates of residual variance may be offset (as in this case) by the increased complexity of the larger models. In other words, three-parameter models are not always better than two-parameter models. Based on the small differences in the AIC and the principle of parsimony, the two-parameter models should be chosen to fit the data. This choice reinforces the issues of stability discussed in Section 3.3.1.3.1.

Finally, the basis for choosing one model as opposed to another may not be based solely on the goodness of fit of the model within the range of doses for the observed data, but rather on the ability of the model to produce reasonable extrapolation outside that range. While Greer et al.

(2002) suggest that the linear-log model can be extended outside the observed range by linear extrapolation, there is no theoretical or empirical justification for this recommendation. Further, the linear-log model was not used to calculate valid statistical confidence intervals. Thus, the EPA has chosen to use the BMDS Hill model to analyze the RAIU data. The Hill model fits the data as well as the linear-log model, estimates a BMDL, and allows calculation of confidence intervals on that dose estimate.

3.3.1.3.3 New Section 4.2.1.3.2.3: Evaluation of Sample-Time and Dose-Duration Dependence

A number of reviewers on the 2002 peer panel were concerned about the short duration of the Greer et al. (2002) study and the potential for the exposure duration to influence the estimate of a perchlorate dose associated with a significant degree of iodide uptake inhibition. Thus, the time and dose-duration dependence of the Hill model parameters, k, β_0, $\beta_0 - v$, and v were evaluated by EPA. Results are provided in Table 3-9.

The most striking effect was the consistent decrease of the scale parameter k with both duration (one to 13 days of exposure) and time (8 hr versus 24 hr on the same day) from samples 8B2 to 24B2 to 8B14 to 24B14. While the parameters depend on both the model for n and the exclusion of outliers, by far the largest decreases in k are from samples 8B2 to 24B2.

There is a weak change with β_0 increasing from samples 8B2 to 24B2; but there is less discrimination among sample sets 24B2, 8B14, and 24B14. The dose-independent component $\beta_0 - v$ shows a general dependence on duration of exposure, increasing substantially from samples 8B2 and 24B2 to sample 8B14 and 24B14. There does not appear to be a strong dependence of $\beta_0 - v$ on time within a day, nor does there appear to be much dependence of the dose-dependent component v on either duration or time. In the full data, roughly 65 to 87 percent of thyroid iodide uptake can be attenuated by sufficiently high perchlorate doses. However, the values $v = 1.045$ and $\beta_0 - v = 0.068$ for sample 8B14 with unconstrained n appear to be anomalous. With outliers removed, the values of v are somewhat lower, by roughly 60 to 80 percent and show little dependence on duration or time.

The Hill and exponential regression models using the SYSTAT software produced similar results to those obtained with the BMDS software. Detailed results are provided in Marcus (2003b). Parameter estimates for β_0 are similar for both sample sets on exposure day E2 but are somewhat larger in the sample sets after two weeks of exposure on day E14. The estimates of

Table 3-9. New Table 4-13. BMDS Estimates of Parameters k, β_0, v, and n for All Four Sample Sets on Thyroid Iodide Uptake in Greer et al. (2000, 2002) and Merrill (2001a)

		n unconstrained		n ≥ 1		n ≡ 1	
Parameter	**Sample set**	**Full data**	**w/o out.+**	**Full data**	**w/o out.+**	**Full data+**	**w/o out.+**
k*	8B2	0.1228	0.175	0.1213	0.1939	0.1213	0.2602
	24B2	0.0965	0.1138	0.0963	0.1289	0.0963	0.1294
	8B14	0.0866	0.0923	0.0769	0.0923	0.0769	0.0972
	24B14	0.0702	0.0815	0.0702	0.0815	0.0688	0.0824
β_0	8B2	0.958	0.771	0.95	0.779	0.95	0.797
	24B2	1.026	0.871	1.034	0.938	1.034	0.939
	8B14	1.113	0.911	0.99	0.911	0.99	0.936
	24B14	0.968	0.917	0.968	0.917	1.04	0.93
v	8B2	0.808	0.562	0.788	0.606	0.788	0.732
	24B2	0.853	0.622	0.818	0.793	0.808	0.797
	8B14	1.045	0.657	0.763	0.657	0.763	0.722
	24B14	0.679	0.677	0.679	0.677	0.821	0.707
$\beta_0 - v$	8B2	0.15	0.209	0.162	0.171	0.162	0.065
	24B2	0.173	0.149	0.17	0.145	0.166	0.142
	8B14	0.068	0.254	0.227	0.254	0.227	0.214
	24B14	0.289	0.25	0.289	0.24	0.219	0.224

* mg/kg-day
+ without outliers

the perchlorate dose-dependent fraction β_1 are similar for three of the sample sets at about 0.63 but are slightly larger for sample set 24B2. As shown in Table 3-10, the estimates for β_2 in the exponential model show a consistent decrease both with sample time and duration of exposure, just as the parameter k did in the Hill function models (Table 3-9). All of the parameters in the models are highly significant; typical t-statistics of ~3 to 4 suggest a good model fit but one with wide confidence intervals as might be expected due to the small sample size (Marcus, 2003b).

Table 3-10. New Table 4-14. Nonlinear Regression Estimates of Parameters β_0, β_1, β_2 in the Exponential Model for All Four Sample Sets on Thyroid Iodide Uptake in Greer et al. (2000, 2002) and Merrill (2001a)

Sample Set[#]	8B2	24B2	8B14	24B14
Parameter				
β_0	0.302	0.297	0.321	0.325
β_1	0.625	0.699	0.632	0.635
β_2*	0.1315	0.116	0.1014	0.0884
Intercept	0.926	0.996	0.955	0.96

[#]Full data for sample sets 8B2, 24B2, 8B14, and without two outliers for sample set 24B14.

Thus, these statistics strengthen the validity of the concerns expressed at the peer review meeting regarding the short duration of the Greer et al. (2002) study. The clear duration dependence of key parameters in the regression models and the lower benchmark dose level estimates at longer durations confirm the reviewers' concerns that Greer et al. (2002) has limitations with respect to the reliability of inferences that might be drawn from these data for estimating benchmark responses at longer exposures in adult subjects. Caution should be employed to address extrapolations to chronic exposures in adults. Additional issues with respect to population attributes such age and sex will be addressed in the next section.

3.3.1.3.4 New Section 4.2.1.3.2.4: Variability and Evaluation of Confounding by Age and Sex

As the peer panel members noted at the meeting, Greer et al. (2002) overlooked the very large inter-individual variability in baseline uptake values. The use of ratios relative to baseline as the response variable does not eliminate the baseline level as a source of variation but it does simplify comparison with the results published in Greer et al. (2002) and is easily interpreted. However, the variation in the baseline is large, ranging from a minima of 0.068 at 8 hours for subject MJ (at dose 0.007 mg/kg-day) and GH (at dose 0.020) to an 8-hour maximum of 0.254 for subject LB (at dose 0.007 mg/kg-day). Likewise, the variation ranges from a minima at 24-hours of 0.098 for subject SG (at dose 0.100 mg/kg-day) and MJ (at dose 0.007 mg/kg-day) to a

24-hour baseline maxima of 0.337 for subject LB and 0.329 for subject AH (at dose 0.100 mg/kg-day).

As shown in Marcus (2003b) (Figures 5 through 9), the inter-subject longitudinal correlations appear to be quite high. It is obvious that successive measurements on the same subject are not independent, especially at the higher doses. The correlations at low doses appear to be small. Thus, when combining the data sets, the response variables and parameter estimates can not be assumed to be statistically independent. In the interest of simplifying the analyses, EPA did not consider multivariate analyses in which all of the data for each subject at all combinations of sample time (8 or 24 hours) and duration (1 or 13 days of exposure or 14 days post-exposure) but analyzed each sample set separately.

Another concern expressed by some of the 2002 peer panel members was the lack of control for confounding by age and sex in the subjects studied by Greer et al. (2002). It was questioned whether the use of randomized block analyses would be useful, e.g., stratified by sex and age. Weight and age might also be used as covariates, along with dose. In response, the Agency was concerned that any subdivision of the data would further decrease the power of the model to detect differences in dose-response. However, the Agency did perform some preliminary analyses to evaluate the concern for confounding.

The Agency first looked at each sex separately. Because the sample size for each sex separately is considerably lower than for the combined sample, the estimates of BMD and other parameters have much greater uncertainty. Consequently, the BMDL estimates for each gender are expected to be much smaller than those for the combined sample.

Another concern in retrospectively analyzing Greer et al. (2002) as though it was a randomized clinical trial is that the doses may not have been as randomly assigned to the subjects as would have been desirable. Marcus (2003b) took 45 years of age as the upper limit of women of child-bearing age and evaluated the distributions across dose groups. Only 3 of the 10 subjects at the 0.020 mg/kg-day dose were of age ≤ 45 years and 7 of 10 were in the older category. At the 0.500 mg/kg-day dose, the opposite distribution occurred: 9 of the 10 subjects were aged 45 or younger as compared to only 1 in the > 45 years category.

The age-versus-dose difference is nearly statistically significant, even with this small sample: the P value of the Pearson chi-squared statistic equals 0.055; the P value of the likelihood ratio chi-squared test is 0.037; and the Cochran test for trend is 0.066. When the

sample is restricted to females only, the significance levels of the Pearson, likelihood ratio, and Cochran trend test are 0.073, 0.029, and 0.100, suggesting that there may be some confounding between dose level and age. The allocation of younger versus older male subjects to dose groups appears to be more balanced.

3.3.1.3.5 New Section 4.2.1.3.2.5: Summary of EPA Benchmark Analyses of RAIU Data

The EPA based its choice of model and sample set for the Greer et al. (2002) RAIU data on considerations of stability, parsimony, addressing outliers, goodness-of-fit tests, and ability to calculate confidence intervals. Based on the above analyses of the outliers in the data and goodness of fit (Section 3.3.1.3.2), the EPA chose the BMDS Hill model with n restricted to be equal to 1 as the most appropriate model to address the RAIU data. The 24-hr sample set without the two outliers on exposure day E14 was considered the most appropriate data set for consideration of chronic risk estimate derivation due to the clear trend towards lower benchmark estimates and in parameter dependence observed with both sample time (8 or 24 hours) and exposure duration (after 1 or 13 days of exposure). The BMDL estimates for a BMR of 5% for this data set are 0.00237 mg/kg-day for absolute risk and 0.00171 mg/kg-day for extra risk. Both of these round to an estimate of 0.002 mg/kg-day.

3.3.1.4 New Section 4.2.1.3.3: EPA Analysis of Serum Hormone Data

Rather than two-way ANOVA with a categorical variable for time (before, during and after perchlorate) and for dose (high, middle, or low) in a two-way ANOVA as performed by Greer et al. (2002), EPA chose to analyze the serum hormone data with different types of regression analyses. Based on the evaluation of the RAIU data (Marcus, 2003b) and on comments of the 2002 peer review panel, the objective of these analyses was to explore various covariates. Dose rate (mg/kg-day), exposure duration, circadian rhythms in serum hormone fluctuations and gender were used as independent variables or covariates (Marcus, 2003c).

Dose rate and duration of exposure (sampling times) were specified as part of the design of the main study and may be regarded as independent variables. The uptake study was somewhat *post hoc* and age-sex-dose were shown to be not independent covariates in females (Marcus, 2003c). The actual covariates in the RAIU study were age and weight, but it was not clear in Greer et al. (2002) that the subjects were recruited so as to obtain a representative sample of

these in either the main or the uptake studies. Interactions among these covariates were also evaluated. Circadian rhythm was approximated by the use of sin and cos functions to simulate periodicity.

The strategy was to first fit a full model with all plausible main effects and interaction terms, then to simplify the models by step-wise removal of the least informative of the remaining independent variables or covariates and interaction terms.

Dose rate and dose rate with an interaction with duration were statistically significant predictors of TSH in almost all of the models. Dose rate demonstrated a statistically significant negative relationship with TSH. When dose rate was included in a fixed effects linear model then the effect of the product of dose rate times duration (an interaction) was often negative but of similar magnitude and the relationship became more statistically significant.

The interactions of dose rate with duration or circadian rhythm suggest that duration of exposure and time of day modify the effects of perchlorate dose. There may also be a time of sampling dependency. This is consistent with Greer et al. (2002) who did note a dependency of T3 on time of sample. This observation also supports recent findings in laboratory animals that suggest that the pattern of exposure can inhibit iodide uptake with a pattern that can alter the typical circadian rhythm of thyroid hormones (Zoeller, 2003c) . Unless the sample point is designed to test for a pattern in the time course this may be responsible for the seemingly counter-intuitive findings of increases in T4 and decreases in TSH. Preliminary data suggest that developmental effects in the brain of rats may be "tuned" to a specific pattern and alteration of its periodicity results in significant gene expression changes in the brain (Zoeller, 2003c).

An important feature of the models is that they fit the data much better by assuming that different subjects had different baseline values that persisted during the study, i.e., consistently ranked higher or lower relative to the mean for the subjects. This is consistent with the observation by Greer et al. (2002) who noted that certain individuals appeared to be distinct throughout the duration of the 14-day exposure. The EPA used mixed random effects models to address the individual expression of perchlorate effect (Marcus, 2003c). Dose rate was a consistently better predictor of TSH or log(TSH) in the mixed effects models than the product of dose rate and duration. The relationship of TSH to dose is modified by duration of exposure and by circadian rhythm. The circadian rhythm was modified by dose rate and duration of exposure.

Thus even a single hormone alone (TSH) is a complex system and these EPA analyses suggest that these data should be reanalyzed with nonlinear models. In the fixed effects models neither dose rate alone nor the dose rate × duration product was a significant predictor of T4, but the circadian modification of these terms as dose rate × cosine(f) or dose rate × duration × cosine(f) were marginally significant and suggestive of a possible relationship. Free T4 also showed a marginally significant positive relationship to dose rate in an extremely reduced stepwise model with only sine(f) and dose rate as covariates. The positive relationship of fT4 to perchlorate dose rate may not be a plausible finding and the involvement of circadian effects may play a role. Any analysis of relationships between TSH and T4 would certainly need to account for these interactions. EPA also evaluated the relationship between TSH and T4 with RAIU. This revealed a poor relationship and may be due to measurement error or the influences of the above interactions.

In conclusion, EPA believes that it is seeing signals for effects of perchlorate on TSH and T4 in the Greer et al. (2002) data, but the meaning of these signals is not clear. Because of the interactions between dose rate, duration of exposure, and possibly circadian effects as well, it may be necessary to also take into account the times of dosing (at approximately 0800, 1200, 1600, 2000 hours of each exposure day as well as the blood sampling times in order to understand these findings, i.e. a better understanding of the kinetics. It is worth noting that a TSH overshoot, i.e., increases in TSH values were observed at the post-exposure sample points in both Greer et al. (2002) and Lawrence et al. (2000, 2001) studies, also suggestive of some disturbance in this dynamic system. The differences between normal dosing schedules of experimental animals and humans (Greer's subjects as well as free-living populations) might be worth examining.

3.3.1.5 New Section 4.2.1.3.4: EPA Conclusions Regarding Analysis of Greer et al. (2002) Study

In summary, the Agency performed a detailed analysis of the model and findings used in Greer et al. (2000, 2002) in response to recommendations of the 2002 external peer review panel. Specific analyses as recommended by the 2002 external peer panel that were addressed included (1) exploration of the influence of any apparent outlier(s); (2) use of more sophisticated and alternative model structures that allowed for the evaluation of the goodness of fit; (3) calculation of confidence intervals to attempt to address variability in the data; (4) evaluation of the

influence of age and gender with a randomized block design; and (5) evaluation of the influence of the duration of the study on estimates of radioactive iodide uptake inhibition. In response, the EPA conducted an evaluation of the statistical methods employed by the authors, developed alternative models, and performed additional analyses to explore patterns of the association between perchlorate dose and inhibition of radioactive iodide uptake (RAIU) or serum hormones. The linear-log model used by Greer et al. (2002) was not used to calculate true confidence intervals that would help to address the variability noted in the data. The assumption used by Greer et al. (2002) for extrapolation of the function outside of the observed range was not considered plausible, and no data were available to test the model at doses below 0.007 mg/kg-day. Thus, several plausible alternate models for estimating the dose-response function were employed by the Agency. The EPA chose the Hill equation implemented in the BMDS software for its description of the data. This model was shown to fit the data in the observed range as well as the linear-log model and provided formal statistical extrapolation and proper calculation of lower confidence limits. The outlier issue was addressed in detail, and two cases were excluded from one of the sample sets (the 24-hour sample on exposure day 14) based on general criteria.

A clear sample time (8- or 24-hour) and duration (after 1 or 13 days of exposure) dependence of key model parameters and resultant benchmark dose estimates was demonstrated. The lower benchmark dose estimates after the 13-day exposure reinforces concerns expressed by the 2002 peer review panel that the 2-week duration of exposure for this study was likely insufficient to characterize effects of chronic exposure to perchlorate. The post-hoc imposition of a randomized block design was not implemented due to concern for the small sample size. An analysis stratified by age or sex was not used because the estimates for a benchmark dose level for each age or sex would be lower than those estimated on the combined data. The EPA reanalyses also demonstrated that there may be some confounding between dose level and age.

Based on its overall analysis, the EPA chose the BMDS Hill model with n restricted to be equal to 1 as the most appropriate model to address the RAIU data. The 24-hr sample set without the two outliers on exposure day E14 was considered the most appropriate data set for the derivation of an estimate of chronic risk due to the clear trend towards lower benchmark estimates and in parameter dependence observed with both sample time (8- or 24-hours) and exposure duration (after 1 or 13 days of exposure). The BMDL estimates for a BMR of 5% for

this data set are 0.00237 mg/kg-day for absolute risk and 0.00171 mg/kg-day for extra risk. Both of these round to an estimate of 0.002 mg/kg-day. This value should be used to characterize the results of this study in any consideration for the point of departure of an RfD derivation. The additional analyses support concern for the extrapolation of the results of this study to the general public without consideration of additional factors for the inherent uncertainty. Additional discussion on this topic can be found in Chapter 7.

The EPA analyses of the hormone data of Greer et al. (2002) suggest complex interactions that may need to be pursued with more rigorous control of dosing pattern and diurnal hormone fluctuation before fully understood.

3.4 DISCUSSION OF EXPOSURE MEASURES AND BOUNDING ON DOSE-RESPONSE ESTIMATES

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question B.4 and in the Summary Section (Chapter 9, Page 9-1) of the peer review report.

Comment(s)*: Despite the limitations of the ecological and clinical studies discussed in the previous sections, several reviewers recommended that EPA explicitly compare effect levels observed in laboratory animal studies to those observed in humans. One reviewer remarked that the human studies appear to show the absence of health risks (particularly for thyroid tumors). One reviewer noted that he saw no better alternative than the two approaches already employed by the EPA: to construct exposure measures from the epidemiological studies and to use PBPK models to calculate internal doses to compare across species. The panel also debated the differential sensitivity of rats and humans. One reviewer indicated that rats and humans have dramatically different thyroid physiology with perturbations in rats leading to far greater effects. Another reviewer cautioned against inferring that rats are more sensitive to perchlorate exposure than humans. No researchers have established the exact amount of thyroid hormone decrements that result in adverse neurodevelopmental effects in rats or humans; therefore, no conclusions should be drawn as to whether rats are more sensitive to the thyroid disrupting effects of perchlorate exposure than humans, at least in terms of neurodevelopmental and adult neurological sequelae.*

EPA Response(s) and Recommendation(s) for Revision(s): The discussion of interspecies variability with respect to the two different sets of adverse sequelae, i.e., neurodevelopmental effects or neoplasia, has been discussed in revisions recommended for Chapter 2. Consideration

of interspecies differences is also addressed in revisions recommended to the weight of evidence discussion and comparative risk assessment section of Chapter 7. The Agency does point out, however, that none of the human studies considered outcome measures specific to either set of sequelae — indices of neither cognitive or neurodevelopmental deficiencies nor of thyroid tumors were evaluated.

In written comments, one peer reviewer offered that the lack of adequate exposure measures in the epidemiological studies preclude the identification of specific dose-effect relationships. Discussing the study by Li et al. (2000a,b), he noted that whereas the study was able to detect effects of gender, birth weight, and the day of life on which the blood sample was taken for neonatal T4, if the study had used population measures of gender (proportion), birth weight (average), and day of life (average) instead of individual measures, no relationship with T4 would have been observed. Thus, the failure to observe a significant shift in average monthly T4 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 endpoint with greatest homology between species is iodide uptake inhibition; and, for this index, the rat and human are remarkably similar. These considerations will be addressed in Chapter 7 in the section regarding the point of departure.

Comment(s)*: The panel suggested that the EPA consider the clinical data as the point of departure and consider the ecological studies as providing insight in to the effects of long-term exposures. Another reviewer noted that the 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 the comparative risk analyses in Chapter 7, assuming that a defensible calculation can be made.*

EPA Response(s) and Recommendation(s) for Revision(s): EPA has revised its discussion of the weight of evidence to reflect the utility of the human epidemiological and clinical data more explicitly, included the new results for estimates from the Greer et al. (2002) study in the synthesis of the data for the analysis that supports the point of departure, and revised Section 7.1.5.1 (Comparison with Derivation Considering Human Data). These revisions will be made in Chapter 7 (Dose-Response Assessments for Human Health) in the final document and discussed in Chapter 7 of this response document.

3.5 CONSISTENCY OF ASSOCIATIONS WITH MODE OF ACTION AND CONTROL FOR CONFOUNDING

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question B.5.

Comment(s)*: Summarizing the reviewers pre-meeting comments, the discussion leader 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. It was 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.*

EPA Response(s) and Recommendation(s) for Revision(s): EPA will note the general consistency in its revision to the introduction of Chapter 7. No additional revisions in response to this comment are recommended.

3.6 ADDITIONAL COMMENTS AND EPA RECOMMENDATIONS

This section provides the disposition of comments and recommendations for revisions to a number of general comments made by the peer review panel.

Comment(s)*: One reviewer 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.*

EPA Response(s) and Recommendation(s) for Revision(s): EPA agrees that three sets of revisions are necessary in Table 4-5 (now Table 4-15 in the revised final document). These include the corrections to the Schwartz (2001) description as provided above and an expanded discussion of both the "findings" and "problems/comments" fields for the Crump et al. (2000) based on Section 3.2.1. A new entry will be included for the Greer et al. (2000, 2002) study based on Section 3.3.1. The revised table is provided in Appendix A of this response document.

Comment(s)*: One reviewer recommended that the revised document include some text on the prevalence of goiter among populations exposed to perchlorate, considering that goiter is widely observed among individuals with iodine deficiencies. Other general insights focused on iodide*

uptake inhibition and dietary iodine deficiency. One reviewer 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 further inhibition. The same reviewer noted that iodide uptake inhibition may have serious consequences, especially considering iodine deficiency among pregnant mothers is one of the most preventable causes of mental retardation in the world. Another reviewer agreed and suggested that humans with iodine deficiency may be a susceptible population, but he added that most U.S. residents' dietary intake is far higher than the recommended levels. The other reviewer agreed that iodine deficiency is not a widespread problem in the US, but noted that some sub-populations in the U.S. do not meet their iodine dietary intake requirements, particularly during pregnancy and lactation. Upon reviewing the draft of the peer panel report, one peer reviewer commented that the Agency should consult with expert endocrinologists when characterizing the incidence of iodine deficiency among the population.

EPA Response(s) and Recommendation(s) for Revision(s): A discussion of goiter prevalence was added to the analysis of the Crump et al. (2000) study findings. The Agency notes that a discussion and table from the January 2001 report by the National Academy of Sciences concerning the dietary reference intake of iodine was in the 2002 ERD on Page 3-27.

APPENDIX 3A

SUMMARY OF HUMAN POPULATION STUDIES

Table 3A-1. Table 4A-5 now Table 4-15. Summary of Human Population Studies (Park, 2001)

	Publication	Study Population	ClO^{4A-} Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
1.	Gibbs JP, Ahmad R, Crump KS, et al JOEM 1998; 40:1072-1082. *Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function.*	Kerr-McGee workers in voluntary medical surveillance 1994A-98; 170 out of 254 did survey; 130 did single shift evaluation	Airborne exposure to AP in 8 homogenous exposure groups: 0.04A-627 µm/m^3 using closed face cassettes	1 day 1-27 yr.	T3U, T4, FTI, TSH, liver, kidney and hematol fcn T4: 7.5 µg/dL TSH: 2.0 µIU/ml	Indication of increase in TSH over work shift: 2.2 -> 2.5. In workforce, T4 declines and TSH increases from low to high exposure but also from low exposure to unexposed; see inconsistent TSH trends using two lab groups; for both thy and liv outc, SDs increased in high dose group: for thy and liv fcn, averages for low vs high AP very different but %iles up to 75th are not. Implies big excursion at high exposure end.	Possibly half of eligibles did not participate in shift study; possibly confounded by shift duration. Did not evaluate ITR. Suggestion of inappropriate unexposed comparison group. In this steady state and cross-sectional population, difficult to assess thyroid regulatory status. SDs suggest heterogeneity of effect. **Indications of chronic effects.**
2.	Lamm SH, Braverman LE, Li FX, et al. JOEM 1999; 41:248-260. *Thyroid health status of ammonium perchlorate workers: a Cross-sectional occupational health study.*	American Pacific workers: 37 AP and 21 azide workers: full feasible participation; all from same site with same other work attributes	Airborne exposure in 3 AP groups based on visible dust level; total and respirable AP by individual closed-face samplers 10-11 hrs on subset from each exp group; levels: total dust (mg/day): .01, .34, 6.57, 59.4; resp fraction (mg/day): .02, .09, .60, 8.6	1 day n=58; 6 days n=2	Urine AP, T3, T4, FTI, TSH, THBR, and hematologic fcn T4: 7.0 pg/dL TSH: 2.6 JLrU/InI	18% of total airborne Mb is respirable (range *8-25);* urinary excretion of P shows much higher absorbed dose in unexposed workers than expected from air samples: (mg): .88, 4.0, 10.9, 33.6 (assuming 8 hr halflife). Thy, hematol by current exp group: no association (T3, T4]; absorb dose greatly exceeds resp total inhaled dose [F51. See aberrant clearance in 1 of 2 6-day subjects fF2]. Authors conclude no AP health effects.	Some misclassification apparent among exposure groups based on absorbed dose; non-inhalable contribution may constitute important deficit in air sampling results. Steady-state, cross-sectional population difficult to interpret. Thy, hematol results based on current, non-cumulative AP exposure are uninterpretable for chronic effects. Possible increasing trend for max(T3) with exposure group.
3.	Lawrence JE, Lamm SH, Braverman LE. J Endocrinol Invest 1999; 22:405-407. *The use of perchlorate for the prevention of thyrotoxicosis in patients given iodine rich contrast agents.*	Radiocontrast patient series	Therapeutic high oral doses (1000 mg) in day prior to contrast agent	1 day	Misc. thyroid parameters —	Recommend in high risk patients (low iodide areas and elderly) a combination of perchlorate and contrast agent.	Not relevant to and uninformative on chronic exposure effects in adults and acute effects in infants.

Table 3A-1 (cont'd). Table 4A-5 now Table 4-15 (cont'd). Summary of Human Population Studies (Park, 2001)

	Publication	Study Population	ClO^{4A-} Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
4.	Li FX, Squartsoff L, Lamm SH. JOEM 2001; 43:630-634, *Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water.*	Medicaid population at risk for thyroid disease in Nevada in 1997-98.	Perchlorate in drinking water in one county (P= 8.9-11.6 µg/L) versus all others	Lifetime	ICD 240-246; ICD 193: thy cancer	Exposed county (Clark) with Las Vegas compared to another county with a city (Reno/Washoe) as well as with all other counties. No significant excesses found for exposed county for the 8 outcomes studied. Actually, the comparison counties (one with a city, and all others) for all important outcomes differed more between them than with the exposed county. For the 6 more prevalent outcomes (n=3069) the exposed county had higher rates than the unexposed (Washoe) county.	Based on period-prevalence rates. Two outcomes with small numbers are not informative: congenital hypothyroidism (n=22) and thyroid cancer (n=44). **The difference in the comparison counties suggests that uncontrolled confounders or uncertain estimates are affecting this analysis and that the study is uninterpretable for all but large effects.** Confounders might include age, gender, body mass, diet, iodine intake, ethnicity, occupational exposures.
5.	Crump C, Michaud P, Tellez R et al. and Crump KS, Gibbs JP. JOEM 2000; 42:603-612. *Does perchlorate in drinking water affect thyroid function in newborns or school-age children?*	School children from 1 or 2 schools in three cities in Chile (n=53,49,60 in 0, low and high P cities); all newborns 2/96-1/99 in same cities (n=8888,468,428)	Geological Na-P in drinking water (0, 5.5, 111.6 µg/L)	Recent and lifetime for 6-8 yr-olds; gestation	T3, T4, free T4, FTI, TSH, hematol, liver, kidney, prev:goiter, prev:family H_x thy disease T4:10.0 µg/dL TSH: 3.0 µIU/mL	Did comparisons across cities. Urinary I/creatine low in city-2 lifetime residents: (1,092, 862, 963); goiter high in city-2 recent residents: (17.7, 26.5, 23.3%) and high in city-3 lifetime residents: (22.2, 19.5, 26.0 based on 8, 8, 13 cases); family H_x of the disease high in city-3: OR=4.9 (11.1, 9.8, 30.0); highly significant increase in T4 with increased P (1.25, 1.34, 1.50). Highly significant decrease in log (TSH+1) in newborns in city-3–high P (.91, .91, .66) [T9], which is in the unexpected direction. There was a diverse age-at-screen distribution across cities.	Dietary, ethnic, birthwt, SES confounders of thy fcn uncontrolled; observe trends in unexpected directions; suggesting confounding. Unknown if some Chileans boil drinking water. **Significant paradoxical effects indicate uncontrolled confounding and inappropriate thy fcn model in relation to P in this population**. Possible role of ambient temperatures.
6.	Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. Thyroid 2000; 10:659-663. *The effect of short-term low-dose perchlorate on various aspects of thyroid function.*	9 healthy, male volunteers K-perchlorate – 10mg/day	Potassium perchlorate 10 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematology T4: 7.0 µg/dL TSH: 1.0 µIU/mL	Assumed identical P doses. Upward trend for T3 at BL, 7-, 14-, and 28-days (136, 140, 151, 157; trend not tested). See depressed I-uptake at 14 days (40%) with rebound at 28 days; non-24 hour urinary- and serum-I was unchanged throughout. Authors conclude: no thyroid impact because of large I-storage.	Hematol, liver test results clinically "normal" but no data presented. **Inappropriate assessment: clinical rather than epidemiological**. T3 effect not addressed; dietary I not controlled or reported**. Suggests long term iodine depletion.**

Table 3A-1 (cont'd). Table 4A-5 now Table 4-15 (cont'd). Summary of Human Population Studies (Park, 2001)

	Publication	Study Population	ClO^{4A-} Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
7.	Lawrence JE, Lamm S, Braverman LE. Thyroid 2001. 11:295 (letter) *Low dose perchlorate (3 mg daily) and thyroid function.*	8 healthy volunteers	Potassium perchlorate 3 mg/day	14 days	T3, T4, FTI, TSH, THBR, RAIU, liver, hematol	No signif changes (data not presented) except for depressed I-uptake at 14 days (10%) with significant rebound (22%) at 28 days;	**Implies some I depletion over 2 weeks at 3 mg/day** (seen by other investigators at 1.4 mg/day).
8.	Greer, MA, Goodman, G, Pleuss, RC, Greer, SE. Env. Health Perspect. 2002; 110:927-937. *Health effects assessment for environmental perchlorate contamination: the dose-response for inhibition of thyroidal radioiodine uptake in humans.*	37 healthy adult volunteers	Potassium perchlorate 0.007, 0.02, 0.1 or 0.5 mg/kg-day	Samples after 1 (E2) or 13 (E14) days of exposure; also 2 weeks postexposure (P15)	T3, T4, fT4, TSH, RAIU	Dose-dependent decrease in RAIU. EPA performed new benchmark analyses to address model deficiencies in original manuscript. A BMDL of 0.002 mg/kg-day estimated for 5% BMR. Some evidence for hormonal perturbations with duration dependence but pattern not explained	Sample-time and duration-dependence of model parameters and benchmark dose analyses suggest study duration insufficient to evaluate chronic study effects; age and sex dependence evident and not addressed.
9.	Lamm SH, Doemland M. JOEM 1999; 41:409-411. *Has perchlorate in drinking water increased the rate of congenital hypothyroidism?*	Newborns in CA and NV in 1996-97 in 7 counties	Perchlorate in drinking water: 4-16 µg/L	Gestation	Congenital hypothyroid-ism based on neonatal screen (expected= $35/10^5$) —	Compared counties. Hispanic-adjusted prevalence ratios by county: 0.6 (n=8) to 1.1 (n=136); none statistically significant.	No county-specific levels of P; no individual consumption. Should have used other CA and NV counties for expected rates. **Identification of cases is limited by screening procedure that does not consider age at screen, ethnicity and birthweight**. Unable to address transient developmental sequelae.
10.	Li Z, Li FX, Byrd D, et al. and Lamm. JOEM 2000; 42:200-205. *Neonatal thyroxine level and perchlorate in drinking water.*	Newborns in Reno (n=5,882)and Las Vegas (n=17,308) NV 4/98 – 6/99 with birthwt 2.5-4.5kg and age at screen < 5 days and non ICU	Perchlorate in drinking water of Las Vegas: 0 up to 15 µg/L, measured monthly	Gestation	T4 T4:17.0 µg/dL	Compared cities. Significant period effect (seasonal) (ΔT4=.60) when adj for birthweight (.85/kg), age at screen (day 1,2,3 vs. 4: −1.275, .408, 758) and gender (.727). No city * period interaction implies no P effect. Age * exposure interaction not investigated. Did regressions on monthly means (T4, cum.P); also, used 10 percentile T4 as an outcome–no effect. See jump in T4 at newborn return visits in days 2-4.	**These T4 levels are much higher than in other neonate studies (7-10)**. Birthweight may be intervening variable: P causing reduced birthwt via impaired thy fcn. Loss of power in regressions using monthly means instead of individual obs. Early return visits have selection bias: reason for early return.

Table 3A-1 (cont'd). Table 4A-5 now Table 4-15 (cont'd). Summary of Human Population Studies (Park, 2001)

	Publication	Study Population	ClO^{4A-} Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
11.	Li FX, Byrd DM, Deyhle GM et al. and Lamm. Teratology 2000; 62:429-431. *Neonatal thyroid-stimulating hormone level and perchlorate in drinking water.*	Newborns in Reno and Las Vegas NV 12/98 – 10/99 with birthwt 2.5-4.5 kg	Perchlorate in drinking water of Las Vegas: 0 up to 15 µg/L, measured monthly	Gestation	TSH TSH: 10.0 µIU/mL	Compared cities. TSH levels, adjusted for gender and age at screen (2-7 vs. 8-30): no difference for LV vs. Reno.	TSH log transformation for variance stabilization could suppress TSH differences in the high range; inadequate control for age at screen (LV vs Reno), ethnicity and birthwt (2.5-4.5 kg); birthwt may be intervening variable. TSH levels may not be relevant vs T4**. Insensitive to developmental issues and short-term time variability of P exposure.**
12.	Brechner RJ, Parkhurst GD, Humble WO et al. JOEM 2000; 42:777-782. *Ammonium perchlorate contamin-ation of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona.*	Newborns 10/94A-12/97 in two Arizona cities whose T4 screen was below state-wide daily 10%ile	Perchlorate in drinking water <16 µg/L	Gestation	TSH TSH: 13.4 µIU/mL	Compared cities. TSH higher in newborns from exposed city (median: 19.9 vs 13.4); age at screen distribution very different between two cities: exposed screened sooner. Stratifying on age at screen (0, 1-4, 5+ days) and Hispanicity, see signif increase (p=.017); adj effect not reported.	TSH levels (13-20) higher than reported for other newborns (7-10).] Selection on T4 level is problematic due to strong age dependence of T4 surge at birth thus causing variable percentile discrimination with age (8-40% were screened depending on age). This effect could increase TSH of the exposed city relative to unexposed city but the effect of the bias is difficult to predict. Uncontrolled other confounding e.g., birthwt, gest. age, iodine intake, SES**.**

Table 3A-1 (cont'd). Table 4A-5 now Table 4-15 (cont'd). Summary of Human Population Studies (Park, 2001)

	Publication	Study Population	ClO^{4A-} Source and Levels	Duration	Outcomes studied	Findings	Problems/Comment
13.	Schwartz J.Dissertation, UC Berkeley, 2001. *Gestational exposure to perchlorate is associated with measures of decreased thyroid function in a population of California neonates.*	99% of California newborns screened for thy disease in 1996	Perchlorate in drinking water classified in 3 levels and assigned by zip code: 1-2,3-12, 13+ µg/L	Gestation	T4, TSH, presumptive positive; congenital hypothyroidism T4: 160 mg/dL TSH: 7.6 µIU/mL	Compared across four levels of estimated exposure. Has detailed covariates: birthweight, age at screen in hours, ethnicity in 20 groups; birth multiplicity; ANCOVA model with extensive control of most confounders finds highly significant decrease in T4 (mean=16.6 µg/dL) with P level (0, −0.9., −1.12, −1.82) and large effects for birthweight (−7.2 for birthweight 1500-2500), age (−5.0 for hours 7-18) and ethnic groups (−1.0 to −3.0); see initial T4 fall followed by surge by 12 hours and stays elevated until 36 hours; initial onset of TSH surge unresolvable in time; stays elevated till 18 hours. Significant P effect on TSH (0, .029, .03, .128) but birthweight effects models (−.09 for < 1.5 kg). Model for presumptive positives shows strong age at screen and ethnicity effects; for congenital hypothyroidism, insignificant effect.	[T4 is reported at levels 10,000-fold higher than in other studies.] Definition of presumptive positive: for each laboratory tray: all infants with T4 levels below 9 µg/dL plus the lowest 5% of those remaining infants immediately above 9 µg/dL. NO P-ITR reported, e.g., P * age (especially on surge amplitude), P * birthweight; possible selection bias in identification of TSH subjects. Age at screen was included in logistic model, but was not significantly associated with congenital hypothyroidism (and was therefore removed from the model). **This study had best control for exposure and presents strong evidence of perchlorate health effects in neonates from drinking water contamination with perchlorate.**
14.	Soldin OP, Braverman LE, Lamm SH. Therapeutic Drug Monitoring 2001; 23:316-331. *Perchlorate clinical pharmacology and human health: a review.*	Review of animal and human evidence				This review, co-authored by two major participants in industry funded perchlorate research, argues that there is now sufficient evidence to recommend safe levels for regulatory purposes, i.e., at this time there is no need for further refinement of the physiological issues underlying the existing epidemiologic study designs or for new initiatives in evaluating such issues in human populations.	Not considered in this review are issues such as (1) short term effects of variable exposure during pregnancy, (2) the effects of maternal iodine depletion on T4 or TSH surge response at birth, (3) the equilibration of this system under chronic exposure and the masking of potential deficiency states, and (4) the special situation of populations with inadequate iodine intake.

I = iodine; P = perchlorate; AP = ammonium perchlorate; exp = exposure; thy = thyroid; liv = liver; hematol = hematologic; ITR = interaction; outc = outcomes; SD = standard deviation; H_x = history; [Tn] = table in paper; [Fn] = figure in paper.

4. TOXICOLOGICAL EFFECTS IN LABORATORY ANIMAL STUDIES

Topic Area C at the March 2002 external expert workshop was devoted to peer review of the available laboratory animal studies conducted to evaluate the toxicity of perchlorate as well as of the EPA analyses and conclusions regarding those data. The studies included the contract laboratory reports that were part of the original testing strategy and submitted to the Agency to address database requirements. As originally agreed upon by interested parties and as represented in the testing strategy outlined in Table 3-8 of the 2002 ERD, these studies fulfilled necessary data gaps for derivation of an oral reference dose (RfD). In the original testing strategy (Table 3-8 of the 2002 ERD), these studies were performed to evaluate endpoints that were deemed *a priori* to represent likely adverse outcomes due to the mode of action for perchlorate toxicity. These studies were submitted to the Agency to address this testing strategy. The studies were performed by the DoD or by contractors to the DoD and the defense industry consortium know as the Perchlorate Study Group (PSG). The role of the Agency was to perform an evaluation of the adequacy of these submitted data and to integrate all available data into an overall assessment of the risk posed by ingested perchlorate.

This chapter presents the disposition of comments received on the various studies submitted to the Agency and on the data analyses that EPA performed to evaluate and draw conclusions from them. In some cases when the study was submitted after the 1999 peer review and had not undergone peer review, the 2002 expert peer panel was also charged with evaluating the adequacy of the design and performance of that study. The review status of these studies and of any new studies performed in 2003 that address concerns of the 2002 expert peer panel will be clearly identified herein.

Discussion of the studies and peer panel comments on the EPA interpretation of the data are found in the 2002 peer review report in Sections 4, 7, and 9 (U.S. Environmental Protection Agency, 2002b). This document chapter responds to comments in any of those sections regarding the analysis and interpretation of any data on the individual endpoints. The synthesis of the laboratory animal data together with the available human data is discussed in Chapter 7 of this response document. The disposition of comments on each of the individual studies will be

discussed in a separate section. These responses and recommendations are suggested primarily for revision of Chapter 5 (Laboratory Animal Studies) in the 2002 ERD and some changes will also be reflected in Chapter 7 (Dose-Response Assessments for Human Health).

4.1 COMMENTS ON DEVELOPMENTAL TOXICITY

This discussion pertains to evaluation of the available data on developmental toxicity other than neurodevelopmental endpoints. Typically two developmental studies in different species are required to derive an RfD estimate with high confidence (see Table 3-6 in Section 3.5 of the 2002 ERD). Two developmental studies that were recently submitted as contract reports were also peer-reviewed and had their analysis discussed. The first developmental study was performed on rabbits (Argus Research Laboratories, Inc., 1998a). This study also appeared in the literature (York et al., 2001a). However, being submitted for publication after the Pathology Working Group (PWG) review of thyroid histopathology (Wolf, 2000, 2001; Experimental Pathology Laboratories, Inc., 2000), its conclusions on the thyroid effects and developmental endpoints were the same as in the contract report which is much more detailed. Because the published paper did not reflect the results of the PWG and the contract report is more detailed, the paper will not be discussed further in this document.

The rationale for using rabbits in the original 1997 strategy was to evaluate a second species beyond that (the rat) already used in the developmental neurotoxicity study (Argus). The Argus Research Laboratories, Inc. (1998 study was reviewed by the 1999 panel so that the comments on study adequacy and design in the 2002 peer report represent the second peer review of this study. Upon review of the effects seen in the rabbit study, the 1999 peer review panel recommended that the developmental study be repeated in rats. The Argus Research Laboratories, Inc. (2000) is a report of the study that was performed in rats and submitted to the Agency in response to that recommendation. The 2002 peer panel provided a peer review of the study design, its adequacy, and of the EPA analysis. In general, the panel indicated that the two new studies were well conducted and that they considered an appropriate number of animals to detect effects. Specific comments regarding the studies are found below.

The historical data available regarding the developmental toxicity of perchlorate were discussed in Section 5.4.1 of the 2002 ERD. The newer studies in rabbits and rats were

discussed in Section 5.4.2 and 5.4.3. These comments represent those discussed at the peer panel and provided in Chapter 4 of the 2002 peer report (U.S. Environmental Protection Agency, 2002b), as well as written comments provided by the assigned panel member for developmental toxicity (Dr. Michael Collins) and by the public as part of the review process. Any recommendations made regarding these studies therefore pertain to text for those sections in the upcoming revised document.

4.1.1 Review of Developmental Studies Prior to the 1999 Peer Review

Comment(s): *The discussion leader 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; and Sherwood, 1971). For each study he emphasized three key points to consider when interpreting these studies: (1) 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); (2) fetuses in the studies were not examined for a wide range of developmental effects; and (3) 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.*

EPA Response(s) and Recommendation(s) for Revision(s): EPA concurs with the comments made regarding the limitations of the historical data. A new summary to the section on historical studies will be included in the revised final document and is provided here in Section 4.1.1.

4.1.1.1 Recommended New Summary "Section 5.4.1.1 Conclusions Regarding Historical Data Available on Developmental Toxicity"

The utility of these historical data are of limited use for hazard identification of the potential developmental toxicity of perchlorate and not appropriate for dose-response evaluation. Although these studies may have been regarded as adequate for the purpose intended at the time they were conducted, the studies were not performed in a manner consistent with current testing guidelines (Office of Prevention, Pesticides, and Toxic Substances, Health Effects Testing Guidelines 870.3700; U.S. Environmental Protection Agency, 1996a); and the fetuses in these studies were not evaluated in a manner consistent with current assessment guidelines (U.S. Environmental Protection Agency, 1991). The examinations were limited in that a wide range of developmental effects was not studied; additionally, the window of exposures in some studies did not include the most critical time frames for organogenesis.

4.1.2 Regarding the Study of Developmental Toxicity in New Zealand White Rabbits (Argus Research Laboratories, Inc., 1998a)

Comment(s): *The discussion leader 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 (excluding the thyroid effects).*

EPA Response(s) and Recommendation(s) for Revision(s): These were the same conclusions reached by EPA in Section 5.4.2.2 (Developmental Endpoints) of the 2002 ERD so that no change is needed. As discussed in the 2002 ERD, there were remarkable histopathological changes in the thyroids at around 0.1 mg/kg-day (with a BMDL of 0.008 mg/kg-day and 0.42 mg/kg-day for colloid depletion and hyperplasia) and effects on T4 (NOAEL at 0.1 mg/kg-day and LOAEL at 1 mg/kg-day determined by ANOVA). While no discussion occurred regarding these effects, the panel did note that fetal life stages were consistently more susceptible across the available studies.

4.1.3 Regarding the Segment II Developmental Study in Rats (Argus Research Laboratories, Inc., 2000)

Comment(s): *The discussion leader identified the range of doses (0.1 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 at sites at sternal centers and forelimb phalanges in the highest dose group). The discussion leader found two elements of the study design unusual: (1) that the dosing began prior to cohabitation which he noted in written comments could either have ensured inhibition of NIS or alternatively ensured upregulation and (2) that there was a notable gap between the highest dose at 30 mg/kg-day and 1 mg/kg-day. He noted also that the EPA did not agree with the conclusions of the Argus report — Argus considered the high dose a NOAEL; whereas the EPA designated it a LOAEL.*
In pre-meeting comments the discussion leader noted that his conclusions corresponded to that of the EPA. At the meeting another reviewer also supported the designation of the high dose as a LOAEL. The discussion at the meeting ended with that conclusion. In written comments this reviewer noted that the EPA document incorrectly listed the NOAEL at 3 rather than at 1 mg/kg-day. He noted also that the fetuses were only stained with alizarin red S and not double-stained with alcian blue for cartilage. He agreed with the EPA that this made it difficult to visualize cartilage development. This reviewer did not agree with the EPA that alopecia was a significant finding. This reviewer agreed with the suggestion by another reviewer that malformations observed in the various developmental studies appeared to result from hypothyroidism. This

reviewer also emphasized in the summary session of the meeting (Section 9) that the available studies suggest that fetuses are more susceptible to perchlorate toxicity than are the maternal organisms.

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees that the LOAEL for developmental toxicity in rats is properly designated at 30 mg/kg-day and with the majority of these points made by the expert peer reviewer. The Agency has corrected the designation of the NOAEL to be 1 mg/kg-day. The EPA does not feel that a change in the discussion of its concern regarding alopecia is required. The discussion points out that an effect in the dams was observed. Keeping this discussion does not change the overall evaluation or designation of effect level for developmental toxicity. The rationale for the study range was provided in the description of the study design provided in the 2002 ERD. *The following sentence will be included*: It is noted that the actual impact of the unique dosing regimen on the maternal thyroid hormone status at start of conception was not measured. *The following two (2) sentences to will be added to the end of Section 5.4.3.2 (Developmental Endpoints) are recommended*: The peer review panel in 2002 also had concern for the lack of double-staining with alcian blue because this made it difficult to visualize cartilage development. This is a concern especially in light of the aforementioned reduction in ossification sites for sternal centers and forelimb phalanges. *The last sentence of Section 5.4.3.3 (Conclusions Regarding Developmental Toxicity in Rats) will be changed and additional text will be included as*: The results of reduced ossification sites, while not definitive, are suggestive of developmental toxicity. Concern for these effects is warranted in light of the lack of staining to visualize cartilage, the unknown effect of the unique dosing regimen with respect to maternal hormone status at start of conception, and because of the limitation posed by the slightly reduced numbers of fetuses per litter. The 2002 peer review panel agreed with the EPA designation of the 30 mg/kg-day as the LOAEL for developmental effects observed in this study. The NOAEL for developmental toxicity is 1 mg/kg-day.

4.2 COMMENTS ON REPRODUCTIVE TOXICITY

This discussion pertains to evaluation of the reproductive toxicity study performed by Argus Research Laboratories, Inc. (2000). Evaluation of the data from this study at the 1999 external peer review was limited to preliminary findings from the F1 generation. Thus, the 2002

peer panel performed a review of the final published study. This study has also been reported in the literature (York et al., 2001b). However, despite being submitted for publication after the PWG review of thyroid histopathology (Wolf, 2000, 2001; Experimental Pathology Laboratories, Inc., 2000), its conclusions on the thyroid effects and reproductive endpoints were the same as in the contract report which is much more detailed. Because the published paper did not reflect the results of the PWG and the contract report is more detailed, the paper will not be discussed further in this document.

The Argus Research Laboratories, Inc. (2000) study was evaluated in Section 5.5 of the 2002 ERD. These comments represent those discussed at the peer panel and provided in Chapter 4 of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b) as well as written comments provided by the assigned panel member for reproductive toxicity (Dr. Thomas Collins) and by the public as part of the review process. Any recommendations made regarding these studies therefore pertain to text for those sections in the upcoming revised document. It should be noted that concerns and questions regarding findings in this study were sent by this reviewer (Dr. Thomas Collins), through the contractor (Environmental Research Group), to the study author (Dr. Raymond York). The questions, responses, and reviewer comments on the responses are provided in Section J of the 2002 peer review report (U.S. Environmental Protection Agency, 2002b). With the few exceptions noted below, Dr. Collins concluded that the responses provided by Dr. York adequately addressed the concerns that he had raised.

4.2.1 On Reproductive Endpoints

Comment(s): *The discussion leader offered generally favorable comments on the study design and methods other than that the pups were not weighed until day 1 of lactation — a shortcoming that he did not consider critical. His summary noted that nearly every endpoint revealed little evidence of reproductive effects at any dose level. Though some changes were observed in pregnancy rates and in the number of stillborn pups, these and other findings were not statistically significant so he agreed with the EPA and Argus designation that the high dose (30 mg/kg-day) was a NOAEL for most reproductive effects (with possible exception of selected male endpoints per next comment).*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees that the NOAEL for reproductive toxicity testing is appropriately designated at 30 mg/kg-day. The concerns raised in post-meeting comments will be addressed in response to the next comment.

4.2.2 Concerns Regarding Tissue Fixation and Sperm Evaluation

Comment(s): *The discussion leader raised concerns at the meeting and in post-meeting written comments that posed additional questions to the principal investigator. He noted a dose-related decrease (not statistically significant) in sperm density and spermatid density in the F1 generation. He presented his own calculations that showed a dose-related decrease (not statistically significant) in the daily sperm production of the F1 generation and that daily sperm production in the two highest dose groups of the F1 generation were notably lower than routine measures made in his laboratory with the same device. He also noted that the number of animals with low sperm counts increased with dose in the F1 generation. While he acknowledged that selected parameters of male reproduction (sperm motility and morphology) were evaluated in a satellite to the 90-day study (Springborn Laboratories, 1998), he recommended further analysis of the sperm data before he would be convinced that the high dose was appropriately designated as a NOAEL for reproductive toxicity. Post-meeting comments (Section J) also raised concerns regarding fixation of testicular tissues.*

EPA Response(s) and Recommendation(s) for Revision(s): An EPA scientist that specializes in reproductive toxicology evaluated the post-meeting questions posed to the study author, responses from the author, and peer reviewer comments on those responses that are provided as Section J to the 2002 peer review report (Darney, 2003; U.S. Environmental Protection Agency, 2002b). This third EPA reviewer reinforced the initial reviews by two other EPA reproductive experts (Clegg, 1999; Rogers, 2000) and concurred that there were no significant reproductive effects identified in this study (Darney, 2003). *The following section will be added at the end of Section 5.5.1 (General Toxicity Results and Evaluation of Reproductive Parameters) on page 5-85:*

A number of specific concerns were raised at the 2002 peer review meeting and in post-meeting technical questions posed by the designated expert, Dr. Tom Collins. The EPA disposition of these comments and on the responses provided by the study author (Dr. York) that are found in Section J of the 2002 peer review report (U.S. Environmental Protection Agency, 2002b) are summarized here and provided in Darney (2003).

The concern was raised by Dr. Collins on the peer panel that the fixative used in the Argus report induces artifacts that may obscure subtle changes in testicular histology causing them to be missed. He noted that testing guidelines (FDA, EPA, OECD, and ICH) suggest using Bouin's fixative or another suitable fixative to preserve testicular tissues for evaluation. Because the testicular tissues were not preserved using these more accepted fixatives, Dr. Collins submitted that *"the 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." Dr. Darney of the EPA agreed with Dr. Collins and offered that Argus research labs should be encouraged to use better methods for testicular histology, including Bouin's fixative with hematoxylin and eosin (H&E) with PAS staining; however, she found that the lack of significant effects on other measures of testis function in the Argus study (Argus Research Laboratories, Inc., 1999) suggested that the original conclusion regarding the lack of significant effects on testis histology was sound.

Regarding sperm evaluation, Dr. York clarified that the Argus lab used computer assisted sperm analysis (CASA) to define "percent motile sperm" as is specified in the OPPTS 870.3700 test guidelines (U.S. Environmental Protection Agency, 1996a). Dr. Darney noted that those guidelines actually specify determinations of the percentage of "*progressively* motile sperm" because it is possible to have a specific effect on the *quality* of sperm motion without an effect on the percentage of motile sperm. Nevertheless, a review of all the data from both parental and F1 males suggests that additional effects on sperm motion are unlikely in this case because there are no significant declines in any male reproductive endpoints. She also noted that the percentage of motile sperm in the controls was in line with values in the literature for the rat. Therefore, Dr. Darney agreed with Dr. Collins that the response provided by Dr. York was "acceptable."

Nevertheless, EPA notes that the Argus lab should be encouraged to validate a method for reporting the percentage of progressively motile sperm in future two-generation reproductive studies. Dr. Darney felt that the reply provided by Dr. York that the Argus lab could not validate straight-line velocity (VSL), the distance traveled from the first point to the last point divided by time, did not make sense because the lab was apparently able to validate velocity of the average path (VAP) and straightness (STR), and these measures depend on the ability and accuracy of determining VSL.

Although there was some variability in the data on sperm density, the EPA reviewer concluded that there was no significant decline in this measure. The peer panelist (Dr. Collins) was not entirely convinced regarding his additional concerns that the sperm density was dramatically different between the parental and F1 generation, stating *"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."* He noted that, although an examination of the historical control data indicated that the counts obtained for the animals at the

high dose were within the range of historical control values, the dose-related decrease in sperm number observed in the F1 generation remained puzzling. Therefore, the peer panel reviewer concluded that the sperm density data should be considered questionable, it should not be discounted. Dr. Darney of the EPA noted that the statistical analyses appeared sound and that there was no convincing evidence for a significant reproductive effect in the F1 males. She concluded that although there was low confidence in these sperm density data, the conclusion with respect to the 30 mg/kg-day as a NOAEL remained sound.

EPA further evaluated the testis weight data and the age at preputial separation (an index for puberty) for the F1 males in light of recent reports that severe hypothyroidism during perinatal development can affect testis development in the rat (Cooke and Meisami, 1991). Such effects would be expected to increase testis size and sperm counts in the testis and epididymis. There were no alterations in these parameters (Darney, 2003).

In summary, the third EPA scientist with expertise in reproductive toxicology who evaluated the post-meeting questions posed to the study author, responses from the author, and peer reviewer comments on those responses that are provided as Section J to the 2002 peer review report (Darney, 2003; U.S. Environmental Protection Agency, 2002b). This third EPA expert reviewer reinforced the initial reviews by two other EPA reproductive experts (Clegg, 1999; Rogers, 2000) and concurred that there were no significant reproductive effects identified in this study (Darney, 2003). Therefore, the highest dose tested in this study (30 mg/kg-day) remains designated as the NOAEL for the reproductive toxicity of ingested perchlorate.

4.3 COMMENTS ON ENDOCRINE AND NEUROENDOCRINE TOXICITY

This section presents responses to comments received at the peer workshop and as part of the public comment process regarding primarily how perchlorate exposure affected circulating thyroid hormones (T4 and T3) and pituitary hormone (TSH) across all of the studies in Chapter 5 of the 2002 ERD.

4.3.1 On Concordance Among Hormonal Endpoints and Sources of Inconsistency

Comment(s): *Because of the mode of action for perchlorate, the discussion leader expected concordance 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 (comments on thyroid histopathology were saved and discussed in Section 4.4). The discussion leader found a high degree of concordance among the thyroid hormone endpoints in a recent laboratory animal study (Argus Research Laboratories, 2001) such as dose-dependent decreases in thyroid hormones (T4 and T3) and increases in TSH, but acknowledged that earlier studies did not observe similar results across all three hormones. The discussion leader suspected that the lack of concordance across the hormonal endpoints in the earlier studies may have resulted from poor measurement techniques.*

Two other reviewers found these inconsistencies somewhat troublesome, but the discussion leader again asserted that they likely resulted from how the researchers use the radioimmunoassay (RIA) kits to measure thyroid hormone levels. He explained that many measurements documented in the laboratory animal studies (e.g., Argus 1998a and 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 these studies did not document inter-assay and intra-assay variability, the precision of the RIA measurements is unknown, complicating the efforts to interpret results. Because of these concerns, the discussion leader suspected that poor measurement techniques caused the lack of concordance among thyroid endpoints within studies and the lack of consistency in outcomes across the two 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.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees with the discussion leader. As shown in Table 4-1, there was a strong qualitative consistency across all the studies. Despite differences in dosages and experimental design, perchlorate decreases serum thyroid hormones (T4 and T3) and increases serum TSH. This pattern of effects on serum hormones was found in the 14-day Caldwell et al. (1995) study, at both time points (14- and 90-day sacrifices) of the subchronic study (Springborn Laboratories, 1998), the first neurodevelopmental study (Argus Research Laboratories, Inc., 1998a), the neurodevelopmental "Effects Study" (Argus Research Laboratories, Inc., 2001), the mouse subchronic study (Keil et al., 1999), and the rabbit developmental study (Argus Research Laboratories, Inc., 1998c). The only exception to this pattern of effects was in the two-generation study performed on rats (Argus Research Laboratories, Inc., 1998b) in which marginal effects were observed in the opposite direction for TSH. Qualitative differences across the database are likely due to differences in laboratories and study design.

Table 4-1. New Table 5-7. Qualitative Consistency of Effects of Perchlorate on Thyroid and Pituitary Serum Hormones*

Study	Time Point	T4	T3	TSH
Rat 14 Day (Caldwell et al., 1995)	14-Day	⇓	⇓	⇑
Rat Subchronic (Springborn, 1998)	14-Day	⇓	⇓	⇑
	90-Day	⇓	⇓	⇑
Rat Neurodevelopmental (Argus, 1998a)	PND5	⇓	⇓	⇑
Rat Argus"Effect Study" (Argus, 2001)	Dams - GD21	⇓	⇓	⇑
	Dams - PND10	⇓	⇓	⇑
	Dams - PND22	⇓	⇓	⇑
	F1 - GD22	⇓	⇓	⇑
	F1 - PND5	⇓	⇓	⇑
	F1 - PND10	⇓	⇓	⇑
	F1 - PND22	⇓	⇓	⇑
Rat 2-Generation Study	P0 Males	⇓	--	⇑
	P0 Female	--	--	--
	P1 PND21	⇓	--	⇓
Mouse Subchronic	14-Day	⇓	⇓	⇑
	90-Day	⇓	⇓	NA
Rabbit Developmental (Argus, 1998b)	Gestation Day 28	⇓	⇓	⇑

*see Tables 5-2and 5-4 of the 2002 ERD provided herein in Appendix 4B as Tables 4B-1 and 4B-2 for details. NOAEL and LOAEL estimates were determined by Agency ANOVA for the individual studies.
NA = not available

With respect to the two neurodevelopmental studies (Argus Research Laboratories, Inc., 1998a, 2001), the differences in the NOAEL and LOAEL for thyroid hormones 0.1 and 1.0 mg/kg-day) and TSH (3.0 and 10.0 mg/kg-day) on PND5 in the 1998 study versus the LOAEL for all three serum hormones at 0.01 mg/kg-day on PND21 in the Argus (2001) study may be due to differences in sample points (postnatal age or days of treatment), dose spacing, or species differences in addition to the technical limitations discussed by the peer reviewer.

The Agency will include the above discussion and table in the revised final document as part of the new Section 5.3.4.1 (Perchlorate Treatment Reduce Circulating Levels of Thyroid Hormones). This section is in the new summary Section 5.3.4 (Conclusions Regarding the Neurotoxic Effects of Developmental Exposures to Perchlorate) as provided below in Section 4.6.

4.3.2 On the Shape of the Observed Dose-Response

Comment(s): *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 indicated that many studies over the years have identified non-monotonic dose-response behavior, such as U-shaped or inverted U-shaped 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 that 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 agreed but added that the lack of consistency is more troubling than the reported shape of the dose-response curve. The discussion leader offered different insights on the observed dose-response behavior. Based on the proposed mode of action, he indicated that a monotonic dose-response relationship for changes in thyroid hormones is expected. However, he emphasized that the nature of down-stream effects of these changing hormone levels can not be predicted given that the mechanisms by which such effects occur have not been fully established. The absence of a non-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.*

EPA Response(s) and Recommendation(s) for Revision(s): Based on careful review of pre- and post-meeting written comments provided by the peer reviewers, none of which addressed the shape of the dose-response for effects of perchlorate on serum hormones, the Agency believes that this discussion was misdirected in the summary of peer panel report. The concerns regarding the shape of the dose-response curve were aimed at the brain morphometry results as discussed below. The Agency will include the discussion of the consistency of effect on thyroid hormones as provided above in the new document.

4.3.3 On Statistical Analyses of Thyroid Hormone Levels

Comment(s): *EPA asked the panelists to comment on the statistical methods used to evaluate the thyroid hormone levels, asking specifically if the panel supported a recommendation from the 1999 peer panel that EPA use ANOVA for these evaluations rather than t-tests that do not take into account the litter of the individual animals. The chair of the panel indicated that he supported the recommendations made by the 1999 peer-panel biostatistician. The discussion leader agreed, noting that multiple t-tests are clearly inadequate. He supported the use of ANOVA because repeated measures of thyroid hormone levels in many studies are not available. Moreover, due to concerns about inconsistent use of the RIA kits, he cautioned EPA against pooling measurements from multiple studies into a single statistical analysis.*

Another reviewer recommended approaches other than ANOVA, particularly if EPA was 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 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 alternative approach, the discussion leader 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).

EPA Response(s) and Recommendation(s) for Revision(s): The Agency concludes that the ANOVA approaches as specified by the 1999 peer panel biostatistician are appropriate.

With respect to the second comment, the EPA notes that pharmacodynamic hypotheses are evident in the proposed mode of action. As discussed in Chapter 2, considerations of pharmacodynamics were the basis of specifying two different endpoint sequelae of perchlorate exposure: neurodevelopmental effects of thyroid hormone deficiency and neoplasia for TSH upregulation. These considerations were superimposed by the Agency when attempting to integrate the diverse data represented by the different experimental designs (dosing regimen and duration) across life stages and endpoints. However, the tight regulatory nature of the HPT feedback is such that the hormones are closely coupled as are the histopathological indices (see below). The limitations of the data sets and existing experimental techniques, e.g., histopathology evaluated in a “freeze frame” nature at certain sample points, precludes discerning critical events of perturbation of the HPT axis and resultant pathogenesis and prevents a more quantitative description or approach to the risk analysis.

The EPA has always suggested that a comprehensive biologically based dose-response (BBDR) model that would provide for the quantitative interface of iodide uptake inhibition with thyroid hormone deficits and subsequent TSH upregulation would be useful. The original

proposal with the AFRL was for development of a BBDR model. The development of the dosimetry models discussed in Chapter 6, however, was a substantive effort over more than 5 years. Development and validation of a biologically based model would require considerable additional experimental studies and model development. The Agency is aware that an attempt to develop a more comprehensive BBDR model is underway at the University of Georgia by cooperative agreement with the Agency for Toxic Substances and Disease Registry (ATSDR), but this effort will take a number of years yet for development and validation.

4.4 COMMENTS ON THYROID HISTOPATHOLOGY INCLUDING CANCER

This section presents response to comments received at the peer workshop and as part of the public comment process regarding the histopathological effects observed in thyroid glands across all of the studies in Chapter 5 of the 2002 ERD and the analysis of the tumors that occurred in the F1-generation pups of the two-generation reproductive study (Argus Research Laboratories, Inc., 1999). Additional discussion of the potential for perchlorate to cause thyroid tumors can be found in Chapter 2 of this document regarding the mode of action. Historical data on the potential for perchlorate to cause tumors are found in 5.1.1. (Cancer Studies) of the 2002 ERD.

4.4.1 General Comments

This section provides responses to some general comments made about the study and with regard to the histopathological endpoints. Specific comments on the EPA analysis of the tumor data are provided in Section 4.4.2.

4.4.1.1 Diet Used in Argus (2001) Study

Comment(s): *This comment was brought to the attention of the peer panel by public comment (Jonathan Borak, Yale University, on behalf of Lockheed Martin) as included in Section J of the peer review report. Based on this presentation, the discussion leader for this section expressed concern that the rats in the "Effects Study" had been fed a "certified rodent diet 5002" that was not used typically in toxicological studies although he acknowledged that no adverse thyroid pathologies were observed in the control animals. The specific concern presented by Dr. Borak was that the feed was soy-based and could contain goitrogens which could "exacerbate or confound" the effects of perchlorate on the thyroid. A second reviewer did not agree and stated*

that the particular feed was cereal-based and widely used in laboratory animal toxicological studies. This issue was not resolved at the peer review meeting, but the discussion leader and a third reviewer recommended that it be investigated.

EPA Response(s) and Recommendation(s) for Revision(s): In response, the EPA notes that the effects observed in the Argus (2001) study are completely consistent with the effects observed in other studies in the perchlorate database (e.g., Springborn Laboratories, Inc., 1998; Caldwell et al., 1995). Rodents in those studies were not fed the diet in question. The Agency also calls attention to the post-meeting comments by a fourth peer panel member (Dr. Tom Zoeller) that addressed this issue. Dr. Zoeller notes that the diet used by Argus laboratories in its 2001 "Effects Study" is very commonly used in laboratory rodent studies. He went on to further note that if there were the kind of confound that the authors (Borak et al.) propose, and as described by Ikeda et al. (2000), it would be very obvious in the literature considering the values for TSH. The Ikeda et al. (2000) report indicates that animals on soy-enriched diets exhibited TSH levels of about 125 ng/ml. Dr. Zoeller noted that this is greater than 10-fold higher that what is considered to be elevated TSH levels in rats as produced by agents such as methimazole and propylthiuracil. Dr. Zoeller himself noted that a study he recently completed in his own laboratory that combined methimazole and 0.5% perchlorate showed TSH levels in the 10 to 20 ng/ml range, much lower than those reported by Ikeda et al. (2000). Dr. Zoeller concluded that "*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. (2000)*." Further, the Agency notes that Chang and Doerge (2000) have shown that rats fed a diet fortified with genistein at levels as high as 500 ppm starting *in utero* through 20 weeks resulted in inhibited thyroid peroxidase (TPO) but did not exhibit any changes in serum hormones (T3, T4 or TSH) or thyroid histopathology. The EPA therefore concludes that this issue is not a reason to preclude inference of perchlorate effect on the thyroid or to use the Argus data to inform a dose-response relationship.

4.4.1.2 Are Colloid Depletion, Hypertrophy, and Hyperplasia Adverse Effects?

Comment(s): *The discussion leader questioned whether the observed histopathologies (colloid depletion, hypertrophy and hyperplasia) should be considered adverse effects, especially when some of the outcomes are apparently reversible and are not associated with compromised*

thyroid function. This reviewer indicated that colloid depletion was basically an adaptive effect but acknowledged that the onset of hyperplasia suggests that the thyroid has lost its ability to compensate adequately. He noted that the hyperplasia was essentially reversible in a satellite group of the 90-day study (Springborn Laboratories, Inc., 1998) that was sacrificed after stopping the exposure for 30 days. This reviewer questioned the biological significance of these diagnoses. The discussion leader noted that sustained hyperplasia would lead to thyroid follicular neoplasms, but he did not think that the studies provided evidence for advanced effects. Another reviewer disagreed, noting that reversible hyperplasia does not necessarily suggest that adverse effects will not occur. He noted that some non-genotoxic carcinogens (e.g., phenobarbital) may cause transient hyperplasia with tumors occurring later. The discussion leader agreed but emphasized that "delayed" tumors would only occur in the presence of continued exposures.

EPA Response(s) and Recommendation(s) for Revision(s): As described in the beginning of Chapter 5 of the 2002 ERD, a Pathology Working Group was recommended by the 1999 external peer review panel. This recommendation was made to obtain an independent peer review by a second pathologist for many of the studies and to evaluate the thyroid histopathology across all of the studies with the same nomenclature and scoring system. The thyroid slides from the subsequent second neurodevelopmental "effects" study (Argus Research Laboratories, Inc., 2001) were read by one of the pathologists that participated on the PWG and used the same scoring system. The three histopathological lesions were chosen by the PWG to represent changes indicative of an impact on the hypothalamic-pituitary-thyroid (HPT) axis and function of the thyroid gland.

As also noted in the introduction to Chapter 5 on Page 5-2 and illustrated in Figures 5-1 and 5-2 of the 2002 ERD (provided here in Appendix A as Figures 4A-1 and 4A-2), there is considerable overlap of the distributions among the three indices. In any one study, colloid depletion is not necessarily more sensitive than hypertrophy or hyperplasia. For example, the BMDL for hyperplasia was many times lower than the BMDL for colloid depletion in the thyroid from two-generation study where thyroid tumors were observed in the F1 pups. The BMDL for hypertrophy in thyroids from the subchronic study of Sprinborn Laboratories, Inc. (1998) was much lower than that for colloid depletion at both time points (14- and 90-day sacrifices). The EPA suggests that this overlap among the BMDL estimates for each of the three indices is indicative that all three are monitoring the same tightly controlled feedback mechanism. Distinguishing the temporal aspects of thyroid hormone decrement that depletes the stored colloid with subsequent upregulation of TSH causing hypertrophy and hyperplasia is not

possible using current histopathology and experimental design. That is why the "early biological effect" illustrated in the MOA figure (see Figure 2-1) has both thyroid hormone and TSH in the same compartment despite the different sequelae that are the consequence of each. It is critical to consider the timing of the dosing and also of the observations in order to arrive at any integration for these data.

The comments made by the discussion leader suggest that the observed histopathology is only important for consideration of thyroid tumors. Rather, as suggested by the choice of histopathology at the 0.1 mg/kg-day dose in the F1 pups from the first neurodevelopmental study (Argus Research Laboratories, Inc., 1998c) to motivate the point of departure in 1998 (U.S. Environmental Protection Agency, 1998a), EPA asserts that the histopathological change within the gland is serving as a biomarker for perturbations in the HPT axis that may be detrimental to the developing fetus. It is unlikely that the degree of colloid depletion, hypertrophy, and hyperplasia that was observed at this sacrifice point for the pups occurred within 5 days from neonatal exposure so the rationale was that the histopathology was reflecting *in utero* perturbations of the fetal HPT axis.

This assertion was reinforced by the finding of thyroid tumors at the 30 mg/kg-day dose in pups of the F1 generation in the Argus Research Laboratories, Inc. (1999) two-generation reproductive study and by the pathology in the pups observed in the second developmental neurotoxicity study (Argus Research Laboratories, Inc., 2001) performed to repeat the finding of the first. Thyroid tumors are rare — a background incidence of only 1.1% (38/3419) has been calculated from the terminal sacrifice data of 67 National Toxicology Program (NTP) studies in this sex and strain of rat. The tumors due to perchlorate treatment occurred in the pups at 19 weeks with both a statistically significant decreased latency and a statistically significant increase in incidence (Dunson, 2001b; U.S. Environmental Protection Agency, 2002a). Given that over 80% of a tumor lifespan occurs before it is visible with histopathology, it is likely that these tumors were initiated at a young age if not *in utero* (Cotran et al., 1999).

The EPA proposed that the decrease in latency and increase in incidence of these rare tumors pointed to the potential for *in utero* programming or recalibration of the HPT axis, as has been observed for other endocrine disruptors (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). The 2002 expert peer panel agreed with the concern for *in utero* programming and suggested that a "womb to tomb" design should be considered for future studies. It was also

pointed out by the 2002 expert panel that the fetus is consistently more susceptible compared to adults based on evaluation of both thyroid histopathology (Figures 5-1 and 5-2; Tables 5-1 and 5-3 in the 2002 ERD) and serum hormone analyses (Tables 5-2 and 5-4 in the 2002 ERD). The summary tables and figures of thyroid histopathology across the studies have been provided in Appendix A and tables that summarize the serum hormone results in Appendix B of this response document for ready reference.

Thus, while this reviewer also pointed out that the hyperplasia and thyroid weight changes observed in the adults animals at the 10 mg/kg-day level were reversible after the 30-day stop exposure period following the 90-day study (Springborn Laboratories, Inc., 1998), the EPA counters that the reversibility observed in adult animals after a subchronic study is considerably less reassuring regarding characterization of the dose-response for tumors given the lack of chronic data. The fetus or pup is consistently more susceptible to the perturbations of the HPT axis as evident by both histopathology and serum hormones, and the pups manifest rare thyroid tumors at 19 weeks at a dosage of only 3-fold above the level associated with reversibility after short-term dosing in adults. It is further noted that thyroid hormone (T4) and thyroid stimulating hormone remained affected in the adults at this 120-day recovery time point. Because the target concern is for neurodevelopmental effects which are themselves permanent after transient perturbation of thyroid hormones the reversibility is irrelevant.

In summary, the EPA maintains that these three histopathological indices should be considered together and that they indicate perturbation of the HPT axis that are of potential consequence as biomarkers for neurodevelopmental sequelae as well as for the potential for tumors. Further, it is appropriate to designate adversity (e.g., NOAEL or LOAEL) as typically applied for histopathological lesions using benchmark analysis where possible. A benchmark response level of 10% is typically used for histopathological endpoints.

4.4.1.3 Diagnoses of Thyroid Adenomas

Comment(s): *The discussion leader expressed concern about whether the thyroid adenomas identified in the two-generation reproductive toxicity study (Argus Research Laboratories, Inc., 1999) were truly neoplasms and he asked the EPA to identify the criteria that the PWG used to make these diagnoses. This reviewer noted that the Society of Toxicological Pathology (STP) criteria, which are based strictly on histopathology, may lead to false positive diagnoses — a concern based on a personal 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. Thus, this reviewer questioned whether the thyroid adenomas identified by*

the PWG were instead advanced, but reversible, hyperplasia. Another reviewer had a different opinion and indicated that the EPA had presented strong evidence that the highest perchlorate dosage in that study (30 mg/kg-day) produced cancer in the rat. He added that the presence of tumors supported EPA's inference that perchlorate exposure leads to neoplastic outcomes. The discussion leader later agreed, adding that the presence of tumors in only the highest dosage group suggests that rats exposed at this level for a lifetime would get thyroid tumors.

EPA Response(s) and Recommendation(s) for Revision(s): Dr. Wolf, the EPA pathologist who performed the second review of 100% of the materials in response to the thyroid PWG recommendations, clarified at the March 2002 peer-review meeting that the PWG used the Society of Toxicologic Pathology (STP) Standardized System of Nomenclature and Diagnostic Criteria (SNNDC). Dr. Wolf added that the PWG included both external experts on the thyroid as well as pathologists familiar with the pathology of the gland from the NIEHS' National Toxicology Program (NTP). Finally, Dr. Wolf pointed out 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. The EPA considers that this response adequately addresses the comment, and the EPA position is in agreement with the panel conclusions that the thyroid tumors found by the PWG in pups of the two-generation study were indeed neoplasms.

4.4.1.4 Rodents as Models for Neoplastic Outcomes in Humans

Comment(s): *The discussion leader 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 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.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA disagrees with this comment based on several issues. First, the proposed mode of action for perchlorate was endorsed by two expert peer panels and the potential for thyroid neoplasia is a well-defined set of sequelae in the conceptual model (See Figure 2-1). Second, the EPA has standing guidance that was vetted by external scientific peer review, recommending that rodent thyroid tumors

should be considered relevant for human risk assessment (U.S. Environmental Protection Agency, 1998b). Third, the prevalence of thyroid tumors at terminal sacrifice in this sex and strain of rat as evidenced by all of the data in the NTP archives is not very high (1.1%), which is why the observation of tumors at an early age with a statistically increased incidence (6.7%) in the two-generation study raised the concern for *in utero* programming and for the lack of chronic data on this compound. It appears that *in utero* exposure may recalibrate the HPT axis and make young animals more susceptible. Adult rats exposed at the same dose that caused tumors in the young animals (30 mg/kg-day) exhibited only hyperplasia and did not exhibit tumors. The 2002 expert panel agreed with the concern for *in utero* programming and the lack of chronic data. As discussed in Chapter 2, the historical chronic data that showed thyroid tumors after perchlorate exposure were performed with a single high dose that precludes evaluation of dose response. Thus, the dose-response for thyroid tumors of chronic exposures essentially remains poorly characterized. Fourth, none of the human studies of perchlorate have evaluated the potential for thyroid-tumor outcomes or even thyroid histopathology in general. Fifth, the number of mutations is not reflective of relative ease of carcinogenesis but rather speaks to the ease of transformability of the test system. There are *in vivo* human examples such as mutations in BRCA1, p53, and retinoblastoma where there is an increased ease of carcinogenesis based on only a single mutation in the normal allele. Based on these considerations, the EPA reasserts the relevance of the thyroid tumors in rats as relevant for human risk assessment. *EPA recommends that these points above be listed explicitly in a new section entitled 5.1.3. Conclusions Regarding Potential Carcinogenicity*. This section has been previously provided in response to comments in Chapter 2.

4.4.2 Specific Comments on Bayesian Statistics Used for Tumor Analyses

This section is devoted to discussion of the analysis of the tumors that occurred at 19 weeks in the F1-generation of the two-generation reproductive study (Argus Research Laboratories, Inc., 1999). The study is described in Section 5.5 of the 2002 ERD. Evaluation of the thyroid histopathology is described in Section 5.5.2, and the analysis of the tumors is described in Section 5.5.2.2 of the 2002 ERD.

4.4.2.1 Comments on the Software Used to Implement the Analysis

***Comment(s)*:** *Several reviewers commented on EPA / NIEHS's Bayesian analysis of the thyroid tumor incidence. One reviewer found the analyses elegant; another commended EPA on its use of the Bayesian hierarchical model. One concern was that the software (BUGS) used for this analysis is not based on the most sophisticated approach for the numerical simulations (i.e., it uses correlated draws rather than independent ones). Other concerns follow in comments below.*

EPA Response(s) and Recommendation(s) for Revision(s): EPA believes this was addressed by one of the contributing assessment authors, Dr. Dunson of the NIEHS, at the workshop but includes the response for completeness herein. The software that was used for analysis of the thyroid tumor incidence and latency was S-PLUS using independent draws. The BUGS software was used for the Bayesian analysis of the motor activity data because Markhov-chain Monte Carlo simulation methods were needed to accomplish the multidimensional integration required with those data.

4.4.2.2 Rationale for Bayesian Analyses

Comment(s): *While the reviewer acknowledged that Bayesian analysis is a powerful statistical tool, one reviewer 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 that Bayesian approaches could have instead be used 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, when the Bayesian approach employed by the EPA/NIEHS considered outcomes observed in historical control groups.*

EPA Response(s) and Recommendation(s) for Revision(s): The thyroid tumors were the only outcome examined using the Bayesian analysis approach in the two-generation reproductive study (Argus Research Laboratories, Inc., 1999); therefore, there is no need to adjust for the testing of multiple hypotheses. The motivation for using the Bayesian analysis was the strong weight of evidence from previous studies in the National Toxicology Program (NTP) archives that thyroid tumors are very rare in this sex and strain, and especially so at this age. The prevalence of thyroid tumors at terminal sacrifice in this sex and strain of rat as evidenced by all of the data in the NTP archives is not very high (1.1%), thus, the thyroid tumors were unanticipated simply because they are so rare. However, adenomas are completely consistent with the proposed mode of action; and all three histological lesions (colloid depletion,

hypertrophy and hyperplasia) also occurred in these same animals. Discarding information from previous studies would have resulted in an extremely conservative analysis. Although historical control data were used, it is important to incorporate a control in each study to evaluate and account for heterogeneity among experiments.

4.4.2.3 On the Extrapolation of Cancer Incidence Across Age

Comment(s): *Another reviewer expressed concern about assumptions that the EPA/NIEHS made to compare the tumor incidence observed in rats after 19 weeks in the two-generation reproductive study (Argus Research Laboratories, Inc., 1999) to that observed among rats at terminal sacrifice in 2-year bioassays from historical laboratory controls. Citing his personal experiences with 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. Based on these concerns, this reviewer viewed the Bayesian analyses as a modeling exercise and questioned whether EPA/NIEHS can state the probability of cancers occurring at 19 weeks with as much confidence (p = 0.005) as reported in the 2002 ERD.*

EPA Response(s) and Recommendation(s) for Revision(s): We agree that there is a moderate degree of uncertainty involved in choosing a value for the relative risk of tumors at 19 weeks versus 2 years. Therefore, we accounted for a high degree of uncertainty in the choice of this relative risk by not assuming that this relative risk was known but instead allowing for a high prior variance in calculating the confidence (p = 0.005) value. As described in Dunson (2001b), we also conducted sensitivity analyses in which we chose reasonable alternative values and obtained similar results.

4.5 COMMENTS ON NEUROTOXICITY

This discussion pertains to evaluation of the available data concerning the effects of developmental exposure to perchlorate on the nervous system. The studies include the 1998 developmental neurotoxicity screen performed by Argus Research Laboratories, Inc. (1998a) which was described in Section 5.3.1 of the 2002 ERD and included evaluation of both brain morphometry and neurobehavior (motor activity). Based on evaluation of the results of that study, the 1999 peer panel recommended a second study to repeat the findings observed in both brain morphometry and motor activity. The additional studies performed in response to those recommendations that were submitted to the Agency by the DoD and defense industry, are described in Section 5.3.2 (Motor Activity Study [Bekkedal et al., 2000]) and Section 5.3.3 (The

2001 "Effects Study") of the 2002 ERD. The motor activity study was performed by the United States Navy in conjunction with the Air Force Research Laboratory, and the "Effects Study" was again performed by Argus Research Laboratories. A new study was performed in 2003 using the tissue blocks that remained from the Argus 2001 study (Consultants in Veterinary Pathology, Inc., 2003). This new study was performed as a response to the 2002 peer panel and public comments under contract to the Agency by Consultant in Veterinary Pathology, Inc. This study is described in the recommendations for new text that the Agency has used to revise the document.

4.5.1 Comments on Studies of Changes in Brain Morphometry

This section of the response document pertains to evaluation of the available data on the effects of perchlorate on brain histopathology and morphometry. These studies were considered critical to the 1997 testing strategy because neurodevelopmental effects from thyroid hormone disruption were anticipated based on the established effects of decreases in thyroid hormone from other compounds and disease states.

The analysis and interpretation of the available data regarding perchlorate's effects on brain morphometry were the subject of significant comment at the workshop and particularly of comments submitted by interested parties and the public. The 2002 peer-panel discussion is found in Sections 4.5, 7 and 9 of that report (U.S. Environmental Protection Agency, 2002b). Pre- and post-meeting comments submitted by the assigned reviewers (Drs. Aschner, Paule and Zoeller) were also considered and additionally addressed some public comments. Specific concerns raised will be described in greater detail below.

There are now three studies that have evaluated the effects of perchlorate on the developing brain of rat pups exposed *in utero* and perinatally. Two laboratory studies have been conducted that exposed pregnant dams to ammonium perchlorate in drinking water and that evaluated their pups for effects on brain and thyroid (Argus Research Laboratories, Inc., 1998a, 2001). These two studies were developed and performed under contract to the defense industry or by the Department of Defense and then submitted to the Agency for evaluation. These studies were reviewed at the 1999 and 2002 peer panel workshops, respectively. It should be noted that because Argus Laboratories identifies the day of birth as PND1, that the age of PND12 or PND22 actually corresponds to PND11 or PND21 using the US EPA convention that date of birth equals PND0 (U.S. Environmental Protection Agency, 1998b).

The 1998 study included limited histopathological and brain morphometry evaluations. A subset of pups (6/sex/dose for a total of 30 male and 30 female pups) on PND12 were selected for brain morphometry. Pups in Subset 4 (6/sex/dose for a total of 30 male and 30 female pups) were assigned for brain weight and neurohistological examination on days ranging from PND81 to PND86. As described in Section 5.3.1.1 of the 2002 ERD, a number of indications that perchlorate had affected the brains of developing pups were evident. Morphometric analyses of the pups on PND12 revealed a 23.4% increase in the size of the corpus callosum in females and a 30.2% increase in males at the high dose (10 mg/kg-day) that were not statistically significant. Pups from Subset 4 sacrificed on PND82 also showed an increase in the size of the corpus callosum in males (20.9% increase), but no effect was observed in the females at the high dose. There was also a 3.4% increase in the brain weight of males, a 9.2% increase in the size of the frontal cortex, and a 10.2% increase in the size of the caudate putamen.

The EPA concluded at that time that these effects may be significant and that analyses of the brains from the next lowest dosage were warranted. York (1998d) responded and performed morphometry in the pups dosed at 3.0 mg/kg-day of Subset 1 (PND12) at PND12. A statistically significant increase in the anterior/posterior cerebellum size, decrease in the caudate putamen (females), and decrease in hippocampal gyrus (males) was observed.

While the Argus Laboratories did not consider these observations to be treatment-related because they were not dose-dependent, a preliminary re-analysis by EPA restricted to the corpus callosum showed a statistically significant effect of treatment at the high dose and designated it as a LOAEL (Crofton, 1998c). The corpus callosum was chosen for that limited analysis because it appeared to have some significant effects. Additional EPA analyses presented at the 1999 peer review (Geller, 1999a) corroborated the preliminary findings in the corpus callosum and found effects in other regions of the brain, including the hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen.

Because of concern for these effects, the 1999 external peer review panel requested either additional sectioning of the existing brains from this study or a new study to be performed evaluating brain morphometry (Harry, 2001). The remaining block materials were determined to be of insufficient quality for additional sectioning and histological evaluation so that a protocol for the second study known as the "Effects Study" (Argus Research Laboratories, Inc., 2001) was developed for additional brain morphometry at PND9 and PND21.

The "Effects Study" protocol used doses of 0.01, 0.1, 1.0, and 30 mg/kg-day, started 2 weeks before mating in contrast to the previous 1998 study that started dosing on GD0. The dose levels were chosen to be (1) below the LOAEL dose in the 1998 database and (2) three-fold higher than that used in the previous study. There were 16 animals/sex/litter/dose. Coronal sections were used to replicate the 1998 study and to ensure that multiple brain regions could be effectively assessed. EPA analyzed these data using a multivariate approach known as profile analysis to address the repeated measures issue posed by the measurement of numerous brain regions in individual animals (Geller, 2001d). Profile analysis is routinely used in the social and biological sciences to examine large databases to identify and make sense of patterns of responses on correlated measures, such as the multiple scales that comprise intelligence tests (Brazma and Vilo, 2000; Tabachnik and Fidell, 2001).

The results reported from statistical analyses of data in the original contract report from Argus Research Laboratories, Inc. (2001) for the "Effects Study" were similar to those identified in their 1998 study when both sexes were considered together. This finding is notable given the differences in study design in dosing period, age of the animals, and dose spacing. Significant effects of perchlorate exposure at all dose levels were also obtained in the PND9 brains despite the poor tissue quality (Argus Research Laboratories Inc., 2001; Geller, 2001d).

The profile analyses performed by EPA on the "Effects Study" showed also a widespread pattern of altered brain growth in various regions of the brain and an effect of perchlorate at all dose levels. While a similar pattern was observed for the PND9 brains, the 2002 EPA analyses excluded these data because the integrity of some of the cerebellum sections were viewed as inadequate due to disruption or damage. It should be noted that this lack of tissue integrity was considered to be an inherent problem with the examination of such small brains (PND9) and fragile tissue rather than any question of the performance of the contractors conducting the morphometry (Harry, 2001).

The third study involved additional sectioning and measurement of brains from the Argus Research Laboratories, Inc. (2001) study and was performed under contract to the Agency (Consultants in Veterinary Pathology, 2003). The EPA requested these additional data to address some of the concerns described below. The new EPA 2003 analyses performed in response to the comments and using these new data are described below and in more detail in Geller (2003). More detailed descriptions of the EPA analyses of the 1998 and 2001 studies are

found in the 2002 ERD in Sections 5.3.1.1 and 5.3.3.4 and their associated technical memorandums (Crofton, 1998c; Geller, 1999a; Harry, 2001; Garman, 2001a,b,c; Geller, 2001d).

4.5.1.1 Methodological Concerns Regarding the "Effects Study" (Argus Research Laboratories, Inc., 2001)

This section addresses various concerns raised by the panel and in comments submitted by interested parties or the public. These comments were regarding aspects of experimental design of the studies submitted to the Agency or of the EPA analyses performed with those data**.** Specific criticisms that have been raised regarding the design and performance of these studies are addressed in separate sections below.

4.5.1.1.1 Regarding Use of Linear Measurements

Comment(s): *After noting that the Argus 2001 "Effects Study" was generally well-conducted with QA/QC assurance, used an adequate number of animals, and was fairly extensive, the discussion leader identified several aspects of the Argus 2001 study that he viewed as flaws. The peer panel had differing opinions concerning these flaws and the extent to which they may have affected the findings. One specific flaw was the use of linear measurements of brain dimensions that are subject to artifacts from fixation, sectioning, and positioning of the grid for viewing the sections. This reviewer indicated that volumetric measurements are preferred.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA response to this concern, as provided at the meeting, is two-fold. First, the EPA guidelines for the assessment of neurotoxicity call for linear measurements (U.S. Environmental Protection Agency, 1998b). These guidelines were vetted by scientific experts and embody recommendations for state-of-the-science applications in regulatory risk assessment. Second, linear measurements were performed in the 1998 study. Given that the objective of the 2001 "Effects Study" was to repeat the previous one to see if findings were observed again, linear measurements were recommended by the representatives of the DoD, the Perchlorate Study Group (PSG, a consortium of defense industry members that sponsored some of the studies, including the Argus 2001 study), EPA, and NIEHS all of whom were consulted on experimental design and who reviewed the protocol before it was sent to the contractor to be performed. The EPA further notes that there has not been any systematic study to compare volumetric with linear measurements suggesting that volumetric analyses would afford greater accuracy or differential sensitivity.

4.5.1.1.2 Regarding Tissue Sectioning

Comment(s): *The discussion leader also questioned whether inconsistent sections may have biased results and how EPA decided which samples to include and exclude from its statistical analyses. Given the overall dimensions of brains in rat pups, the discussion leader was particularly concerned that small deviations in sectioning could lead to substantial errors in fine-scale measurement. This reviewer also pointed out that the analyses of brain sections were not blinded. Another of the assigned neurotoxicity peer reviewers was not convinced that these concerns invalidated the data. This reviewer explained that the methodological issues (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 lead to detection of effects that do not exist. He found no evidence that these factors introduce any systematic bias. He and the third peer reviewer assigned to assess neurotoxicity noted that they found no evidence of systematic errors introduced by the study methodology (e.g., use of different section practices for different dose groups), and therefore recommended that EPA not discard the data due to the random errors that the study design may have caused.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees with the two neurotoxicity peer reviewers on the panel who suggested that random errors would be expected to decrease the ability of the statistical analyses to find significant effects. The purpose of the landmarks used for each region is to provide consistency in measurements and to decrease the variability introduced by potential deviations in sectioning. The laboratory that performed the sectioning has considerable experience and credentials supporting their proficiency for performing these types of studies. In fact, the laboratory was chosen by the PSG for the contract based on their good reputation.

With respect to the samples used in the statistical analyses, Dr. Geller of the EPA clarified at the peer review meeting that the Agency used all of the brain sections for its primary analyses. The restricted analyses that were performed were done to evaluate whether omission of the data from sections in tissue block level II (viz., posterior corpus callosum and all hippocampal measures), i.e., those that were alleged to be sectioned with bias (see Section 4.5.1.1.3 below), would provide different results. The restricted analyses showed that significant effects of perchlorate exposure remained at all dose levels (Geller, 2001d). The EPA concluded that it was unlikely that systematic bias would occur in sectioning for all brain regions and that the restricted analysis supported those conducted with all of the sections.

Consideration of blind reading was given when the protocol for the study was designed and it was not felt to be a major issue of concern given the reality and practicality of the large

number of images (Harry, 2001). The decision to not have the morphometry performed in blind fashion was consistent with standard guidelines for toxicologic pathology (Society of Toxicologic Pathology, 2002). Further, the notion of blind reading is somewhat a misnomer in this case. These are measurements and not subjective diagnoses as in histopathological examination. According to the best practices guideline for toxicologic pathology (Society of Toxicologic Pathology, 2002), re-evaluation of masked slides may be necessary for specific tissues or lesions to ensure consistent use of diagnostic criteria. It is not recommended for the collection of quantitative data from numerous sites across a complex organ such as the brain. It is commonly accepted that, when collecting defined quantitative data such as brain morphometry, one does this kind of evaluation with knowledge of which animal is being examined. This is done for data management. There would be a far greater risk of inaccurate transcription and decoding if the morphometry was done blind than the possible bias from open measurement. Finally, it was decided that as in this case, when collecting numerous measurements from each animal using a specific and defined approach, it was highly unlikely that such a large number of measurements would be entered in a consistently biased way across all brain regions**.**

4.5.1.1.3 Regarding Coronal Versus Sagittal Sectioning

Comment(s): *Comments submitted to the peer panel and the Agency by stakeholders as part of the review process have asserted that morphometric changes in the corpus callosum would be more accurately measured if the brains had been sectioned along sagittal rather than coronal planes.*

EPA Response(s) and Recommendation(s) for Revision(s): As agreed upon by the representatives for DoD and the PSG, EPA, and the NIEHS when discussing the design of this study, the choice of coronal sections was made to better serve the objective of repeating the Argus 1998 study (Harry, 2001). Further, a preference for sagittal sections would only be valid if the corpus callosum was the only brain region affected by perchlorate exposures. Multiple brain regions were significantly altered in both the Argus 1998 and 2001 studies. Several of these brain regions are, in fact, better assessed in coronal sections than in sagittal sections. For example, the frontal cortex, parietal cortex, hippocampus, and striatum have well-defined characteristics in coronal sections. Additionally, the landmarks that can be used to define specific locations for linear measurements of these regions can be reliably obtained in coronal

sections whereas some brain regions would not be assessed with any degree of accuracy with sagittal sections. In contrast, the corpus callosum can be reliably evaluated in coronal sections. Finally, the US EPA guidelines for developmental neurotoxicity testing recommend coronal sections (U.S. Environmental Protection Agency, 1998b) which is why they were chosen for the Argus 1998 study.

4.5.1.1.4 Variability in Measurements

Comment(s): *The discussion leader expressed concern about the variability in the brain section measurements, particularly among pups in the same litter. He cited some observations (Table 1 of Argus 2001, as reproduced on page C-48 of the 2002 peer panel report) in which the Argus 2001 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 felt that this variability was due to the linear measurements cited as a flaw in the methodological section above.*

EPA Response(s) and Recommendation(s) for Revision(s): As noted in the 2002 peer panel report on Page 4-12, an EPA scientist (Dr. Crofton) responded to this comment at the review meeting. The data cited by the discussion leader present the range of measurements and not the variability. An appropriate expression to portray variability is the coefficient of variation (CV) which presents the standard deviation as a percentage of the mean. In a survey of brain morphometry data from these types of developmental neurotoxicity studies that have been submitted to the Agency, it was noted that brain morphometry has lower variability than other endpoints such as body weight (Crofton et al., 2001). It was also noted that there is a range of variability among brain regions. The cerebellum, cortex, and hippocampus show less variability than the corpus callosum (Crofton et al., 2001).

Table 4-2 presents the CV calculated for the measurements of the corpus callosum, striatum, and cerebellum at each dose level for both the Argus Research Laboratories, Inc. (2001) study and for the new study performed with additional sections from that study (Consultants in Veterinary Pathology, Inc., 2003) that is described below in Section 4.5.1.1.3.1 (new Section 5.3.3.4 in the revised final document). For the Argus 2001 data set, the mean CV was calculated from the right and left measurements of striatum and anterior and posterior corpus callosum, and from the cerebellum measurements. For the CVP 2003 data set, the mean CV from all of the corpus callosum and striatum measurements across the plates was used. The CV for corpus callosum for both studies is well within the range of data submitted in the twelve

Table 4-2. New Table 5-4. Coefficients of Variation[1] (%) for PND22 Male Rats in Different Brain Regions

	Corpus Callosum		Striatum		Cerebellum
Dose Group (mg/kg-day)	Argus (2001)[1] (%)	CVP (2003)[2] (%)	Argus (2001) (%)	CVP (2003) (%)	Argus (2001) (%)
0	17.5	15.8	5	6.5	4.3
0.01	20.7	13.5	6.7	3.8	4.3
0.1	27.6	21.6	3.5	2.2	4.3
1	22.3	17	5.7	4.3	2.8
30	19.6	19.1	5.4	6	2.9

[1]Calculated as: mean/standard deviation
[2]Argus Research Laboratories, Inc. (2001).
[3]Consultants in Veterinary Pathology (2003).

DNT studies evaluated by Crofton et al. (2001); and the CV for the measurements of striatum and cerebellum are markedly lower, ranging from only 3 to 7 %.

Thus, EPA intends to include this table and discussion to support the conclusion that both studies achieved an acceptably minor degree of variability for these brain region measurements. These will be added to a new Section 5.3.3.4.2.3 (Variability of Measurements) of the revised final document.

4.5.1.1.5 Systematic Bias

This concern was raised originally by the NIEHS at meetings held with the EPA to evaluate the results of the 2001 Argus study. Since that time the issue has been confused with that of variability and used to suggest that the study is "flawed." The issues of variability have been addressed above. To clarify the concern regarding systematic bias, this is characterized as the potential that the depth at which brain sections from tissue block level II (viz., corpus callosum and hippocampal measures) varied with dose group. This was based on an analysis of the males on PND21 that showed the dorsal landmarks for dose groups 1 and 5 (control and 30 mg/kg-day) were sectioned more anterior than dose groups 2, 3, and 4. In the sections from this tissue block level, the linear dimensions of the corpus callosum vary with depth in the anterior to posterior plane through the brain. If different dose groups were sampled at different depths through the brain, then brain region size could vary due to sampling site in addition to, or

rather than, due to perchlorate exposure. Some of the confusion likely arose from the fact that different landmarks for homology were used as criteria when evaluated by the NIEHS (Harry, 2001) than when evaluated by Consultants in Veterinary Pathology (Garman, 2001a,b,c).

As described in Section 4.5.1.1.2, the restricted analyses that omitted data from sections of the tissue in block level II (viz., posterior corpus callosum and all hippocampus) to address this potential bias showed that, when the sections of the region in question were omitted, significant effects of exposure to all doses of perchlorate were still present in the remaining data. In arriving at the conclusions for the analyses presented in the 2002 ERD, EPA concluded, and NIEHS agreed, that it was unlikely that a systematic bias would occur in sectioning for all brain regions and that the analysis indicated an effect at all dose levels tested. As also noted above, two of the three peer reviewers assigned to neurotoxicity on the 2002 external panel agreed, noting that it would be difficult to introduce a systematic bias into multiple measurements. Nevertheless, the discussion leader for this section on the peer panel was not convinced by the statistical approach taken to address the data quality. Comments submitted to the Agency by stakeholders alleged bias. To address these concerns further, the EPA contracted Consultants in Veterinary Pathology, Inc. (CVP), to obtain measurements from new sections of the existing tissue blocks in order to re-evaluate the PND21 male rat brain morphometry data from the Argus 2001 study. New sections were obtained from tissue in block level II and from another tissue block, Level I, because no concern was expressed regarding sections from that area of the brain.

The additional analyses will be described in the following section and are provided in detail in an accompanying memorandum (Geller, 2003). This description is provided in Section 4.5.1.2 and will be incorporated into the revised document as a new Section 5.3.3.4 (Additional Study and Analyses of Brain Morphometry).

4.5.1.2 New Section 5.3.3.4 (Additional Study and Analyses of Brain Morphometry)

In response to the suggestion that a systematic bias potentially occurred in the sections from a specific tissue block in the Argus Research Laboratories, Inc., (2001) study (Harry, 2001), the EPA performed statistical analyses that restricted the profile analysis to brain regions that omitted sections from the tissue block level II that was in question (viz., posterior corpus callosum and hippocampal measures). This analysis showed that significant effects were still present in the remaining brain regions at all dose levels (Geller, 2001d). In arriving at its conclusions for the analyses presented in the 2002 ERD, the EPA concluded, and the NIEHS

agreed, that it was unlikely that a systematic bias would occur in the sectioning for all brain regions and that the restricted analysis supported those conducted with all of the sections. The EPA also agreed with comments made by two of the three expert peer reviewers assigned to evaluate the neurotoxicity data on the 2002 peer review panel who suggested that random errors would be expected to decrease the ability of the statistical analyses to find significant effects. Nevertheless, the discussion leader for this section on the peer panel was not convinced by the statistical approach taken to address the data quality. Comments submitted to Agency by stakeholders alleged bias. To address these concerns further, the EPA contracted Consultants in Veterinary Pathology, Inc. (CVP), to obtain measurements from new sections of the existing tissue blocks in order to re-evaluate the PND22 male rat brain morphometry data from the Argus 2001 study. New sections were obtained from tissue in block level II and from another tissue block, Level I, because no concern was expressed regarding sections from that area of the brain. The study and analysis of the new sections are described in the following sections.

4.5.1.2.1 New Section 5.3.3.4.1 (Methods Used for New EPA 2003 Analyses of Brain Morphometry)

CVP was asked to perform two tasks: (1) to re-section and re-measure the control brains from the level I and level II tissue blocks and (2) to re-examine the existing step-sections from levels I and II taken from the PND22 male rats treated with perchlorate. In the re-sectioned and re-measured control brains, CVP matched section depth to anatomical atlas plates and measured striatum, anterior, and posterior corpus callosum in each section. They then assigned each step-section from the perchlorate-treated animals a depth based on anatomical-atlas plate numbers and re-measured the striatum, anterior, and posterior corpus callosum (Consultants in Veterinary Pathology, Inc., 2003).

The rationale for this study was that control data on brains unexposed to perchlorate were first needed to ascertain if the expected shape of various regions varied with the anterior-posterior axis used in coronal sectioning. The second task allowed the EPA to analyze data from the exposed animals that were rigorously controlled for depth of sectioning.

Statistical analyses of these new 2003 brain morphometry data were conducted by both CVP and the EPA. The CVP approach consisted of univariate comparisons for each brain region re-sampled at each anatomical atlas plate number, i.e., according to sections of comparable brain depth. Because the number of statistical analyses run in this type of approach increases the risk

of introducing Type I error (finding effects where none exist) while ignoring the inherent correlational structure in the data, EPA's re-analysis of the 2003 data set once again followed the strategy used in the 2002 risk characterization: to examine all of the samples available from each animal simultaneously in a multivariate analysis of variance (MANOVA) statistical approach called profile analysis (SAS, Inc., PROC GLM, REPEATED PROFILE) (Johnson and Wichern, 1988; Tabachnick and Fidell, 2001). Step-down univariate tests were then also performed to serve as a point of comparison where indicated (Geller, 2003).

As described earlier in the document, profile analysis makes between-groups comparisons using a vector composed of the linear measures of the brain regions from each animal (i.e., within-subject measurements). Multivariate analysis reduces the number of statistical tests evaluating main effects, reducing the risk of Type I statistical error, while taking into account any correlations inherent in the data set.

Profile analysis' primary test for parallelism of the vectors establishes whether the pattern of results between treatment groups is the same or different. Profile analysis determines whether there are exposure-related changes in the pattern of brain growth, i.e., brain growth in one region relative to another, while precluding prior expectations about specific areas of the brain or about the direction and degree of these changes. Thus, this simple determination allowed the EPA to examine the entire set of data without an *a priori* expectation regarding the nature of the effect in one brain region or another.

The new 2003 EPA analyses also matched tissue sections by depth to address the issue of whether different section levels introduced a systematic bias. The data used in this analysis included the morphometric measurements from anterior corpus callosum and striatum taken at a brain depth identified as plate 17 (block level I) and from posterior corpus callosum taken at plate 31 (block level II) (Consultants in Veterinary Pathology, Inc., 2003). These plates were selected because they had the most samples across dose groups, and the study pathologist indicated that they were reasonably representative of the brain areas examined (Garman, personal communication, 2003). In addition to the data from the repeated sectioning and measurements, measurements were also included from the Argus 2001 cerebellum data because no problem with sections had been indicated in those samples. A criterion of $n \geq 6$ was set for inclusion in the analysis to match the minimum data requirements from the Developmental Neurotoxicity Study Health Effects Test Guidelines (U.S. Environmental Protection Agency, 1998b). Because profile analysis requires data from each animal for each brain structure, the

analysis was necessarily restricted to 49 of 79 animals in the study for which a complete data set was available: Control, data from 15 rats out of 16 tested; 0.01 mg/kg-day, 9 of 16; 0.1 mg/kg-day, 4 of 15; 1.0 mg/kg-day, 10 of 16; 30.0 mg/kg-day, 11 of 16.

4.5.1.2.2 New Section 5.3.3.4.2 (Results of New EPA 2003 Analyses of Brain Morphometry)

Morphometry data from this first task demonstrated how the striatum and the anterior and posterior corpus callosum vary with anterior-posterior depth of sectioning in the control brain. The morphometry data from the control brains demonstrate what is known about the variation of size for striatum and corpus callosum with anterior-posterior depth of sectioning (Figures 4-1 and 4-2).

The extant morphometry measurements in the CVP 2003 study of the brain regions from animals dosed with perchlorate reflect the pattern of variation in size for the corpus callosum and striatum with anterior-posterior depth of section shown for the control animals (Figures 4-1 and 4-2). Inspection of these figures reveals that differences in brain region size in the exposed animals compared to control animals are present at a range of depths throughout the structures. The control measurements were consistently lower than those from perchlorate-exposed animals in both the posterior corpus callosum (Figure 4-1, anatomical-atlas plate numbers 29 to 33) and the striatum (Figure 4-2, anatomical-atlas plate numbers 16 through 18), suggesting that the effect of perchlorate exposure on brain morphometry in these regions is robust and independent of variation in depth of sectioning.

The statistical analyses conducted by CVP in 2003 consisted of univariate comparisons for each brain region re-sampled at each anatomical atlas plate number, i.e., according to sections of comparable brain depth. For example, control striatum measurements made at atlas plate number16 were compared to striatum measurements from atlas plate number 16 in the 0.01, 0.1, 1.0, and 30 mg/kg dose groups; similar comparisons were then made at atlas plate number 17. These analyses demonstrated significant effects of perchlorate exposure in striatum and corpus callosum at doses ≥ 0.01 mg/kg-day ammonium perchlorate in drinking water.

As stated earlier, guidelines on the assessment of neurotoxicity (U.S. Environmental Protection Agency, 1998a) specify that alterations in brain structure should be considered adverse and relevant to human health risk assessment. Alterations in brain structure are consistent with the mode-of-action for perchlorate, i.e., transient decrements in tissue

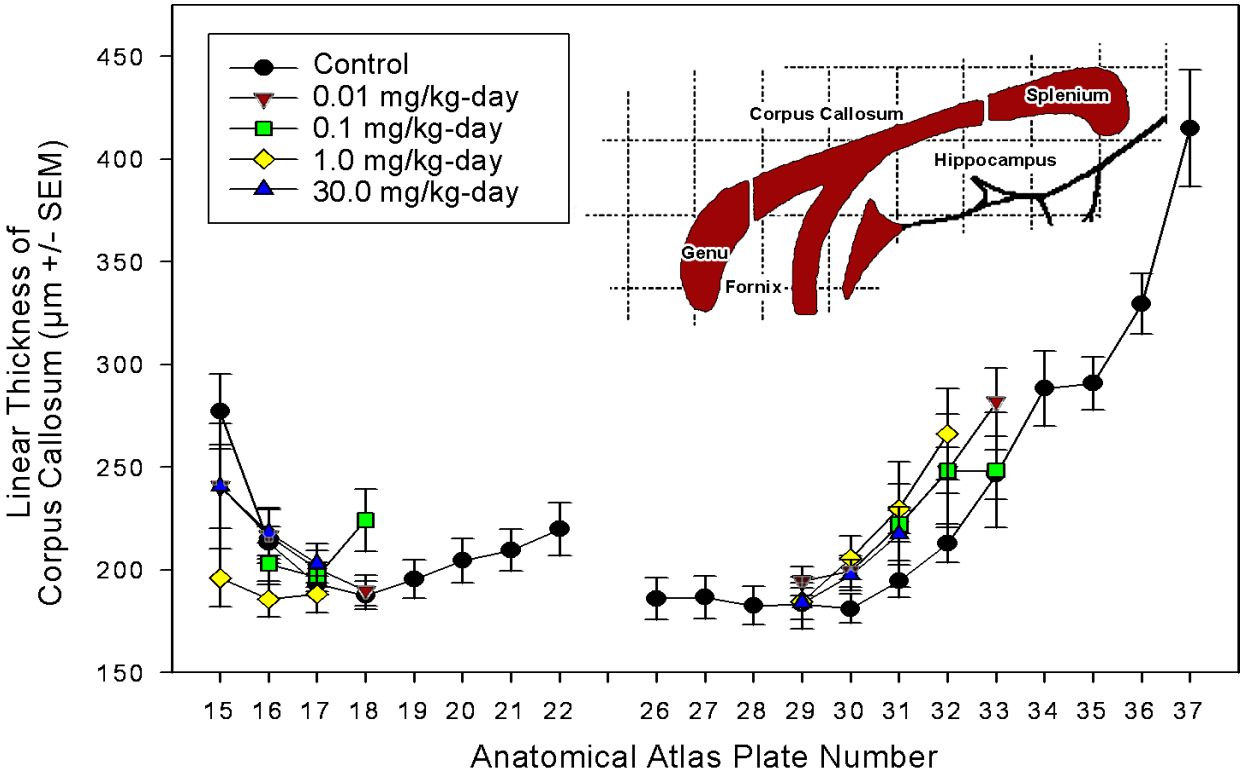

Figure 4-1. New Figure 5-16. Linear morphometric measurements across the brain region called the corpus callosum (CC) submitted in data from a new study by Consultants in Veterinary Pathology, Inc. (2003). These data represent new sections in the coronal plane from tissue blocks of the Argus (2001) study. Plots represent measurements (means ± standard errors) of the CC thickness for at least 6 animals/plate/dose group (Geller, 2003). The pattern of the control data (black circles) shows the expected contour of the CC with an enlarged genu at the anterior end (i.e., at the lower atlas plate numbers to the left-hand side of the x-axis), a fairly narrow "waist" region where there is little change in thickness with depth (e.g., anatomical atlas plate numbers 26 - 30), and rapid expansion toward the posterior end near the splenium (see inset taken from Figure 5-14 in the 2002 ERD). The white lines indicated on the inset mark the approximate position of sections at atlas plate numbers 17 and 31, those used in the new 2003 analyses.

concentrations of thyroxine (T4) and tri-iodothyronine (T3) during development can result in neurodevelopmental effects. Therefore, the significant findings reported in the CVP 2003 report strongly reinforce the argument that EPA made using the Argus (2001) data: that adverse effects of ammonium perchlorate are present at the lowest dose level tested. Because statistically

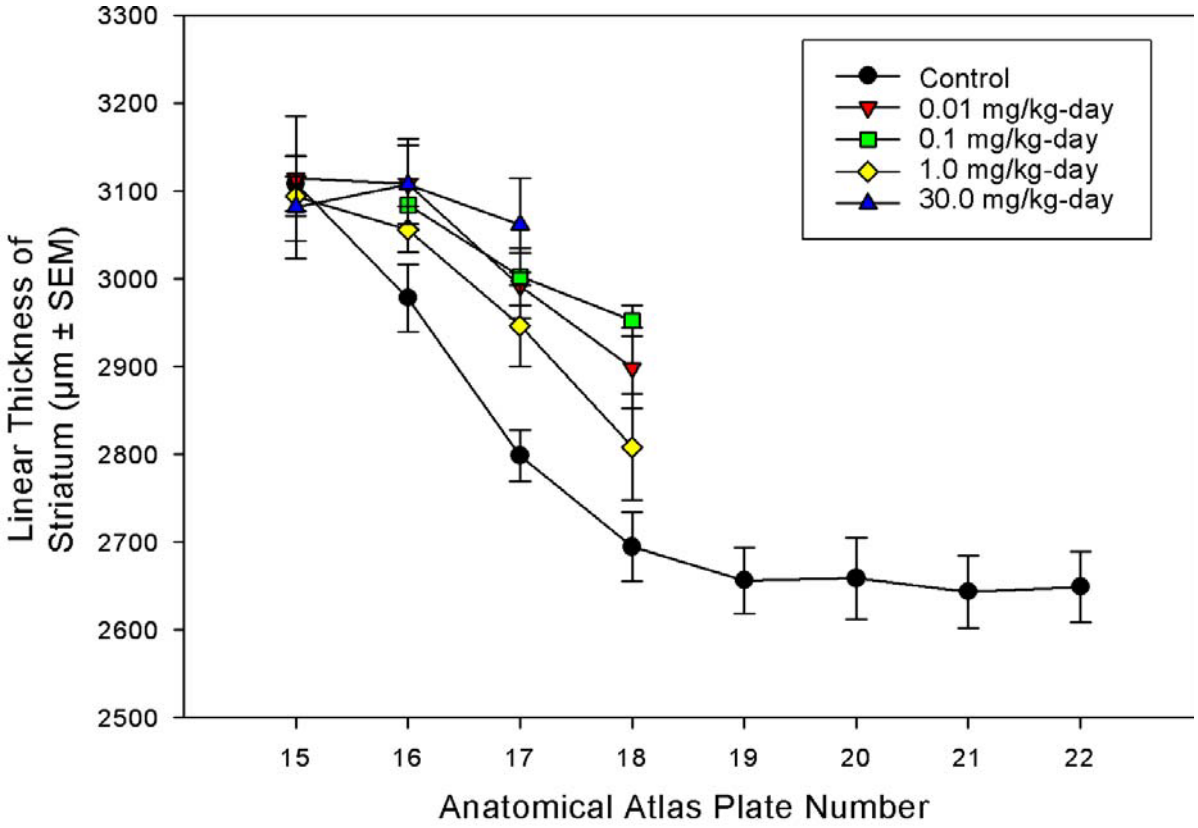

Figure 4-2. New Figure 5-17. Linear morphometric measurements across the brain region called the striatum submitted in data from a new study by Consultants in Veterinary Pathology, Inc. (2003). These data represent new sections in the coronal plane from tissue blocks of the Argus (2001) study. Means ± standard errors the striatum measurements for at least 6 animals/plate/dose group (Geller, 2003) are shown. The pattern of the control data (black circles) show the expected contour of the striatum, larger at the anterior end (i.e., at the lower atlas plate numbers to the left hand side of the x-axis), then narrowing to a consistent thickness in atlas plate numbers 19 through 22.

significant changes in brain morphometry were observed at all dose levels tested, these studies have identified a LOAEL an no NOAEL.

4.5.1.2.3 Mutivariate EPA Analyses (Profile Analyses)

While the analysis in the CVP (2003) report was compelling, EPA once again used the profile analysis strategy to eliminate the possible introduction of Type I error posed by the repeated measures used in the CVP analyses. Multivariate analyses of the brain morphometry

data indicated significant effects of treatment on the pattern of regional brain growth (Geller, 2003). There were significant effects of treatment with perchlorate on brain morphometry, overall, and for all doses versus control (Figure 4-3). These effects were present whether or not the cerebellum data were included. They were also present whether or not data from the treatment group receiving 0.1 mg/kg-day were included (which had fewer than the $n \geq 6$ criterion). Thus, profile analysis on the new 2003 data which were controlled for depth of section, confirm the results of analyses of the data from the Argus (2001) "Effects study" and once again indicate that dose levels of 0.01 mg/kg-day and above produce changes in the development of multiple brain structures. Based on these observations, the 0.01 mg/kg-day level is designated by the EPA as a LOAEL.

4.5.1.2.4 Univariate EPA Analyses

To facilitate comparison with the Argus 2001 and CVP 2003 reports, step-down univariate analyses were conducted as indicated by the multivariate results. Univariate analyses were carried out using a 1-way analysis of variance (ANOVA) and Dunnett's t-test for pairwise comparisons to controls (SAS, Inc., Cary, NC; PROC GLM, MEANS/DUNNETT).

Analysis of variance showed significant effects of treatment in cerebellum, striatum, and posterior corpus callosum at all dose levels. Univariate analysis using the data set of matched sections in a step-down from the profile analysis showed significant differences between control and treated animals in the size of the cerebellum and striatum at all dose levels (Geller, 2003). For the striatum, pairwise tests versus control values showed that all dose groups were significantly different from control animals: perchlorate-exposed animals showed a 5 to 9% increase in the size of this region. For the cerebellum, the measurements from animals dosed at 0.01, 0.1 and 1.0 mg/kg-day were significantly different from control: all doses levels were approximately 10% larger than control values. Univariate differences in cerebellum and striatum were also present when all data from each of these brains regions were used rather than only that included in the profile analysis (Table 4-3, new Table 5-5 in the revised final document). The univariate analysis of the posterior corpus callosum showed an 11 to 18% increase in size in the groups dosed with perchlorate, but only the 1.0 mg/kg-day group showed a significant difference. *A specific discussion of variability found in the measurements of the corpus callosum was provided in Section 4.5.1.1.2 (Variability in Measurements). That discussion on Page 4-28 to 4-29 and Table 4-2 (Table 5-4 of the revised final document) of this response*

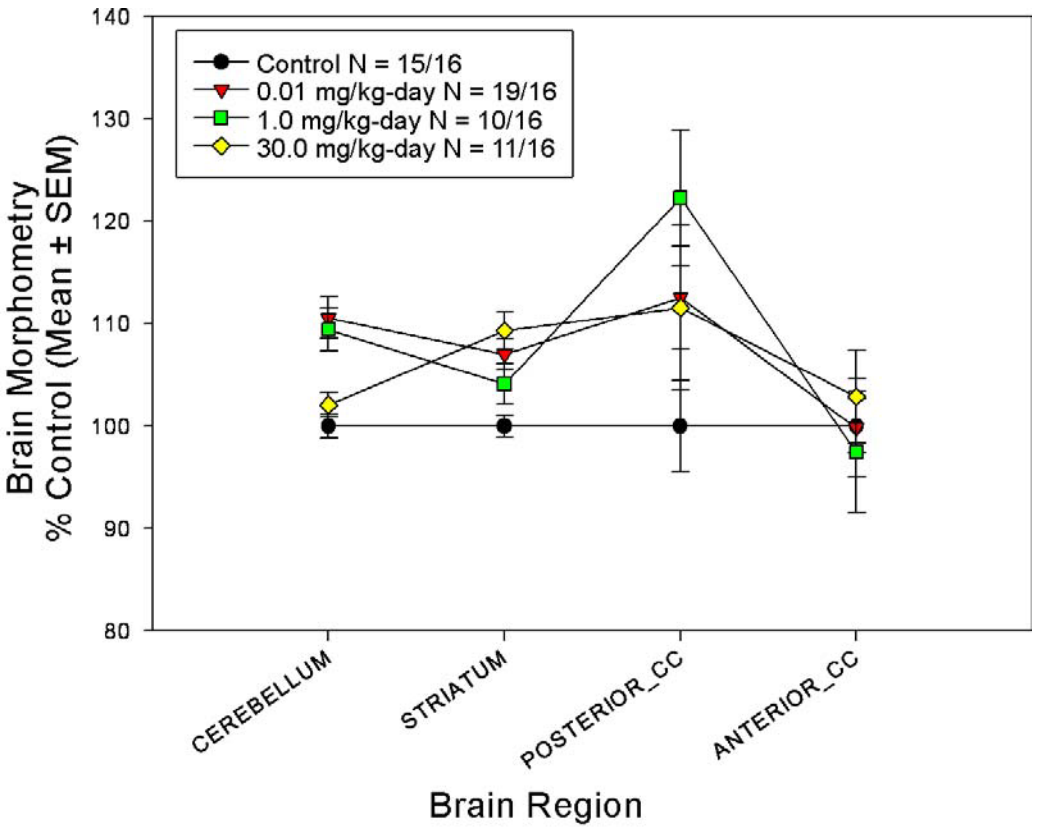

Figure 4-3. New Figure 5-18. Results of 2003 EPA profile analysis of morphometry in selected brain regions (Geller, 2003). Data plotted are the normalized morphometric measures. Profile analysis used raw measurement values. The percent (%) of the control are plotted to aid visualizing the results. Significant treatment effects were found by the profile analysis (Parallel profile: F = 6.09, p < 0.0001; Equal profile: F= 8.93, p < 0.0001). The data for the striatum and anterior and posterior corpus callosum were from the study conducted by Consultants in Veterinary Pathology, Inc. (2003). Cerebellum data are from Argus Research Laboratories, Inc. (2001). Significant treatment effects were also present when the cerebellum data were excluded from the analysis. Data from the 0.1 mg/kg-day group were omitted from the plot because the number of samples (n = 4) did not meet the criterion for minimum number of sections (n ≥6) in the repeated measures analysis. Significant treatment effects were also present when data from this treatment group (0.01 mg/kg-day) were included.

document are to be placed in the new Section 5.3.3.4.2.3 (Variability of Measurements) of the revised final document at this juncture.

Table 4-3. New Table 5-5. Results of Univariate Analyses of Variance on Data from Cerebellum and Striatum (Consultants in Veterinary Pathology, Inc., 2003; Geller, 2003)

Statistical Analysis	Data Used	$N_{analysis}/N_{total}$	Dose
Univariate ANOVA	Cerebellum	79/79	Treatment effect: $F = 12.41$, $p < 0.0001$
Dunnett's t	Cerebellum		Doses 0.01, 0.1, 1.0 mg/kg-day different from control at $\alpha = 0.05$
Univariate ANOVA	Striatum, Plate 17	64/79	Treatment effect: $F=6.44$, $p < 0.0002$
Dunnett's t	Striatum, Plate 17		All dose groups different from control at $\alpha = 0.05$

4.5.1.2.5 New Section 5.3.3.4.3 (Conclusions of the New EPA 2003 Analyses)

The EPA and the contractor, CVP, both reviewed and analyzed a new 2003 set of data that was controlled for depth of sectioning from two different tissue block levels (Levels I and II). The new 2003 analyses of these data (Consultants in Veterinary Pathology, Inc., 2003; Geller, 2003) support the findings in other studies (Argus Research Laboratories, Inc., 1998a,2001) and their associated analyses (U.S. Environmental Protection Agency, 1998a,2002; Geller, 1999a, 2001d), showing a dose-dependent effect on brain morphometry in weanling pups due to developmental exposure to ammonium perchclorate at 0.01 mg/kg-day and higher. The new analyses included profile analysis of all brain regions together against the control profile measurements or step-down univariate ANOVAs with Dunnett's t-test for control of pairwise comparisons of individual brain regions.

A specific discussion of the shape of the dose-response curve and of a comparison of the findings across various studies will be included in this conclusion section at this juncture for revision of the document. In this response document, these two sections are provided separately below in Sections 4.5.1.2 and 4.5.1.3.

4.5.1.3 Comments on Shape of the Dose-Response

This section addresses comments in the 2002 peer panel report found in Section 7 as well as those submitted to the Agency by interested parties regarding the biological significance of the shape of the dose-response curve in one brain region in particular, the inverted U-shape observed in the corpus callosum. *As indicated above, the responses to comments on this topic*

will be included in the revised final document Section 5.3.3.4.3. (Conclusions of the New EPA 2003 Analyses), so they are denoted here with blue text.

Comment(s): *The peer panel had various comments on the biological significance of the shape of the inverted U-shape dose-response curve observed in the corpus callosum. One reviewer indicated that the dose-response curve implies that high doses of perchlorate may protect rats against neurodevelopmental effects. The three reviewers specifically assigned to address neurotoxicity had a different opinion. Because the mechanisms of thyroid hormone action on the reported brain morphometry changes have not been identified, one reviewer assigned to neurotoxicity indicated that he has no basis for dismissing the data because a linear or monotonic dose-response curve was not observed. The second neurotoxicity reviewer 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 included specific hypotheses about mechanisms that may account for the U-shaped dose response. The third reviewer assigned to neurotoxicity also found no inherent problem with non-linear dose-response curves, but he was troubled by the fact that the dose-response trends were not consistently observed across both sexes.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees with the reviewers assigned to neurotoxicity that U-shaped curves have been well documented in the literature and are not to be excluded or dismissed. Comments on consistency across studies are addressed in the next section. The following text is to be in the revised final document in the new Section 5.3.3.4.3 (Conclusions of the New EPA 2003 Analyses) to address the topic of the shape of the dose-response function.

It is unfortunate that dialogue and submitted comments from interested parties have focused to such a degree on the plausibility of the "U-shaped" dose-response shown by the univariate analyses of certain individual brain regions. This has distracted significantly from an appreciation of the attributes and results of the profile analysis performed by the EPA.
As reflected in the discussion below, the trajectories for growth of any given region in the brain at various points in time (e.g., different days of perinatal development) are likely to quite distinct. The profile analysis provided a statistical approach that did not superimpose any *a priori* expectations of direction and magnitude of effects in a given region at any particular sample point. This was viewed by the EPA as the most appropriate approach to evaluate the available morphometry data and thereby to assess the dynamic system that is the developing brain.

Further, the EPA chose profile analysis to be a conservative and rigorous analysis in that it requires all of the regions to exhibit a dose dependence while addressing repeated measures rather than utilizing one region as assessed by a t-test to derive statistical significance. The profile analyses of both the Argus 2001 study and the new 2003 study performed by CVP (Consultants in Veterinary Pathology, Inc., 2003) indicate a dose-dependent pattern of change across all regions that shows the 0.01 mg/kg-day level was statistically significant from controls. It was the profile analysis that was used to designate this as a LOAEL for effects on brain structure in the 2002 ERD. The EPA did not rely on univariate analyses in only one region to designate this effect level although such determinations are routinely made in regulatory toxicology and pharmaceutical development (Sheets, 2002).

The univariate analyses were performed to allow a comparison with the statistical analyses performed by the contractor and to ascertain any insights on specific brain regions. These univariate analyses found U-shaped and inverted U-shaped dose-response functions in some regions of the brain. A comparison of the patterns across studies is discussed below.

Both U-shaped and inverted U-shaped functions have been widely reported previously in response to hormonally active agents for *in/ex vivo* and *in vitro* assays and assessments (reviewed in Davis and Svendsgaard, 1990; NRC, 1999; Melnick et al., 2002; Welshons, et al., 2003). The World Health Organization notes in its principles and approaches for neurotoxicity risk assessment that biphasic (U-shaped or inverted U-shaped) curves are common and likely to reflect multiple mechanisms of action, the presence of homeostatic mechanisms, differences in pharamocokinetics with dose, or activation of compensatory or protective mechanisms (World Health Organization, 2001).

Inverted U-shaped responses have been reported for a broad variety of compounds and endpoints including behavioral, neurochemical, and neurophysiological assessments in a wide range of species. For example, treatment with corticosterone produced an inverted U-shaped response for motor activity in sparrows and mice (Breuner and Wingfield, 2000; Sandi, et al., 1996) and for hippocampal function in the rat (Diamond, et al., 1992). Developmental lead exposure produced an inverted U-shaped response for hippocampal receptor binding in the rat (Lasley, et al., 2001) and U-shaped curves for neurotransmitter release and hippocampal function in the rat (Gilbert et al., 1999; Lasley et al., 2001; Lasley and Gilbert, 2002). Estradiol induced inverted U-shaped responses for prostate, testis, and epididymal weights in the rat (Putz, et al., 2001a, 2001b). Gonadal dysgenesis in the frog was altered by atrazine with an inverted

U-shaped (Hayes, et al., 2003). TCDD produces a U-shaped cancer response as reviewed in Andersen and Barton (1998). A number of other compounds demonstrating non-monotonic dose response curves (i.e., U-shaped or inverted U-shaped functions) are reviewed in Davis and Svendsgaard (1990) and Calabrese and Baldwin (1998).

As discussed in Chapter 3 (of the 2002 ERD and revised final document), the development of the mammalian nervous system is a highly complex process which has very specialized morphological and biochemical patterns of organogenesis that evolve as a precisely-timed mutistage process guided by chemical messengers (World Health Organization, 2001). As organogenesis proceeds, cells become more differentiated and migrate to their appropriate locations. Other critical milestones include the formation of synapses, development of connectivity between structural components, and myelination of axons. The temporal and spatial organization of this development process is a precise and complex process, in which the basic framework is laid down sequential process and in which each step is dependent upon the proper completion of the previous one. A relatively minor disturbance resulting in a perturbation of the developmental interactions between selective cells for a limited time may result in a major deleterious outcome (World Health Organization, 2001). Given these complexities, the trajectories of individual brain regions are difficult to predict but certainly non-monotonic (e.g., U-shaped) curves can be expected.

Non-monotonicities are often attributed to the influence of multiple processes underlying a response (Andersen and Barton, 1999; Barton and Andersen, 1998). Because many different processes mediate brain development, the existence of U-shaped or inverted U-shaped curves is not unexpected. Different brain regions undergo growth, proliferation, programmed cell death, and myelination at different times. Autism in humans appears to involve at least four phases of brain growth abnormality that illustrates the temporal considerations necessary for evaluating any change in brain structure at any point in time — a reduced head size at birth is followed by a sudden and excessive increase later in the second phase, brain growth rate then slows in the third phase to a decline in the fourth phase (Courchesne et al., 2003; Lamhart, 2003).

The combination of the timing of development of a brain region with the degree and timing of the hypothyroxinemia or hypothyroidism experienced in the gestational or post-natal milieu, as well as the animal's age at sacrifice could result in differently shaped dose-response functions for different brain regions. These considerations coupled with considerations of the differences in pharmacokinetics across the range from 0.01 to 30 mg/kg-day suggest that a non-monotonic

dose-response functions (i.e., U-shaped or inverted U-shaped curves) are entirely biologically plausible.

4.5.1.4 Comments on Consistency Across Studies

This section addresses comments in the 2002 peer panel report found in Sections 4 and 7 regarding the consistency of findings between the Argus 1998a and 2001 studies with respect to observed effects on brain morphometry. A*s indicated above, the responses to comments on this topic are to be included in revised final document in the new Section 5.3.3.4.3. (Conclusions of the New EPA 2003 Analyses), so that they are denoted here with blue text.*

Comment(s): *The reviewers had different opinions on the implications of consistencies, and lack thereof, between laboratory animal studies. On the one hand, the discussion leader was very concerned about 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 (1998a) found a significant and much larger effect in the females; some results differed between the right and left hemisphere of the brain; and the previous and recent studies had inconsistent results in the different age groups and treatment groups considered. This reviewer said that these inconsistencies, coupled with his concerns about the study methods, left him little confidence in the observed effects. On the other hand, the other two reviewers assigned to neurotoxicity indicated that certain neurodevelopmental events are known to take place over distinct (and sometimes narrow) windows of time so that it is not unreasonable to observe inconsistent brain morphometry effects at two different postnatal days. One of these reviewer further noted that perturbations in thyroid hormone levels may affect various brain regions differently, and one should not necessarily require consistent effects be observed across multiple regions. The third reviewer offered that inconsistencies in rodent studies can simply result from studies being conducted in different seasons and suggested that EPA superimpose this consideration when evaluating consistency across studies.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA disagrees with the one reviewer who felt that there were considerable inconsistencies in the brain morphometry data across the two previous studies (Argus Research Laboratories, Inc., 1998a; 2001). As Dr. Geller noted at the peer review meeting, while some differences were observed between right and left brain hemispheres, no systematic biases were observed. Further, the differences between the two hemispheres were generally smaller than the brain size differences that appeared to result from perchlorate dosing. Differences between the sexes have been noted in the serum hormones so that changes in the brain and dose-response may reflect these differences. The differences

also likely reflect differences in variability of the data as reflected in post-hoc mean comparisons.

The Agency agrees with the other two reviewers who noted that strict consistency is not necessarily even to be expected, especially not across brain regions. Echoing EPA commentary at the 2002 peer meeting, the following text on a comparative analysis (Geller, 2003) shows that there was in fact considerable consistency across the studies despite differences in dosing regimen and sampling points.

Table 4-4 shows a comparison of the shape of the dose-response curve for specific brain regions obtained in the 1998 versus 2001 studies performed by Argus Research Laboratories, Inc. Despite the difference in exposure duration and the considerable difference in dose spacing between the two studies, a remarkable consistency in the shape of the dose-response between the two studies for various brain regions is evident. The U-shaped response for the corpus callosum and cerebellum in the Argus 2001 versus Argus 1998 study may be due to the three-fold difference in the highest dose tested (30 mg/kg-day versus 10 mg/kg-day).

Table 4-4. New Table 5-6. Comparison of Results of EPA Analyses of Brain Morphometry Data From Two Studies Submitted by Argus Research Laboratories, Inc. (1998a, 2001)

Brain Region	Argus (1998a)[1]	Argus (2001)[2]
Corpus callosum	↑	∩
Cerebellum	↑ (anterior/posterior)	∩ (vertical thickness)
Striatum (caudate putamen + globus pallidus)	∪ (caudate only)	∪
Hippocampus	∪ (hippocampal gyrus)	∪ (CA3)

[1] Dams exposed from GD1 through sacrifice; pups sacrificed at PND12; doses = 0, 3, 10mg/kg-day; 1 male and 1 female/litter, 6 litters/dose.
[2] Dams exposed 2 weeks before mating through sacrifice; pups sacrificed at PND22; doses 0, 0.01, 0.1,1.0, 30 mg/kg-day; 1 male and 1female/litter, 16 litters/dose.
↑ – increase in size
↓ – decrease in size
∩ – inverted U-shaped dose response
∪ - U- shaped dose response

4.5.1.5 General Comments and Conclusions

This section describes additional comments on the study of brain morphometry not directed at the results of the individual studies but at more general issues regarding the nature of the endpoint.

4.5.1.5.1 Is Hypothyroidism expected to increase the size of selected brain regions?

Comment(s)**:** *The neurotoxicologists on the peer panel debated the biological plausibility that hypothyroidism causes increased dimensions in specific brain regions. The discussion leader, for example, expected hypothyroidism to result in decreased sizes of brain regions, based on studies published in the literature. Another reviewer did not share this expectation. He cautioned that the published studies linking hypothyroidism to decreased sizes of brain regions are based largely on subjects with severe hypothyroidism or on subjects given thyroid hormone replacement to treat severe hypothyroidism, not on subjects experiencing the level of impaired thyroid function believed to result from perchlorate exposure. This reviewer noted that, because researchers have yet to quantify the dose-response relationship for how changes in thyroid hormone levels affect linear measurements of brain sections, he has no clear expectation of 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; one reviewer noted that such an understanding currently does not exist.*

Nevertheless, the discussion leader suggested that the EPA examine the existing data, or possibly the control data from the literature, to determine if 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, or 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 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.

EPA Response(s) and Recommendation(s) for Revision(s): While it is established that thyroid hormone is essential for proper brain development, the state of the science at this time does not provide for a precise mapping of the growth for each individual brain region across different days of neonatal development. As discussed in Section 4.5.1.2, the trajectories of each region at any point in time are likely to be different. Thus, the EPA agrees with the peer reviewer who noted that a more complete mechanistic construct of the events is not possible. Specific regions at a given point in time were not chosen explicitly due to this insufficient

knowledge base. Again as discussed above, the reason for the profile analysis was to invoke the totality of the information from all of the brain regions to direct specification of an effect level. The EPA did not rely only on one brain region because the knowledge to specify which region, the direction of the change (i.e., larger or smaller) for that region at a given postnatal day, and the expected magnitude of change are not well established. However, the national and international community has concluded that any changes in brain structure should be considered adverse (U.S. Environmental Protection Agency, 1998b; World Health Organization, 2001).

The EPA disagrees with the reviewer that no thyroid hormone changes were associated with the dosages that caused changes in brain morphometry. The Agency notes that there were statistically significant thyroid hormone changes in both the maternal animals on GD21 as well as in the pups of the Argus 2001 "Effects Study" at the same dose levels (0.01 mg/kg-day and higher) that the brain morphometry effects were observed. The LOAEL for both T4 and TSH in the dams on GD21 was 0.01 mg/kg-day. The LOAEL for both T4 and TSH on PND22, the day on which the pups were sacrificed for brain measurement, was also 0.01 mg/kg-day. It is also clear that an external (serum) hormone marker of the thyroid hormone status at a specific time during development may not correlate with changes in brain that derive from alterations in tissue hormone levels during critical points in development of that structure. Correlation analyses across time points were precluded since some blood samples were pooled and blood was not drawn from the same animals on the PND22 sacrifice that were used for the morphometric analyses.

4.5.1.6 Are Changes in Brain Morphometry Adverse?

Comment(s): *One reviewer asked if the observed brain morphometry changes have been associated with any functional, cognitive, or other types of adverse effects. The discussion leader replied that the 2002 ERD did not correlate the brain morphometry findings with observations from any other endpoint; he suggested that EPA evaluate whether such correlations exist. A third reviewer cited the following quote from the Argus (2001) study indicating that it did not consider other endpoints: "Detailed microscopic analysis. . . .failed to indicate any evidence of treatment-related neuropathologic effects." Another reviewer indicated that EPA considers any alteration in brain structure as an adverse effect, regardless of whether its potential impacts, if any, have been identified.*

EPA Response(s) and Recommendation(s) for Revision(s): As stated in the WHO principles and approaches for neurotoxicity risk assessment, it can be assumed that developmental

neurotoxicity effects in laboratory animals indicate the potential for altered neurobehavioral development in humans although the specific types of developmental effects seen in laboratory animals will not necessarily be the same as those that may be produced in humans (World Health Organization, 2001). And, as will be discussed in the EPA conclusions below (Section 4.6), the standing Agency guidance on risk assessment of potential neurotoxicity states that *"Neurotoxicity is an adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical."*

The Agency agrees with the written comments of the discussion leader who pointed out that neuropathology did also occur in the 1998a Argus study. This reviewer noted that the mean value for the thickness of the corpus callosum in females of the high-dose group (10 mg/kg-day) was significantly greater than controls on PND12. The histopathology performed on PND92 revealed significant effects in brain weights and in the frontal cortex and corpus callosum in the males of the high-dose group. The Agency agrees with the conclusions of the peer reviewer that these effects were not random variation as asserted by the contract lab (Argus Research Laboratories, Inc., 1998a) but were rather an indication of a histopathological correlate to the observed brain morphometry changes.

4.5.1.7 Additional General Comments and Conclusions

Comment(s): *The peer reviewers made additional comments on the brain morphometry studies that do not fall under the categories listed above. First, the discussion leader was concerned that the 2002 ERD relied too heavily on the Argus (2001) study without integrating the findings from the previous study (Argus Research Laboratories, 1998a). Another peer reviewer recommended that EPA's statistical analyses include some adjustments for multiple comparisons to determine if a dose-related signal exists across the two brain morphometry studies. Third, though not disagreeing that statistical analyses may be helpful, the discussion leader emphasized that no statistical analyses can correct for the methodological weaknesses identified in the Argus (2001) study. He wondered if any mechanistic argument could explain the U-shaped dose-response curve.*

Two peer reviewers offered their conclusions regarding the brain morphometry studies and the Agency's interpretation of them. The discussion leader acknowledged that both brain morphometry studies (Argus Research Laboratories, Inc., 1998a;2001) provide evidence suggesting an association between perchlorate and changes in brain morphometry in the rat. However, given the limitations of the study methodology and inconsistencies in results, he asserted that one can not be certain the effects are not the result of sampling error, selection bias, or some other artifact and thus found the studies inconclusive. Another peer reviewer disagreed and indicated that the EPA had appropriately designated the changes in brain structure as a LOAEL given the Agency guidance on such effects.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has no additional response to these comments that are not already addressed in the sections above.

4.5.2 Comments on Motor Activity Studies

This discussion pertains to evaluation of the available data on neurobehavioral endpoints found in Section 4.5.2 of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b). Effects of perchlorate on motor activity were assessed in two studies. The Argus Research Laboratories, Inc. (1998a) developmental neurotoxicity study contained a battery of behavioral tests (passive avoidance, water maze, auditory startle). A second study was performed to repeat the findings of effects on motor activity observed in the Argus 1998a study in response to recommendations at the 1999 peer review (Bekkedal et al., 2000).

While the testing laboratory (Argus Research Laboratories, Inc.) discounted effects in the pups on post-natal day 14 (PND14), which amounted to a 95% increase in number of movements and time of movements over that observed in controls because they were not statistically significant, EPA concluded in the 1998 ERD that such increases in motor activity in developing animals were clearly of concern and questioned the high degree of variability in the data (Crofton et al., 1998). The BMDL estimates for number of movements and time spent moving were 1.04 and 0.66 mg/kg-day, respectively. The 1999 peer panel agreed with the EPA and recommended that an additional study of motor activity be performed.

The US Navy laboratory at Wright-Patterson Air Force Base performed an additional study of motor activity in response (Bekkedal et al., 2000). The 2002 peer panel was charged with providing a peer review of this study and then with commenting on EPA's interpretation and analysis of both studies. Responses to peer-panel comments at the meeting as well as to those written by assigned panel members (Drs. Paule, Aschner, and Zoeller) or the public are addressed in this section.

The EPA analyses for the 1998a Argus study are found in Section 5.3.1.4 (Behavioral Evaluations) on Pages 5-43 to 5-47 of the 2002 ERD. The study performed by Bekkedal et al. (2000) and EPA's Bayesian analysis for the evaluation of the two motor activity studies combined are found in Section 5.3.2 on Pages 5-47 to 5-52 of the 2002 ERD. Recommendations for changes pertain to these sections.

Comment(s): *The study leader noted that the reviewers found the Bekkedal et al. (2000) motor activity study to be rigorous and EPA's interpretations appropriate. One reviewer suggested that consultation with the study authors regarding the timing with which the behavioral measurements were collected could be informative to the considerable variability observed in the data. Another reviewer noted that the statistical approaches employed by the authors of the study had limited power given the high variability and number of animals. This reviewer found EPA's statistical analyses compelling and stated that the analyses provided clear confidence in the significance of the effects in the Argus 1998 study and demonstrated a replication of a perchlorate effect. He agreed that the results indicate that behavioral effects occurred in both studies, making the two studies consistent. He noted that these functional studies support an effect in the cerebellum, an association that would corroborate the morphometric findings. This reviewer added that perfect replication across two such behavioral studies is not expected, given season differences and variability in motor activity with time of day.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA does not recommend any changes to the analyses or interpretation of the motor activity data based on these comments. The analyses did take into account the timing of the responses as the testing intervals were used explicitly. The precise time of day for each test was not provided. The Bayesian approach offers several advantages, notably addressing the repeated measures issues and variability as well as providing for formal combination of the data across studies. The EPA agrees with the reviewer comments that the two studies show a consistent outcome with respect to a functional effect of perchlorate and one that is supportive of the findings in brain morphometry. The NOAEL designated at 1 mg/kg-day by the Bayesian approach that integrates both studies agrees with the benchmark approaches performed previously on the Argus 1998a study.

4.6 CONCLUSIONS REGARDING THE NEUROTOXICOLOGICAL EFFECTS OF DEVELOPMENTAL EXPOSURES TO PERCHLORATE

Analogous to the new section recommended to be included on conclusions regarding the potential carcinogenic risk posed by perchlorate exposure, the EPA will also add a new section (*5.3.4. Conclusions Regarding the Neurotoxicity Effects of Developmental Exposures to Perchlorate*) at the end of Chapter 5 to present summary statements and conclusions on the neurotoxicological effects of developmental exposures to perchlorate. The purpose of this new section is to provide an integration of the various endpoints that signal the neurotoxicological potential for perchlorate into one location in the document. This section will rely on more

detailed summaries of the data from the individual studies elsewhere. The new section is provided in the blue text that follows.

This section summarizes the evaluation of the potential for pechlorate exposure to pose a significant risk to the developing nervous system based on the evidence described in greater detail in the previous three sections. This summary is the result of careful evaluation and consideration of the relevant data by a team of expert neurotoxicologists in the EPA's National Health and Environmental Effects Research Laboratory (Boyes et al., 2003). The review was specifically requested in response to comments made at the expert peer review of the 2002 ERD.

The data reviewed include the two developmental neurotoxicity studies (Section 5.3.1 and 5.3.3) conducted by the Air Force Research Laboratory (AFRL) or contractors to the Department of Defense (DoD) and the Perchlorate Study Group (PSG), a consortium of defense industry members (Argus Research Laboratories, Inc., 1998a; 2001). In addition, supplemental materials regarding the brain morphometry studies were examined, including the new 2003 study performed under contract to the EPA (described in Section 5.3.3.4.3) and provided by Dr. Robert Garman, Consultants in Veterinary Pathology, Inc. (Consultants in Veterinary Pathology, Inc., 2001a; 2001b; 2003). Two neurobehavioral studies were also evaluated, the one conducted as part of the original developmental neurotoxicity (DNT) study (Argus Research Laboratories, Inc., 1998a) described in Section 5.3.1 and the second by the United States Navy (Bekkedal et al., 2000) study as described in Section 5.3.2. Additionally, the EPA evaluated comments received by the Agency in two extramural peer reviews of its previous ERD documents (U.S. Environmental Protection Agency, 1998b; U.S. Environmental Protection Agency, 2002a; Environmental Protection Agency, 2002b).

The section steps through the Agency's rationale for arriving at inferences from the available data and then applies the current principles of risk assessment for neurotoxicity to arrive at the conclusions in Section 5.3.4.6.

4.6.1 New Section 5.3.4.1: Perchlorate Treatment Reduces Circulating Levels of Thyroid Hormones

As described in Chapter 2 (Chapter 3 of the 2002 ERD and revised final document) on the mode of action and confirmed by data in the studies described throughout this chapter, perchlorate causes reductions in the concentrations of thyroid hormones in blood and in target tissues at various life stages. Perchlorate causes these effects by inhibiting active uptake of

iodide at the NIS in various tissues, including the thyroid gland, GI tract, mammary gland and placenta (Wolff, 1998; Dohan et al., 2003). Iodide is required by the thyroid for the production of thyroid hormone. Thyroid hormones are required for the development of many organ systems. The nervous system is particularly sensitive to alterations in the levels of thyroid hormone during prenatal and postnatal growth.

Despite differences in dosages and experimental design, perchlorate decreases serum thyroid hormones (T4 and T3) and increases serum TSH. As was shown in Table 4-1 of Section 4.3 in the response document (Table 5-7 in revised final document), there was a strong qualitative consistency across all the studies. The same pattern of effects on serum hormones was found in the 14-day Caldwell et al. (1995) study, at both time points (14- and 90-day sacrifices) of the subchronic study (Springborn Laboratories, 1998), the first neurodevelopmental study (Argus Research Laboratories, Inc., 1998a), the neurodevelopmental "Effects Study" (Argus Research Laboratories, Inc., 2001), the mouse subchronic study (Keil et al., 1999), and the rabbit developmental study (Argus Research Laboratories, Inc., 1998c). The only exception to this pattern of effects was in the two-generation study performed on rats (Argus Research Laboratories, Inc., 1998b) in which marginal effects were observed in the opposite direction for TSH. Qualitative differences across the database are likely due to differences in laboratories and study design.

With respect to the two neurodevelopmental studies (Argus Research Laboratories, Inc., 1998a,2001), the differences in the NOAEL and LOAEL for thyroid hormones 0.1 and 1.0 mg/kg-day) and TSH (3.0 and 10.0 mg/kg-day) on PND5 in the 1998 study versus the LOAEL for all three serum hormones at 0.01 mg/kg-day on PND22 in the Argus (2001) study may be due to differences in sample points (postnatal age or days of treatment), dose spacing, or species differences in addition to the technical limitations discussed at the 2002 peer review.

4.6.2 New Section 5.3.4.2: Hypothyroxinemia Alters Neural Development

It is well established in both laboratory animals and in humans that reductions in the levels of thyroid hormones impair the proper development of a number of organ systems including the nervous system (Schwartz, 1983; Dussault and Ruel, 1987; Thompson and Porter, 2000). Infants or children with low circulating thyroid hormones, such as those suffering from dietary-iodine deficiency or congenital hypothyroidism, have impaired neurological development (Rovet, 1999; Halpern et al., 1991; Boyages and Halpern, 1993). Children lacking adequate thyroid hormones

during brain development have a variety of neurological problems including difficulty making coordinated motor movements, speech and hearing impairments, and cognitive problems. Severe hypothyroidism leads to endemic cretinism with pronounced mental retardation (DeLong, 1989; Timiras and Nzekwe, 1989; Halpern et al., 1991; Boyages and Halpern, 1993; Porterfield and Hendrich, 1993). Neurological endpoints are among the most sensitive and critical health outcomes of thyroid disruption in developing organisms (Rovet, 1999; Haddow et al., 1999; Pop et al., 1999; Zoeller et al., 2002; Porterfield, 2000). A key issue for evaluating risks of environmental exposures is determining the consequences of mild degrees of hypothyroxinemia on neurological development. In this regard, children show IQ deficits with as little as a 25% decrease in maternal thyroxine during pregnancy (Man et al., 1991; Pop et al., 1999; Haddow et al., 1999; Morreale de Escobar et al., 2000). Indeed, these studies are part of the evolving controversy regarding the adequacy of current clinical norms when evaluating the potential for population impacts of thyrotoxicants to those with subclinical disease or for maternal screening (Brucker-Davis et al., 2001; Meier et al., 2001; Morreale de Escobar et al., 2000; Andersen et al., 2002).

4.6.3 New Section 5.3.4.3: Laboratory Animal Models of Brain Development and Neurotoxicity

Laboratory animal models confirm that hypothyroidism produces adverse health outcomes. Current data suggest that the sensitivity of laboratory animal models to thyroid disruption depends upon whether the health outcome being considered is cancer or neurological development. There is good evidence that rats are more sensitive than humans to some antithyroid effects of exposure to thyrotoxicants, such as formation of thyroid tumors (McClain, 1995; Capen, 1997; Hill et al., 1998). It appears, on the other hand, that the currently used laboratory animal models of neurological development may be either equal or less sensitive to thyroid disruption. Differences in mechanisms that underlie tumor formation and neurodevelopmental impairments are likely responsible for the relative sensitivity of these health outcomes. This is why the model of the mode of action for perchlorate proposed by the Agency has two different sets of sequelae depending on the perturbation: neurodevelopmental events follow deficits in thyroid hormones alone and neoplasia follows perturbation of the HPT axis sufficient to also upregulate TSH.

Adult rodents are more susceptible than humans to thyroid tumors caused by chemical exposure. Non-genotoxic thyroid tumors in rodents are caused by hypersecretion of TSH. The rodent thyroid gland at greater risk of tumors than is the human (McClain, 1995; Capen, 1997; Hill et al., 1998). Up-regulation of TSH is a critical precursor in formation of thyroid tumors and is relevant, although conservative, in assessing human health risks (Hill et al., 1998; US Environmental Protection Agency, 1998b).

It is critical to note that a different relationship to serum hormones exists for neural development because the mechanisms for disruption of nervous system development differ from those of tumor formation. The precursor events in the disruption of nervous system development by thyrotoxicants are not the same as for tumor formation. The brain requires adequate tissue levels of triiodothyronine (T3) for normal development, which in turn is dependent on adequate circulating levels of both the thyroid hormones, T3 and thyroxine (T4). Decrements in circulating T4 may impair neurological development even when TSH levels are normal. Thus, the critical event for impaired neurological development is a decrease in tissue levels of thyroid hormones, not a change in circulating levels of TSH (DeVito et al., 1999; Morreale de Escobar et al., 2000). Even transient perturbations in tissue thyroid hormones during development can lead to permanent adverse outcomes (Porterfield, 2000; Howdeshell, 2002).

A biomarker for changes in tissue thyroid hormones is circulating serum levels of thyroid hormones. Decreases in circulating hormone levels will eventually lead to inadequate tissue levels of T3, the active hormone, and to subsequent decreases in thyroid-hormone-mediated development. Alterations in neurodevelopment caused by hypothyroxinemia can occur in the absence of clinical signs of hypothyroidism or alterations in serum T3 or TSH (Goldey and Crofton, 1998; Morreale de Escobar et al., 2000; Haddow et al., 1999; Pop et al., 1999; Lavado-Autric et al., 2003). This suggests that changes in circulating thyroid hormone levels should be viewed as a “hallmark” precursor event for changes in neurodevelopment. This is in contrast to the serum TSH increases that are a critical precursor for tumor formation (Capen, 1997; Hill et al., 1998). Changes in serum thyroid hormones and TSH both result from the anti-thyroid effects of perchlorate on iodide uptake.

There are numerous experimental models of hypothyroidism including those using rodents, amphibians, birds, and fish (Colborn, 2002; Damjanovski et al., 2002; McNabb, 1989; Power et al., 2001). Rodent models of developmental hypothyroidism have been employed successfully for many decades and express many of the disease hallmarks observed in humans

including reduced growth rates, motor impairments, hearing loss, and cognitive dysfunctions (Cabello and Wrutniak, 1989; DeLong, 1989; Eyers, 1971; Timiras and Nzekwe,1989; Halpern et al., 1991; Boyages and Halpern, 1993; Porterfield and Hendrich, 1993). Thyroid hormone replacement therapy prevents or ameliorates the severe neurological deficits and demonstrates the essential role of thyroid hormone in neural development in animals and humans (e.g., Uziel et al., 1981; Escobar-Morreale et al., 1996; Sprenkle et al., 2001; Rovet, 2002). However, subtle impairments in neuropsychological endpoints persist in children with congenital hypothyroidism despite thyroid-hormone therapy within the first few weeks of life (Rovet, 2002).

The similarity between laboratory animal models and humans also extends to the molecular level where thyroid hormone influences gene expression during brain development. Many of the genes that encode proteins critical for cell migration and synaptogenesis, dendritic arborization, myelination, and basic architectural layout of the CNS are thyroid-hormone dependent (Bernal, 2002). Sequencing of these thyroid hormone-related genes has demonstrated a substantial homology across species. The precise timing, duration, and sequencing of gene expression under the control of thyroid hormones are essential to normal brain development (Uziel, 1986). Even transient disruptions in the complex network of gene expression at critical stages can lead to a failure to develop normal neuronal systems and can induce long term consequences (Goldey and Crofton, 1998; Morreale de Escobar et al., 2000; Haddow et al., 1999; Pop et al., 1999; Rovet, 2002). Perturbations in the expression of thyroid-responsive genes have also been implicated in developmental insults (such as fetal alcohol syndrome) that have clear and long lasting neurological manifestations (Cudd et al., 2002; Yang and Zoeller, 2002; Scott et al., 1998).

Experimental work in laboratory animals has focused on the neurological impairments that result from severe depletion of thyroid hormones during development (Uziel et al., 1981; Hendrich and Porterfield, 1996; Morreale de Escobar et al., 1993). Human exposure to environmental thyrotoxicants will not likely produce the severe degree of thyroid hormone disruption (i.e., hypothyroidism) typically involved in clinical models, but instead milder degrees of hypothyroidism or hypothyroxinemia. In laboratory animal models, whether there is a threshold level of thyroid hormone disruption necessary for altering gene expression and subsequent changes in brain structure and function is currently unknown. However, the essential role of thyroid hormone in brain development makes it critical to consider the possibility that mild degrees of hypothyroxinemia may impair neurological development. Such concern is

consistent with the observation that as little as a 25% decrease in maternal thyroxine during pregnancy can cause IQ decrements in offspring (Man et al., 1991; Pop et al., 1999; Haddow et al., 1999; Morreale de Escobar et al., 2000). These data suggest that human neurological development may be sensitive to degrees of hypothyroidism or hypothyroxinemia lower than those that have been to date evaluated in typical laboratory-animal studies.

4.6.4 New Section 5.3.4.4: Perchlorate Treatment Alters the Size of Structures in the Developing Brain

The hypothesis that developmental exposure to ammonium perchlorate in drinking water would lead to reductions in circulating thyroid hormones and consequently to alterations in development of the nervous system was evaluated in two studies. As discussed in Chapter 3 (of the 2002 ERD and final revised document), a neurodevelopmental toxicity study was considered critical to the database for perchlorate because of its mode of action. The first study (Argus Research Laboratories, Inc., 1998a) was limited in scope with regard to number of dose levels, dosing period, and number of litters. Despite the limited scope, statistically significant changes in the size of several brain regions were observed in perchlorate-treated offspring (U.S. Environmental Protection Agency, 1998a). Brain regions altered by perchlorate treatment included the striatum, hippocampus, corpus callosum, and cerebellum. The 1999 external peer review panel recommended that a second study be conducted to confirm and extend the findings of perchlorate exposure on developing brain structures.

The second study was conducted under contract to the PSG and submitted to the Agency for evaluation (Argus Research Laboratories, Inc., 2001). The experimental design for the second study included a lower dose level as recommended by the 1999 peer panel, dosed maternal animals prior to gestation, and evaluated larger sample sizes. The EPA profile analysis results of the second study confirmed that developmental perchlorate treatment caused a statistically significant pattern of change across all of the brain regions in a dose-dependent fashion to levels as low as 0.01 mg/kg-day. Univariate analyses of individual regions revealed changes in several brain regions in the exposed offspring, including the striatum, hippocampus, corpus callosum, and cerebellum, that were different from controls with statistical significance at all dose levels, i.e., including 0.01 mg/kg-day and above.

The morphometry methodology of the second study was criticized by DoD and other stakeholders who alleging that inconsistency in the anterior-posterior position of the brain

section for the corpus callosum from tissue block level II occurred in a systematic fashion and biased the results based on artifactual measurements. In order to evaluate this concern, EPA contracted Consultants in Veterinary Pathology (CVP) to re-section and re-measure brain tissue from that tissue block and another that did not incur the concerns from the 2001 study.

The goal of the CVP study was to measure the size of specified brain structures while more strictly controlling the anterior-posterior depth of the brain sections for the corpus callosum in tissue block level II and the striatum in tissue block level I. It was possible to accomplish this by carefully determining the anterior-posterior level of each brain section using a variety of brain landmarks, including those proximal to the measured structures. Only data from a single histological plate number for each brain structure were included in the analysis in order to eliminate any bias related to depth or plane of sectioning. Data on the cerebellum were also included because this region was not indicated to be a concern with respect to sectioning bias in any of the comments. While this re-analysis was limited due to the amount of the tissue remaining in the blocks, it served to tightly control for plane of section.

Profile (multivariate) analyses of these data from the restricted set of histological plates indicated that there were significant effects of treatment on the pattern of regional brain dimensions (Geller, 2003) at all doses. Univariate analysis of the data also revealed statistically significant differences in the size of the cerebellum and striatum between control and treated animals at 0.01 mg/kg-day and higher levels of perchlorate.

The EPA concludes that the data available to the Agency from the two studies on brain morphometry as submitted by the defense industry (Argus Research Laboratories, Inc., 1998a; 2001), as well as an additional study performed under contract to the EPA (Consultants in Veterinary Pathology, Inc., 2003), despite their limitations, are consistent with changes in the size of regions in the brain of developing pups exposed to ammonium perchlorate in drinking water at concentrations of 0.01 mg/kg-day and higher.

4.6.5 New Section 5.3.4.5: Perchlorate Treatment Alters Behavior of Offspring

The behavioral consequences of developmental exposure to ammonium perchlorate were also evaluated in two studies. The second study was, as for the brain morphometry, recommended to validate the findings of the first.

The first study was conducted by Argus Research Laboratories (Argus Research Laboratories, Inc., 1998a) and the second by USN/DoD researchers (Bekkedal et al., 2000). In the first study, concentration-related increases were obtained in PND14 male rat pups in two related measures of ambulatory activity. These increases in motor activity, which at the highest concentration reached 95% (time-spent-in movement) and 65% (number of movements), were not found to be statistically significant owing to excessive variability in the behavioral data (e.g., coefficients of variation in the control group exceeded 100%). As a result, it was recommended by the 1999 extramural peer review panel that a second study be undertaken. A similar experimental design was used for the USN study (Bekkedal et al., 2000) although the motor activity measurement device was not identical to that used in the first study. Nevertheless, perchlorate produced similar concentration-related increases in the number of ambulatory movements of the rat pups.

The effects of developmental perchlorate exposures on ambulatory activity were re-analyzed by NIEHS statisticians using Bayesian statistics (Dunson, 2001a). Two of the advantages of Bayesian statistics are the following: (1) they do not require assumption of normally distributed data, as do conventional parametric statistics and (2) the availability of prior data can be used to refine probability distributions for more accurately determining statistical significance. Accordingly, this approach was used to re-analyze the data from the two studies, both separately and when the data from the two studies were combined. The results of these analyses showed similar concentration-related increases in ambulatory activity in both studies. Analysis of the combined data sets further strengthened the conclusion that developmental exposure to ammonium perchlorate produced concentration-related increases in ambulatory behavior in the offspring. Developmental exposure to ammonium perchlorate caused changes in the behavior of treated offspring in both studies. Based on these results, a NOAEL was conservatively estimated to be approximately 1 mg/kg-day.

In comparing the results of developmental exposure to ammonium perchlorate with those on morphometric measures of the brain, it is not clear why changes in behavior were observed at higher dose levels (lower limit on the 10% response level at 1.0 mg/kg-day) than were changes in the size of brain structures (0.01 mg/kg-day). Three factors that could contribute to the situation include the following: (1) excessive variability in the results of the motor activity studies may have rendered this assay relatively insensitive to disruption by perchlorate treatment, particularly at the lowest dose (0.1 mg/kg-day), (2) the neurological substrates of motor activity

have not been identified and may not involve the brain structures altered at lower dose of perchlorate, (3) focused testing of learning, memory or other higher-order cognitive functions may have been more sensitive to disruptions than the motor activity assays employed.

4.6.6 New Section 5.3.4.6: Conclusions Regarding the Developmental Neurotoxicity of Perchlorate Based on Current Principles

The findings discussed in the sections above (4.6.1 through 4.6.5) were considered within the context of the Agency's Neurotoxicity Risk Assessment Guidelines (U.S. Environmental Protection Agency, 1998b). These guidelines represent the consensus opinion of neurotoxicologists with respect to inferences that can be drawn from contemporary neurotoxicity testing studies, such as the developmental neurotoxicity studies described above, for application in regulatory risk assessment. Of note, these guidelines state that:

> *Neurotoxicity is an adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent. . . .Structural neurotoxic effects are defined as neuroanatomical changes occurring at any level of nervous system organization; functional changes are defined as neurochemical, neurophysiological, or behavioral effects. . . .Neurotoxic effects. . . .can be observed at many different levels, so only a single endpoint needs to be found to demonstrate a hazard, but many endpoints need to be examined to demonstrate no effect. For example, to judge that a hazard for neurotoxicity could exist for a given agent, the minimum evidence sufficient would be data on a single adverse endpoint from a well-conducted study. In contrast, to judge that an agent is unlikely to pose a hazard for neurotoxicity, the minimum evidence would include data from a host of endpoints that revealed no neurotoxic effects. . . .In some cases, it may be that no individual study is judged sufficient to establish a hazard, but the total available data may support such a conclusion.*

In accordance with this guidance, the dose-dependent changes observed in the multivariate profile analysis of the measured brain structures in two different studies of developmental exposure to perchlorate, and statistically significant changes observed by univariate analysis of both the striatum and cerebellum in these same studies, constitute what can be considered adverse neurotoxic outcomes.

Also according to principles of neurotoxicity risk assessment, changes in behavior may be considered adverse when considerations of the nature and magnitude of the effects are evaluated. Developmental exposure to perchlorate caused up to 95% increases in behavior activity of the

treated offspring. When these factors are considered, the increases in motor activity observed following developmental exposure to perchlorate in two studies are considered to be adverse.

Integrating and considering these data together provides two lines of evidence that the developing nervous system is adversely affected by exposure to perchlorate. At this time, the data from the morphometric analyses and the behavioral studies do not lead to a clear understanding of cause and effect between alterations in brain structure and behavioral function. This is not surprising because these studies were not designed to characterize these relationships, but were designed instead for neurotoxicity screening. Although each of the studies evaluated has limitations, the pattern of evidence across the studies in combination with the larger scientific knowledge of the role of thyroid hormones in neural development is sufficient to conclude that developmental exposure to perchlorate constitutes a potential risk of adverse neurodevelopmental effects.

4.7 COMMENTS ON IMMUNOTOXICITY

This discussion pertains to evaluation of studies performed or completed since the 1999 peer review workshop and to evaluation of the EPA interpretation and analyses of these data. The new studies included the reports submitted by Keil et al. (1999) and BRT Burleson Research Technologies (2000a,b,c). This discussion is found in Section 4.6 of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b).

4.7.1 On Keil et al. (1999)

Comment(s): *The designated discussion leader had generally favorable comments concerning both the two immunotoxicity studies completed since the 1999 peer review and on EPA's interpretation of these studies with the exception of the EPA's proposed uncertainty factor to account for database insufficiencies regarding this endpoint (see specific comment below). Regarding the summary provided in Table 5-2 of the 2002 ERD, he noted that either no effects were observed or that the effects were not consistent across dosage groups and exposure durations (i.e., 14-day 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 — the sheep red blood cell (SRBC) antibody response. He then commented on the one parameter that exhibited some consistency in its effect, decreased macrophage phagocytosis that was observed in all dose groups. It was noted that the change was not in a dose-dependent fashion in the 90-day study and that the effect was not detected in the satellite group sacrificed at 30 days after exposure had ceased. Given that the decreased macrophage phagocytosis was not observed across all experiments and was not accompanied by any sign of*

compromised host resistance as evidenced by a lack of response to Listeria monocytogenes and that there no consistent effects for many of the parameters in this study, the discussion leader concluded that this study indicated that perchlorate results in minimal immunotoxic effects.

EPA Response(s) and Recommendation(s) for Revision(s): While the Agency agrees that the SRBC assay has been established as one with particularly high predictive power (Luster et al., 1988) and is used to screen chemicals for humoral (antibody-mediated) effects, the EPA disagrees with the conclusions of the discussion leader regarding the results of the Keil et al. (1998; 1999) studies for this assay. As noted by the 1999 expert panel member for immunotoxicology, an enzyme-linked immunosorbant assay (ELISA) is not the traditional procedure performed for this assay and recommended that the more established and accepted plaque forming assay (PFC) be performed. That is why evaluation of the SRBC study was repeated by BRT-Burleson Research Technologies (2000a,b,c). See the EPA response below for other recommendations and conclusions regarding potential humoral immunotoxicity due to perchlorate.

The Agency also disagrees with the interpretation by the discussion leader of the *in vitro* macrophage phagocytosis assay. First, as explained by Dr. Smialowicz of the EPA at the 2002 peer review workshop, the 1999 panel did not feel that an *in vitro* assay was an appropriate test for phagocytic capacity of macrophages and recommended an *in vivo* clearance assay be used in future studies. This peer panel recommendation was not heeded in the repeat studies performed (Keil et al., 1999) so that the adequacy of that characterization remains questionable. Secondly, while a decrease in the phagocytic activity of macrophages could be expected to be reflected in the *L. monocytogenes* host-resistance assay, there are additional concerns regarding the performance of this assay also. Based on the inconsistence of the host-resistance and phagocytosis results, it appears that the laboratory performing these assays had limited previous experience with these assays. A decrease in the *in vitro* phagocytosis of *L. monocytogenes* was observed at 3 and 30 mg/kg-day in the 14-day ("C" study) and in the 90-day ("A" study). Macrophage phagocytosis was also decreased in the 90-day "N" study. However, no effect in 14-day "G" and 90-day "D" study was observed. No effect was observed in the two 120-day studies ("B" and "E") whereas one would expect to see suppressed phagocytosis in the 120-day studies given that these mice were dosed an additional 30 and 106 days from the 14-day and 90-day exposures (Keil et al., 1998). In the 1999 "re-run" of the in vitro phagocytosis assay (Keil et al., 1999), a decrease in phagocytosis was observed at the 1.0 and 30.0 mg/kg-day dose

levels in the 14-day study and at 0.1, 1.0, 3.0 and 30.0 mg/kg-day in the 90-day study (Table 28). However, there were no decrease in phagocytosis at any dose in the 120-day study (Table 29). Thirdly, experience has shown that chemical-induced immune suppression does not translate to expression of suppression of each and every immune function endpoint examined. In general, it appears that different chemicals "target" different immune functions (Luster et al., 1988). Thus, a testing battery typically consists of more than one assay to ensure adequate evaluation of specific roles of the immune system.

4.7.2 On BRT-Burleson Research Technologies (2000a,b,c) and the Local Lymph Node Assay

Comment(s): *To review this study, the discussion leader first noted that mice had either no significant change, 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. Regarding the local lymph node assay (LLNA) for evaluation of contact hypersensitivity that showed an exacerbated effect in some groups after 14-days of exposure, he noted that no clear dose-response relationship was observed and consistent findings were not observed following 90-days of exposure. Moreover, the study reported a lack of negative controls. As a result, the discussion leader questioned the results of the LLNA findings and concern for contact hypersensitivity. The discussion leader suggested that these effects, i.e., signs of an enhanced response, could be viewed as protective or favorable. He noted further later in the discussion that the LLNA might not be a good model for such effects occurring in humans. Another reviewer disagreed with this 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.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA disagrees that the assays of antibody response to sheep red blood cells (SRBC) were consistent across the Keil et al. (1998,1999) and BRT-Burleson Research Technologies (2000a,b,c) studies. As pointed out by Dr. Ralph Smialowicz at the meeting, there was no effect demonstrated by an enzyme-linked immunosorbant assay (ELISA) in the Keil et al. (1998,1999) studies. Further, as discussed above, the 1999 expert peer immunotoxicologist expressed concerns about the performance of the assay and recommended that the more established and accepted plaque forming colony (PFC) assay be performed. The PFC assay subsequently done by BRT-Burleson Research Technologies (2000a,b,c) demonstrated an effect. There is no reason to expect effects at day 14 based on the observation of effects at 90 days. Prolongation of the exposure to 90 days resulted in the two highest dose levels (2.0 and 50.0 mg/kg-day) showing an adjuvant-like

increase in the PFC response. Due to the technical performance issues raised by the 1999 expert panelist regarding the Keil et al. (1998) study that were not rectified in the Keil et al. (1999) study, the Agency maintains its 2002 conclusion that there is a finding for effects on humoral immunity based on the SRBC results of the 90-day study performed by BRT-Burleson Research Technologies (2000a,b,c) with a LOAEL and NOAEL at 2.0 and 0.2 mg/kg-day, respectively.

Regarding the comment on the relevance of the LLNA, the EPA has to agree with the second reviewer. The reason that this study was suggested by the 1999 expert peer immunotoxicologist was that the data of Keil et al., (1998) suggested the potential for hypersensitization. The LLNA is widely accepted in the regulatory arena for evaluation of this endpoint and a symposium at the Society of Toxicology was devoted to the topic in 2002 (National Institutes of Health, 1999; Kimber et al., 2002; Basketter et al., 2002). Further, to suggest that the findings for contact hypersensitivity are somehow advantageous are simply unfounded.

Because of remaining concerns regarding the interpretation of the LLNA data, the immunotoxicity study performed by Burleson et al. (2000a,b,c) was reviewed by another EPA expert on immunotoxicology, Dr. Ian Gilmour. This new reviewer expressed concern that perchlorate may enhance contact hypersensitivity in the skin (Gilmour, 2003), stating that the results clearly showed both enhanced production of SRBC responses and a parallel increase in the local lymph node assay (LLNA) responses. This latter effect should be regarded as detrimental because the LLNA is most commonly used to identify contact sensitizers. Effects were seen to be significant at 0.06 mg/kg-day, and a NOAEL was not established. Gilmour (2003) strongly recommended that the LLNA be repeated to confirm the results and urged "caution about environmental and occupational exposures to AP until these findings can be verified and safe limits established."

Thus, EPA's response to the 2002 panel comments on the LLNA is that the Agency maintains its concern for the potential of this endpoint. The lack of a negative control in the study precluded a definitive designation of adversity. The Agency also notes that the LOAEL of 0.06 mg/kg-day is within an order of magnitude of the other toxicity observed at 0.01 mg/kg-day with no NOAEL established. Finally, the following sentences in Chapter 5 (lines 31 on Page 5-108 through line5 on Page 5-109) has been revised to read as below:

It is not known that an enhancement of the LLNA translates into a more severe contact hypersensitivity reaction.

The LLNA measures the induction phase of the chemical sensitization process (i.e., accumulation of and proliferation of lymphocytes in the local lymph nodes), while the elicitation phase involves a qualitative reaction of the skin (redness and erythema) as a consequence of re-exposure to the chemical. Studies are needed to determine if ammonium perchlorate itself is a contact sensitizer as determined by the LLNA, as described above, and whether the degree of the LLNA response to ammonium perchlorate itself or to a known contact sensitizer can be linked to a quantifiable adverse outcome.

4.7.3 On EPA's Overall Interpretation of Immunotoxicity

Comment(s): *The discussion leader generally supported EPA's interpretation of the immunotoxicity studies but 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: (1) the doses at which perchlorate affects the thyroid hormone levels are much lower than the doses at which contact hypersensitivity were observed; (2) the contact hypersensitivity findings have inconsistencies and were inappropriately controlled, thus leaving questions as to whether perchlorate causes the observed effect; (3) the relevance of skin rashes and agranulocytosis in Grave's disease patients treated with perchlorate is questionable given that no such effects have been observed in rodents or in humans receiving lower doses of perchlorate; and (4) the only immunotoxic effect that exhibited some consistency (decreased phagocytosis) appears to be reversible; and (5) contact hypersensitivity effects that occur in rodents may not be a good model for such effects occurring in humans.*

The EPA responses to each of these comments are as follows. Regarding the first comment that there is a large disparity between the levels associated with thyroid hormone disruption and immunotoxicity, the EPA responds that the LOAEL for the LLNA assay (0.06 mg/kg-day) is within the same order of magnitude as the various LOAEL estimates for serum hormones. Second, the EPA has reaffirmed its concern for the LLNA findings suggestive of contact hypersensitivity as described above. The findings of an effect in the BRT-Burleson Research Technologies (2000a,b,c) study reinforced concerns based on findings of delayed-type hypersensitivity in Keil et al. (1998) as was called attention to by the 1999 peer panel member. Third, the testing strategy included immunotoxicity not only because of the literature on skin rashes and agranulocytosis, but also because immune function is established to depend on thyroid hormone (Blalock, 1994; Fabris et al., 1995; Klecha et al., 2000). Evidence from the assays should be evaluated in the context of contemporary understanding of these assays and not whether they map perturbation of the immune system directly to these other documented effects. Fourth, whether or not an immune effect is reversible has no bearing on its designation of

adversity, particularly when the objective is to arrive at an estimate of a continuous lifetime risk. The notion that the LLNA test is not relevant when it is a widely used screening assay with acceptance in the regulatory arena was refuted in the above response to comment.

In summary, the EPA remains concerned regarding uncertainty in the characterization of the potential for perchlorate to pose an immunotoxicological risk. With respect to the magnitude of the UF applied for uncertainty in the characterization of potential immunotoxicity, the EPA notes that only a partial factor of 3 was applied for a database deficiency on an endpoint that was specifically indicated in the original testing strategy for evaluation and then again by the 1999 peer review panel. The panel member has erroneously represented the reason for the inclusion of a database deficiency factor. The relationship of the immunotoxicological endpoint to other endpoints (e.g., thyroid hormone) is not the issue — rather the rationale for application of this particular UF is whether a key endpoint has been adequately characterized, i.e., does an uncertainty remain regarding adequacy of the database on a given endpoint. The Agency maintains that one does remain. Nevertheless, the EPA has reconsidered these recommendations regarding the UF applied for database deficiencies in its newly proposed assessment as discussed in Chapter 7 of this document.

APPENDIX 4A

SUMMARY TABLES AND FIGURES OF THYROID HISTOPATHOLOGY ACROSS LABORATORY ANIMAL STUDIES (AS PROVIDED IN 2002 ERD)

Table 4A-1. Table 5-1. Benchmark Dose (BMD)[a] and Benchmark Dose Lower Confidence Limit (BMDL)[a] Estimates Calculated From the Wolf (2000, 2001) Thyroid Histopathology Data (Geller, 2001a)

Study Name, Time Point Wolf (2000; 2001) Table Number	Ammonium perchlorate dose levels test (mg/kg-day)	Colloid Depletion				Hypertrophy				Hyperplasia			
		BMD	BMDL	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]	BMD	BMDL	χ^2 [b]	Exp[c]
1 Caldwell Tbls. 1 and 2	0, 1.25, 5, 12.5, 25, 50, 125, 250	13.29	0.72	0.97	4.37	Not done[d]				35.29	0.78	0.2	0.88
2 Subchonic, 14-day Tbls. 3 and 6	0, 0.01, 0.05, 0.2, 1.0, 10.0	2.55	0.28	0.2	0.74	0.75	0.017	0.54	0.78	NOE[e]			
3 Subchronic, 90-day Tbls. 4 and 7	0, 0.01, 0.05, 0.2, 1.0, 10.0	0.13	0.03	0.7	0.5	0.21	0.008	0.74	0.55	8.36	2.09	1	7.87
4 Subchronic, 120-day Tbls. 5 and 8	0, 0.05, 1.0, 10.0	NOE				NOE				NOE			
5 Neurobehav., F0 Fem Tbl. 9	0, 0.1, 1, 3, 10	NOE				NOE				NOE			
6 Neurobehav., PND5 Tbls. 10 and 11	0, 0.1, 1, 3, 10	0.45 0.53	0.009 0.33	0.46 0.67[f]	0.94 1.0	0.92 1.27	0.24 0.88	.024 0.26[f]	0.81 1.0	15.18 11.02	1.86 3.62	0.70 0.32[f]	0.36 1.0
7 Neurobehav., adult Tbls. 12 and 13	0, 0.1, 1, 3, 10	0.72	0.029	0.23	0.89	3.48	NC	0.72	0.29	NOE			
8 2-gen., P1 Tbls. 14 and 15	0, 0.3, 3, 30	1.97	0.11	0.68	3.84	Poor fit[g]				7.89	2.44	0.41	0.72
9 2-gen., P2 Tbls. 16 and 17	0, 0.3, 3, 30	2.16	0.9	0.06	1.16	0.99	0.15	0.67	0.7	4.62	0	0.14	0.31
10 2-gen., F1-weanling Tbls. 18 and 19	0, 0.3, 3, 30	2.51	0.8	0.17	1.2	0.21	0.057	0.4	0.79	2.74	0.66	0.85	0.52
11 2-gen., F2-weanling Tbls. 20 and 21	0, 0.3, 3, 30	Poor fit				1.19	0.32	0.25	0.52	NOE			
BMDL Range: Rat Studies		**0.009 - 0.90**				**0.008 - 0.74**				**0.0004 - 3.62**			
12 Dev tox., rabbit dams Tbl. 22	0, 0.1, 1, 10, 30, 100	0.12	0.008	0.19	0.36	Poor fit				1.53	0.42	0.13	0.61
13 Immunotox. Mice, combined studies Tbl. 23	0, 0.1, 1, 3, 30	26.07	5.15	1	7.88	1.62	0.97	0.58	0.84	24.92	4.48	1	7.86

[a] Units of mg/kg-day.
[b] χ^2 p-value.
[c] Exponent in Weibull model fit not restrained to ≥ 1.0 unless indicated.
[d] Not done: Because of non-routine staining, cytological characteristics were not adequate to make determination of hypertrophy on these samples (Wolf, personal communication).
[e] No observed effect (NOE): Either no incidence of endpoint noted in animals tested or no notable difference between dosed and controls.
[f] Exponent in Weibull model fit restrained to ≥ 1.
[g] Poor fit: $p < 0.05$ for χ^2 test.

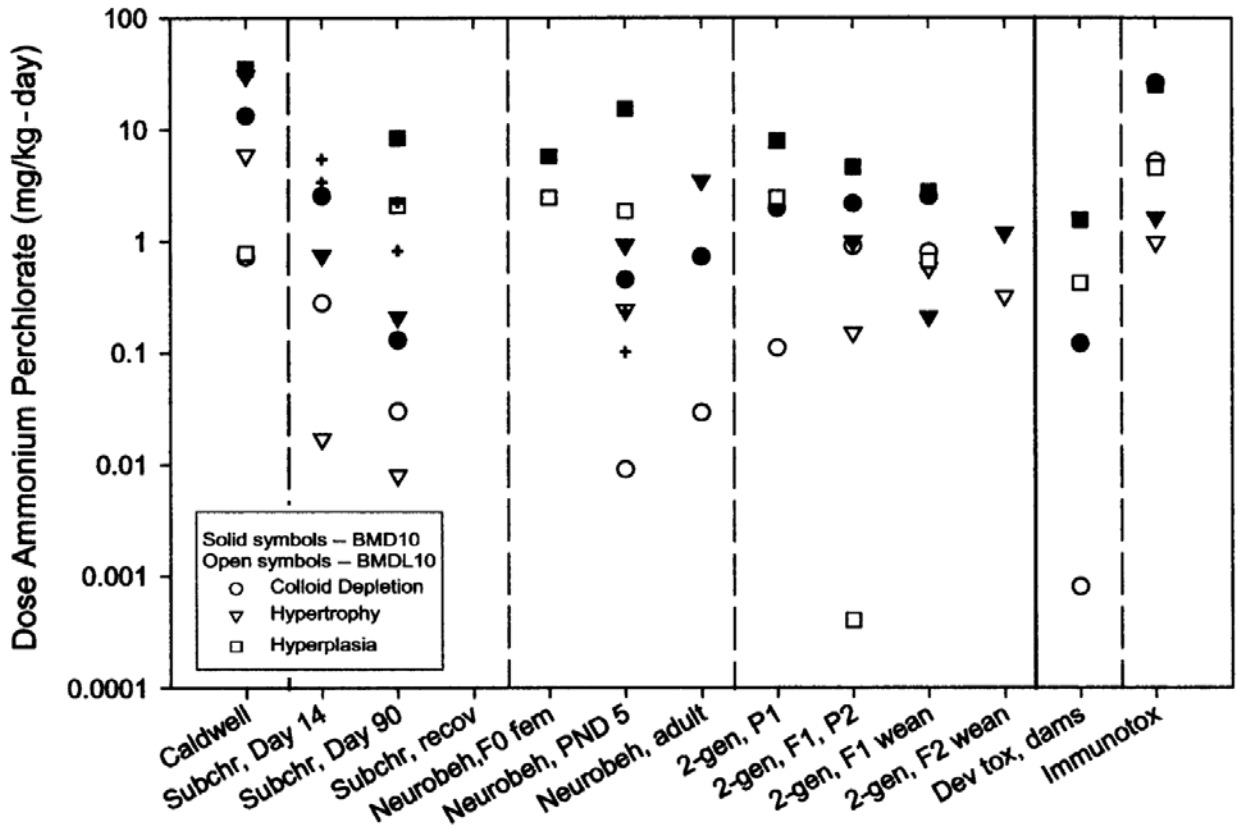

Figure 4A-1. Figure 5-1. Benchmark dose (BMD) and benchmark dose lower limit (BMDL) estimates recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Data on incidence of colloid depletion, thyroid hypertrophy and thyroid hyperplasia were submitted to the EPA for the perchlorate risk characterization. Values used are presented in Table 5-1 (Geller, 2001a). Greater value represents the BMD and lesser value represents the BMDL. The + denotes BMD and BMDL from previous EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a). Values to the left of the vertical solid line are from the rat studies. Values to the right are from the developmental study in rabbits (Argus Research Laboratories, 1998c) and the mouse immunotoxicity studies (Keil et al., 1998). Study denoted by "Caldwell" refers to Caldwell et al. (1995); "Subchronic" to Springborn Laboratories, Inc. (1998); "Neurobeh" to the 1998 developmental neurobehavioral study (Argus Research Laboratories, 1998a); and "2-gen" to the completed 2-generation reproductive toxicity study in rats (Argus Research Laboratories, 1999).

Table 4A-2. Table 5-3. Benchmark Dose (BMD)[a] and Benchmark Dose Lower Confidence Limit (BMDL)[a] Estimates From Thyroid Histopathology in the "Effects Study" (Argus Laboratories, Inc., 2001; Geller, 2001b)

Study Population "Effects" Study (Argus, 2001)	Colloid Depletion				Hypertrophy				Hyperplasia			
	BMD	BMDL	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]	BMD	BMDL	χ^2 [b]	Exp [c]
GD 21 Dams	5.1	1.01	1	17.9	15.46	1.19	1	6.25	28.54	8.51	1	5.03
GD 21 Male pups	0.69	0.12	1	8.82	NOE [4]	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 Female pups	0.18	0.04	0.6	2.08	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
GD 21 M + F pups	0.65	0.12	0.16	7.8	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Male pups	0.88	0.29	0.12	7.37	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 Female pups	0.82	0.18	0.12	7.78	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND4 M + F pups	0.84	0.33	0	7.5	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Dams	0.62	0.13	0.59	2.65	2.65	1.01	0.22	17.86	2.24	0.92	0.49	1
PND9 Male pups	1.29	0.71	0.59	6.4	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 Female pups	0.33	0.13	0.61	1.3	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND9 M + F pups	0.93	0.48	0.36	3.77	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 Dams	1.21	0.62	0.34	4.9	15.6	1.24	1	6.34	3.59	0.99	0.66	1
PND21 Male pups	17.3	1.36	1	5.85	NOE	NOE	NOE	NOE	26.97	5.45	0.58	5.06
PND21 Female pups	16.4	1.24	1	5.94	NOE	NOE	NOE	NOE	NOE	NOE	NOE	NOE
PND21 M + F pups	17.3	2.17	1	5.92	NOE	NOE	NOE	NOE	54.17	13.7	0.24	1

[a] Units of mg/kg-day.
[b] χ^2 goodness of fit criterion
[c] Exponent in log-logistic function restricted to be ≥ 1.0.
[d] NOE = No observed effect.

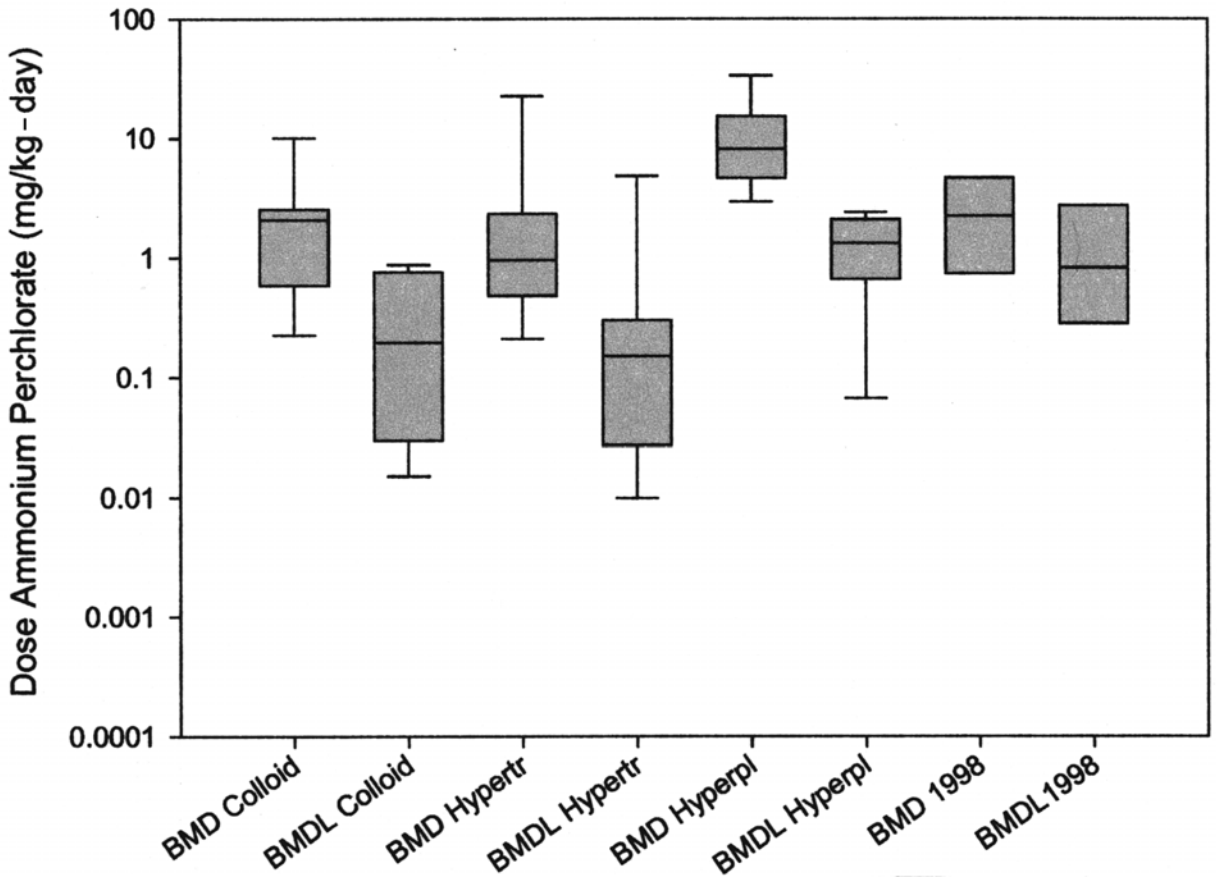

Figure 4A-2. Figure 5-2. Distribution of BMD and BMDL estimates shown by "box and whisker" plots of colloid depletion (colloid), hypertrophy (hyptry), and hyperplasia (hyppls) from rat studies recalculated for thyroid histopathology based on 2000 Pathology Working Group review (Wolf, 2000; 2001). Values are presented in Table 5-1. Study #4 was excluded since it was a 30-day recovery experiment and #5 was excluded due to lack of monotonicity. The boundary of the box closest to zero indicates the 25th percentile, a line within the box denotes the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. The two rightmost boxes plot values from the combined rat studies from the 1998 EPA risk characterization (U.S. Environmental Protection Agency, 1998d; Geller, 1998a).

APPENDIX 4B

SUMMARY TABLE OF EFFECTS ON SERUM HORMONES ACROSS LABORATORY ANIMAL STUDIES (AS PROVIDED IN 2002 ERD)

Table 4B-1. Table 5-2. A Comparison of NOAELs and LOAELs from the Original 1998 Analysis and the 2001 Re-Analyses for Hormone and Morphometry on Thyroid Follicular Lumen Size (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses NOAEL	Original Analyses LOAEL	Re-Analyses[a,b] NOAEL	Re-Analyses[a,b] LOAEL
Rat 14-Day (Caldwell et al., 1995)	14-Day *(males - 0.0, 0.11, 0.44, 1.11, 2.26, 4.32, 11.44, 22.16) (females - 0.0, 0.12, 0.47, 1.23, 3.06, 4.91, 11.47, 24.86)*	T3	M	0.11	0.44	0.11	0.44
			F	—	0.11	—	0.12
		T4	M	—	0.11	—	0.11
			F		1.25		0.12
		TSH	M	0.44	1.11	0.44	1.11
			F	0.12	0.47	**—**	**0.12**
		hTg	M	—	0.11	—	0.11
			F	—	0.12	—	0.12
		rT3	M	0.44	1.11	**0.11**	**0.44**
			F	5	12.5	**0.12**	**0.47**
Rat Subchronic Study (Springborn, 1998)	14-Day *(0, 0.01, 0.05, 0.2, 1.0, 10.0)*	T3	M	—	0.01	—	0.01
			F	10	—	10	—
		T4	M	1	10	**—**	**0.05**
			F				
		TSH	M	0.05	0.2	**0.01**	**0.05**
			F	0.01	0.05	**—**	**0.01**

Table 4B-1. (cont'd). Table 5-2. A Comparison of NOAELs and LOAELs from the Original 1998 Analysis and the 2001 Re-Analyses for Hormone and Morphometry on Thyroid Follicular Lumen Size (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses NOAEL	Original Analyses LOAEL	Re-Analyses[a,b] NOAEL	Re-Analyses[a,b] LOAEL
Rat Subchronic Study (Springborn, 1998) (cont'd)	90-Day *(0, 0.01, 0.05, 0.2, 1.0, 10.0)*	T3	M	—	0.01	—	0.01
			F	—	0.01	—	0.01
		T4	M	—	0.01	—	0.01
			F			—	0.01
		TSH	M	0.05	0.2	0.05	0.2
			F				
Rat Subchronic Study (Springborn, 1998)	120-Day *(0, 0.05,1.0, 10.0)*	T3	M	1	10	1	10
			F				
		T4	M	—	0.05	**—**	**0.05**
			F			**1**	**10**
		TSH	M	10	—	**—**	**0.05**
			F				
Rat Developmental Neurotoxicity Study (Argus, 1998a)	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	1	3	**0.3**	**3**
			F				
	PND90 *(0, 0.1, 1.0, 3.0, 10.0)*	Lumen size	M	Data not available for original analyses		**10**	**—**
			F			**10**	**—**
	PND5 *(0, 0.1, 1.0, 3.0, 10.0)*	T4		1	3	**0.1**	**1**
		T3		0.1	1	0.1	1
		TSH		3	10	3	10
	PND90	T4, T3, and TSH		No data available			

Table 4B-1. (cont'd). Table 5-2. A Comparison of NOAELs and LOAELs from the Original 1998 Analysis and the 2001 Re-Analyses for Hormone and Morphometry on Thyroid Follicular Lumen Size (Crofton and Marcus, 2001; Marcus, 2001; Crofton, 2001a)

Species/Study	Time Point, Age *(Doses, mg/kg-day)*	Endpoint	Sex	Original Analyses NOAEL	Original Analyses LOAEL	Re-Analyses[a,b] NOAEL	Re-Analyses[a,b] LOAEL
Mouse Hormone and Immunotoxicity (Keil et al., 1998)	14-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	3	30	—	**0.1**
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[c]
		TSH	M	No data			
	90-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	0.1	3	—	0.1[c]
		T3	M	Data not available at time of 1998 analysis		—	**0.1**[d]
		TSH	M	30	—	—	**0.1**[d]
	120-Day *(0.0, 0.1, 1.0, 3.0, 30)*	T4	M	30	—	30	—
		T3	M	Data not available at time of 1998 analysis		30	—
		TSH	M	30	—	—	—
Rabbit Developmental Toxicity (Argus, 1998b)	Gestation Day 28 *(0.0, 0.1, 1.0, 10.0, 30.0, 100.0)*	T4	F	0.1	1	0.1	1
		T3	F	100	—	100	—
		TSH	F	100	—	100	—

[a]Bold indicates where 2001 analyses is different than 1998 analyses.
[b]Results from the liberal and conservative statistical approaches were the same.
[c]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.
[d]No dose response - 0.1 and 1.0 differ from control; 0.3 and 30.0 do not differ from control.

Table 4B-2. Table 5-4. NOAELs and LOAELs for Effects on Thyroid and Pituitary Hormones from the Argus 2001 "Effects Study" (Crofton, 2001b)

				Effect Level Designation	
Generation	**Hormone**	**Age**	**Sex**	**NOAEL**	**LOAEL**
Dams	T3	GD21	F	1	30
		PND10	F		
		PND22	F		
	T4	GD21	F	—	0.01
		PND10	F	0.1	1
		PND22	F	1.0	30.0
	TSH	GD21	F	—	0.01
		PND10	F	—	0.01
		PND22	F	0.01	0.1
Fetus and Offspring	T3	GD21	Pooled	—	0.01
		PND5	Pooled		
		PND10	Pooled		
		PND22	F	0.01	1.0
			M		
	T4	GD21	Pooled	0.01	0.1
		PND5	Pooled		
		PND10	Pooled		
		PND22	F	no significant effects	
			M	—	0.01
	TSH	GD21	Pooled	0.1	1.0
		PND5	Pooled	no significant effects	
		PND10	Pooled	—	0.01
		PND22	F	0.01	0.1
			M	—	0.01

[a]Dosages of 0, 0.01, 0.1, 1.0, and 30 mg/kg-day.

5. ECOLOGICAL RISK ASSESSMENT AND EVIDENCE FOR INDIRECT EXPOSURE

This chapter addresses recommendations by the panel or comments received from the public and interested stakeholders regarding the Agency's analyses of the ecological effects data on perchlorate presented in Chapter 8 (Screening Ecological Risk Assessment for Perchlorate) and also regarding the evaluation and presentation of the evidence for indirect exposure to perchlorate presented in Chapter 9 (Evaluation of Evidence for Indirect Exposures) of the 2002 ERD. These responses and recommendations are suggested primarily for revision of those two chapters. In addition, refinements regarding the discussion of these topics are also recommended by the Agency for Chapter 10 of the 2002 ERD (Major Risk Characterization Conclusions) and these are found in this response document in Chapter 8 with a similar title.

It should be noted that the Agency considers the data now available on perchlorate sufficient to support using the methods of Stephan et al. (1985), and thus the revised title of Chapter 8 is recommended to no longer include "screening" as a qualifier. The title of the chapter is to be changed to *"Ecological Risk Assessment for Perchlorate."* This chapter has undergone a significant set of revisions. This response document will first highlight the major comments and then provide the associated text revisions.

5.1 REVIEW OF RELEVANT STUDIES

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Questions D.1 and D.2.

Comment(s)*: The discussion leader for the peer panel 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.*

EPA Response and Recommendations for Revisions: While only a few of the cited studies have currently been published in the peer-reviewed literature, the cited memos, internal reports, draft reports, and laboratory reports have been formally submitted to EPA or DOD and the PSG (stakeholders in this work) and are part of the public record on perchlorate. As such, these studies were appropriately included in this assessment. In his written comments, the discussion leader (WA) identifies some specific limitations in these studies, including that the studies were not conducted in accordance with Good Laboratory Practices; that the reports or memos did not always give complete details of the methods, QA/QC, or statistics used in the studies; and that nominal concentrations were used instead of measured concentrations. However, the studies did follow specific and appropriate testing guidance; and the reviewer admits that the methods, statistical analyses, and conclusions drawn from the studies were appropriate for a screening level report although the data remain limited. The discussion leader agrees that while the "Condike Report" (Condike, 2001) presents some "interesting data on fish, plant, and sediment pore-water concentrations for comparison with other field results," it is too preliminary and presents insufficient information on study design, methods, or results to interpret the significance of this study's results. Also, additional information from this study has not become available since the peer review. Therefore, no changes to the document are needed in response to these comments.

Comment(s)*: The reviewers' other responses to this question primarily addressed the disparate findings between the various studies of perchlorate uptake by plants. Specifically, one reviewer 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). Each of the two reviewers also identified reasons why both the laboratory studies and the field studies may not be characterizing plant uptake accurately.*

EPA Response and Recommendations for Revisions: The reassessment document also addresses the question of disparate findings between the various studies of perchlorate uptake in plants and identifies reasons why particularly the laboratory studies may not be characterizing plant uptake accurately. Particularly problematic is that the laboratory studies were designed to study plant uptake as a method for bioremediation of perchlorate rather then to study plant

bioaccumulation of perchlorate. Despite this, a comparison of the field and laboratory data for terrestrial vegetation yields bioaccumulation factors in the same order of magnitude range (i.e., ≈1 to 100), and on this basis a conservative assumption that concentrations in leaves can exceed water concentrations by a factor of 100 was made. In aquatic systems, when concentrations in physical media were greater than the reporting limits for biological media, the concentrations in aquatic vegetation were similar or somewhat greater than those in water or pore water, while concentrations in other biota were generally less. We do not agree with the reviewer's assessment that the methods used to collect sediment and pore-water samples in the Parsons (2001) study may dilute the concentrations in sediments. Because of the solubility of perchlorate, concentrations of perchlorate in the overlying water are likely to be similar to those in the pore waters. Even so, such a problem would decrease the estimates of bioaccumulation from sediments to aquatic vegetation. Therefore, no changes in the document are needed in response to these comments.

5.2 ADEQUACY OF ASSAYS TO DETERMINE ECOLOGICAL EFFECTS OF CONCERN

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question D.3.

Comment(s)*: 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, it was 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, it was 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), both reviewers strongly supported further studies of amphibians.*

EPA Response and Recommendations for Revisions: Part of the challenge of this ecological risk assessment has been including ongoing research on perchlorate. Since the external review draft was published, the results of several additional studies on both aquatic and terrestrial ecological receptors have become part of the public record either because they have been

published in the peer-reviewed literature or have been submitted as reports to EPA or DOD. Published studies include two papers by Goleman et al. (2002a,b) on *Xenopus laevis;* one by Patino et al. (in press) on the fish *Danio rerio*; and one by Thuett et al. (2002) on deer mice (*Peromyscus maniculatus*). Reports include one by Parsons (2002) presenting new data from standard acute and chronic bioassays designed to fill the data gaps for development of freshwater water quality criteria for perchlorate and several final reports on studies by the group at Texas Tech University (TTU) to the Strategic Environmental Research and Development Program (SERDP). These studies include one on uptake and thyroid status of the fish *Gambusia holbrooki* and *Ictalurus punctatus* (Theodorakis et al., 2002a); one on uptake and elimination in larval *Rana catesbeisana* (Carr et al., 2002a); two on the use of fish and amphibians to assess exposures and effects at Longhorn Army Ammunition Plant (Theodorakis et al., 2002b,c); one on uptake into various plant species (Anderson, 2002a); one on effects on the earthworm *Eisenia foetida* (Anderson, 2002b); one on effects on development and growth, thyroid function, and reproductive status of larval and adult frogs at Longhorn Army Ammunition Plant (Carr et al., 2002b); one on effects on metamorphosis of *Xenopus laevis* in bioassays that used water collected from Longhorn Army Ammunition Plant (Carr et al., 2002c); and one on bioaccumulation and effects on the mammals *Procyon lotor* and *Didelphis virginiana* at Longhorn Army Ammunition Plant (Smith et al., 2002); and a laboratory study of effects on metamorphosis of *Xenopus laevis* in bioassays that used filtered water from Lake Superior spiked with sodium perchlorate (Tietge and Degitz, 2003).

We agree that Goleman et al. (2002a,b) and these other reports have added significantly to our understanding of the potential effects of perchlorate, as have these other papers and reports. Therefore, we have added discussions of these reports and papers to the appropriate sections of the revised final ecological risk assessment.

Comment(s)*: The reviewers identified additional future research areas. One indicated that EPA should consider evaluating herbivorous avian species, given the fact that plant uptake of perchlorate has been reported. This suggestion was not based on any perceived sensitivity of these species to perchlorate. Another reviewer 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. Further screening with other algae species and with macrophytes was also recommended.*

EPA Response and Recommendations for Revisions: Although studies of herbivorous avian species, other algae species, or macrophytes are not currently available for perchlorate, studies of herbivorous terrestrial mammals including the deer mouse (*Peromyscus maniculatus*), raccoon (*Procyon lotor*), and Virginia opossum (*Didelphis virginiana*) have been conducted and reported as described in the previous response. As also described previously, results from a number of other studies are now available; and discussions of these reports and papers have been added to the appropriate sections of the revised ecological risk assessment.

5.3 GOALS AND OBJECTIVES OF ECOLOGICAL ASSESSMENT

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question D.4. The two peer reviewers who commented on the ecological risk assessment offered different responses, and each will be addressed.

Comment(s): *One reviewer 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) that 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 2002 ERD).*

EPA Response and Recommendations for Revisions: Discussions of the Goleman et al. (2002a,b) papers and other studies that are now available have been added to the appropriate sections of the ecological risk assessment. The discussion of effects on aquatic organisms has been revised to emphasize the remaining uncertainties regarding the effects on amphibian metamorphosis. A lower benchmark for chronic effects has been established because of these and other findings.

Comment(s): *The other reviewer 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.

EPA Response and Recommendations for Revisions: Although measured body burdens may assist in integration of the findings between the laboratory toxicity tests and field studies, body burdens are not generally measurement endpoints for most standard bioassays except when bioconcentration factors (BCF) are of interest. However, some of the new studies described previously have measured body burdens in laboratory exposures, and discussions of these studies have been added to the ecological risk assessment. The reviewer's comment about effects on anion transport suggest another mechanism of toxicity that may be applicable to plants or algae, but the only other data currently available is for the cyanobacterium, *Microcystis aeruginosa*, and is discussed in Parsons (2002). Parsons (2002) describes the studies that produced these data as not acceptable for water-quality-criteria development. However, we have added a discussion of these data to the ecological risk assessment where appropriate and have made an independent evaluation of these data.

5.4 COMMENTS ON ANALYSES, CONCLUSIONS, AND CHARACTERIZATION OF UNCERTAINTY IN THE ECOLOGICAL RISK ASSESSMENT

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question D.5.

***Comment(s)**: While the discussion leader originally had a favorable opinion of EPA's analyses, the opinion changed after reviewing the laboratory toxicity study of Goleman et al. (2002a) that was published after EPA released the 2002 ERD. It was recommended that EPA revise its analyses to incorporate this study's findings. Specific comments included some on EPA's analyses of the data available prior to the publication of Goleman's study and implications of more recent data. The discussion leader noted that the Revised ERD integrated all data that were available on exposure and effects into an initial, screening-level ecological risk assessment and 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 2002 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). The reviewer concluded, therefore, that EPA's analyses of the existing effects data were sound.*

EPA Response and Recommendations for Revisions: No changes in the document are needed in response to this comment that are not addressed elsewhere.

Comments on the implications of more recent data (Goleman et al., 2002). *The two reviewers 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 2002 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 2002 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.*

EPA Response and Recommendations for Revisions: EPA recognizes the implications of this study but also recognizes that there are both scientific questions about the validity of the study and policy questions about the use of the study in water-quality-criteria development. Therefore, discussions of the Goleman et al. (2002a, 2002b) papers were added to the ecological risk assessment where appropriate. In addition, both the scientific and policy questions have been discussed in order to determine how these results will be used in the ecological risk assessment. Discussions of additional, more recent studies using *Xenopus* that have addressed the major concerns about the Goleman study have also been added.

Comment(s): *Though concerned about the implications of the Goleman study, the discussion leader 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.*

EPA Response and Recommendations for Revisions: Our evaluation and discussion of the Goleman et al. (2002a,b) papers have included the three aspects of the study outlined by the discussion leader. We also have added discussions of additional, more recent studies using *Xenopus* that have addressed the major concerns about the Goleman study.

5.5 REVIEW OF AVAILABLE DATA TO CHARACTERIZE ENVIRONMENTAL TRANSPORT AND TRANSFORMATION

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question D.6.

Comment(s): *The peer reviewers agreed with EPA that the available data characterizing the fate and transport of perchlorate are limited. The discussion leader 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 of the peer review report (US EPA, 2002b). The peer reviewers' specific comments on these data follow.*

Transport of perchlorate. *The discussion leader 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). The second reviewer agreed and added that the available studies provide conflicting information on perchlorate transport. For instance, 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 (TF) 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.*

EPA Response and Recommendations for Revisions: Although more detailed knowledge on the transformation of perchlorate in the environment would decrease some uncertainties associated with the potential for exposure to perchlorate, there is significant data that indicate perchlorate is persistent. Therefore, this ecological risk assessment has used a conservative assumption, based on the available data, that transformation of perchlorate is limited in the environment. More detailed knowledge would only decrease the estimates of exposure resulting

from this assumption. However, we have changed the ecological risk assessment in Chapter 8 where appropriate to clarify that this is a conservative assumption because of uncertainty about the transformation of perchlorate.

5.6 REVIEW OF AVAILABLE DATA TO CHARACTERIZE SOURCES OF INDIRECT EXPOSURES

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel regarding Charge Question D.7.

Comment(s)*: The peer reviewers provided few comments on this charge question. The discussion leader indicated that future research is needed to develop more sensitive analytical methods, not only for water but also for biotic tissues, soils, and sediment. The other reviewer agreed, indicating that ion chromatography analyses potentially suffer from matrix interference and interfering ions. 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 noted that humans may be indirectly exposed to perchlorate in vegetables grown on land irrigated with perchlorate-contaminated water. Finally, it was recommended that future studies characterize the mechanisms by which plants take up perchlorate. With a mechanistic understanding of uptake, EPA may be able to predict uptake behavior in plant species that have not been sampled.*

EPA Response and Recommendations for Revisions: EPA recognizes that detection limits associated with the analytical methods used to measure perchlorate in various physical and biological media have been a problem in past studies of perchlorate; consequently we have indicated when high detection limits have influenced our ability to assess ecological exposure to perchlorate. As noted by the second reviewer, ongoing research, reflected in the introduction chapter to the revised document, has improved the sensitivity and selectivity of the available analytical methods (i.e., liquid chromatography with mass spectrometry). However, many of the available studies were conducted before the newer analytical methods were fully developed and suffer from the problem of high detection limits. Currently, use of the bioaccumulation factor of 100 allows for a conservative prediction of the uptake behavior of an unsampled plant species. However, a more mechanistic understanding of perchlorate uptake by plants would reduce the uncertainties associated with this prediction. Text to clarify this has been added where appropriate. Discussion of more recent studies have also been included (Anderson, 2003;

Urbansky and Brown, 2003; Collette et al., 2003; and Sundberg et al., 2003) as well as a description of a forthcoming report from EPA (Hutchinson et al., 2003) and plans for studies by the USDA, SERDP and US Army Corps of Engineers.

5.7 ADDITIONAL PUBLIC COMMENTS

This section provides the disposition of comments and recommendations for revision based on comments made at the meeting (transcript provided in Appendix H of U.S. Environmental Protection Agency, 2002b) or received in writing from the public that have not been addressed in previous comments from the external peer panel.

Comments (D. Garrison, USAF)*: The commenter addressed the endpoints used to derive ecological screening benchmarks. He suggests that the goal is to assess potential effects on receptors at the community or population level. In deriving an ecological screening benchmark, a requirement for endpoint selection is that it is based on an ecologically relevant effect, such as survival, reproduction, or growth. 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, he suggests that ecological benchmarks for perchlorate appear to be based on endpoints with no known or implicated ecological relevance. The alteration of 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. This 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. This commenter also said that the EPA paid little attention to the dissimilar results between rats and rabbits and failed to address interspecies variability.*

EPA Response and Recommendations for Revisions: Even though an observed endpoint (e.g., alteration of thyroid function in mice or redness and swelling of larval fish tissues suggesting internal hemorrhaging) may not have an obvious relationship to the usual endpoints of survival, growth, and reproduction, use of such an endpoint is still appropriate if a causal link can be hypothesized between the endpoint and the usual population-based endpoints. This is particularly true in the absence of chronic tests and full life-cycle tests with the same or similar species that might mitigate the concerns raised by the observed endpoint. We believe that this is true of both endpoints discussed in this comment. Because there is apparent interspecies

variability in effects concentrations, the lower benchmark is more likely to be protective of the majority of herbivorous species. Therefore, no changes in the document are needed in response to these comments.

Comments (R. Palachek, Parsons Engineering Science, Inc.)*: The commenter stated that dry-wet-weight and percent moisture are available for all tissue samples collected in the Parsons (2001) study. Another issue involved plant accumulation of perchlorate. He observed that the highest plant accumulation values for the Las Vegas Wash site were in the leaves from plants in areas where groundwater was very shallow. Therefore concentrations in groundwater need to be considered in addition to soil concentrations. The commenter also discussed some additional toxicity data and analyses that were conducted by Parsons Engineering Science, Inc., for the U.S. Air Force. He points out that these data will follow GLP and will present measured concentrations. He stated that these data will be used to develop a surface-water-quality standard using the EPA protocol.*

EPA Response and Recommendations for Revisions: We thank the commenter for pointing out that dry-weight and percent moisture data are available in the Parsons (2001) study. No change in the document is needed in response to this comment. We have also reviewed an external review draft of the document (Parsons 2002) referred to in this comment. We have considered the data from Parsons (2002) and data from the other papers and reports discussed previously and determined that sufficient data are now available to develop a freshwater aquatic life water quality criterion for perchlorate independently of the conclusions of Parsons (2002) following the Stephan et al. (1985) guidance document. Because sufficient data are now available, we have changed the benchmark values developed by our document from Tier II values to values derived based on standard Agency methodology (Stephan et al., 1985).
We note, however, that the Agency's interpretation of the available data differs from that of Parsons (2002) and that the chronic benchmark for aquatic life that is derived in the revised final document (i.e., 0.12 mg/L) is lower by nearly two orders of magnitude than the Final Chronic Value (FCV) which they derive (9.26 mg/L). The derivation of the Agency's benchmark is fully explained in Section 5.8.4.1.1 below. Moreover, in light of the additional data that has become available for perchlorate since completion of the peer review draft of the document, the Agency now considers the ecological risk assessment to be no longer just a screening-level risk assessment, although significant uncertainties remain. The changes to the ecological risk assessment reflect this.

Comments (R. Porter, Mitretek Systems)*: The commenter was involved in field sampling at the Las Vegas Wash site and makes some personal qualitative observations about the apparent lack of effects on small mammals and mosquito fish at that site. The commenter refers to some new studies by Jim Carr's laboratory were they were unable to duplicate the results of Goleman et al. (2002a, 2002b) with site-contaminated water that was brought into the lab and used in the Xenopus assays.*

EPA Response and Recommendations for Revisions: It is difficult to corroborate such qualitative observations, even by designing a quantitative field study. Moreover, the observations pertain to only two species. Therefore, no changes to the document are needed in response to this comment. As discussed previously, we have included a discussion of Goleman et al. (2002a,b) and Carr et al. (2002b,c) in the document and made an independent assessment of these studies. We also have added a discussion of an additional study using *Xenopus* (Tiegte and Degitz, 2003) which addresses major concerns about the Goleman study.

5.8 MAJOR TEXT REVISIONS TO THE ECOLOGICAL ASSESSMENT (CHAPTER 8)

This section provides the updated text that includes the major revisions to Chapter 8 of the 2002 ERD which have been made by the Agency in response to the comments outlined above. Minor changes that occur elsewhere in the chapter are not reflected in these excerpts. The first major change is that the title of the Chapter has dropped "screening" as an adjective. The new title of the Chapter is *"Ecological Risk Assessment for Perchlorate."* Changes will be identified by redline text under their major headings and subheadings. The entirety of Section 8.3.2 (Characterization of Effects) is provided in Section 5.7.5 of this response document due to the wide scope of this revision and the resultant change in status of the ecological risk assessment.

5.8.1 Changes to Section 8.1.2 Scope, Complexity, and Focus

Lines 23 through 26 describing the assessment as a screening level have been deleted. Section numbers are now recommended to identify the individual sets of data discussed.

5.8.1.1 Changes to New Section 8.1.2.2

Test Battery Reports: The following text is added: For terrestrial receptors, other reports to the Strategic Environmental Research and Development Program (SERDP) from the Institute of Environmental and Human Health (TIEHH) at Texas Tech University present further data on the toxicity of perchlorate to plants (Anderson et al., 2002a) and to earthworms (Anderson et al., 2002b).

5.8.1.2 Changes to New Section 8.1.2.4

Algal Toxicity Testing: The following text is added: Older data that do not follow EPA methods for algal bioassays are available for species of green algae in the order Chlorococcales (Krebs, 1991) and for *Scenedesmus quadricauda* and *Microcystis aeruginosa* (Bringmann, 1975; Bringmann and Kuhn, 1977; 1978a; 1978b; 1979; 1980a; 1980b).

5.8.1.3 Changes to New Section 8.1.2.5

Frog Embryo Teratogenesis Assay: Xenopus (FETAX) Study: The title has been changed to ***Amphibian Studies*** to reflect the new and more comprehensive data available. The following revised discussion of Goleman et al. (2002a.b) and of data from new EPA studies performed in the National Health and Environmental Effects Research Laboratory (NHEERL) is now included: In addition, Goleman et al. (2002a, 2002b) published the results of bioassays that are a modification of the FETAX assay and are designed to be chronic (i.e., 70 days), partial life-cycle (i.e., egg to tadpole to metamorphosed froglet) tests for thyroid-mediated effects on development, metamorphosis, and sex ratios in amphibians. Other reports to SERDP from TIEHH have investigated uptake and elimination of perchlorate in American bullfrog (*Rana catesbeiana*) tadpoles (Carr et al., 2002a); surveyed effects on tadpoles and adult frogs (i.e., American bullfrog and chorus frog [*Pseudacris triseriata*]) at Longhorn Army Ammunition Plant, Texas (Carr et al., 2002b); compared effects observed by the *Xenopus* chronic assays between perchlorate-contaminated water collected from Longhorn Army Ammunition Plant and perchlorate-spiked, FETAX medium (Carr et al., 2002c); determined lethal concentrations of sodium perchlorate and ammonium chloride to *Xenopus laevis* in an acute exposure (Carr et al., 2002d); and surveyed bioaccumulation and effects of perchlorate on caged American bullfrog tadpoles at contaminated sites at Longhorn Army Ammunition Plant (Theodorakis et al., 2002b).

Also, a memo from Joseph Tietge and Sigmund Degitz of the EPA's National Health and Environmental Effects Laboratory, Duluth, describes the results of 14-day flow-through laboratory tests with *Xenopus laevis*. In these tests, sodium perchlorate was added to filtered Lake Superior water; and Tietge and Degitz (2003) assessed effects on metamorphic development and thyroid histology of initial Nieuwkoop-Faber (NF) stage 51 and 54 *Xenopus* tadpoles exposed for 14 days. Measured perchlorate concentrations ranged from 0.0 (i.e., control) to 4.0 mg/L.

5.8.1.4 Changes to New Section 8.1.2.6

Phytotransformation Studies: The following text is added: Further, a report, *Uptake of the perchlorate anion into various plant species* (Anderson et al., 2002a), examined uptake of perchlorate by cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), and soybeans (*Glycine max*) from sand and by duck weed (*Lemna minor*) from water. This report also compared perchlorate concentrations in vegetation and soil/water samples from two contaminated sites at Longhorn Army Ammunition Plant.

5.8.1.5 Changes to New Section 8.1.2.7

Biotransport Investigation Studies: The following text is added: Another study examined bioaccumulation of perchlorate in larger terrestrial mammalian omnivores at Longhorn Army Ammunition Plant (Smith et al., 2002).

5.8.1.6 New Data in New Section 8.1.2

5.8.1.6.1 New Section 8.1.2.8 Added

Report for Development of Freshwater Aquatic Life Criteria. Parsons (2002), external review draft, *Scientific and Technical Report for Development of Freshwater Aquatic Life Criteria for Perchlorate*, dated July 2002, reviews the available aquatic toxicity data for perchlorate and details additional aquatic toxicity and bioconcentration studies aimed at filling out the data base needed to calculate a numerical ambient water quality criteria (AWQC) for perchlorate following U.S. EPA guidance (Stephan et al., 1985). The report includes standard acute bioassays for the midge, *Chironomus tentans*; rainbow trout, *Oncorhynchus mykiss*; bluegill sunfish, *Lepomis macrochirus*; oligochaete, *Lumbriculus variegatus*; clam, *Corbicula*

fluminea; and green frog, *Rana clamitans*; bioconcentration studies for *L. macrochirus* and *C. fluminea*; and a chronic bioassay for *C. tentans*. The report also proposes AWQC for the protection of aquatic organisms and their uses based on the available data and following Stephan et al. (1985).

5.8.1.6.2 New Section 8.1.2.9 Added

Additional Studies of Fish. A study by Patino et al. (in press) investigated thyroid and reproductive effects in the adult zebrafish (*Danio rerio*). Reports to SERDP from TIEHH examined uptake of ammonium perchlorate by and the thyroid status of two species of native fish (i.e., the mosquitofish, *Gambusia holbrooki*, and channel catfish, *Ictalurus punctatus*) in laboratory exposures (Theodorakis et al., 2002a); surveyed bioaccumulation and effects of perchlorate on caged western mosquitofish (*Gambusia affinis*) at contaminated sites at Longhorn Army Ammunition Plant (Theodorakis et al., 2002b); and surveyed perchlorate body burdens, thyroid histology, and the reproductive status of native fish collected from Longhorn Army Ammunition Plant (Theodorakis et al., 2002c).

5.8.6.1.3 New Section 8.1.2.10 Added

Laboratory Mammalian Studies. As described in Chapter 7, laboratory studies have been conducted with rats. In addition, Thuett et al. (2002) report the results of a similar laboratory study that used a native rodent species, the deer mouse (*Peromyscus maniculatus bairdii*).

5.8.2 Changes to Section 8.2: Problem Formulation

The following section was inserted as Section 8.1.2.4 and the previous Section 8.1.2.4 through Section 8.1.2.6 were renumbered as Section 8.1.2.5 through Section 8.1.2.6.

5.8.2.1 New Section 8.2.1.4: Population Productivity of Herbivorous or Detritivorous Aquatic Organisms or Wildlife

Herbivorous and detritivorous aquatic organisms or wildlife are included as an endpoint entity because of the apparent bioconcentration of perchlorate in the foliage of aquatic macrophytes. Aquatic herbivores may feed directly on aquatic macrophytes and include birds, such as ducks, the muskrat (*Ondatra zibethicus*), and certain types of insects or crustaceans, such

as crayfish. Aquatic detritivores, particularly various invertebrates, may feed on the detritus formed when these macrophytes die. Population productivity is used as the endpoint property because growth and reproduction are generally sensitive properties and because these animals are valued for their production of food for carnivores.

5.8.3 Changes to Section 8.3.1.2: Aquatic Bioaccumulation

Information in this section is now supplemented by the additional studies of Theodorakis et al. (2002b) and the laboratory studies of bioaccumulation conducted by Theodorakis et al., (2002a), Carr et al. (2002a), and Parsons (2002). Recommended descriptions for these studies are as follows.

Theodorakis et al. (2002b) placed caged adult mosquitofish and bullfrog tadpoles at four reference sites and two contaminated sites at Longhorn Army Ammunition Plant. Only trace concentrations of perchlorate were detected at the reference sites, while concentrations of 0.00747 ± 0.02781 (detections in 2 of 21 samples) and 0.3694 ± 0.3446 mg/L (detections in 13 of 18 samples) were measured at Fire Station Creek and the INF Pond, respectively. Because of high mortality from predation (i.e., odonate larvae, otters, raccoons) and destruction of cages (i.e., otters, raccoons, alligators), mosquitofish were retrieved from cages at two reference (i.e., Central Creek and Goose Prairie Crockett) and both contaminated sites, while tadpoles were retrieved from cages at only one reference (i.e., Central Creek) and one contaminated (i.e., Fire Station Creek) site. In mosquitofish, perchlorate was detected only at trace concentrations in all four sites. In tadpoles, perchlorate was detected at both the reference (i.e., Central Creek) and the contaminated (i.e., Fire Station Creek) sites at concentrations of 0.0538 ± 0.0836 and 0.0746 ± 0.148 mg/kg. The coefficient of variation for both of these means was greater than 100% because perchlorate was detected in only 2 of 6 tadpoles from Central Creek and 2 of 9 tadpoles from Fire Station Creek. The authors speculate that there may be an undetected, intermittent source of perchlorate in Central Creek.

Theodorakis et al. (2002a) exposed mosquitofish and channel catfish to ammonium perchlorate in the laboratory for 30 days. Test concentrations for the mosquitofish were 0, 0.1, 1, 10, 100, and 1,000 mg/L, while the channel catfish was tested at 100 mg/L, only. Whole body concentrations were measured for the mosquitofish, while concentrations were measured for selected organs, the head, and fillet for the channel catfish. At 30 days, concentrations in

mosquitofish increased from trace in the 0 and 0.1 mg/L test concentrations to 85.4 mg/kg in the 1000 mg/L test concentration. In the catfish, perchlorate was detected at trace concentrations in the gonads and 0.17, 0.49, 0.95, 132, 7.26, and 25.4 mg/kg in the liver, gill, kidney, gastrointestinal tract, fillet, and head, respectively. In all cases the tissue concentrations were less than the water exposure concentration.

Carr et al. (2002a) exposed bullfrog tadpoles to ammonium perchlorate at a concentration of 117 mg/L for 96 hours and then transferred the tadpoles to plain water to observe elimination. Whole-body concentrations of perchlorate increased linearly and at 96 hours reached a concentration of 53 mg/kg. Perchlorate concentrations remained elevated (~10 mg/kg) up to 96 hours after the tadpoles were transferred to plain water.

Parsons (2002) conducted 28-day bioconcentration tests with the bluegill and *Corbicula* clam. Nominal test concentrations were 1.5 and 15 mg/L in the tests with bluegills, while test concentrations were 6.5 and 15 mg/L for *Corbicula*. Bioconcentration factors for the bluegill were 0.73 and 0.40 for the respective test concentrations. Bioconcentration factors for *Corbicula* were < 1.1 and 0.87 for the respective test concentrations.

5.8.4 Changes to Section 8.3.1.4 Uptake by Vegetation

The following two new sections have been added.

Addition to Line 11 page 8-18: Anderson (2002a) observed uptake of perchlorate by cucumber, lettuce, and soybeans from sand and duckweed from water. In various trials using the terrestrial species, perchlorate exhibited some depletion (average 17%) in unplanted controls (i.e., cups of sand without plants). Otherwise, the plants generally depleted the perchlorate from the sand; and while significant concentrations of perchlorate were often found in the roots in the first few weeks, by the end of the trials, perchlorate was completely translocated to the leaves. This occurred whether the medium was a nutrient solution called Hydrosol, deionized water, or a mixture of the two. For duckweed, perchlorate uptake occurred from a 0.10 mg/L solution of sodium perchlorate in Hydrosol (i.e., plant concentrations ranged from 0.079 to 1.7 mg/kg), but did not occur in similar solutions in deionized water or a mixture of Hydrosol and deionized water.

Addition to Line 9 page 8-20: A few additional concurrent collections of plants and soil from the Building 25C site by Anderson (2002a) included a willow (plant concentration

= 19.8 mg/kg wet-weight, soil concentration = 0.129 mg/kg wet-weight, uptake factor = 153), fern (plant concentration = 2.59 mg/kg wet-weight, soil concentration = 0.054 mg/kg wet-weight, uptake factor = 48), and *Juncus* (plant concentration = 2.35 mg/kg wet-weight, soil concentration = 0.076 mg/kg wet-weight, uptake factor = 31).

5.8.5 Revised Section 8.3.2 Characterization of Effects

EPA recommends major revision of this section as provided below.

5.8.5.1 Changes to Section 8.3.2: Characterization of Effects

5.8.5.1.1 Changes to Section 8.3.2.1: Aquatic Organisms

Effects on the richness and productivity of fish, aquatic invertebrate, and aquatic plant communities are jointly characterized through the derivation of acute and chronic aquatic benchmark values. This was accomplished with the procedures for deriving national acute and chronic water quality values for the protection of aquatic organisms and their uses following the guidance of Stephan et al. (1985). Previous drafts of this document have calculated Tier II water quality values (U.S. Environmental Protection Agency, 1993a; Suter and Tsao, 1996), which are derived when data are not sufficient for deriving ambient water quality criteria (AWQCs). Sufficient data are now available to use the methods of Stephan et al. (1985). However, as these values have not been promulgated by the Office of Water nor have undergone full peer review, they cannot be considered national ambient water quality criteria at this time. Moreover, as discussed below, most of the available chronic data are from standard toxicity tests in which the measurement endpoints were mortality or growth. As such, these chronic tests appear to be insufficient to fully assess the potential thyroid-mediated effects of perchlorate on the development of aquatic animals.

Test results potentially useful for deriving acute and chronic aquatic benchmark values were initially available for five aquatic species (Table 5-1). In acute tests (48 and 96 h) with sodium perchlorate, using the water flea *Daphnia magna*, the amphipod *Hyalella azteca*, and the fathead minnow *Pimephales promelas*, the endpoints lethality and inhibition were studied. In seven-day tests with a different water flea (*Ceriodaphnia dubia*) and with *P. promelas*, acute lethality was studied in addition to more sensitive endpoints, including the number of offspring per female (*C. dubia*) and growth rate (i.e., body weight; *P. promelas*). A seven-day test with *C. dubia*

generally is here used in place of a chronic (i.e., twenty-one day) test because test organisms produce three broods during the test; a seven-day test with *P. promelas* is subchronic because of the test's short duration relative to the organism's lifespan (Suter, 1990; Norberg-King, 1990). A 35-day, early-life-stage (ELS) test with *Pimephales*, here used in place of a chronic test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested. However, all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling that was not observed in the larvae exposed to the control water. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic No-Observed-Effect-Concentration (NOEC) has been adequately determined for this species.

Toxicity data for four species of algae and one species of macrophyte are available for perchlorate although only a study of *Selenastrum capricornutum* (EA Engineering, Science and Technology, 1999) is appropriate for criterion development. Other studies include Krebs (1991), which reported an EC_{10} for potassium perchlorate greater than 1,000 mg/L for species of green algae in the order Chlorococcales, and Bringmann (1975) and Bringmann and Kuhn (1977, 1978a, 1978b, 1979, 1980a, 1980b), which reported toxicity thresholds of 360 mg/L for *Scenedesmus quadricauda* and 79 mg/L for *Microcystis aeruginosa.* Anderson et al. (2002a) observed growth of duckweed (*Lemna minor*). No significant inhibition in growth of duckweed was observed at concentrations of perchlorate up to 0.10 mg/L in water. However, the greatest tested concentration in this study was much less than the threshold concentrations reported the studies of algae.

Parsons (2002) has added test results for six aquatic species, consisting of six acute tests and one chronic test for the insect *Chironomus riparius*. The acute tests examined lethality endpoints while the chronic test examined survival to emergence, growth, and reproductive output. To the meet the data requirements set out by Stephan et al. (1985), Parsons (2002) also conducted laboratory bioconcentration tests for one fish, *Lepomis macrochirus*, and one invertebrate, *Corbicula fluminea*. Average bioconcentration factors were less than one for both species (i.e., 0.40 - 0.73 L/kg for *L. macrochirus* and 0.87 - < 1.1 L/kg for *C. fluminea*).

The data base now meets the requirements set out by Stephan et al. (1985). Specifically, acceptable acute tests are available for 10 species of freshwater animals in 9 different families (8 are needed) including, as required, a species of the family Salmonidae (class Osteichthyes),

Oncorhynchus mykiss; a second family in the class Osteichthyes, *L. macrochirus*; a third family in the phylum Chordata, *P. promelas* or *Rana clamitans*; a planktonic crustacean, *D. magna* or *C. dubia*; a benthic crustacean, *H. azteca*; an insect, *C. tentans*; a family in a phylum other than Arthropoda, *C. fluminea*; and a family in any order of insect or any phylum not already represented, *Lumbriculus variegatus*. Data are available to calculate acute-chronic ratios for species of aquatic animals in four different families (three are required) including one fish, *P. promelas*; one invertebrate, *C. tentans*; and one acutely sensitive species, *C. dubia*. Additionally, an acceptable test is available for a freshwater alga or vascular plant, *S. capricornutum*; and an acceptable bioconcentration factor has been determined, *L. macrochirus* or *C. fluminea*.

Steps followed in the derivation of the aquatic benchmark values for perchlorate are presented in Tables 8-2, 8-3 and 8-4. The acute and chronic benchmark values for the perchlorate ion are calculated to be 22.3 mg/L and 10.3 mg/L, respectively. A sodium chloride control test showed that some of the toxicity to *P. promelas* was potentially attributable to the sodium cation. These tests suggest the possibility that if perchlorate were associated with a less toxic cation, the FCV may be lower than is necessary to protect against perchlorate ion toxicity. Further tests with perchlorate may be needed to assess potentially less toxic cations.

Similar chronic (or subchronic) tests were conducted with ammonium perchlorate (Table 5-1). Results, expressed as ClO_4^-, were very similar for *C. dubia;* but *P. promelas* was more sensitive to ammonium perchlorate than to sodium perchlorate. Therefore, data for ammonium perchlorate, except that for *C. dubia*, are not used to calculate the acute and chronic values, because the lack of ammonium controls makes it difficult to determine whether the observed effects were caused by the perchlorate anion or by ammonium. When perchlorate is administered as the ammonium salt, the ammonium ion concentration expressed on an ammonia-nitrogen (in milligrams of NH_3-N/L) basis is 14% of the respective perchlorate ion concentration. A Lowest-Observed- Effect-Concentration (LOEC) for *C. dubia* of 24 mg/L perchlorate (Table 8 1) thus corresponds to 3.4 mg NH_3-N/L. Based on a species mean chronic value (SMCV) of 13 mg NH_3-N/L for *C. dubia* exposed to ammonia alone (U.S. Environmental Protection Agency, 1998h), the former value is probably too low to be responsible for the observed effects[1]. On the

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Ammonia/ammonium toxicity increases as test-water pH increases (U.S. Environmental Protection Agency, 1998e). The value of 13 mg NH_3-N/L corresponds to a pH of 8.0; however, unless the test water pH had exceeded 8.8, it is doubtful that 3.4 mg NH_3-N/L was responsible for the observed effects.

Table 5-1. New Table 8-1. Results of Perchlorate Toxicity Tests in Aquatic and Terrestrial Species

	Test Description		Endpoints (as mg/L. perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 1998)							
Daphnia magna	<24 hr	Acute (48-hr)	490 (406 - 591)	—	—	—	—
Pimephales promelas	12 -13 days	Acute (96-hr)	1,655 (1,507 - 1,1817	—	—	—	—
Ceriodaphnia dubia	<24 hr	Chronic (7-day)	66 (40-144) [48-h]	10	33	18.2	17 (8.1 -20.5)
Pimephales promelas	<24 hr	Subchronic (7-day)	614 (540 - 714) [96-h]	155	280[c]	208[c]	212[c] (175 - 231)[c]
Lactuca sativa		Chronic definitive (28-day, soil)		<80	80	<80	78
Lactuca sativa		Chronic definitive (28-d, sand)		<80	80	<80	41
Lactuca sativa		Chronic definitive (28-d, soil)		40	40	56.6	30
Lactuca sativa		Chronic definitive (28-d, sand)		20	40	28.3	34.3
Eisenia foetida		Acute definitive (7 day/14 day, soil)	4,450/4,450	—	—	—	—
Sodium perchlorate ($NaClO_4$)[b] tests (Anderson et al., 2002a)							
Cucumis sativus		Subchronic (10-d, sand)		10	—	—	—
Lactuca sativa		Subchronic (10-d, sand)		100	10	31.6	—

Table 5-1 (cont'd). New Table 8-1. Results of Perchlorate Toxicity Tests in Aquatic and Terrestrial Species

	Test Description		Endpoints (as mg/L. perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
Sodium perchlorate ($NaClO_4$)[b] tests (EA Engineering, Science and Technology, Inc., 2000)							
Pimephales promelas	Embryo	Chronic (35-day, early life stage)	> 490 [96-hr]	> 490[d] <28[e]	> 490[d] 28[e]	> 490[d]	> 490[d] <28[e]
Hyalella azteca	7 - 14 days	Chronic definitive (28-day)	assumed > 1000	> 1000	> 1000	> 1000	> 1000
Sodium perchlorate (NaClO4)[b] tests (EA Engineering, Science, and Technology, 1999)							
Selenastrum capriconutum	7 days	Acute (96-hr)	—	500	1200	775	615 (149 - 1,126)
Ammonium perchlorate (NH_4ClO_4)[f] tests (Block Environmental Services, Inc., 1998)							
Ceriodaphnia dubia	<24 hr [8]	Chronic (6-day)	77.8 [6-days]	9.6	24	15	24
Pimephales promelas	< 24 hr [8]	Subchronic (7-day)	270 [7-days]	9.6	96	30	114
Ammonium perchlorate (NH_4ClO_4)[f] tests (Dumont and Bantle, 1998)							
Xenopus laevis	Embryo	96-hr	420 [h]	—	—	—	—
Xenopus laevis	Embryo	96-hr	336 [h]	—	—	—	—
Sodium perchlorate ($NaClO_4$)[b] tests (Parsons 2002)							
Chironomus tentans	larvae (11 days)	Acute (48-hr)	8,140 (6,610 - 10,030)	5640	12700	9830	—
Oncorhynchus mykiss	Fry	Acute (96-hr)	2,010 (1,810 - 2,220)	1460	2760	2210	—
Lepomis macrochirus	Juveniles	Acute (96-hr)	1,470 (1,270 - 1,710)	547	1260	971	—
Lumbriculus variegatus	Adults	Acute (96-hr)	3,710 (3,500 - 3,900)	2500	5760	4440	—
Corbicula fluminea	Adults	Acute (96-hr)	6,680 (5,300 - 8,400)	4850	10800	8370	—

Table 5-1 (cont'd). New Table 8-1. Results of Perchlorate Toxicity Tests in Aquatic and Terrestrial Species

	Test Description		Endpoints (as mg/L. perchlorate in water or mg/kg in soil or sand)[a]				
Test Species	**Age**	**Duration**	**Acute LC_{50} (95% CL)**	**NOEC**	**LOEC**	**ChV**	**IC_{25} (95% CL)**
			Sodium perchlorate ($NaClO_4$)[b] tests (Parsons 2002)				
Rana clamitans	Tadpoles (7 - 8 months)	Acute (96-hr)	5,500 (4,700 - 6,400)	2400	5800	4440	—
Chironomus tentans	Larvae (1-day)	Chronic - Survival to emergence (42-day)	146[i] (134 - 159)	58.5	118	83.1	—
Chironomus tentans	Larvae (1-day)	Chronic - Growth (42-day)	363[i] (158 - 834)	118	233	166	—
Chironomus tentans	Larvae (1-day)	Chronic - Reproduction (42-day)	211[i] (131 - 338)	118	233	166	—

[a] Notation: LC_{50} = Concentration lethal to 50% of individuals; NOEC = No-observed-effect concentration; LOEC = Lowest-observed-effect concentration; ChV = Chronic value; IC_{25} = Concentration inhibiting a process (e.g., growth, reproduction) by 25%; CL = confidence limits.
[b] Sodium chloride control showed no adverse effects of sodium ion except as indicated. Reported values are based on nominal concentrations.
[c] Sodium chloride control showed significant adverse effects attributable to sodium cation at highest test concentration. Effects observed at this perchlorate concentration may have been caused in part by sodium ion toxicity.
[d] Standard endpoints: survival, growth, biomass
[e] Although there were no effects on standard endpoints at any tested concentration, the investigators reported that all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling, which was not observed in the larvae exposed to the control water.
[f] Ammonium control was not used; adverse effects of ammonium ion cannot be ruled out at all effect concentrations. *C. dubia* and *P. promelas* results are based on measured concentrations. *Xenopus laevis* results are based on nominal concentrations. Confidence limits are not reported.
[g] Not reported; assumed based on standard protocols.
[h] IC_{50} for malformations.
[i] This is the EC_{50} for chronic effects.

Table 5-2. New Table 8-2. Procedure for Deriving Acute and Chronic Aquatic Benchmark Values[1] for Perchlorate

Step	Value (mg/L ClO_4-)	Rationale
Calculate GMAVs for each genus and rank GMAVs from lowest to highest.	see Table 8-3	Stephan et al. (1985)
Select four lowest GMAVs and use to calculate FAV.	FAV = 44.7	If less than 59 GMAVs, use four lowest GMAVs, calculate cumulative probability, P, for each GMAV as R/(N+1), then calculate S^2, L, A, and FAV using equations from Stephan et al. (1985).
Calculate acute aquatic benchmark value.	acute aquatic benchmark value = 22.3	Acute aquatic benchmark value is equal to one-half the FAV: 44.7 ÷ 2 = 22.3
Calculate SMACR for each species and rank species based on SMAVs	see Table 8-4	Stephan et al. (1985)
Select the SMACRs for species whose SMAVs are close to the FAV and calculate the geometric mean of their SMACRs.	FACR = 4.34 (unitless)	If SMACR seems to increase or decrease as the SMAVs increase, the FACR should be calculated as the geometric mean of the SMACRs for species whose SMAVs are close to the FAV (Stephan et al., 1985). Only the SMAV for *C. dubia* is close to the FAV.
Divide FAV by FACR to calculate FCV	FCV = 10.3	Stephan et al. (1985), Calculation: 44.7 ÷ 4.34 = 10.3
Calculate chronic aquatic benchmark value.	chronic aquatic benchmark value = 0.12	Chronic aquatic benchmark value ordinarily is equal to FCV. However, because of evidence that the tests used to derive the FCV did not account for thyroid-mediated effects on amphibian development, a lower value is established. See text.

[1] GMAV = Genus mean acute value, FAV = final acute value, SMAV = Species mean acute value, SMACR = species mean acute chronic ratio, FACR = final acute chronic ratio, FCV = final chronic value.
[2] These values do not constitute National Ambient Water Quality Criteria.

Table 5-3. New Table: 8-3. Ranking of Genus Mean Acute Values (GMAVS) for Perchlorate

Type	Species	Acute EC_{50} values (mg/L)	GMAV (mg/L)	Rank
invertebrate	*Ceriodaphnia dubia*	77.8, 66	71.7	1
invertebrate	*Daphnia magna*	490	490	2
fish - Cyprinidae	*Pimephales promelas*	1,655, 614, >490	793	3
invertebrate	*Hyalella azteca*	>1,000	>1,000	4
fish - Centrarchidae	*Lepomis macrochirus*	1470	1470	5
fish - Salmonidae	*Oncorhynchus mykiss*	2010	2010	6
invertebrate	*Lumbriculus variegatus*	3710	3710	7
amphibian	*Rana clamitans*	5500	5500	8
invertebrate	*Corbicula fluminea*	6680	6680	9
invertebrate	*Chironomus tentans*	8140	8140	10

Table 5-4. New Table 8-4. Calculation of Species Mean Acute Chronic Ratios for Species with Acute and Chronic Values — Species Are Ranked Based on Their Chronic Values

Species	Acute LC_{50} (mg/L)	Chronic value (mg/L)	ACR	SMACR	Rank
Ceriodaphnia dubia	66	18.2	3.63	4.34	1
	77.8	15	5.12		
Pimephales promelas	1655	28	59.1	13.2	2
	614	208	2.95		
Chironomus tentans	8140	83.1	98	98	3

other hand, the LOEC observed with *P. promelas* at 96 mg/L (Table 8-1) corresponds to 14 mg NH_3-N/L, which exceeds a SMCV of 3.09 mg NH_3-N/L (U.S. Environmental Protection Agency, 1998h). Therefore, ammonium exposure alone could have been responsible for the effects of ammonium perchlorate observed in *P. promelas*. Similarly, in the nonresident species *Danio rerio* (zebrafish), Patino et al. (in press) found effects only at a concentration (i.e., 677 mg ammonium perchlorate/L) where toxicity was largely attributable to ammonia.

The acute and chronic benchmark values derived above based on sodium perchlorate are probably protective even if ammonium perchlorate is the form released. Calculated NH_3-N concentrations corresponding to those values are below the acute and chronic ambient water quality criteria for ammonia, regardless of pH (U.S. Environmental Protection Agency, 1998h). While FAV and FCV are not calculated for plants, it appears that there is little perchlorate or ammonium toxicity to the alga *Selenastrum* in toxicity studies (Table 8-1).

Because of its unique mode of action, there has been concern that perchlorate could cause effects that would not be measured by traditional toxicity tests. This problem has been accentuated by Goleman et al. (2002a, 2002b), who published the results of laboratory toxicity tests designed to assess effects associated with perchlorate inhibition of thyroidal iodide uptake in amphibians. These toxicity tests used embryos and larvae of the clawed frog *Xenopus laevis* and measured percentages of mortality, hatching, bent tails, edema, and abnormal swimming after 5 and 70 days exposure; snout-vent length, hindlimb length, percentage forelimb emergence, and percentage completing tail resorption after 70 days exposure; and thyroid function and gonadal sex ratios after 70 days exposure. The tests were conducted with nine concentrations of ammonium perchlorate in FETAX (i.e., frog teratogenesis assay-*Xenopus*) medium: 0.005 ± 0.002 mg/L measured, 0.018 ± 0.003 mg/L measured, 0.147 ± 0.006 mg/L measured, 1.412 ± 0.032 mg/L measured, 14.4 ± 0.07 mg/L measured, 133 ± 2.5 mg/L measured, 425 ± 45 mg/L measured, 585 mg/L nominal, and 1175 mg/L nominal. FETAX medium is an artificial water created by adding 10.7 mM NaCl, 1.14 mM $NaHCO_3$, 0.4 mM KCl, 0.14 mM $CaCl_2$, 0.35 mM $CaSO_4$, and 0.62 mM $MgSO_4$ to deionized water that has been passed through a carbon filter.

The 5- and 70-day LC_{50}s for ammonium perchlorate were 510 ± 36 mg/L and 223 ± 13 mg/L, respectively (Goleman et al., 2002a). The LOEC for hatching success was 1175 mg/L, while that for percent bent tails, edema, and abnormal swimming was 425 mg/L. However, the 5-day LC_{50} for ammonium perchlorate was equivalent to a molar concentration of ammonia that was similar to

that in a test with ammonium chloride (Carr et al., 2002d; 5-day LC_{50} for NH_4Cl = 118 mg/L), indicating that ammonia toxicity contributed to the observed lethal effects on *Xenopus*. However, in addition to these lethal effects, Goleman et al. (2002a) reported reduced hindlimb length, percentage forelimb emergence, and percent completing tail resorption at a concentration of 0.018 mg/L, which is much lower than the concentrations associated with ammonia toxicity. Also, these effects are consistent with inhibition of metamorphosis associated with perchlorate effects on thyroid activity. At 70-days, whole-body concentrations of thyroxine (T_4) were reduced at a concentration of 14.1 mg perchlorate / L, while thyroid epithelial cell heights and gonadal sex ratios were skewed toward fewer males at concentrations greater then 0.059 mg/L (Goleman et al., 2002b). Therefore, these studies raise concerns about the effects of low levels of perchlorate on amphibian metamorphosis.

Parsons (2002) questioned both the scientific validity and regulatory applicability of the Goleman et al. (2002a, 2002b) studies. That report suggests that Goleman et al. (2002a) should not be used for AWQC development for five reasons: *(1) X. laevis* is not a native species, (2) FETAX solution is an unusual dilution water, (3) there was possible ammonia toxicity in the acute test, (4) the exposures were not flow-through, and (5) the 70-day test is not a full chronic test. Some of these reasons are not valid, as discussed below; but other data sustain the concern that the results obtained by Goleman et al. (2002a, 2002b) may be unreliable.

First, although *X. laevis* is not native to the United States (e.g., it's natural range is South Africa), it is a resident species that is listed in Appendix 1 of Stephan et al. (1985). According to the Global Invasive Species Database, feral populations of *X. laevis* occur in at least California (Invasive Species Specialty Group, on line). More importantly, *X. laevis* is the model for amphibian development (Kavlock 1998), an endpoint of interest in the toxicology of perchlorate, and in this respect, is representative of native frogs. Moreover, if native frogs are shown to exhibit similar effects, it would be appropriate to use this *X. laevis* data.

Second, "unusual dilution water" is defined in Stephan et al. (1985) as "dilution water in which total organic carbon or particulate matter exceeded 5 mg/L." Stephan et al. (1985) also states that data should usually be rejected if from "tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts." However, salts are added to deionized water to make FETAX medium, as described above (Kavlock 1998, Parson 2002). A valid scientific question, though, is whether the absence of some other elemental nutrient, such

as iodine, which is critical to development, may have contributed to the observed perchlorate effects (J. Carr, Texas Tech University, personal communication). Perchlorate exerts its effect on development by inhibiting thyroidal iodide uptake, but inhibition may be reduced as the available iodine increases.

Third, although there was possible ammonia toxicity in the acute test with ammonium perchlorate, there are alternate data from an acute test with sodium perchlorate where toxicity of the cation (i.e., Na^+) was not a concern. The 5-day LC_{50} from the acute test with sodium perchlorate was >1200 mg/L (Carr et al., 2002d).

Fourth, flow-through chronic tests are required in Stephan et al. (1985) to avoid problems with the loss of the test material from solution and accumulation of waste products, such as ammonia, with time. Perchlorate is stable in solution, and the method used in Goleman et al. (2002a), 50% renewal of test and control solutions every 72 hours, seems sufficient to maintain the test concentrations of perchlorate. However, this static renewal method may be insufficient to prevent accumulation of waste products, particularly when ammonia was added as part of the test material. Moreover, Goleman et al. (1992a, 1992b) fed their *Xenopus* once every 72 hours, apparently to avoid the problem with accumulation of waste products (i.e., waste products result from excretion by the test animals and microbial degradation of uneaten food). This diet may have been insufficient for tadpole growth (J. Tietge, EPA, ORD, Duluth, MN; personal communication).

Fifth, a chronic test may be valid if it does not include the entire life cycle of the test organism but does include life stages of the organism that are generally sensitive to the test material. The 70-day test in Goleman et al. (2002a) tested *Xenopus* from the egg to metamorphosing tadpole stages, and the metamorphosing tadpole is believed to be the most sensitive life stage of amphibians to perchlorate (Goleman et al. 2002a). Therefore, absent the other concerns raised, a test of this type should be considered a valid chronic test.

A number of more recent studies have been conducted that are instructive as to the concern about the elemental content of the FETAX medium. Carr et al. (2002c) compared the results of toxicity tests where *X. laevis* was exposed either to laboratory solutions of ammonium perchlorate (14.0 mg/L or 0.038 mg/L) in FETAX medium or unamended surface waters collected from four sites at Longhorn Army Ammunition Plant. These included two contaminated sites, HOLP and Harrison Bayou catfish pond (HBCP), and two reference sites, HPRD and Star Ranch Pond

(STAR). Measured concentrations of ammonium perchlorate in water samples from HOLP used in toxicity tests in April 2000, August 2000, and February 2001 ranged from 0.086 mg/L to 5.46 mg/L, while measured concentrations in water samples from HBCP used in a toxicity test in April 2001 was 0.014 mg/L. In the toxicity tests with laboratory solutions, tail length and developmental stage (i.e., Nieuwkoop-Faber or NF stage) were significantly different relative to controls after 45 days at a concentration of 14.0 mg/L. In the toxicity tests with surface waters from the contaminated sites, no significant differences were observed in tail length or NF stage at intervals from 14 to 45 days. As discussed by Carr et al. (2002c), these differences between the two sets of toxicity tests may result from the amount of iodide available to the tadpoles. In the laboratory-solution toxicity tests, the only source of iodine was food (i.e., frog brittle, 4.8 µg I / 0.1 g ration fed every 72 hours). In addition to the food, surface water (up to 180 µg I /L) was a source of iodine in the surface-water toxicity tests. This suggests the effects observed at very low concentrations (0.018 mg/L) of perchlorate in Goleman et al. (2002a) may be in part an artifact of the test solutions used, and the results of these tests were not used to derive an AWQC for this reason.

Carr et al. (2002b) collected bullfrog (*Rana catesbeiana*) tadpoles at a contaminated site at Longhorn Army Ammunition Plant during April 2000 [HOLP (INF treatment holding pond): Perchlorate = 1.97 mg/L] and a reference [HPRD (holding pond reference ditch): Perchlorate = not detected]. Hindlimb length (HOLP: 5.02 ± 0.60 mm versus HPRD: 26.2 ± 2.36 mm) and the ratio of hindlimb length to snout to vent length (HOLP: 0.21 ± 0.02 versus HPRD: 1.12 ± 0.09) were much less at the contaminated site than at the reference site (i.e., ≈ 5 times less). Chorus frog (*Pseudacris triseriata*) tadpoles were also collected at three sites where perchlorate was not detected (B25C-2, B25C-3, and B25C-8) and one site where perchlorate was detected at 10 mg/L and analyzed for thyroid histology. Tadpoles from the three nondetect sites did not exhibit follicular hypertrophy or colloid depletion, while tadpoles from the 10 mg/L site had significant colloid depletion and follicular hypertrophy.

Theodorakis et al. (2002b) placed caged bullfrog tadpoles (*Rana catesbaena*) in two contaminated and four reference locations at Longhorn Army Ammunition Plant for four to six weeks. However, because of high mortality, in part because of predators, tadpoles survived at only one reference site (Central Creek) and one contaminated site (Goose Prairie Creek). Perchlorate was detected only in trace concentrations at both sites, while the tadpoles had metamorphosed to

froglets at the contaminated site and had retained their tails and not grown forelimbs at the reference site. Moreover, perchlorate was quantified in tadpole tissues at the reference site (0.0538 mg/kg), but detected only in trace concentrations at the contaminated sites, suggesting that sites had been misidentified as contaminated and uncontaminated or that the samples had been mislabeled.

In summary, the research by Carr et al. (2002b) on native frogs (i.e., bullfrogs and chorus frogs) suggests that perchlorate affects metamorphosis in natural freshwaters from Longhorn Army Ammunition Plant in the range of 2.0 to 10 mg/L. Other findings with bullfrogs in Longhorn Army Ammunition Plant waters were anomalous (Theodorakis et al., 2002b), and results with *X. laevis* using these waters showed no apparent effects in an exposure range of 0.1 - 5 mg/L. However, it is also unclear whether the concentrations of iodine observed in surface water at Longhorn Army Ammunition Plant are representative of other surface waters. Moran et al. (2002) published data from a survey of I and I^{129} in 36 rivers across North America. Of those rivers, only one (i.e., Rio Grande at Brownsville, Texas, 212 µg I / L) exceeded the concentration of 180 µg I / L observed by Carr et al. (2002c) at Longhorn Army Ammunition Plant; and only four additional rivers (i.e., the Rio Grande at El Paso, Texas, 79 µg I / L; San Joaquin River at Vernal, Utah, 66 µg I / L; Pecos River at Pecos, Texas, 57 µg I / L; Brazos River, Texas, 32 and 43 µg I / L) exceeded 30 µg I / L. The other rivers ranged from 0.5 to 21 µg I / L. Moran et al. (2002) suggest that the concentrations at the high end of the range (i.e., > 30 µg I / L) are from rivers with watersheds in arid regions where water use for irrigation is high and that the total dissolved solids content of these rivers has increased from nonpoint source runoff and return flow from irrigated agriculture. Additionally, they state that the concentrations in this high end range have not been reported previously for freshwater (Moran et al. 2002). Therefore, the potentially complicating role of iodide concentration in the determination of perchlorate effect levels remains an uncertainty in this risk assessment. Additional research with perchlorate in freshwaters containing a range of natural or added concentrations of iodine would be needed to address this uncertainty.

Results are also available from 14-day flow-through tests using filtered Lake Superior water. Tietge and Degitz (2003) measured sodium perchlorate effects on metamorphic development and thyroid histology of initial NF stage 51 and 54 *Xenopus* tadpoles. In this study, sodium perchlorate inhibited metamorphic development after 14 days at concentrations of 0.25 mg/L or

greater. For initial NF stage 51 tadpoles, the percentage of tadpoles reaching NF stage 57 after 14 days exposure ranged from 71 to 91% in the control and concentrations of 0.018 and 0.062 mg/L but was only 18% at 0.247 mg/L. For initial NF stage 54 tadpoles, the percentage of tadpoles reaching NF stage 60 after 14 days exposure ranged from 38 to 56% in the control and concentrations of 0.018 and 0.062 mg/L but was only 12% at 0.247 mg/L. Alterations of thyroid histology (i.e., decreased luminal space and glandular hypertrophy) were also noted after 14 days at the lowest concentration tested (i.e., 0.018 mg/L).

The results of Goleman (2002a,b), Carr (2002b), and Tietge and Degitz (2003) provide strong evidence, in spite of certain limitations, that the chronic aquatic benchmark value of 10.3 mg/L, calculated following Stephan et al. (1985) and based on standard laboratory toxicity tests, does not provide sufficient protection from the thyroid-mediated effects of perchlorate on amphibian development. Therefore, based on the effects on *Xenopus* development observed by Tietge and Degitz (2003), which we consider to be the most reliable study of perchlorate effects on amphibian development currently available, an interim chronic aquatic benchmark of 0.12 mg/L [geometric mean of LOAEL (0.247 mg/L) and NOAEL (0.062 mg/L)] is established. If the histopathological results they observed are demonstrated by longer term studies to lead to adverse effects on development, it is possible that this benchmark should be lowered further.

5.8.5.1.2 Changes to Section 8.3.2.2: Terrestrial Organisms

5.8.5.1.2.1 Changes to Section 8.3.2.2.1: Plants

The primary available phytotoxicity information comes from 28-day seedling growth tests of lettuce (*Lactuca sativa*) performed in soil and sand cultures with sodium perchlorate (EA Engineering, Science and Technology, Inc., 1998; see Table 8-1). Exposure was to sodium perchlorate solution added to the solid media, and the results are expressed as milligrams per kilogram soil or sand, wet weight. Growth was a more sensitive response than germination or survival. In an initial test with nominal concentrations of 80, 180, 420, 1000, and 2400 mg/kg, the quartile inhibitory wet-weight concentrations (IC_{25}s) for growth in soil and sand were 78 mg/kg and 41mg/kg, respectively. Survival was reduced 26% at 420 mg/kg in soil and 39% at 180 mg/kg in sand. In a second test, in which the nominal test concentrations were 10, 20, 40, 80, and 160 mg/kg, the IC_{25}s for growth were 30 mg/kg for soil and 34.3 mg/kg for sand. Survival was

reduced 64% at 160 mg/kg in soil and 37% at 80 mg/kg in sand. In sand, germination was reduced 20 to 40% at concentrations of 10 mg/kg or greater.

Anderson et al. (2002a) observed germination of cucumber and lettuce. No effect on germination of cucumber was observed at concentrations of perchlorate up to 10 mg/kg. Germination of lettuce was reduced 60% at 10 mg/kg relative to controls and treatments up to 1 mg/kg.

To account for interspecies variance, a factor of 10 is applied to the NOAEL for lettuce germination of 1 mg/kg from Anderson et al. (2002a). This results in a benchmark value of 0.1 mg/kg as a wet-weight concentration in soil.

5.8.4.1.2.2 Changes to Section 8.3.2.2.2: Soil Invertebrates

Anderson et al. (2002b) conducted 14-day toxicity tests with *Eisenia foetida* and observed significant decreases in survival and average weight between concentrations of 2,000 mg/kg and 5,000 mg/kg on filter paper, sand, or artificial soil, but did not calculate toxicity values. The only available toxicity data for soil invertebrates are from a 14-day acute lethality test of the earthworm (*Eisenia foetida*) performed in artificial soil irrigated with sodium perchlorate. The LC_{50} at both 7 and 14 days was 4,450 mg/kg as a wet-weight concentration in soil (EA Engineering, Science and Technology, Inc., 1998; see Table 5-1). No factors or other models are available to extrapolate from that LC_{50} to chronic effects on survival, growth, or fecundity or to extrapolate from this species to the soil invertebrate community as a whole. Therefore, factors, which have been applied to aquatic communities (U.S. Environmental Protection Agency, 1993; Suter and Tsao, 1996) were used to obtain a conservative estimate of a soil benchmark value for soil community effects. Following these methods, a factor that accounts for interspecies variation varies according to the number of specified taxonomic groups for which genus mean acute values were available. In this case, because only one value is available (i.e., the LC_{50} for the earthworm); the appropriate value is 242. No chronic toxicity data are available to calculate an acute-chronic ratio; a default value of 17.9 (which provides 80% confidence based on other toxicants; Suter and Tsao 1996) is substituted. On this basis, a soil benchmark value for soil community effects is calculated as follows:

Threshold = LC_{50} ÷ (factor for interspecies variation × acute-chronic ratio)

= 4,450 mg/kg ÷ (242 × 17.9)

= 1.0 mg/kg as a wet-weight concentration in soil.

The equivalent aqueous phase benchmark is 1.0 mg/L. This approach requires the assumptions that the variance among soil species is approximately the same as among aquatic species, and that the distribution of acute-chronic ratios across chemicals is approximately the same for both communities. The interspecies variation factor is the one for a test species that has not been demonstrated to be highly sensitive.

5.8.5.1.2.3 Changes to Section 8.3.2.2.3: Herbivores

The human health risk assessment for perchlorate uses 0.01 mg/kg-day as the LOAEL from which the RfD is derived (Chapter 7). That value is based on perturbations in thyroid and pituitary hormones, thyroid histopathology and changes in brain morphometry in P0 dams on GD21 and F1-generation rat pups on PND5, PND10, and PND22. Because the representative species for the herbivore endpoint (meadow vole) is a rodent, that value is used here as well. These numbers are very similar to those of Thuett et al. (2002) from a laboratory study of the native species, *Peromyscus maniculatus* (deer mouse). Endpoints in this study were litter size; pup survival until PND21; individual weights at PNDs 1, 5, 10, 15, and 20; and mean body and organ weights at necropsy on PND21. The doses of perchlorate were 0, 0.000117, 0.117, and 117 mg/L (i.e., control, 1 nM 1 µM, and 1 mM) in water. Heart weights for individual pups were less than controls at a dose of 0.117 mg/L (1 µm) in water. This concentration would be a LOAEL; whereas 0.000117 mg/L would be a NOAEL. Using a water ingestion rate of 0.34 g/g-day from other laboratory studies of deer mice (U.S. EPA, 1993), the LOAEL dose is equivalent to 0.04 mg/kg-day.

The population-level implications of these developmental effects are unknown; however, it seems likely that such effects on the thyroid, pituitary, and brain could diminish survivorship and fecundity and diminish population production. To account for interspecies variance and LOAEL to NOAEL extrapolation, an uncertainty factor of 10 is applied to obtain a dietary benchmark value for herbivores of 0.001 mg/kg body weight-day, or ~0.01 mg/kg as a wet-weight concentration in plant tissue (see exposure assumptions in Section 8.3.1.5).

5.9 MAJOR TEXT REVISIONS TO THE EVALUATION OF EVIDENCE FOR INDIRECT EXPOSURES (CHAPTER 9)

The major revisions in Chapter 9 are recommended to occur in Section 9.2.3 (Extrapolating to Food Plants) and the Summary (Section 9.3) of the 2002 ERD. Because of the extent of the revision and importance of this emerging topic, the revisions recommended by the Agency are provided in their entirety. The revised summary is also provided.

5.9.1 Changes to Section 9.2.3: Extrapolating to Food Plants

Because so much U.S. produce is fertilized with perchlorate-free chemical commodities, the risk from exposures via fertilizers is small. Some crops (e.g., corn, wheat, and rice) are fertilized with materials that are unquestionably perchlorate-free. Additionally, there is no reason to suspect that any perchlorate contamination is associated with growing grains. However, the risk of exposure resulting from irrigation with perchlorate-tainted water in the American Southwest is unknown. (Additionally, more recently, a variety of sites have appeared around the country where perchlorate has been detected in deep soils or in groundwater. However, the EPA has no data regarding whether water at these sites is used for any agricultural purposes.) At present, there are no federally-sponsored efforts to survey fruits and vegetables for perchlorate in a widespread manner. That notwithstanding, a number of independent investigators have begun to look at food crops, especially lettuce. It is important to keep in mind that many of the studies on uptake by plants have been based on concentrations higher than those encountered in irrigation water. Furthermore, some products derived from Chilean saltpeter are known to be among those used on California citrus crops. The role that such fertilizers play in the growing of domestic and imported produce is almost entirely uncharacterized.

One of the few peer-reviewed studies of perchlorate uptake by edible plants is the forthcoming work to be released by the National Exposure Research Laboratory in the ORD of EPA with lettuce *(Lactuca sativa)* grown in a greenhouse with irrigation water fortified with perchlorate (Hutchinson, 2003). Lettuce is of particular importance for assessing the risk of perchlorate to the food supply because much of the winter lettuce produced in the U.S. is irrigated by water that is fed by the Las Vegas Wash, which is contaminated with perchlorate. During growth, lettuce transpires a significant amount of water through its outer leaves, likely leaving behind any stable unmetabolized ions that are dissolved in the irrigation water. Additionally,

much of the above-ground lettuce plant is consumed without cooking or processing. These characteristics would present a potential risk if lettuce efficiently accumulates perchlorate.

In this study, Hutchinson (2003) irrigated lettuce plants grown in sand and varied the concentrations of perchlorate applied for a period of 95 days following planting. Plants were treated three times per week with the perchlorate-fortified water (up to 10 mL). Whole lettuce plants were sacrificed approximately once per week over the growth period and divided into above- and below-sand biomass prior to analysis by an analytical method adapted from Ellington and Evans (2000). In the day-95 samples, recoveries of total added perchlorate were 87, 73, 69, and 84 % at perchlorate treatment levels of 0.5, 1.0, 5.0, and 10.0 mg/L, respectively. (Note that treatment levels were significantly higher than the ambient perchlorate level in irrigation waters of interest, e.g., the Colorado River water.) Growth and vigor of the plants were maintained by once per week application of a commercial nutrient solution. For day-95 samples, the perchlorate concentration factors ranged from 17-28 for the outer leaves and from 3-9 for the inner leaves (i.e., the lettuce head). Note that, for this species of lettuce, the head is typically eaten and the outer leaves are not. The uptake of perchlorate appeared to be continuous and linear throughout the plant growth cycle. Concentration factors did not appear to be correlated with perchlorate treatment level.

This narrow screening-level study of Hutchinson (2003), while demonstrating that perchlorate from irrigation water can accumulate in lettuce, is far from a complete depiction of the risk posed by this potential exposure route. For example, extrapolations to other food crops would not be valid. Also, comparison with field conditions is difficult due to differences in environmental conditions (e.g., soil versus sand, light and wind conditions, impact of rainfall, etc.). Nonetheless, we note that there have been recent press reports of perchlorate being detected in lettuce known, or presumed, to have been grown in the field with perchlorate-tainted irrigation water. For a summary of these reports, see the website of the Environmental Working Group at: http://www.ewg.org/reports/suspectsalads/es.php. This includes a report that perchlorate was detected at an average of about 70 ppb in 4 of 22 samples of lettuce bought in grocery stores during the winter of 2003. Each bag of lettuce was sampled once without replicate. In this case, it is impossible to know where the lettuce was grown or the contamination level (if any) of the irrigation water. It has also been reported by the press that in 1997 a vegetable grower in CA found perchlorate in some of 8 samples of vegetables that they tested after learning that their wells

were contaminated with perchlorate at an average of about 40 ppb. In contrast to journal articles and technical reports, these press reports do not contain the type and extent of scientific details that are needed to judge the significance of these studies. Also, it is not possible to evaluate the extent of quality assurance that was employed. However, the press-reported findings noted above are not obviously inconsistent with the findings of Hutchinson (in preparation) and the analytical methods appear to be sound.

Even if many food plants can be shown to absorb and retain perchlorate, it is important to note that the primary source of the contaminant is irrigation water polluted from defense-related activities. Because affected sites appear to be reasonably localized geographically, most of the country's agricultural products (e.g., corn, wheat, rice, milk) should be perchlorate-free. On the other hand, some types of produce are supplied almost entirely by regions dependent on contaminated irrigation water. Therefore, current information suggests that these sites might represent possible exposure routes for much of the nation via foods such as lettuce (and possibly some citrus crops). However, at this point, the sampling has not been representative, and the sample size has not been adequate to draw confident conclusions. Also, the distribution of the produce mostly in question—in terms of individual human exposure relative to other sources of lettuce or to lettuce consumption in general—has not been characterized. Unfortunately, these issues preclude any realistic assessment of potential impact resulting from human consumption.

Historically, much of the emphasis on fertilizer pollution from agricultural runoff has been on fertilizers applied to the soil. However, potassium nitrate is usually applied to the leaves of citrus trees when a potassium deficiency is found by analyzing leaf tissue. Such foliar application would not necessarily contribute significantly to runoff pollution of waterways, but could lead to the absorption of contaminants through the leaves and wood. There are no reliable data on the sources of potassium nitrate used for citrus crops. While it is known that absorption of anions similar to perchlorate (e.g., pertechnetate) are affected by the ionic strength and composition of the surrounding solution, relatively little is known about the factors that influence perchlorate influx via roots or leaves.

In order to begin elucidating factors that affect the uptake of perchlorate via the root system, the U.S. Department of Agriculture (USDA), the Strategic Environmental Research and Development Program (SERDP), and the U.S. Army Corp of Engineers have begun funding studies regarding mechanisms of perchlorate absorption and accumulation in plant tissues; but the

work is unlikely to lead to immediately applicable general principles. Due to the complexity and number of variables that affect the absorption and accumulation of chemical species in plant tissues, early studies must necessarily be limited in scope. In particular, most botanical or biophysiological studies have relied or will rely on hydroponic or sand farming rather than introducing the complications associated with soils. In addition, the way in which the perchlorate is delivered to the plants may or may not reflect the agricultural setting; in this way, conclusions of such studies may not be directly applicable without substantial correction for other factors. For example, some research that has demonstrated perchlorate accumulation in cucumbers (Todd Anderson, personal communication, 2003) has been designed to promote maximal uptake with exposure concentrations reaching the point of acute phytotoxicity; this is most unlike actual farming exposures.

While soils are generally not expected to retain or otherwise affect the mobility of perchlorate (Urbansky and Brown, 2003 and references therein), they contain organic matter, insoluble minerals, and soluble salts (ions) that affect how plants absorb nutrients or other chemical species as has been pointed out elsewhere (Collette et al., 2003). The agricultural setting is susceptible to a host of other influences from hydrology (water movement), geology (landforms, rock structure), meteorology (weather), and pedology (soil). Accordingly, the results are not necessarily applicable in an agronomic or agrologic (i.e., real-world farm) setting where the aforementioned factors interact and potentially confound the botanical, physiological, and physicochemical phenomena. At least initially, any attempts to determine a relative source contribution from produce will probably have to rely on statistically valid sampling protocols of actual produce combined with models for the distribution and consumption of that produce rather than laboratory or microcosm studies. The time and cost involved in investigating all possible variables for relevant crops are likely to be prohibitive for the short-term. Field surveys are more likely to provide immediately applicable generalizations.

Despite the general difficulties, there are some specific issues and recent results that should be considered for evaluating risk and exposure. The ultimate fate of absorbed perchlorate in plants is largely unknown and is probably species-dependent. Some species may phytodegrade perchlorate while others may simply store it.

For most plants, it may be that xylem-supplied tissues, such as leaves, are the final repository rather than phloem-supplied tissues, such as fruits. Nevertheless, the SERDP-sponsored research

(Phil Smith et al., 2002) led to a recent report of perchlorate in blackberries; but this finding has not been independently confirmed. At the time, the investigation had been geared towards ecological exposures for small- to medium-sized mammals, and so a more rigorous examination in terms of human exposure was not performed. The matter is of interest because it is the first time a fruit has been found to contain perchlorate. The authors did not see accumulation in grapes at the same site, however.

Preliminary findings from USDA-sponsored research are consistent with results from other investigations on the accumulation of perchlorate or similar anions in foliage. Specifically, perchlorate can be found in the leaves and stems of soybeans (*Glycine max*), but not in the seeds, which are the human-consumable portion (Todd Anderson, personal communication 2003). While many plants appear to exclude unmetabolizable anions from their seeds or nuts, it appears that some grasses will accumulate perchlorate in their seeds (Smith et al., 2001). The significance of such a finding for food crops is debatable at the present time. The EPA has no systematic data regarding grass crops (e.g., sorghum, wheat, corn) nor regarding the possible exposure to perchlorate via contaminated water. Because entire plants may serve as forage or silage, it will be important to understand how perchlorate is introduced to, accumulated in, and translocated in these plants and whether there is a potential for effects in secondary consumers. A chemicokinetic model has been developed for the tobacco plant (Sundberg et al., 2003); and similar models may likewise be developed for food, forage, and silage crops. Despite gaps in knowledge, one point must be emphasized: the capability (or even proclivity) of a plant to take up perchlorate is immaterial so long as there is no exposure. Similarly, exposure is equally immaterial if absorption does not occur under real-world conditions.

The issues described above (and more) have begun to be examined by the EPA, but there are many remaining unknowns (U.S. Environmental Protection Agency, 2001b). Until such time as quantitative studies are performed on various species to determine what factors influence the absorption, accumulation, and distribution of perchlorate in plants, it is not possible to estimate whether foods can meaningfully contribute to the body burden or to the risk posed to humans from perchlorate contamination. Even if they do, there is considerable assurance in knowing that fertilizers and water supplies are generally not providing perchlorate to plants. Consequently, prudence demands that only a small number of U.S. food crops be targeted for further study. On the other hand, it is not known to what extent other countries rely on natural saltpeters to

fertilize food crops. Moreover, it is not known whether fruits and vegetables absorb and retain the perchlorate ion. Therefore, it is not possible to say whether fruits and vegetables grown outside the U.S. serve as possible exposure routes at this time. Depending on the season, imported oranges, apples, and grapes and their juices are consumed throughout the U.S.; during the winter, some fruits and vegetables available in grocery stores in some parts of the U.S. may be exclusively imported.

Because there are minimal data on perchlorate in imported or homegrown produce and minimal data from controlled experiments on uptake in fruit crops, it is impossible to assess whether these foods contribute to perchlorate ingestion in humans or whether drinking water is the only important dietary source. At the present time, the available data point towards drinking water as the principal exposure pathway for humans; nevertheless, a number of possible and some likely exposure routes remain largely unexplored in a systematic and nutritively significant fashion. Unfortunately, the state of the knowledge will likely discomfort and distress both consumers and regulators. Nonetheless, to make any recommendations regarding the consumption of produce without more systematic investigations would be unwarranted because it would be based on a lack rather than an abundance of well-characterized data. It would fail to account for impacts on nutrition, and it could be entirely unjustifiable in terms of actual exposures.

5.9.2 Changes to Section 9.3: Summary

After some initial findings suggested that fertilizers should be considered as a significant source of perchlorate, more thorough and better designed studies which were subsequently conducted have ruled this out. Current fertilizer manufacturing practices and raw material sources make it unlikely that perchlorate contamination could occur widely and without discovery. While some plants absorb and even accumulate perchlorate in specific tissues, there are many unknowns with regard to the edible portions of nutritionally and agriculturally important crops. Many factors influence transport of ions in the agricultural field, and the current understanding of plant physiology and botany suggest that perchlorate uptake would be reduced (compared to simple greenhouse studies) as a result of such factors. Even if perchlorate uptake occurred in some food crops, perchlorate contamination is localized geographically outside of most major agricultural regions, minimizing possibility of uptake in all but a few types of produce. Difficulties in analyzing many plant or animal tissues originally were obstacles to executing appropriate studies,

but these problems have generally been solved. Systematic studies of crops and food commodities that represent different plant types (e.g., tuber, leafy, fruit) and which are eaten disproportionally by potentially susceptible populations such as neonates (e.g., carrots and milk) are warranted. Indeed, a recent article at the time that this document was being produced showed perchlorate unambiguously detected in seven of seven supermarket milk samples (Kirk et al., 2003).

As discussed in Chapter 2, the NIS also exists in mammary tissue for concentrating iodine in milk to ensure the availability of this essential nutrient to infants. As pointed out by Kirk et al. (2003), milk is an important dietary ingredient for expecting and lactating mothers. This report by Kirk et al. (2003) increased the appreciation of food and drink as potential indirect exposure vectors for perchlorate intake. The findings reinforce the need for systematic studies to provide quantitative knowledge of the extent of indirect exposures to perchlorate. Such studies are being conducted by the USDA and FDA.

6. USE OF PBPK MODELING

Topic Area E at the March 2002 external expert workshop was devoted to peer review of the physiologically-based pharmacokinetic (PBPK) models that were developed by the Air Force Research Laboratory (AFRL) and applied to interspecies extrapolation by the Agency in the 2002 ERD. The development, validation, and application of these models by the AFRL was described in Chapter 6 of the 2002 ERD. As mentioned elsewhere in this document, the modeling effort was circumscribed to pharmacokinetics — the description of steady-state disposition (uptake, distribution and elimination) of iodide and perchlorate. The interface between the effect of perchlorate on iodide uptake inhibition in various tissues (GI, skin, thyroid and mammary gland) with serum hormones was not accomplished due to data and time constraints.

The principal models developed for the description of perchlorate exposures in laboratory animal models were those of the adult male rat (Merrill, 2001c), the pregnant dam and fetal rat (Clewell, 2001a), and the lactating dam and neonate model (Clewell, 2001b). The human model (Merrill, 2001d) was developed using the data on euthyroid adult subjects from the Greer et al. (2002) study that underwent QA/QC (Merrill, 2001a) with some of the other available human data used in validation exercises (Merrill, 2001b). Because these models were submitted to the Agency as consultative letters, the peer panel members assigned to this topic were tasked to provide peer review of the development and validation of the model structures. The Agency was not provided the full code for these models nor has it exercised the models independently of its collaboration with the AFRL.

In the 2002 ERD, the EPA chose a dose metric as the basis for interspecies extrapolation of the laboratory animal data and also proposed a parallelogram approach to extrapolate across the life stages (pregnant dam, fetus, and lactating neonate). Simulation exercises for the interspecies extrapolation were also provided to the Agency as a consultative letter (Merrill, 2001e). The choice of dose metric for interspecies extrapolation and the model applications proposed by the EPA were also subjects of the peer review. Specific comments on the applications were made in Section 7 (charge question F.2) of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b). The specific comments will be addressed in this chapter and the implications for their applications in Chapter 7.

The Agency is aware that some of the experimental studies used to develop the models (Yu et al., 2002; Greer et al., 2002) and papers on the PBPK models themselves (Merrill et al., 2002; Clewell et al., 2001; 2003a,b) have since been published in the peer-reviewed literature. The AFRL will also soon submit for publication a manuscript on the human model (Merrill, personal communication) and on the mode of action described as competitive inhibition (Clewell et al., 2003c). The Agency will update these references in Chapter 6 and notes that no substantive differences have occurred in the model structures as previously described in the 2002 ERD. Some responses and recommendations for changes with respect to the application of these models will also be reflected in Section 7.1.2 (Dosimetric Adjustment of Exposures Associated with Effect Levels) in Chapter 7 (Dose-Response Assessments for Human Health).

6.1 COMMENTS ON MODEL STRUCTURES

Summarizing the pre-meeting comments, the discussion leader indicated that the PBPK model structures were technically sound, account for the major anatomical compartments, and are based on standard approaches and equations. He and several reviewers, however, voiced a number of concerns about certain model representations, particularly perchlorate uptake into cells, and about parameter selections. These are addressed in specific comments in the sections below.

As noted above, these PBPK models were not developed by the EPA but rather by the AFRL at Wright-Patterson Air Force Base and its associated contractors. The Agency participated in meetings with respect to model development and was supportive of their application in the assessment process, but has had little discretion to exercise response to these peer review recommendations. Thus, these responses reflect the status of the models as currently published or under development by the AFRL. The EPA has considered these comments and the current status of the models in its approach to dosimetric adjustment and in revising its characterization of the confidence the Agency now has in applying these models for interspecies extrapolation.

6.2 COMMENTS ON REPRESENTATION OF IODIDE UPTAKE INTO THYROID CELLS

As noted in the introduction above, the PBPK model structures described the disposition of iodide and perchlorate in parallel. These comments address concerns regarding the representation of iodide uptake into the thyroid cells.

Comment(s): *Several peer reviewers questioned why the PBPK model structures 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 recommended that the PBPK models resolve the relative importance of these two uptake processes. Another reviewer commented further on passive uptake of chemicals into cells when discussing disposition of perchlorate (see Section 6.1.2 below).*

EPA Response(s) and Recommendation(s) for Revision(s): Although the EPA did not develop these models, the Agency agrees with the approach taken by the AFRL modelers. Permeability area cross-products and partition coefficients were used to describe the movement of iodide and perchlorate between the capillary bed, tissue, and inner compartments of the thyroid gland (e.g., follicle or colloid), GI tract, and skin which results from inherent electrochemical gradient within the tissues. Passive diffusion using partitions and blood flows were used to describe the movement of both anions into the kidney, liver and fat because these tissue do not contain NIS. The structure containing both active transport and diffusion is considered necessary to describe the various phases of iodide uptake. The Agency's only other comment is that it must be kept in mind that these PBPK models are semi-empirical and not as detailed mechanistically as some of the peer panelists would have preferred. However, the model predictions are able to predict steady-state blood concentrations which at this juncture reflects the level of resolution of assays and most routine measurements. As discussed in Chapters 2 and 4, there is some uncertainty regarding the nature of the interaction of perchlorate with the NIS.

Comment(s): *One reviewer indicated that the 2002 ERD did not adequately justify the use of Michaelis-Menten kinetics to model iodide uptake into the thyroid. Another reviewer, 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.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that the discussion in Section 2.1 regarding the interaction of perchlorate at the NIS has addressed this comment.

6.3 COMMENTS ON REPRESENTATION OF PERCHLORATE UPTAKE INTO THYROID CELLS

As noted in the introduction above, the PBPK model structures described the disposition of iodide and perchlorate in parallel. These comments address concerns regarding the representation of perchlorate uptake into the thyroid cells. Overall, the peer review panel indicated that the PBPK models should have more refined representations of the active and passive uptake into cells. Two reviewers clarified that they have no question that perchlorate interacts with the NIS, thus inhibiting iodide uptake, but that their primary concern was to what degree that perchlorate actually enters cells. Another reviewer 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. This topic was also discussed previously herein as a concern regarding the model for the mode of action in Chapter 2.

Comment(s): *Several reviewers recommended that EPA verify whether the NIS actively transports perchlorate into thyroid cells — an assumption made in all four of the PBPK model structures. One reviewer noted that she is unaware of any research that unequivocally demonstrates that the NIS translocates perchlorate into thyroid cells, while she has reviewed several papers that suggest that translocation does not occur. Regarding recent publications that report concentrations of perchlorate in the thyroid (Yu et al., 2001), this reviewer suspected that the perchlorate detected was bound to cell membranes rather than resided 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 the NIS or is translocated by the protein), though she did not think that such an experiment has already been conducted.*

Although several reviewers agreed that the NIS apparently does not actively translocate perchlorate into cells, one reviewer cited evidence that perchlorate is likely entering cells by other mechanisms. Referring to the study that administered 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 varius 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 2002 ERD does not provide a complete, convincing account of all cellular uptake mechanisms.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that the data are equivocal with respect to the exact nature of perchlorate interaction at the NIS. The Agency's more detailed response to address these issues is presented in Section 2.1.

Comment(s): *Given that the NIS does not translocate perchlorate into thyroid cells, the reviewers questioned whether the PBPK models and the 2002 ERD should describe the cellular uptake process via NIS as "competitive inhibition". The discussion leader 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 (Km). He questioned, therefore, how the model developers could derive a Km for the PBPK models (see Pages 6-23 to 6-25 in the 2002 ERD). Another reviewer agreed, noting that perchlorate does not have a Km but rather an inhibition constant (Ki) that has been widely published. The reviewers recommended that the PBPK models include a revised kinetic description of iodide uptake inhibition. In another comment, one reviewer indicated that the value of this parameter should fall roughly between 20 and 30 μM.*

EPA Response(s) and Recommendation(s) for Revision(s): As discussed in Chapter 2, the difference between non-competitive and competitive inhibition may not make a dramatic difference to predicted levels of perchlorate AUCB.

Comment(s): *Two reviewers 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 indicated that passive transport of anions into cells can be an important process, especially when concentrations in the extracellular matrix are extremely high (e.g., following an exposure). Another reviewer agreed in principle, but added that passive transport in humans would only be relevant when perchlorate achieves extremely high serum concentrations — concentrations that may not be physiologically relevant.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that the inclusion of both active and passive transport in the models for both perchlorate and iodide is appropriate.

6.4 COMMENTS ON MODEL PARAMETERIZATION

Several peer reviewers evaluated the parameters and their assigned values assigned in the PBPK models. Specific comments are as follows.

Comment(s): *In addition to the concern that perchlorate does not have a Km but rather a Ki (see above), the peer reviewers discussed the derivation of other relevant parameters, including the maximum velocity capacity (Vmaxc) in various tissues, permeability areas, plasma binding coefficients, and clearance values. One reviewer said that appropriate parameters were selected for iodide, but he indicated that the 2002 ERD did not adequately address how these parameters were selected for perchlorate. The other reviewer assigned to evaluate the PBPK models said that the parameters were 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).*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees that the approach to the model development used by AFRL was defensible for description of steady-state perchlorate disposition. The Agency also agrees with the reviewers that the models would benefit from additional time-course and experimental data. These time course data may be very important to descriptions of periodic perchlorate dosing and its effects on variations in regulatory rhythms or cycles of circulating serum hormones.

6.5 COMMENTS ON MODEL APPLICATIONS

The discussion leader indicated that the overall value of the models depends largely on their ultimate application. He indicated that the models will not be useful for quantifying cellular concentrations of perchlorate until 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 and for estimating human equivalent exposures.

6.5.1 Comments on EPA's Choice of Dose Metric for Interspecies Extrapolation

The response to these comments will be reflected in changes to Section 7.1.2 (Dosimetric Adjustment of Exposures Associated with Effect Levels) in the 2002 ERD.

Comment(s): *In summary comments in Sections 7 and 8 of the peer panel report, the discussion leader indicated that the area-under-the curve of pechlorate in blood (AUCB) is an acceptable dose metric and is the most reasonable measure of internal dose that allows for defensible extrapolations across species and different life stages, though he noted that other reviewers were not convinced that this dose metric is the best predictor of adverse health effects.*

Though not disagreeing that AUCB as the dose metric allows for defensible interspecies extrapolations, two reviewers advocated the use of a dose metric more predictive of toxic effects. One reviewer noted that decreases in thyroid hormone levels or increases in TSH may be better indicators of adverse effects than circulating perchlorate levels. Another reviewer agreed and added that pharmacodynamic modeling can help to differentiate metrics more related to adverse effects (e.g., excess cell mitoses per unit time) from those with no risk implications.

The discussion leader added that the use of other dose metrics (e.g., AUC of perchlorate in thyroid tissue, 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. The other reviewer assigned to evaluate the PBPK models echoed many of these comments and added others. Regarding the cellular uptake processes, the second reviewer recommended that the PBPK models include more refined representations of these processes. If this can not be achieved, the reviewer suggested that EPA prominently acknowledge in the revised documents that the PBPK models are not based on a mechanistic understanding of the uptake processes.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency notes that the model structures have not changed substantively in their published forms and agrees that in light of these peer panel comments that the uncertainties in the description of uptake processes should be further discussed. The concern over the description of uptake processes exacerbates the concern that was shared by the AFRL and the EPA and described in Section 7.1.2.1 of the 2002 ERD (Merrill, 2001e; U.S. Environmental Protection Agency, 2002a). EPA concurred with the recommendations of the AFRL at that time that the thyroid parameters in the fetus and neonatal rat should not be used in the formal risk assessment. Fetal and neonatal thyroid were never analyzed for perchlorate concentration. In the case of the fetal rat, kinetic parameters were determined by fitting model simulations of fetal thyroid concentration to available iodide data and assuming that the perchlorate:iodide ratio would be similar to that of the mother. Given the concerns expressed by the panel that perchlorate can inhibit the NIS in the placenta and in mammary tissue, the validity of this assumption is now more seriously questioned. In the case of the neonatal rat, no data were available for thyroid concentrations for either perchlorate or iodide.

While these considerations diminish the confidence of the model descriptions for perchlorate interaction in the thyroid, it may not change the accuracy of the description of

circulating levels significantly due to the small tissue and blood volume of thyroid. Thus, the Agency still has considerable confidence in model descriptions for the adult male rat and pregnant dam but less confidence in the fetal and neonatal model structures. This revised text will be added to Section 7.1.2.

As discussed in Section 6.5 (Application of PBPK Model Structures to Interspecies Extrapolation) of the 2002 ERD, the models do not link perchlorate and iodide kinetics to perturbations in thyroid hormone. The existing data and current model structures were not designed to address the complex issues involved with hormone homeostasis of the hypothalamic-pituitary-thyroid (HPT) feedback axis. While the Agency has always advocated for the development of a biologically-based dose response (BBDR) model as the preferred approach the integration of diverse data sets, it notes (as it did in 2002) that development of such a model will take considerable more time. Additional and extensive experimental studies will be required, especially in light of the comments on the nature of the interaction of perchlorate at the NIS, to characterize with any confidence the nature of perchlorate interaction with the gland. Obtaining time-course data to characterize the HPT feedback system will also be arduous.

Thus, the development and validation of a BBDR model to explore more mechanistic descriptions of thyroid hormone and their feedback across species is viewed as fairly far off on the horizon even with the use of existing model templates for other chemicals that effect thyroid hormones (Kohn et al., 1996). It should also be noted that the relationship of circulating thyroid hormone levels to concentrations at specific receptors of the brain should also be considered critical to any BBDR model development. Serum hormones are merely external markers for concentrations at receptors in the target tissue that are most relevant and which require characterization. Typical assumptions of venous equilibration of blood concentrations with the brain compartment may be inadequate to describe important tissue interactions and mechanisms of response.

6.5.2 Comments on EPA's Parallelogram Extrapolation of Life Stages

This topic was discussed at the 2002 peer panel workshop in Section 7. response to these comments will be reflected in changes to Section 7.1.2 (Dosimetric Adjustment of Exposures Associated with Effect Levels) in the 2002 ERD.

Comment(s): *The only comment relevant to the proposed parallelogram approach for life stage extrapolation addressed the use of PBPK models to interpret effects observed in rats on PND4. Noting that impaired thyroid function at this life stage would most likely result from decreased transport of iodide across the placenta, one reviewer wondered how the interspecies extrapolations with PBPK models accounted for any potential differences in placental physiology between rats and humans. One reviewer responded 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. This reviewer indicated that EPA's approach for using the outputs from the rat models to extrapolate between different life stages is adequate, and he saw no other defensible approach for estimating human equivalent exposures (HEE) for various life stages. Another reviewer noted that NIS is expressed in placentas of both rats and humans, despite notable physiological differences across these species.*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA agrees that the only approach to life stage extrapolation is the parallelogram approach that was proposed in Chapter 6 by the Agency. A limitation noted in the 2002 ERD that remains, however, is that human data to verify the predictions are lacking for the fetal and neonatal models.

7. HUMAN HEALTH DOSE-RESPONSE ASSESSMENT

Discussion devoted to the operational derivation of the reference dose (RfD) for human health risk assessment occurred under Topic Area D at the March 2002 external peer review workshop. The discussion largely focused on designation of the point of departure and the application of uncertainty factors and is found in Chapter 7 of the peer panel report (U.S. Environmental Protection Agency, 2002b). This chapter provides the disposition of comments on these topics that were received from the panel and from the public as part of the 2002 review process.

These responses and recommendations primarily revise the first section of Chapter 7 (Dose-Response Assessments for Human Health) in the 2002 ERD. Response to comments on the individual study attributes have been addressed in Chapter 3 for the human data and in Chapter 4 for the laboratory animal data. The emphasis of this chapter is the integration of diverse data sets from studies with different experimental designs and endpoints across various life stages to arrive at a weight of evidence for the key events of pathogenesis by perchlorate. This synthesis is then used to designate a point of departure for operational derivation of the RfD as a health risk estimate for the general population.

Response to recommendations of the peer panel and to public or stakeholder comments are addressed in this document in the following separate sections. Conclusions and considerations regarding the weight of the evidence and identification of key events are discussed in Section 7.1. That section also discusses the designation of the point of departure. These topics correspond to Section 7.1.1. (Key Events and Weight of the Evidence) and 7.1.3 (Point-of Departure Analysis) in the 2002 ERD and in the revised assessment. The choice of a dose metric on which to base interspecies extrapolation is discussed in Section 7.2 which corresponds to Section 7.1.2. (Dosimetric Adjustment of Exposures Associated with Effect Levels) in the 2002 ERD and in the revised assessment. Consideration of uncertainty factors in presented in Section 7.3 which corresponds to Section 7.1.4. (Application of Uncertainty Factors) in the 2002 ERD and in the revised assessment. Section 7.4 corresponds to Section 7.1.5.3 (Possible Susceptibility) and provides the response to comments regarding the characterization of susceptibility. A number of additional revisions already discussed in previous chapters (e.g,

Section 2.3 on the carcinogenic potential of perchlorate) are summarized in Section 7.5 (Additional Comments). These will be used to modify other sections of Chapter 7 of the 2002 ERD for the revised assessment.

Recommendations regarding revision to the operational derivation of the draft harmonized reference dose (Section 7.1.5 of the 2002 ERD) and comparisons of risk estimates based on the human or tumor data will be discussed in Section 7.6 of this document as a summary.

The Agency is recommending that a missing header for Section 7.1 (Data Synthesis and Development of Harmonized RfD) be inserted at Line 12 on Page 7-2 of the 2002 ERD to revise the assessment. The remaining subsections (e.g., 7.1.1. Key Events and Weight of the Evidence) will then follow correctly as intended in this first section of the revised assessment.

7.1 CONCLUSIONS AND CONDITIONS REGARDING KEY EVENT, WEIGHT OF THE EVIDENCE, AND CHOICE OF POINT OF DEPARTURE

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel or from public and stakeholder submissions regarding the topic as posed in Charge Question F.1 — whether the Agency's 2002 ERD conclusions and conditions regarding the key event and weight of the evidence for effects after oral exposure to perchlorate were appropriate and consistent with the information on mode of action. The synthesis of the information and support for the proposed point of departure was also discussed in this section. They will be presented herein as separate sections with recommendations for revisions to existing sections in the 2002 ERD.

7.1.1 Consistency Between Observed Effects and Mode of Action

Summarizing the pre-meeting comments, the discussion leader indicated that a clear majority of the reviewers who responded to this question found the proposed mode of action for perchlorate to be consistent with the observed neurodevelopmental and neoplastic effects. Responses to comments on specific issues within this topic are provided as separate sections below.

Comment(s): *Elaborating on this general response, one reviewer indicated that the 2002 ERD clearly states how perchlorate exposure initiates the perturbance of the hypothalamic-pituitary-thyroid (HPT) axis which leads to neoplastic and neurodevelopmental effects, thus supporting the harmonized approach to evaluating noncancer and cancer toxicity. Other reviewers indicated that EPA could more convincingly link the mode of action to observed effects as specified in separate comments below.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that with the changes to the conceptual model (Figure 2-1) and with the additional figure showing differences in dosimetry for various life stages proposed in Chapter 2 that the revised document will appropriately address this comment. Additional responses are provided in separate sections that follow.

The Agency also recommends that the title for this section (7.1. Data Synthesis and Development of Harmonized RfD) be inserted on Page 7-2 as noted above. Figure 7-1 and its legend will be replaced with a copy of Figure 2-1 of this response document as discussed in Chapter 2.

7.1.1.1 Lack of Pharmacodynamic Modeling

Comment(s): *Two reviewers acknowledged that the 2002 ERD links perchlorate exposure to the key event (iodide inhibition) but argued that the document does not provide specific details on mechanisms linking the key event to the neurodevelopmental or neoplastic endpoints. One reviewer indicated that the full sequence of events between perchlorate exposure and neoplasia, particularly in humans, is not described explicitly. Another reviewer noted that the lack of pharmacodynamic modeling leaves the relevance of the mode of action uncertain. A detailed pharmacodynamic model based on existing applications (reference not provided) would link iodide uptake inhibition to decrements in thyroid hormone production and circulation which would have provided a more convincing link between the proposed mode of action and the observed toxic effects.*

EPA Response(s) and Recommendation(s) for Revision(s): As discussed in Section 4.3, the Agency does not disagree that a biologically based dose response (BBDR) model would be the preferred approach to the integration of these diverse data sets. However, because the existing PBPK modeling effort by the Air Force Research Laboratory (AFRL) took many years and fell short of accomplishing this interface, the development and validation of such a model is viewed as fairly far off on the horizon even with the use of existing model templates for other chemicals that effect thyroid hormones (Kohn et al., 1996). Although at this time there are no routine experimental endpoints of thyroid hormone action in terms of molecular mechanisms in the

developing brains to determine the adverse neurodevelopmental consequences of thyroid toxicants, work is underway to evaluate genes expressed in the fetal brain that appear to be responsive to maternal thyroid hormone (Zoeller, 2003b). This type of work may be used to extend and to further verify the conceptual model proposed by EPA in the future. The relationship of circulating serum thyroid hormone levels to concentrations at specific receptors of the brain should also be considered critical to any BBDR model development. Serum hormones are merely external markers for concentrations at receptors in the target tissue that are most relevant and which require characterization. Typical assumptions of venous equilibration of blood concentrations with target tissue compartments (e.g., the developing fetal brain) may be inadequate to describe important tissue interactions and mechanisms of response.

The Agency believes that the mechanistic events leading to both the neurodevelopmental and neoplastic sequelae were articulated as the basis of the mode of action and are described in Chapter 3 of the 2002 ERD. Summaries of more recent reviews regarding the action of thyroid hormone on development of the brain and on thyroid hormone receptors will be included. It is noted that some of these reviews highlight that effects on the brain may occur in the absence of marked effects on circulating serum hormone levels (Bernal, 2002; Howdeshell, 2002; Zoeller, 2003b). Further, the relevance of both neurodevelopmental and neoplastic outcomes to human health risk assessment are established by existing Agency guidance (U.S. Environmental Protection Agency, 1998a,b). Additional response to these comments are provided in Section 4.3.

7.1.1.2 Dose Associated with Iodide Uptake Inhibition

Comment(s): *To link the proposed mode of action to toxic effects, one reviewer recommended that the revised final ERD should clearly indicate the doses at which iodide uptake inhibition have been observed in laboratory animals and humans. Focusing on the abstract of the Greer study (Greer et al., 2000), three reviewers noted that the abstract reports measurable iodide uptake inhibition in humans dosed at 0.02 mg/kg-day. These reviewers did not specify whether they considered the 0.007 mg/kg-day group because results at that dosage were not reported in the abstract but rather only in the QA/QC report (Merrill, 2001a). These reviewers also noted that Table 7-5 of the 2002 ERD presents estimates of iodide uptake inhibition as a function of dose for the various PBPK model structures. One reviewer indicated that the estimates of iodide uptake in humans appear to be quite consistent with the low-dose finding reported in the abstract of the Greer study. Noting the that the mode of action proposed by EPA ultimately links toxic effects to iodide uptake inhibition, one reviewer recommended that the revised final ERD more prominently acknowledge the exposure doses at which this inhibition was observed.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has developed a new figure (see Figure 7-3 in Section 7.1.1.4) which shows that inhibition of the NIS occurs in laboratory animals and humans at approximately the same levels. This was the basis for the Agency's concern about neurodevelopmental effects observed in rats. Given the importance and complexity of neurological development in humans it prudent to maintain that human neurodevelopment is at least as sensitive as that of the rat. The essential role of thyroid hormone in brain development makes it critical to consider the possibility that mild degrees of hypothyroxinemia due to iodide uptake inhibition at the NIS may impair neurological development. As discussed in Chapter 4, neurodevelopmental effects in rats are considered very relevant for human risk assessment. Further, as noted by the 2002 peer panel in some of the discussion in Chapter 4, insufficient data exist to determine the shape of the dose-response curve (i.e., to determine if there is a threshold) for neurodevelopmental effects. Given the similarity in iodide uptake inhibition between the species, the Agency has also indicated the NOAEL (0.002 mg/kg-day) for iodide uptake inhibition in euthyroid adult human subjects in a new figure recommended for the chapter (Figure 7-4 in Section 7.1.2) in the section on point-of-departure analysis. This NOAEL was based on the BMDL for a 5% response level that was calculated by the Agency using the data of Greer et al. (2002) study. The BMDL estimation is described in detail in Section 3.3.1.

The Agency believes that the peer reviewers should have referred to both Tables 7-4 and 7-5 in the 2002 ERD for interspecies comparison of iodide inhibition predicted by the PBPK models developed by the Air Force Research Laboratory (AFRL). The tables used in Chapter 7 of the 2002 ERD are being provided here in Appendix A for ready reference so that these become Table 7A-4 and Table 7A-5.

The EPA agrees that these tables show that humans are predicted to behave similarly to rats with respect to the effects of perchlorate on iodide uptake inhibition (e.g., 0.01 mg/kg-day is predicted to result in 1.5% and 2.8% inhibition in rats and humans, respectively). The Agency is somewhat concerned, however, that the 0.002 mg/kg-day dose estimated by EPA from the human subjects in the Greer et al. (2002) study as the BMDL for a 5 % response level falls ~7.5 fold below the human equivalent exposure (HEE) that the PBPK models predict to be associated with a 5% inhibition. The HEE predicted by the PBPK models for a 5% inhibition level in humans would be approximately 0.015 mg/kg-day based on interpolation of the values in Tables

7A-4 and 7A-5 for the inhibition observed in adult male rats 2-hours after an i.v. dose of perchlorate at 0.01 mg/kg-day (1.5% inhibition; HEE of 0.004 mg/kg-day) and 0.1 mg/kg-day (16.3% inhibition; HEE of 0.048 mg/kg-day). This disparity is of particular interest because the Greer et al. (2002) data were those primarily used to develop the human PBPK model (Merrill, 2001c).

This difference between PBPK-predicted HEE estimates and observed values for iodide uptake inhibition may be due to variability in the Greer et al. (2002) study as discussed in Chapter 4 or mis-specification of the PBPK models as discussed in Chapter 6. These issues will be considered in the section on uncertainty factors.

7.1.1.3 Inconsistencies of Findings on Thyroid Hormone Levels

Comment(s): *Referring to Table 5-4 in the 2002 ERD, one reviewer 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 PND22 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 effect thyroid hormones by some mode of action in addition to inhibiting thyroid iodide uptake. Another reviewer agreed that some studies may have inconsistent results but he noted that others (e.g., Argus Research Laboratories, Inc., 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 may 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.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency does not have any additional response to this comment other than that which was provided in Chapter 4. Tables 5-2 and 5-4 from the 2002 ERD provided in Appendix B of Chapter 4 show the ANOVA results for serum hormone analyses across all of the studies. As discussed in Section 4.3 of Chapter 4 and as illustrated in Table 4-1 of this response document, the Agency notes a remarkable degree of qualitative consistency across all of the studies in the pattern of effects on serum hormones due to perchlorate dosing. The quantitative differences in the NOAEL and LOAEL estimates may be due to differences in sample points (postnatal age or days of treatment) and dose spacing in addition to the technical limitations highlighted by the peer reviewers on the 2002 panel.

7.1.1.4 Recommended Revisions to Section 7.1.1 (Key Events and Weight of the Evidence)

The following text represents a new insert and text changes to the 2002 ERD that will be included in the section of the same name in the revised assessment. Major inserts and text change are indicated in blue.

Results of the testing strategy have established that the critical target tissue for perchlorate is the thyroid gland. Changes in thyroid weights and three response indices of thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia) were consistently altered across the array of experimental designs represented by the data base. Likewise, changes in serum hormones representative of perturbation of the HPT axis, i.e., decreases in thyroid hormones (T4 and T3) and increases in the pituitary hormone TSH also exhibited a consistent pattern across the data array. There is some remaining concern over the lack of an adequate characterization of the potential for perchlorate to have effects on the immune system, notably contact hypersensitivity. Effects on the developmental and reproductive systems, thought to be secondary to thyroid hormone perturbation, exhibited NOAEL and LOAEL values that were higher than those associated with thyroid toxicity *per se*.

Figure 7-1 of this response document (Figure 7-2 in the 2002 ERD and the revised assessment) highlights the temporal considerations that have to be superimposed on evaluation of the data from the various studies in laboratory animals and humans in order to characterize the anti-thyroid effects from perchlorate exposure. This poses a challenge to the integration of data across diverse data sets with different experimental designs, notably with respect to outcome measures, exposure duration, and sample points. Conceptually, competitive inhibition of iodide uptake at the NIS by perchlorate is the key event leading to both potential neurodevelopmental and neoplastic sequelae as illustrated the Agency's mode of action model in Figure 2-1. The decrement in iodide uptake leads to subsequent drops in T4 (and T3) that can lead to permanent neurodevelopmental deficits. Corroborating evidence for this likely outcome, given the mode of action of perchlorate, comes from the iodide deficiency literature and recent human and laboratory animal studies showing that maternal hypothyroxinemia (i.e., decrements in T4 with or without concomitant increases in TSH) is linked to poor developmental, neuropsychological, and cognitive outcomes (Haddow, et al., 1999; Pop et al., 1999; Morreale de Escobar, et al., 2000; Lavado-Autric et al., 2003; Zoeller, 2003,a,b).

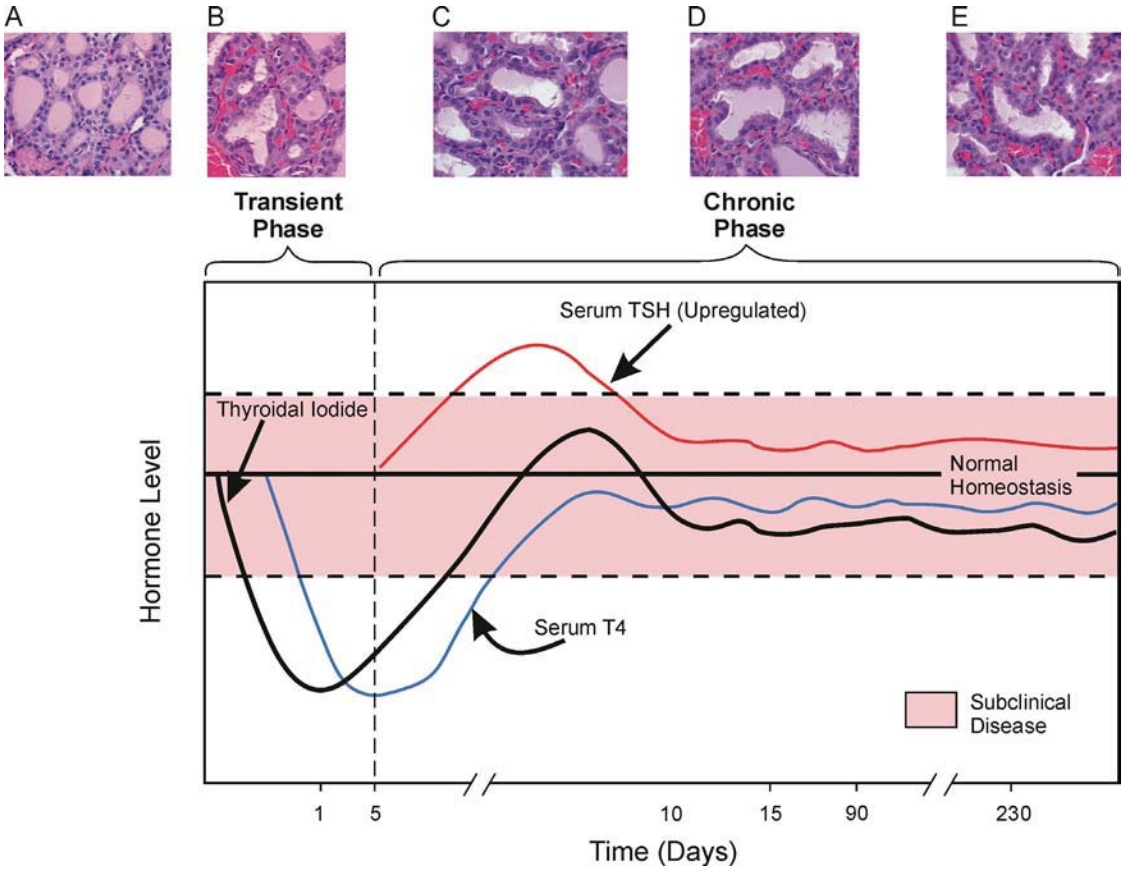

Figure 7-1. Figure 7-2 in revised assessment. Schematic of thyroid and pituitary hormone levels with associated pathology after acute versus chronic dosing with perchlorate. The transient phase is represented by decreases in thyroidal iodide due to the inhibition by perchlorate at the NIS with subsequent drop in T4. The transient drops in T4 can lead to permanent neurodevelopmental sequelae. Once TSH is upregulated via the hypothalamic-pituitary-thyroid feedback, T4 appears to be in normal homeostasis but actually can represent subclinical or undiagnosed disease (hypothyroxinemia). The upregulation of TSH can result in neoplasia. Normal thyroid tissue is represented in Panel A. Panel B shows lace-like colloid depletion which is more pronounced in subsequent panels C, D and E. Panels D and E represent hypertrophy and hyperplasia.

It should be noted that medical concern for hypothyroxinemia remains in the "chronic phase"; i.e., once TSH upregulates in an attempt to regulate the hypothalamic-pituitary-thyroid feedback system back to an apparent homeostasis, because this stress on the system essentially represents a "subclinical" disease state. Hypothyroxinemia per se may be considered an adverse

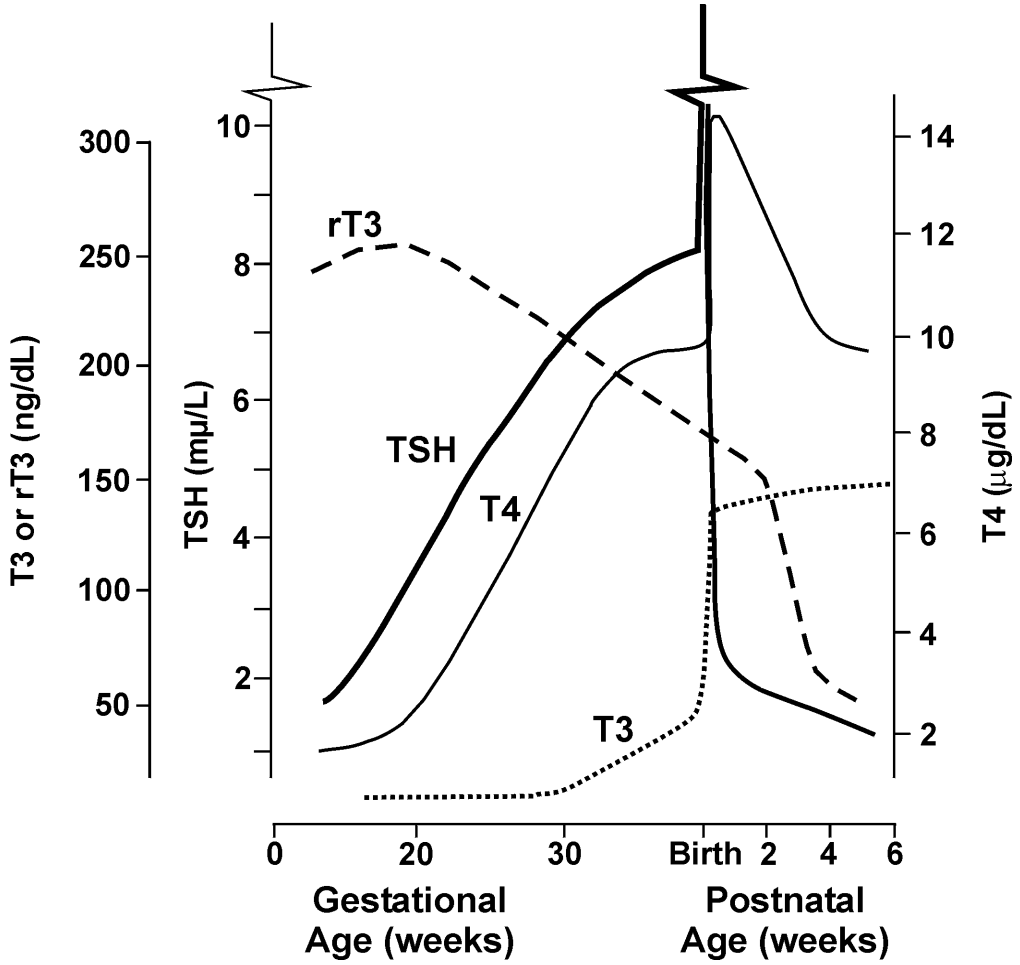

Figure 7-2. Figure 7-3 in revised assessment. Pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation in the human (from Fisher, 1996). A similar pattern is thought to exist in the rat (see text for further details).

outcome in women because the definition of adversity includes the inability of an organism to respond to additional stressors. The system in this case, particularly when considered on a population level, would present a diminished capacity to compensate for other anti-thyroid insults. Since a large percentage of women (4 to 21%) are believed to already be hypothyroid, the importance of this effect to women in general, pregnant women, and fetuses on a population level can not be discounted (Canaris et al., 2000; Calvo et al., 2002). Weiss (2000) has noted that even if the magnitude of effect may be relatively small for most environmental levels, such neurotoxicity is quite significant for public health.

Of notable concern, as previously discussed in Chapter 3, is that the developing fetus is dependent on the mother for its T4 and T3 through parturition, as illustrated for humans in Figure 7-2 (Figure 7-4 of the 2002 ERD and in the revised assessment). A similar pattern occurs in rats. During the period illustrated in Figure 7-2, a number of critical stages in neural development take place, some of which depend on thyroid hormones. The cell precursors of the brain and spinal cord which compose the central nervous system (CNS) begin to develop early in embryogenesis through the process called neurulation. Beginning early in the second week of gestation in rodents (GD9.5 in rats) and the first month of gestation in humans, specific areas of the CNS begin to form with the neurogenesis and migration of cells in the forebrain, midbrain, and hindbrain. This sequence of developmental processes includes proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination (Rice and Barone, 2000; Bernal, 2002; Howdeshell, 2002; Zoeller, 2003b). As discussed in Chapter 3, thyroid hormones play a role throughout this process, regulating proliferation, migration, and differentiation. Alterations in these processes can result in abnormalities of the brain and developmental delays.

Figure 7-4 shows that the key event of iodide uptake inhibition is very similar in rats and humans. This is an important observation. The inhibition in iodide uptake is the most proximate effect caused by perchlorate exposure and to decrements in T4 that could result in permanent neurodevelopmental damage. Because early biological effects are more prevalent in a population at risk than the late events of historical interest (e.g., morbidity and mortality or in this case, hypothyroidism) and may be more specific to the exposure than the outcome itself, molecular epidemiology is currently utilizing the precision afforded by the use of this type of mechanistic data to improve the etiologic classifications of environmental disease (Hattis, 1986; Cullen, 1989; Hulka and Wilcosky, 1988).

As discussed in Section 4.6, the mechanisms for disruption of nervous system development by thyrotoxicants is not the same as for tumor formation. Whereas the rat is considered a sensitive model for thyroid tumor development, it may be as sensitive or even less sensitive than the human as a model for neurodevelopment. Rodent models of developmental hypothyroidism have been employed successfully for many decades and express many of the disease hallmarks observed in humans including reduced growth rates, motor impairments, hearing loss, and cognitive dysfunctions (Cabello and Wrutniak, 1989; DeLong, 1989; Eyers, 1971; Timiras and Nzekwe, 1989; Halpern et al., 1991; Boyages and Halpern, 1993; Porterfield and Hendrich, 1993). While thyroid hormone replacement therapy prevents or ameliorates the severe

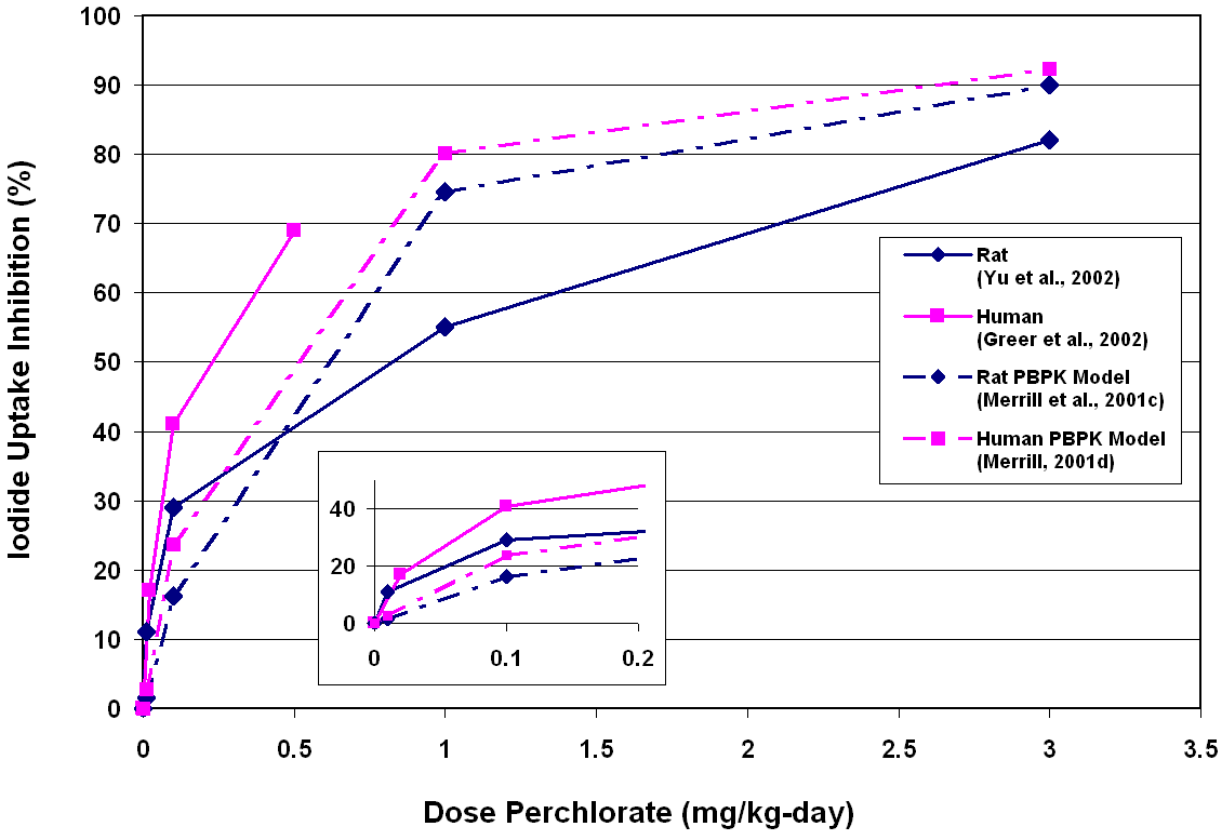

Figure 7-3. New Figure 7-4. Iodide uptake inhibition versus ingested perchlorate in rats (solid blue) and human subjects (solid pink). Predictions from the PBPK models of the Air Force Research Laboratory (AFRL) are presented for adult male rats (dashed blue) and humans (dashed pink). Human data are those of Greer et al. (2002) and the PBPK model is that of Merrill (2001d). Rat data are from Yu et al. (2002) and the adult male rat PBPK model is that of Merrill (2001c).

neurological deficits and demonstrates the essential role of thyroid hormone in neural development in both laboratory animals and humans (Uziel, et al., 1981; Escobar-Morreale et al., 1996; Sprenkle et all, 2001; Rovet, 2002), subtle impairments in neuropsychological endpoints persist in children with congenital hypothyroidism despite thyroid-hormone therapy within the first few weeks of life (Rovet, 2002).

Thus, it is not accurate to assume that the HPT feedback system prevents adverse effects of persistent but small changes in circulating levels of thyroid hormone (Zoeller, 2003b). Indeed, an important question for the perchlorate database is whether subtle reductions in circulating T4

that trigger an increase in TSH release in the dam (or pup) may be detected as thyroid hormone insufficiency in the fetal brain (Zoeller, 2003b; Calvo et al., 2002). This is important for risk assessment purposes because the similarity between laboratory animal models and humans extends to the molecular level where thyroid hormone influences gene expression during development. Many of the genes that encode proteins critical for cell migration and synaptogenesis, dendritic arborization, myelination, and the basic architectural layout of the CNS are thyroid-hormone dependent (Bernal, 2002). The brain requires adequate tissue levels of T3 for normal development, which in turn is dependent on adequate circulating levels of both the thyroid hormones, T3 and T4. Decrements in circulating T4 may impair neurological development even when TSH levels are normal. Even transient perturbations in tissue thyroid hormones during development can lead to permanent adverse outcomes (Porterfield, 2000; Howdeshell, 2002; Zoeller, 2003a,b). Alterations in neurodevelopment caused by decreases in T4 alone (hypothyroxinemia) can occur in the absence of clinical signs of hypothyroidism or alterations in serum T3 or TSH (Goldey and Crofton, 1998; Morreal de Escobar et al., 2000; Haddow et al., 1999; Pop et al., 1999; Lavado-Autric et al., 2003; Zoeller, 2003a).

The upregulation in TSH in the "chronic phase" (see Figure 7-2) also presents an increased potential for neoplasia because stimulation of the thyroid to produce more T4 and T3 can result in hyperplasia. Both the decrement in T4 and T3 and increase in TSH is shown in Figure 2-1 (Figure 7-1 in the revised assessment) at the same step along the continuum. Although separate sequelae (neurodevelopmental versus neoplasia) are shown to follow these perturbations in the HPT axis, decreases in thyroid hormones versus a TSH increases is not separated at this step because the two events are difficult to differentiate with current experimental evidence and diagnostic criteria.

Which of these thyroid responses is the most sensitive to hormone changes has not specifically been studied in the perchlorate testing strategy. The difficulty of trying to capture the dynamics of a disease with "freeze frame" snapshots of histopathology performed at routine sample times has been noted in toxicologic pathology to be a particular challenge for the interpretation of adversity of effects pathology (Ruben and Rousseaux, 1991). As noted in the analyses of the studies in Chapter 5 (of the 2002 ERD and in the revised document), there is a considerable degree of overlap among the three different diagnoses of thyroid histopathology: colloid depletion, hypertrophy, and hyperplasia. If viewed across the database versus within an individual study, colloid depletion may appear to be slightly more sensitive. The fact that

thyroid follicular colloid depletion is a consistent finding not only across this study, but in rodents in general, would suggest that it is a good indicator of sufficient exposure to inhibit thyroid hormone synthesis. From a physiologic point of view this may be logical and supports the mode-of-action model. If there is any reserve thyroid hormone in the colloid, it is depleted before serum hormones are altered. Once serum levels are altered, TSH is upregulated and hypertrophy and hyperplasia are initiated in an attempt by the gland to restore circulating levels of T4 and T3. The diagnosis of colloid depletion has been reported with a similar compound, sodium chlorate, in the rat (Hooth et al., 2001), with many other chemicals in the rat, and with numerous goitrogens and pharmaceutical agents in the mouse. Colloid depletion in association with hypertrophy and hyperplasia suggests sufficient dose of the compound to inhibit colloid synthesis and decreases of circulating serum thyroid hormone levels sufficient to stimulate TSH.

Colloid depletion as the most sensitive indicator is most notable in the pups of the 2001 "Effects Study" on GD21 and then immediately post-parturition on PND4. Alternatively, as discussed in Chapter 5 (of the 2002 ERD and in the revised assessment), it may have been harder to diagnose hypertrophy and hyperplasia in the younger (smaller) and growing glands. The BMDL for colloid depletion increased with post-natal age; and by PND21, hyperplasia was also present. In contrast, all three thyroid indices were present in the PND4 pups of the previous Argus Laboratories, Inc. (1998a) study. This may be due to the difference in dosing of the dams. The dams in the 1998 study were only dosed during gestation and, therefore, likely had a greater decrement in thyroid hormones since they had less time for upregulation of TSH to occur. The dams in the 2001 study were dosed for two weeks during cohabitation, sufficient time as evidenced in the data described in Chapter 6 (of the 2002 ERD and in the revised assessment), for upregulation of TSH to compensate.

However, as also noted in the discussion in Chapter 4 (and in Chapter 5 of the 2002 ERD and in the revised response document), there is notable overlap among the three indices of thyroid histopathology (colloid depletion, hypertrophy, hyperplasia) within a single study. Careful evaluation of each study suggests that whichever index is most sensitive could be dependent on dose spacing in the study, age of animals on test, and sacrifice time point. For example, hyperplasia was the most sensitive of the three in the P2-generation adults (19 week F1-generation pups), and these same pups developed thyroid adenomas. As another example, the BMDL for hypertrophy in thyroids from the subchronic study (Springborn Laboratories, Inc., 1998) was much lower than that for colloid depletion at both the 14- and 90-day scheduled

sacrifices. The Agency suggests that this overlap among the BMDL estimates for each of the three thyroid histopathology indices is indicative that all three are monitoring the same tightly controlled feedback system.

The proposed mode of action mapped in Figure 2-1 (also Figure 7-1 in the revised assessment) is supported by correlations between thyroid hormones and TSH and between thyroid hormones or TSH and an objective measure of lumen size from laboratory animals exposed to ammonium perchlorate. There were positive correlations between T3 and T4, and negative correlations between either T3 or T4 and TSH, as expected based on the mode of action model (Appendix 7A of the 2002 ERD). The positive correlation between TSH and decreased follicular lumen size and the negative correlation between T4 or T3 and decreased follicular lumen size similarly support the proposed model (Appendix 7A of the 2002 ERD). Some of the correlations used in the 1998 assessment were precluded in the 2002 ERD due to the more limited severity scoring system used by the PWG (Wolf, 2000; 2001).

Additional support for the mode of action comes from data that now allow the linkage of both neurodevelopmental and neoplastic sequelae into the conceptual model. Perchlorate exhibits a consistent pattern of causing a decrease in thyroid hormones and increases in TSH across the database (Section 4.3.1). The repeat of observed effects on motor activity and brain morphometry in the new studies allows definitive determination that perchlorate exposure poses a neurodevelopmental hazard as discussed in Section 4.6. Repeatability and variability in statistics, sometimes a concern for evaluation of behavioral assays (Cory-Slechta et al., 2001), were addressed by the Bayesian approach employed for the motor activity analysis (Dunson, 2001a). This approach showed remarkable reproducibility between the results for the two studies despite the deficits previously noted for the Argus Research Laboratories, Inc. (1998a) study. Consistent effects of perchlorate on the size of various brain regions have now been observed in three different studies (Geller, 2003).

With respect to neoplastic sequelae following inhibition of iodide uptake and perturbation of thyroid hormone economy, there is sufficient evidence in the perchlorate database to establish perchlorate as a potential thyroid carcinogen. The neoplastic potential for perchlorate demonstrated at high doses in historical studies was confirmed at lower doses by the thyroid adenomas reported by the PWG (Wolf, 2000; 2001) in the F1-generation pups at 19 weeks (P2 parents) from the two-generation reproductive study (Argus Research Laboratories, Inc., 1999). The genotoxicity battery established that perchlorate is not directly damaging to DNA.

Consequently and consistent with the proposed mode-of-action model, it is concluded that neoplasia caused by perchlorate is likely to result indirectly from its anti-thyroid effects although the exact nature of interaction of perchlorate in the thyroid follicle remains to be unequivocally elucidated.

Thus, in summary, the key event for the anti-thyroid effects of perchlorate leading to either neurodevelopmental or neoplastic sequelae is inhibition of iodide uptake at the NIS that leads to perturbation of the hypothalamic-pituitary-thyroid axis. The evidence for this effect is built upon the observation of consistent changes across a range of experimental designs, including various species. Iodide inhibition has been shown to be similar in rats and humans. Studies of perchlorate treatment have demonstrated consistent effects on thyroid and pituitary hormones, increases in thyroid weight, and increases in three different diagnoses of thyroid histopathology (colloid depletion, hypertrophy, and hyperplasia). Corresponding neurodevelopmental (motor activity and brain morphometry) and neoplastic outcomes were observed in special assays. These outcomes are also consistent with the proposed mode of action and provide further evidence to confirm that inhibition of iodide uptake leading to the perturbation of the thyroid hormone economy should be viewed as adverse.

Due to the age and time-dependent nature of the endpoints in the database that result from the effects of perchlorate on iodide uptake inhibition and thyroid hormone homeostasis and differences in experimental design of the various studies, no one principal study is being chosen for this derivation. Instead, a weight-of-the-evidence approach across the diverse sets of experimental data is taken to arrive at a point of departure in Section 7.1.2 (Section 7.1.3 of revised assessment).

7.1.2 Comments on the Data Used to Designate the Point of Departure

This section summarizes major comments in the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b) found in Section 7.1.2 (Comments on the Use of Brain Morphometry Effects as the Basis for the Point of Departure) and Section 7.1.3 (Comments on the Use of Data Other than Brain Morphometry for the Point of Departure). The reason for combining the two sections in this response document is that the Agency used a weight-of-evidence approach and integrated data on more than one endpoint to arrive at its designation of 0.01 mg/kg-day as the point of departure in the 2002 ERD.

As stated on Page 7-2 of the 2002 peer panel report "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)." Based on the ensuing discussion at the workshop, the Agency appreciates that there is a misconception that only one endpoint was used to arrive a point of departure for operational derivation of the RfD, despite the explicit statements in the 2002 ERD on lines 25 to 26 of Page 7-16 in Chapter 7 that *"Several studies suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for the adverse effects of perchlorate"* and on lines 12 through 19 of Page E-9 in the Executive Summary that *"An administered dose of 0.01 mg/kg-day was supported as a lowest-observed-adverse-effect level (LOAEL) based on effects on brain morphometry in pups from a PND21 sacrifice in a neurodevelopmental study that repeated similar observations made in a similar study 1998 study, hormonal effects indicative of hypothyroidism (decreased T4 and increased TSH) in the dams of those same pups at various developmental stages (GD21, PND4, PND9, and PND21) thyroid histopathology and hormone changes at the 14- and 90-day sacrifice dates in a subchronic study, and indications of immunotoxicity (dermal contact hypersensitivity)."*

Thus, the two sets of comments will be responded to in this one section in the document, and a revised section describing how the Agency arrived at 0.01 mg/kg-day as the point of departure will be provided in the final assessment.

The Agency also appreciates that it failed to communicate clearly the choice of interspecies extrapolation based on the area-under-the-curve in blood (AUCB) for perchlorate in the dams on GD21 from the 2001 developmental neurotoxicity study (Argus Research Laboratories, Inc. 2001). The choice of the maternal blood level associated with serum hormone perturbation as the basis for interspecies extrapolation should have reinforced the notion that more than one endpoint was being used for determination of the point of departure – i.e., changes in the serum hormones of the dams on GD21 that produced pups with changes in brain structure measured on PND21. The choice of this as the basis for interspecies extrapolation was also a result of less confidence in the fetal and neonatal PBPK model structures due to their lack of validation as opposed to the degree of validation for the models of perchlorate disposition in the adult rat and adult human.

Thus, the Agency is revising Section 7.1.3 of the assessment and this new text will hopefully clarify the constellation of effects that cluster at the 0.01 mg/kg-day level, and which is used in a weight-of-evidence approach to suggest this as a level of concern. The entirety of

the new section is provided in this response document as Section 7.1.2.2. Clarification regarding the dose metric and basis for interspecies extrapolation is provided in Section 7.2 of this response document.

7.1.2.1 Summary of the 2002 Peer Panel Comment on the Use of Different Data to Designate the Point of Departure

The EPA believes that each of the comments on data quality below are redundant to a large degree with comments addressed in responses in Chapter 3 for those pertaining to the human data and in comments in Chapter 4 for those pertaining to the laboratory animal data. Thus, the responses in this section will be brief and will provide cross-references to specific sections in those previous chapters. The Agency has also incorporated specific responses in the revisions to either the section on the point of departure or on the dosimetry adjustment below.

7.1.2.1.1 Basing the Point of Departure on Changes in Brain Morphometry

Comment(s): *Based largely on comments made earlier in the meeting, one reviewer 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. Though not disagreeing that the brain morphometry studies have flawed designs, another reviewer 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 produce effects that do not exist. Two reviewers 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 random errors that the study design may have caused.*

Regarding the statistical analyses of the brain morphometry data, one reviewer 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. Another reviewer did not find EPA's statistical analysis compelling, not due to any flaws in the statistical approaches but rather to his concern about the quality of the linear measurements.

In the summary of discussion on this issue, the workshop chair noted that the primary concern for use of brain morphometry to designate the point of departure was whether the data were of acceptable quality and whether the effects were caused by perturbations of thyroid hormones. Two reviewers suggested a blinded reanalysis of the data. Another noted that such reanalysis would not address the validity of linear measurements. A third indicated that these data could be used provided that study methodology and concerns regarding inconsistency could be addressed. Finally the meeting chair suggested that the Agency consider the following options.

(1) Not consider changes in brain morphometry when deriving the point of departure.
(2) Consider the changes in brain morphometry when deriving the point of departure but address concerns about the quality of the underlying data.
(3) Base the point of departure entirely on other endpoints but perhaps account for database insufficiencies regarding neurodevelopmental effects using an uncertainty factor.

After discussing the various strengths and weaknesses of the brain morphometry study, the workshop chair asked the peer reviewers to give their final opinions as provided in Table 2 on Page 7-17 of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b). Two of the three neurotoxicologists on the panel supported the use of the brain morphometry data as the basis of the point of departure. Three of the remaining six peer panel members who specifically commented on this aspect either did not support the use of the brain morphometry data or were not convinced that the study identified adverse effects. The other three of the six who provided comment offered that these data could be used as the point of departure conditional on addressing considerations specified above.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has provided responses to these comments in the discussion in Section 4.5.1. (Comments on Studies of Changes in Brain Morphometry). However, EPA does want to clarify again that the concern was not over different sectioning practices because all of the sectioning was performed by the same laboratory. Only the initial tissue blocks were cut in different laboratories but this was not considered an issue because landmarks were used for each brain region. All brains were fixed and embedded at the same time so that fixation artifacts are not a concern (Harry, 2001).

As discussed in Harry (2001), in the 2002 ERD on Page 5-73, in an associated technical memorandum (Geller, 2001d), and in this response document in Section 4.5.1.1.5, the concern was over a potential for systematic bias of the sections in tissue block level II by dose level, notably for the corpus callosum region. Methodological issues, including blind reading, coronal sections, variability in measurements, and the use of linear measurements were discussed in Section 4.5.1.1. Again, the use of linear measurements in the coronal plane was necessary to repeat the 1998 protocol and are recommended in standing Agency guidance (U.S. Environmental Protection Agency, 1998b). As described in that section, the concern about the use of sagittal sections would only be valid if the corpus callosum had been the only region to show effects. However, a number of brain regions in both the 1998 and 2001 developmental neurotoxicity (DNT) studies showed dose-dependent changes with perchorate treatment.

With respect to the three options outlined by the panel in the summary, the EPA has essentially used both options (1) and (2). As described in Section 7.1.2.1, other data designate 0.01 mg/kg-day as a level of concern for the effects of perchlorate exposure so that the brain

morphometry effects are not the sole basis for making this determination and could be omitted without materially affecting the derivation (option 1). For example, if changes in serum hormones were used as the basis, then the 0.01 mg/kg-day dose level would still be designated a LOAEL. If iodide uptake inhibition were used, then a lower level (0.002 mg/kg-day) would be used as a NOAEL in the derivation but that would be offset by a different UF, and the same estimate would result. These comparative estimates are provided in subsections to Section 7.5 on operational derivation of the RfD.

The Agency believes that the concerns about the data quality (option 2) have been addressed by the new studies performed in 2003 in response to the comments made a the 2002 peer review. These new studies are described in Section 4.5.1.2 and in an associated technical memorandum (Geller, 2003). Consequently, as will be discussed in Section 7.1.2.2, the Agency has chosen to retain the use of the brain morphometry data as part of the weight of the evidence that designates the 0.01 mg/kg-day as the point of departure.

With respect to option (3), the Agency notes that if the brain morphometry data were to be discounted as unreliable or "flawed", then the lack of these studies would represent a significant uncertainty that would require the use of a full 10-fold factor for database deficiencies alone. The developmental neurotoxicity (DNT) screening study was considered a key component of the original testing strategy and important enough to repeat after the 1999 peer review of results of the 1998 DNT study. If these data were to be viewed as insufficient, a major data gap would exist — essentially one that is needed to accurately describe one of the two critical sequelae of thyroid hormone disruption — that of neurodevelopmental toxicity.

7.1.2.1.2 Concerns About Inconsistencies in Brain Morphometry Results

Comment(s): *Two reviewers discussed whether consistency should be expected across certain findings. The first listed various types of inconsistencies that he observed including different results between the sexes, across postnatal days, and between the two developmental neurotoxicity studies (Argus Research Laboratories, Inc. 1998, 2001). The second reviewer agreed that the lack of concordance was troublesome but was not as concerned about the issues raised by the first reviewer because he noted that certain developmental events are known to take place over distinct (and sometimes narrow) windows of time. He indicated that it was 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 brain regions.*

EPA Response(s) and Recommendation(s) for Revision(s): The two DNT studies differed in dosing regimen – the 1998 dosed only during gestation; whereas the 2001 study additionally dosed the dams for two weeks prior to gestation, and there was a 3-fold difference in the highest dose tested (10 mg/kg-day versus 30 mg/kg-day) and a 10-fold difference in the lowest dose tested (0.1 mg/kg-day versus 0.01 mg/kg-day). The studies also tested the pups on different days. Despite these differences in experimental design, Table 4-4 in Section 4.5.1.3 (Consistency Across Studies) shows remarkable consistency in the shape of the dose-response between the two studies for the various brain regions affected by perchlorate treatment.

7.1.2.1.3 On the Shape of the Dose-Response Curve

Comment(s): *One reviewer indicated that the inverted U-shaped dose-response curve implies that high doses of perchlorate may protect rats against neurodevelopmental effects. Other reviewers offered different opinions. Because the mechanisms of thyroid hormone action that result in the reported brain morphometry changes have not been identified, one of the peer reviewers assigned to neurotoxicity indicated that he has no basis for dismissing the data because a linear or monotonic dose-response curve was not observed. Another reviewer assigned to neurotoxicity agreed, saying that inverted U-shaped dose-response curves have been documented, particularly in cases where increased effects initiate compensatory responses, similar to upregulation of thyroid hormone synthesis observed following iodide uptake inhibition. This reviewer also suggested that the revised assessment include specific hypotheses about mechansims that may account for U-shaped dose-response curves. The third reviewer assigned to neurotoxicity also found no inherent problem with non-linear dose-response curves, but he was troubled by the fact that the dose-response curves are not consistently observed across both sexes.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has responded to comments on the shape of the dose-response for the brain morphometry changes in Section 4.5.1.2. The Agency agrees with the three peer reviewers assigned to the topic of neurotoxicity that U-shaped dose-response curves have been well documented in the literature and are not to be excluded or dismissed. Section 4.5.1.2 also discusses current hypotheses that non-monotonic responses can be attributed to the influence of multiple processes underlying a response. Because many different processes mediate brain development at any point in time, the existence of U-shaped or inverted U-shaped curves is not unexpected. The World Health Organization (2001) notes such biphasic curves are common and likely to reflect multiple mechanisms of action, the presence of homeostatic mechanisms, differences in pharmacokinetics with dose, or activation of compensatory or protective mechanisms. Differences in the brain morphometry

between the sexes may be due to differences in serum hormone changes observed between the sexes. Males in the database have been shown to be consistently more sensitive with respect to thyroid hormone perturbations and this same sex difference has been reported in the literature (Meisami, 1984; Bakke et al., 1976).

7.1.2.1.4 On Integration of Brain Morphometry Data with Other Endpoints

Comment(s): *Given the proposed mode of action, one reviewer said that he would have expected that changes in brain morphometry would be accompanied by changes in thyroid hormone levels, but that in the most recent study (Argus Research Laboratories, Inc., 2001) that 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 EPA should consider whether such modest perturbations to thyroid hormone levels would result in altered brain structures.*

Another reviewer 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 Argus 2001 study reported only "snapshots" of thyroid hormone levels, that may not be representative of the circulating hormones 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.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency has responded to comments on this issue in Section 4.5.1.4 and presents its synthesis of the diverse data to arrive at point of departure in Section 7.1.2.1. In Section 4.5.1.4, the EPA corrected the reviewer regarding the serum hormone levels. Statistically significant effects on TSH and T4 occurred in the dams of the Argus 2001 study on GD21 with 0.01 mg/kg-day designated as a LOAEL. The second reviewer's point is well taken in that the changes in thyroid hormone economy for the pups *in utero* may have occurred prior to when these serum hormones changes were evident in the dams or the pups at the only sample point during the gestational period (GD21). The 0.01 mg/kg-day is the same dose level at which significant brain morphometry changes were demonstrated at the two postnatal day sacrifices of pups from these dams. The Agency's evaluation of the consistency in effects between the two studies that measured brain morphometry (Argus Research Laboratories, Inc., 1998a; 2001) was presented in response to the preceding comment and in Section 4.5.1.4 (Consistency Across Studies). Consistency across the studies with respect to changes in serum thyroid hormones was discussed in Section 4.3 and presented in Table 4-1.

7.1.2.1.5 Mechanistic Questions

Comment(s): *Two reviewers noted that the changes in brain morphometry can not 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 also questioned the relevance of the brain morphometry changes in rats to humans. Though other reviewers agreed that the absence of mechanistic links is unfortunate, they did not think that the brain morphometry findings should be criticized for this reason, especially because 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 size of brain dimensions, one reviewer felt uncomfortable disregarding the data because no previous studies have elucidated the mechanisms that may cause these effects. Finally, one reviewer noted that the point of departure that EPA proposed is similar to the effect levels reported in selected ecotoxicological studies — a factor the Agency may wish to consider in the revised assessment.*

EPA Response(s) and Recommendation(s) for Revision(s): As pointed out by panel members in comments regarding serum thyroid hormone levels below, it is well established that thyroid hormone is necessary for proper brain and nervous system development. Individual pup blood levels can not be matched one-to-one with measurements in brain dimensions because the small amount of blood from each animal harvested on the last day of gestation (GD21) must be pooled in order to obtain a sufficient volume with which to run the RIA for serum hormone determination. Analysis of blood from any earlier day in gestation is even more problematic due to this sample size issue. The issues discussed in Chapter 2 regarding the use of RIA kits is also to introduce variability across studies.

However, the protocol of the "Effects Study" (Argus Research Laboratories, Inc., 2001) included serum hormone determinations in both the dam and pups on GD21 in an effort to understand what the fetal levels may have been for the pups during gestation. Table 4B-2 shows that the LOAEL for T4 and TSH in the dams of this study was 0.01 mg/kg-day. In the pups, this level was a LOAEL for T3 and a NOAEL for T4. The LOAEL for T4 was 0.1 mg/kg-day. The NOAEL and LOAEL for TSH were 0.1 and 1.0 mg/kg-day.

Because the pups are more dependent on the maternal thyroid hormones earlier in gestation (see Chapter 2) and because perchlorate can inhibit placental transfer of iodide to the fetus, the levels on GD21 may not represent the greatest insult to thyroid hormone economy that the pups received *in utero*. Thus, the LOAEL for T4 and TSH in the dams at the same level (0.01 mg/kg-

day) as the LOAEL for brain morphometry was considered to be a qualitative correlation that was consistent with the mode of action.

The Agency agrees with the reviewer that the ecotoxicological database is one that reinforces the concern for neurodevelopmental effects. As described in Chapter 5, delayed metamorphosis in *Xenopus* (Tietge and Degitz, 2003) and other thyroid-dependent effects are observed with perchlorate exposure and strongly support the proposed mode of action.

7.1.2.1.6 On Basing the Point of Departure on Human Studies

Comment(s): *Though some reviewers suggested many times during the meeting that the revised assessment should more prominently acknowledge findings from human health effect studies, two peer reviewers questioned the utility of those studies for deriving a point of departure. One reviewer did not think that the Greer et al. (2002) and Lawrence et al. (2000, 2001) studies which examined RAIU and serum hormone levels from a very small subset of healthy, euthryoid adults offered any insights on potentially important sensitive populations (e.g., pregnant women, children, fetuses). The second reviewer 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.*

Three other reviewers indicated that EPA should do a better job of integrating data from the human health effects studies into the revised assessment without necessarily using those data for deriving the point of departure. One of these three wondered if more insights could be drawn from the effects observed among humans with Graves' disease who had been prescribed high doses of perchlorate (e.g., one patient received 3 mg/kg-day for 22 years) although he acknowledged that this dosing was necessary to treat hyperthyroidism. He also suggested that EPA consider basing the point of departure on the data from Greer et al. (2002) and Crump et al. (2000). Another reviewer recommended that these data be used in a sensitivity analysis of the point of departure. The third reviewer estimated an RfD based on Greer et al. study to be 0.0001 mg/kg-day. He derived this based on an estimate of the NOAEL to be 0.001 mg/kg-day and application of a 10-fold UF for intrahuman variability. He recalculated this estimate in written comments and estimated the NOAEL at 0.0025 mg/kg-day and clarified that the RfD could be between 0.00025 mg/kg-day and 0.0008 mg/kg-day, depending on the choice of 3 or 10 as the UF.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency agrees with the reservations expressed by the peer reviewers regarding the utility of the existing human data as the sole basis for the point of departure. As discussed in Chapter 3 of this response document, the peer panel was clear that the ecological epidemiological studies suffer from deficiencies that limit their utility for quantitative dose-response analysis. Crump et al. (2000) is an ecological study, but nevertheless the Agency undertook another thorough reanalysis of the data.

As described in detail in Section 3.2.1, the EPA still finds that this study is of limited use to dose-response evaluation based on concerns over the high background incidence of goiter and a history of thyroid disease in the families that comprised the study population, along with inadequate exposure characterization, a limited sample size, and variability in the serum hormone measures.

The historical data on patients with Graves' disease were not discussed by the panel in depth. This is likely due to the limited reporting of these case studies, limited dose range, limited health evaluation, and the fact that these patients were already ill. The Agency is not recommending changing its stance on the use of the historical case studies.

None of the human studies in the perchlorate database evaluated neurodevelopmental outcome measures. This represents a key shortcoming given their other design limitations. As stated earlier regarding neurodevelopmental effects, it is not accurate to presume that the negative feedback system will prevent adverse effects of persistent but small changes in circulating thyroid hormones. This is especially the case when the effects on thyroid hormones are being evaluated in studies which suffer from significant design limitations due to sample size, population characteristics, and exposure characterization. The Agency agrees with the reviewer who suggested that laboratory animal models are the best choice for evaluating some of these key endpoints, including neurodevelopmental effects, developmental toxicity, reproductive toxicity, and immunotoxicity. The key mechanistic event of iodide uptake inhibition can then be used to compare across species.

With respect to Greer et al. (2002), the Agency notes that its modeled benchmark dose estimate of 0.002 mg/kg-day for iodide uptake inhibition in humans is in good agreement with that suggested by the peer reviewer in his post-meeting written comments. Details of the Agency's analyses, including exploration of the outliers, choice of model and evaluation of goodness of fit, calculation of confidence intervals, and evaluation of duration dependence can be found in Section 3.3.1 of this response document. This benchmark dose estimate will be used in the point of departure analysis and in the calculation of a comparative risk estimate in Sections 7.1.2.1 and 7.5, respectively, below.

7.1.2.1.7 *On Basing the Point of Departure on Iodide Uptake Inhibition*

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

Another reviewer expressed caution over some of the inferences drawn by the other reviewer. She indicated that researchers have not yet established the extent to which NIS inhibition is reversible. She acknowledged that the kinetics of NIS active transport are strikingly similar across species but she was hesitant to make premature judgements 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. A third reviewer noted that the laboratory animals studies found evidence of upregulation while the human studies did not. The second reviewer responded that the same finding could be explained by differences in the vast reserves of thyroid hormones in humans compared to rats and was not therefore not surprised that the 2-week studies in humans found no evidence of upregulation.

EPA Response(s) and Recommendation(s) for Revision(s): Designation of 0.01 mg/kg-day as the point of departure was based on NOAEL and LOAEL or benchmark dose estimates for various toxicological endpoints in the laboratory animal studies and not on any *a priori* determination of the degree of iodide inhibition considered adverse. The Agency disagrees that thyroid hormones were only marginally impaired at this level, again noting the LOAEL in the dams on GD21 for both T4 and TSH at this level.

The Agency agrees that the existing data are insufficient to evaluate the effect of chronic dosing on iodide uptake inhibition and this is precisely why the EPA is concerned about *in utero* programming as evidenced by the thyroid tumors occurring in pups with an increased incidence and decreased latency. The analyses performed on the 2-week dosing study by Greer et al. (2002) in Chapter 3 that show a duration-dependence of both model parameters and benchmark dose estimates reinforce the reviewer's comment that the exposure duration was insufficient to test iodide inhibition that may occur with chronic dosing.

As shown in Section 7.1.1.1, inhibition of iodide uptake at the NIS in animals and humans by perchlorate is remarkably similar, with rats actually slightly less sensitive than humans (see Figure 7-3 above and Table 7-1 in the 2002 ERD provided here in Appendix A as Table 7A-1). All of the interspecies extrapolations were based on steady-state PBPK models for perchlorate

distribution. The longer-term drinking water studies in rats were not used in order to preclude interference with the estimates by upregulation in the rat. The degree of iodide inhibition observed for rats was based on i.v. dosing studies. The correlation between iodide uptake inhibition and AUCB of perchlorate was 0.99. Further, the i.v. data for iodide uptake inhibition in rats were considered to be the most analogous to naive human dosing and most relevant to the transient phase associated with potential T4 decrements. The PBPK model for the human was developed using the data from the Greer study that underwent QA/QC (Greer et al., 2000, 2002; Merrill, 2001a).

It is of note that the HEE based on iodide uptake inhibition (Table 7A-7 or Table 7-7 in the 2002 ERD) calculated from the model for the adult male rat is 0.004 mg/kg-day. This estimate is two-fold higher than the BMDL (0.002 mg/kg-day) that EPA estimated using the RAIU data in adult, euthyroid human subjects from Greer et al. (2002). It appears that a dose level in rats that is associated with serum hormone and brain changes is at about the same level that would be estimated to be a BMDL level for a benchmark response of 5% iodide uptake inhibition in euthyroid adult humans.

However, this comparison must take into account the differences between normal adults and fetuses or neonates in thyroid economy and in the relative sensitivity and reversibility of these different life stages to damage due to thyroid hormone insufficiency. Neurodevelopmental effects may be permanent, and it is not known what decline in circulating levels of thyroid hormone and what duration is required to produce measurable neurological deficits in neonates. Subtle doses of thyroid hormone given to children with congenital hypothyroidism are associated with differences in a number of neurological parameters (Heyerdahl, 2001; Rovet et al., 2002). An empirical study by van den Hove et al. (1999) concluded that the amount of thyroid hormone contained in a neonatal thyroid gland is insufficient to supply the child with thyroid hormone for a single day. This observation reinforces that the rat with its limited stores of thyroglobulin may be a very appropriate model of neurodevelopmental effects in children.

The potential for a lack of reversibility of inhibition exacerbates the concern for the lack of chronic data and decreases the confidence in the interspecies extrapolation because humans express fewer NIS. As stated in Chapter 4, the Agency does not disagree that BBDR models would help to inform this debate but expects that it will take the AFRL or others a significant period of time to develop and validate such structures.

7.1.2.1.8 On Basing the Point of Departure on Serum Hormone Changes

Comment(s): *The use of serum hormones as the basis of the point of departure was discussed briefly by the panel. One reviewer emphasized that EPA should choose this endpoint for the point of departure but should be careful to distinguish changes that are biologically significant from those that are simply statistically significant. Specifically, recognizing that thyroid hormones exhibit considerable diurnal variations, this reviewer recommended that EPA consider only measured hormone levels outside a "normal" range as being potentially adverse. Another reviewer agreed that statistically significant changes that fall within "normal" fluctuations should not be considered adverse effects.*

The reviewers also briefly discussed whether decrements in thyroid hormone levels, specifically T4, can lead to adverse neurodevelopmental effects. One reviewer said that decrements in T4 levels can clearly cause neurological dysfunction. This reviewer added that extensive dose-response data linking these decrements to adverse effects are not available although some clinical thyroidologists have said that humans sustaining 10% to 15% reductions in circulating thyroid hormone levels may show symptoms of hypothyroidism. Another reviewer 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.

EPA Response(s) and Recommendation(s) for Revision(s): The variability in diurnal levels as well as in the measurement of serum hormones using RIA is why the appropriate procedure for analysis is to compare the exposed groups with the control within an experiment. Moreover, the concern over protecting public health is for shifts in thyroid hormone status in a population and not in an individual.

As described in the 2002 ERD, the ANOVA analyses for effects on serum hormones were used after serious consideration. While in clinical studies a normal range typically is defined by a control healthy population, the ANOVA approach is an equally valid approach in that a statistically significant value represents a shift in the mean for the population. The control group defines the range for the unexposed, presumably healthy population, and statistically significant differences indicate that the mean for an exposed group is outside of that normal range. Circadian fluctuations are addressed because the same fluctuations in the control population occur as in the exposed population. A small shift in the mean of a population can have significant consequences to individuals in the tails of the distributions of those populations. Indeed, such an evaluation underlies the basis for the blood lead level used in the National Ambient Air Quality Standard (Davis and Elias, 1996) and has been noted as an important consideration for neurotoxicity (Weiss, 2000).

7.1.2.1.9 On Basing the Point of Departure on Thyroid Histopathology

Comment(s): *Two peer reviewers addressed whether EPA should use the observed histopathology indices (colloid depletion, hyertrophy, hyperplasia) as the basis of the point of departure. The first questioned, by way of an example, whether EPA should view colloid depletion as an adverse effect. He indicated that this effect is better characterized as adaptive. Another reviewer noted 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 proposed 1.0 mg/kg-day may be an appropriate point of departure for thyroid tumorigenesis. He explained that this was the dosage required to observe signs (i.e., hypertrophy) that the thyroid was being stimulated and stressed which he considered a departure from homoestasis. This reviewer also suggested that EPA apply an interspecies uncertainty factor of 0.1 to this point of departure, noting that pertubations of the HPT axis apparently have far different consequences in rats than in humans. As evidence of this, he noted that rats exposed to certain proton pump inhibitors readily develop gastric neuroendocrine tumors; whereas no evidence of such effects have been observed in humans.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency provided an extensive response in Section 4.4 as to why the three indices of thyroid histopathology could not be distinguished from each other given the "freeze frame" nature of the sample points for histology within the dynamic pathobiology and because the HPT system is so tightly regulated that standard histopathology is virtually unable to "see" the distinction of the processes in the gland.

The Agency would not be comfortable designating 1.0 mg/kg-day as the point of departure based on the NOAEL for tumors observed at the 19-week sacrifice of F1 pups from the two-generation reproductive study (Argus Research Laboratories, Inc., 1999) because hyperplasia and hypertrophy were also evident in these animals at significantly lower levels. The benchmark dose estimate for hyperplasia and hypertrophy is 0.0004 mg/kg-day and 0.15 mg/kg-day, respectively, in these animals and reinforces that precursor lesions also inform the determination of the point of departure. The harmonized approach proposed by the EPA takes into account both neurodevelopmental and neoplastic (tumor) endpoints in arriving at its proposed point of departure.

7.1.2.2 EPA Recommended Revisions to Section 7.1.3 (Point-of-Departure Analysis)

This section represents a summary of the responses to the issues raised in the previous section regarding the Agency's rationale for the choice of 0.01 mg/kg-day as the point of departure. This text will replace Section 7.1.3 in the revised document. Emphasis has been

included to reinforce that more than one data set was used to arrive at the point of departure with a cross-reference to the fact that a choice had to be made with respect to which study was used to define the dose metric and to serve as the basis for interspecies extrapolation.

Figure 7-4 (which will be a new Figure 7-5 in the revised assessment) shows the effect levels used by the Agency to arrive at 0.01 mg/kg-day as a level of concern for perchlorate exposure and the point of departure for derivation of the harmonized RfD. This figure highlights that doses of 0.01 mg/kg-day or less can be considered LOAELs for serum hormones effects, thyroid weight increases, and changes in brain morphometry and as NOAELs for thyroid histology in laboratory animals. The LOAEL for immunotoxicity is also within an order of magnitude of the point of departure. Table 7-1 provides information on each of the studies listed in parentheses for any single endpoint and where that effect is discussed in the 2002 ERD or revised assessment.

The designation of effect level was determined with statistical approaches considered to be appropriate for each endpoint. Quantitative dose-response analysis was used because the Agency advocates approaches (e.g., the benchmark dose) that account for the influence of dose spacing, sample size, and variability in the data rather than relying on subjective designation of effect levels. The statistical approaches are described in greater detail in the preceding chapters (of the 2002 ERD and in the revised assessment) but will be listed briefly here. ANOVA was used to determine the effect level designations for the serum hormone analyses. Benchmark dose estimates for a 10% response level, typical for dichotomous outcome measures, were used for the various indices of thyroid histopathology. The NOAEL for the motor activity studies was based on a response level of 10% and was derived using a Bayesian analysis. Effects on brain morphometry were based on profile analysis, a MANOVA that corrected for repeated measures across the different brain regions. A benchmark dose estimate for iodide uptake inhibition was derived using a benchmark response level of 5%, the level usually chosen for continuous developmental outcome measures. Other endpoints, e.g., the NOAEL and LOAEL for thyroid weight changes, developmental toxicity, and reproductive effects, were based on standard statistical comparisons between dose groups such as the t-test.

The left-hand side y-axis on the graph shows both the administered dose or exposure in mg/kg-day and the human equivalent exposure (mg/kg-day) derived by application of dosimetric adjustment for interspecies differences. As will be described in Section 7.2.1, the Agency has

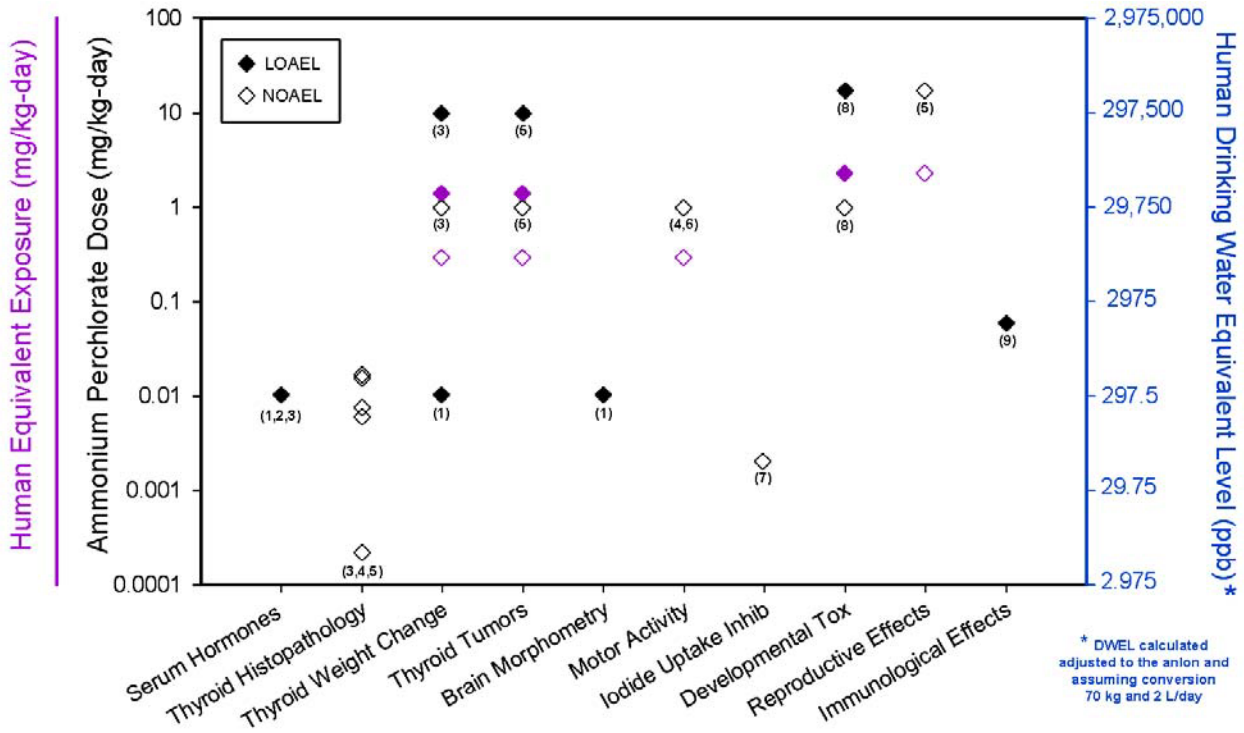

Figure 7-4. New Figure 7- 5. Administered dose (mg/kg-day) or human equivalent exposure (mg/kg-day) of ammonium perchlorate (left-hand y-axis) and designated adversity level (NOAEL or LOAEL) of different effects due to perchlorate treatment in laboratory animals and humans. Human equivalent exposure based on the AUCB as the dose metric and the use of the pregnant or adult male rat as the representative life stage, depending on the endpoint (see text). Numbers in parentheses indicate the studies as listed in Table 7-1 (Table 7-8 in revised assessment). Right-hand Y-axis shows the drinking water equivalent level (DWEL) if the NOAEL or LOAEL were adjusted to the anion only and converted to µg/L (ppb) by assuming 70 kg for body weight and the consumption of 2 L of water per day.

chosen the area under the curve of perchlorate in blood (AUCB) as the dose metric. If the area under the curve for thyroid tissue (AUCT) had been used, the resultant estimates would be on the order of 100-fold lower. The is relationship of HEE to dose level in the rat is not linear at the higher dose levels. The adult rat or pregnant rat is used as the basis for the interspecies extrapolation due to better validation of these models. The HEE estimates for the 1.0, 3.0, 10.0, or 30.0 mg/kg-day exposure dose levels are approximately 0.5, 1.3, 2.20, and 4.20 mg/kg-day.

Table 7-1. New Table 7-4.
Studies Associated with Effect Levels in Figure 7-2 (New Figure 7-5 in Revised Assessment)
Used to Designate the Point of Departure

Effect Level (Number)	Species	Study Duration	Sample Time	Reference	Section in 2002 ERD or Revised Assessment
LOAEL (1)	Rat	2 weeks prior to mating and GD0-PND10	T4 and TSH in dams at GD21; T3 at GD21, T4 at PND22; TSH at PND10 and PND22	Argus Research Laboratories, Inc. (2001)	5.3.3
LOAEL (1)	Rat	2 weeks prior to mating and GD0-PND10	Weight on DL10 in males	Argus Research Laboratories, Inc. (2001)	5.3.3
LOAEL (1)	Rat	2 weeks prior to mating and GD0-PND10	Brain morphometry on PND21	Argus Research Laboratories, Inc. (2001) and Consultants in Veterinary Pathology, Inc. (2003)	5.3.3 5.3.4
LOAEL (2)	Rat	14-day	T3 and TSH at 14-day (interim sacrifice)	Springborn Research Laboratories, Inc. (1998)	5.2.3
LOAEL (3)	Rat	90-day study	T3 and T4 at 90-day sacrifice	Springborn Research Laboratories, Inc. (1998)	5.2.3
NOAEL (3)	Rat	90-day study	BMDL for colloid depletion (0.03 mg/kg-day) and hypertrophy (0.008 mg/kg-day) at the 90-day sacrifice	Springborn Research Laboratories, Inc. (1998)	5.2.3
NOAEL / LOAEL (3)	Rat	90-day study	90-day sacrifice	Springborn Research Laboratories, Inc., (1998)	5.2.3
NOAEL (4)	Rat	GD0 to PND10	BMDL for colloid depletion in PND5 pups at 0.009 mg/kg-day and at 0.029 mg/kg-day in adults on PND90 and PND92	Argus Research Laboratories, Inc. (1998a)	5.3.1
NOAEL (4)	Rat	GD0 to PND90	BMDL for colloid depletion at 0.029 in adults on PND90 and PND92	Argus Research Laboratories, Inc. (1998a)	5.3.1

Table 7-1 (cont'd). New Table 7-4.
Studies Associated with Effect Levels in Figure 7-2 (New Figure 7-5 in Revised Assessment)
Used to Designate the Point of Departure

Effect Level (Number)	Species	Study Duration	Sample Time	Reference	Section in Revised Final ERD
NOAEL (5)	Rat	2-Generation study	BMDL (0.0004 mg/kg-day) for hyperplasia in F1 pups at 19 weeks	Argus Research Laboratories, Inc. (1999)	5.5
NOAEL/ LOAEL (5)	Rat	2-Generation study	F1 pups at 19 weeks	Argus Research Laboratories, Inc. (1999)	5.5
NOAEL (5)	Rat	2-Generation study	F1 and F2 evaluation	Argus Research Laboratories, Inc. (1999)	5.5
NOAEL (6)	Rat	2 weeks prior to mating and GD0-PND10	PND14	Bekkedal et al. (2000)	5.3.2
NOAEL(7)	Human	14-day	24-hour sample on Day 14	Greer et al. (2002)	4.2.1.3 (new)
NOAEL / LOAEL (8)	Rat	Developmental Study	Segment II evaluation	Argus Research Laboratories, Inc. (2000)	5.4.3
LOAEL (9)	Mouse	90-day study	14- and 90-day sacrifices	BRT-Burleson Research Technologies, Inc. (2000a,b,c)	5.6

Abbreviations used in Table: NOAEL = No-observed-adverse -effect level; LOAEL = Lowest-observed-adverse-effect level; BMDL = Benchmark dose lower limit; GD0 = Gestation day 0; GD21= Gestation day 21; DL10 = Lactation day 10; PND5 = Postnatal day 5; PND10 = Postnatal day 10; PND14 = Postnatal day 14; T3 = Triiodothyronine; T4 = Thyroxine, TSH = Thyroid stimulating hormone.

Thus, the NOAELs that appear on Figure 7-4 at approximately 1.0 mg/kg-day show a comparable HEE estimate at 0.5 mg/kg-day, and the LOAELs observed in animals at 10 mg/kg-day have an HEE estimate of 2.20 mg/kg-day.

Several studies suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for the adverse effects of perchlorate. The LOAEL estimates that cluster at this value include serum hormone changes in adult, pregnant, fetal and neonatal animals, thyroid weight changes in pups, and brain changes in pups. The NOAEL value at 0.002 mg/kg-day is for iodide uptake inhibition in humans. Each of these will be discussed separately.

7.1.2.2.1 New Section 7.1.3.1 (Serum Hormone Levels)

With respect to the serum hormone changes, levels of T4 were significantly decreased and TSH levels statistically increased at the 0.01 mg/kg-day level in dams on GD21 in the same study as the significant brain morphometry changes in the PND21 pups (Argus Research Laboratories, Inc. 2001). In this study there was no NOAEL for hypothyroidism in the dams. The pups in the study were also affected at 0.01 mg/kg-day. Effects on T3 occurred at GD21, PND5, and PND9 at this dosage. The 0.01 mg/kg-day dose was the LOAEL for effects on T4 and TSH at PND21 in the male pups and for TSH in both sexes at PND9 as well. This same dose (0.01 mg/kg-day) was also the LOAEL for decreases in T4 and increases in TSH at the 14-day and 90-day time points in the 90-day study (Springborn Laboratories, Inc., 1998).

As discussed in Section 4.6.3 and earlier in this chapter in Section 7.1.2.1.8 the Agency considers circulating serum levels to represent a biomarker for changes in tissue hormones and again emphasizes that even transient perturbations in tissue thyroid hormones during development can lead to permanent adverse outcomes (Porterfield, 2000; Howdeshell, 2002; Zoeller, 2003a,b). Alterations in neurodevelopment caused by hypothyroxinemia (deficits in T4 alone) can occur in the absence of clinical signs of hypothyroidism or alterations in serum T3 or TSH (Goldey and Crofton, 1998; Morreale de Escobar et al., 2000; Haddow et al., 1999; Pop et al., 1999; Lavado-Autric et al., 2003). It is not established whether subtle reductions in circulating T4 that trigger an increase in TSH release in the dam (or pup) may be detected as thyroid hormone insufficiency in the fetal brain (Zoeller, 2003b). Further, it is not accurate to assume that the HPT feedback system prevents adverse effects of persistent but small changes in circulating levels of thyroid hormone (Zoeller, 2003b). Whether there is a threshold level of thyroid hormone disruption necessary for altering gene expression and subsequent changes in

brain structure and function is currently unknown. However, the essential role of thyroid hormone in brain development makes it critical to consider the possibility that mild degrees of hypothyroxinemia may impair neurologic development.

Thus, the ANOVA estimates were chosen for evaluation of the hormone data. While a normal range typically is defined by a control healthy population in clinical studies, the ANOVA approach is an equally valid approach in that a statistically significant value represents a shift in the mean for the population. The control group defines the range for the unexposed, presumably healthy population, and statistically significant differences indicate that the mean for an exposed group is outside of that normal range. Circadian fluctuations are addressed because the same fluctuations in the control population occur as in the exposed population. A small shift in the mean of a population can have significant consequences to individuals in the tails of the distributions of those populations. Andersen et al. (2002) in recent human studies have demonstrated that individual variation in T4 levels are much more narrow than the population variance in T4 which is the basis for the normal reference range. Thus, an individual can experience a decline or excess in serum T4 that is significantly altered but still within the normal reference range. Such an evaluation underlies the basis for the blood lead level used in the National Ambient Air Quality Standard (Davis and Elias, 1996) and has been noted as an important consideration for neurotoxicity (Weiss, 2000).

The notion that continuous data should be considered in the context of the specific dose-response rather than *a priori* categories defined outside of the data under analysis is supported in the benchmark dose literature. Murrell et al. (1998) point out that a continuous quantity measurement such as hormone data should be scaled by the range from background response level to maximum response level (for increasing response functions). The authors go on to note that it is a biological reality that, whatever the mechanism of effect of the toxicant, there is some dose level beyond which no further change in response is seen or is theoretically feasible. In general, there is some type of limitation or saturation phenomenon that occurs at high enough doses (e.g., in the saturation of the symporter capacity, as suggested by the modeling effort in Chapter 6 and the data of Chow and Woodbury [1970] and of Meyer [1998]).

An analogy is the case of quantal data for which an effect is defined as a probability metric in which the response reaches a maximum at one. For continuous measures, the analogous extra effect can be defined as the change in effect from background standardized by the total range of response (Murrell et al., 1998). The total response range is not necessarily the response range of

response (Murrell et al., 1998). The total response range is not necessarily the response range of the observed responses in a study; rather, it is defined by a determination of the minimum and maximum possible responses according to, for example, a model equation fitted to the data as in the case of benchmark analyses. In all BMD analyses, however, the hormone BMDL estimates were shown to be extremely low (Geller, 1998a; Geller, 2001c). This may not necessarily be surprising given that hormones are operative at low doses by definition, but corresponding changes in thyroid histopathology were more consistent with the ANOVA estimates.

7.1.2.2.2 New Section 7.1.3.2 (Changes in Brain Morphometry)

In Section 4.5.1 the Agency addressed in detail various issues pertaining to the quality, consistency, variability, relevance, and interpretation of the changes observed in the developing brains of rat pups exposed to perchlorate *in utero* and perinatally. There were three studies to consider. Two of the studies were developed and submitted under contract to the defense industry or by the DoD and then submitted to the Agency (Argus Research Laboratories, Inc., 1998a; 2001). The third involved additional sectioning and measurement of brains from the Argus Research Laboratories, Inc. (2001) study and was performed under contract to the Agency (Consultants in Veterinary Pathology, 2003). In this study, the sections were controlled for depth of sectioning. EPA then performed analyses using sections matched by anatomical atlas plate number at each dose level. These new EPA analyses were performed in 2003 in response to comments received as part of the peer review process.

As before, the Agency chose to use profile analysis as a rigorous and conservative approach to evaluate the changes in brain morphometry. This MANOVA approach eliminates the possible introduction of Type I error (finding effects when there are none) posed by the repeated measures of various brain regions in each animal. The profile analysis on the new 2003 data which were controlled for depth of section, confirms the results of the previous analyses, once again indicating that dose levels of 0.01 mg/kg-day and above produce changes in the development of multiple brain structures.

An increase in the corpus callosum could plausibly represent a delay in developing brain structures because this area is known to increase in size and then decrease later during development. Neurodevelopmental toxicity suggestive of delays was also demonstrated by effects on motor activity in both the Argus Research Laboratories, Inc. (1998a) and repeated in the Bekkedal et al. (2000) study at 1.0 mg/kg-day (HEE of 0.5 mg/kg-day). The increases in

motor activity represent activity that should have subsided by these test dates. A type of hyperactivity has been noted in monkeys exposed to PCBs (Rice, 2000).

These effects on brain morphometry and motor activity are of particular concern because the relative sensitivity of laboratory animal assays to adequately characterize the types of deficits related to maternal hypothyroxinemia in large population studies is unknown (Morreale de Escobar, 2000; Haddow et al., 1999; Pop, 1999). Screening neurodevelopmental studies may not have the power to ascertain neurological effects that might result from small changes in the thyroid-pituitary hormone economy. As pointed out by Crofton (1998j), the sensitivity of animal models used to explore the role of thyroid hormones in neural development is currently equivocal. Most of the data collected and published to date were with high doses of thyrotoxic chemicals (e.a., methimazole, propylthiouracil) or with thyroidectomy. It is not known whether the available tests are capable of detecting more subtle changes in nervous system development. An analysis presented by Crofton (1998j) suggested that measurements of nervous system development are less sensitive than measurements of T4. Two reasons for this relationship were presented. First, the brain may be protected from perturbations in circulating concentrations of T4, as demonstrated by upregulation of deiodinases in brain tissue that compensate for very large decreases in circulating T4. The second reason, and one of concern in the context development of this model, is that currently available testing methods, particularly screening methods, may not be sufficiently sensitive. Recent data suggest that the battery is insensitive to alterations in thyroid hormones during development (Goldey et al., 1995a,b).

7.1.2.2.3 New Section 7.1.3.3 (Thyroid Histopathology)

Designating the 0.01 mg/kg-day dosage as a level of concern is also supported by thyroid histopathology in the database. A benchmark response level of 10% was used for each of the three indices (colloid depletion, hypertrophy, and hyperplasia). These indices were chosen by the Pathology Working Group to represent changes indicative of an impact on the HPT axis and function of the thyroid gland (Wolf, 2000;2001; Experimental Pathology Laboratories, 2000).

Changes in colloid depletion observed on PND4 in both the 1998 neurodevelopmental study (Argus Research Laboratories, Inc., 1998a) and the newer 2001 “Effects Study” (Argus Research Laboratories, Inc. 2001) were demonstrated. The BMDL estimated for those studies on PND4 was 0.33 mg/kg-day, but an estimate of 0.009 mg/kg-day is also obtained with a model demonstrating adequate fit to the data. The BMDL for colloid depletion in pups on GD21 was

0.12 mg/kg-day, but for female pups alone on GD21 was 0.04 mg/kg-day. The BMDL estimated for thyroid hypertrophy in weanling pups from the two-generation study (Argus Research Laboratories, Inc., 1999) was 0.06 mg/kg-day; whereas the BMDL estimates for colloid depletion were 0.029 mg/kg-day in the dams and 0.009 mg/kg-day in the PND5 pups. The BMDL for adult rats at the 90-day sacrifice in Springborn Laboratories, Inc. (1998) was 0.03 mg/kg-day for colloid depletion and 0.008 mg/kg-day for hypertrophy. The BMDL for hyperplasia in the F1-pups at 19 weeks was 0.0004 mg/kg-day.

The BMDL values for thyroid histopathology designated as NOAEL values on Figure 7-4 represent a range of selected estimates from some specific studies. It can be appreciated from Figure 4A-2 that the median of all studies for both colloid depletion and hypertrophy are approximately 0.1 mg/kg-day (HEE of 0.1 mg/kg-day) and for hyperplasia approximately 1.0 mg/kg-day (HEE of 0.5 mg/kg-day); whereas their 10th percentiles fall more in line with the 0.01 mg/kg-day level.

Of notable concern to this consideration is that the BMDL estimates decrease with duration in the 90-day study. The BMDL estimates for colloid depletion were 0.28 and 0.03 mg/kg-day at the 14-day and 90-day time points in the Springborn Laboratories, Inc. (1998) study. The BMDL estimates for hypertrophy were 0.017 and 0.008 mg/kg-day at the 14-day and 90-day time points. This effect of duration is of concern as it was also evident in the observation of tumors in the F1-generation adults at 19 weeks. The BMDL for hyperplasia and hypertrophy in these pups was 0.0004 mg/kg-day and 0.15 mg/kg-day, respectively. These observations in both studies suggest concern that duration may recalibrate either the homeostatic interactions of the hypothalamic-pituitary-feedback system or the cellular sensitivity and demand for the thyroid hormones.

As discussed in Section 4.4.1.2, the Agency believes that these changes in histopathology represent a biomarker for changes in other tissues (e.g., the developing fetal brain) that may be dependent on and responsive to perturbations in serum thyroid hormone levels. The EPA maintains that the three histopathological indices (colloid depletion, hypertrophy, and hyperplasia) should be considered together and each of the three may indicate perturbation of the HPT axis that is of potential consequence for neurodevelopmental sequelae and not just of the potential for tumors.

7.1.2.2.4 New Section 7.1.3.4 (Iodide Uptake Inhibition)

The single mechanistic human endpoint for comparison in the data array shown in Figure 7-4 is the BMDL estimate for iodide uptake inhibition in humans based on the 14-day Greer et al. (2002) study performed in euthyroid adult subjects. The EPA estimated the BMDL at 0.002 mg/kg-day using a 5% iodide uptake inhibition response level that is slightly higher than the degree of inhibition predicted for adult rats (1.5%) at 0.01 mg/kg-day.

The data in Figure 7-4 indicate that adverse neurodevelopmental effects (changes in brain structure) occur at an applied dose of 0.01 mg/kg-day in rats. Serum hormones in dams, adult animals in short-term studies, and in pups born to dosed animals also occur at this level. Various thyroid histopathological indices also suggest this level to be of concern.

The human study did not evaluate any neurodevelopmental outcomes. Although no serum hormone changes were measured by Greer et al. (2002), it is necessary to carefully consider that the study was limited to 37 euthyroid adults of a limited age range for a short-duration of exposure (14-days). Analyses performed by EPA suggest more complicated models may be necessary to explore interaction of dosing pattern and diurnal variation in hormones before determining that no effect has occurred (Marcus, 2003c).

Concerns were expressed at the 2002 peer review panel and confirmed by recent EPA analyses that the duration may have been insufficient and that confounding with age was also evident. Recent reviews regarding the action of thyroid hormone on development of the brain and on thyroid hormone receptors show that these effects can occur without effects on circulating serum hormone levels (Bernal, 2002; Howdeshell, 2002; Zoeller, 2003b).

7.1.2.2.5 New Section 7.1.3.5 (Other Supportive Endpoints)

As discussed in Section 4.5.2, consistent changes in motor activity in two different studies have been observed and were viewed by the 2002 peer panel and the EPA to be indicative of a functional effect of perchlorate. A BMDL of 1.0 mg/kg-day was estimated using a response level of 10% and Bayesian analysis that combined the data from both studies. This estimate corresponded well with benchmark dose analyses performed previously.

EPA considers these data to be supportive of the changes in brain morphometry and serum hormone levels and as indicative of the potential for perchlorate to cause neurotoxicity. The observation of behavioral changes at higher doses than the serum hormone and brain morphometry changes could be due to excessive variability in the results of the motor activity

studies, which may have rendered the assay relatively insensitive to disruption by perchlorate treatment, or neurological substrates of motor activity may not involve the same brain structures altered at low doses of perchlorate. Focused testing of learning, memory, or other higher-order cognitive functions may prove to be more sensitive to disruptions than the motor activity assays employed. The lack of one-to-one mapping of neurobehavior with neural substrates has recently been noted for human testing but was also considered to not impact confidence in neurobehavioral end points for demonstrating potential neurotoxicity (Bellinger, 2003).

Finally, the NOAEL for immunotoxicity suggested by the dermal contact hypersensitivity assay at 0.02 mg/kg-day can be viewed as supportive, especially because deficiencies in this study raise concern for the characterization and because a LOAEL for the effect was demonstrated at 0.06 mg/kg-day.

7.1.2.2.6 New Section 7.1.3.6 (Consideration of Other Human Data)

The ecological epidemiology studies did not evaluate any neurodevelopmental outcome measures and do not provide any additional insight beyond that of the iodide uptake inhibition reported by Greer et al. (2002). As discussed in Chapter 3 (and Chapter 4 in the 2002 ERD), the EPA has concluded that each of these observational studies suffers to different degrees from a lack of statistical power, of control for confounding, of surveillance of appropriate disease outcome measures, and of exposure characterization (see also Table 3A-1 in Appendix A of Chapter 3). These deficiencies limit their utility to reliably or accurately ascertain the point of departure.

Notwithstanding these shortcomings, these studies suggest a broad range of comparative data. Schwartz (2001) observed statistically significant changes in T4 of neonates as low as 1 ppb. Brechner et al. (2000) demonstrated increases of TSH in newborns to women exposed to perchlorate levels in drinking water in the range of 4 to 16 ppb. Comparable studies by Li et al. (2000a.b) and Lamm et al. (1999) showed no effect on congenital hypothyroidism, T4, or TSH at similar perchlorate levels in drinking water. The study by Crump et al. (2000) suggests that mean perchlorate exposure levels as high as 110 ppb are tolerated without significant changes in serum hormones. The appropriateness of this study population and thus the reliability of the conclusions have been called into question by new EPA analyses, however. Notably, the population has a high background prevalence of goiter, family history of thyroid disease, and increased urinary iodine concentrations.

It becomes difficult to fully evaluate the utility of these human data based on the point of departure alone. Instead, it may be more productive to consider the appropriate uncertainty factor that should be applied to the data identifying a particular point of departure. This would entail consideration of each laboratory animal or human study in context with its design attributes and the specific endpoints studied, and to evaluate the resultant estimates. This comparative derivation is provided in Section 7.5.

7.1.2.2.7 New Section 7.1.3.7 (Summary Regarding the Point of Departure)

Because developmental studies conducted on humans would be clearly unethical, it is necessary to utilize laboratory animal experiments to identify endpoints such as adverse neurodevelopmental consequences of thyroid toxicants. As stated in Section 4.6, while many features of the rodent endocrine system make the rat model well-suited for such neurodevelopmental studies, it is not established to be more sensitive and perhaps may be less sensitive than humans. Thus the challenge to evaluation of the laboratory animal data versus the human data becomes one of carefully considering each of the endpoints and understanding the reliability of the outcome measures used in each study.

Because the rat is considered a model appropriate for studying adverse neurodevelopmental effects of thyrotoxicants, the Agency is compelled to consider with gravity the brain morphometry and serum hormone changes at the 0.01 mg/kg-day dose level. This level is supported by the one mechanistic study available in humans (Greer et al., 2002). That study reinforced that humans are similar with respect to iodide uptake inhibition by perchlorate. It can be viewed that a similar level of iodide inhibition in humans (5%) occurs in rats (1.5%) and that this could potentially be associated with adverse neurodevelopmental outcomes. It is currently unknown whether there is a threshold level of thyroid hormone disruption necessary for altering gene expression and subsequent changes in brain structure and function in the laboratory animal models. Unfortunately, none of the epidemiological studies of perchlorate exposure have studied neurodevelopmental outcomes in the human subjects.

The essential role of thyroid hormone in brain development makes it critical to consider the possibility that mild degrees of hypothyroxinemia may impair neurological development. Thus the perturbations in serum hormones seen in the animals are a concern. Such concern is consistent with the observation that as little as a 25% decrease in maternal thyroxine during pregnancy can cause IQ decrements in offspring (Man et al., 1991; Pop et al., 1999; Haddow

et al., 1999; Morreale de Escobar et al., 2000). These data suggest that human neurological development may be sensitive to degrees of hypothyroidism or hypothyroxinemia lower than those that have been evaluated to date in typical laboratory-animal studies. The results of Andersen et al. (2002) suggest that subtle changes experienced by individuals may be important despite values being within the normal range. Models for the effects of thyrotoxicants on serum hormones will likely need to consider both the pattern of dosing and the diurnal variations of the hormones (Marcus, 2003c; Zoeller, 2003c).

Further, it is not possible to directly compare the lack of hormone changes observed in Greer et al. (2002) versus the statistically significant changes in the laboratory animals without considering that these studies – laboratory and human alike – represent single time-point snapshots of thyroid hormone economy and the status of the HPT axis. Subjects in the Greer et al. (2002) study were exposed to perchlorate for a short duration (14-days), and this may have been insufficient to challenge the HPT axis. EPA analyses show that the benchmark dose estimates decrease after 1 versus 13 days of exposure. The laboratory animal data by virtue of more frequent sampling across life stages, perhaps affords a more complete picture of the dynamic pathogenesis process elicited by perchlorate exposure.

In view of these considerations, the EPA again chooses the 0.01 mg/kg-day as its point of departure for the operational derivation of the RfD. Based on mechanistic underpinnings of the mode of action together with an evaluation of the design and endpoint differences across the studies, the Agency considers the laboratory animal and human data to be mutually supportive of this value.

7.2 USE OF PBPK MODELS FOR INTERSPECIES EXTRAPOLATION AND CHOICE OF DOSE METRIC

These comments have largely been addressed in responses provided in Chapters 2, 4 and 6. The text provided in blue below as Section 7.2.1. will revise Section 7.1.2 of 2002 ERD (Dosimetric Adjustment of Exposures Associated with Effect Levels) in the new assessment.

Comment(s): *The peer reviewers generally supported the use of PBPK models for conducting interspecies extrapolations although some suggested that the development of pharmacodynamic modeling may help to identify dose metrics more closely linked to adverse effects than blood concentration of perchlorate. Commenting specifically on the options that EPA considered for*

the dose metric, one reviewer indicated that the area-under-the-curve in blood (AUCB) of perchlorate 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 of perchlorate in thyroid tissue, 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 agreed and recommended that EPA verify that the Km selected for translocation of iodide through the apical cellular channel is consistent with that documented in a recent publication (Goldstein et al., 1995).

Though not disagreeing that AUCB as the dose metric allows for defensible interspecies extrapolations, two reviewers advocated the use of a dose metric more predictive of toxic effects. One reviewer noted that decreases in thyroid hormone levels or increases in TSH may be better indicators of adverse effects than circulating perchlorate levels. Another reviewer agreed and added that pharmacodynamic modeling can help to differentiate metrics more related to adverse effects (e.g., excess cell mitoses per unit time) from those with no risk implications.

The only other comments addressed the use of PBPK models to interpret effects observed in rats on PND4. Noting that impaired thyroid function at this life stage would most likely result from decreased transport of iodide across the placenta, one reviewer wondered how the interspecies extrapolations with PBPK models accounted for any potential differences in placental physiology between rats and humans. One reviewer responded that human PBPK models were not developed to evaluate pregnancy, fetuses, or neonates and noted that instead only a rat model was developed to evaluate these life stages. This reviewer indicated that EPA's approach for using the outputs from the rat models to extrapolate between different life stages is adequate, and he saw no other defensible approach for estimating human equivalent exposures (HEE) for various life stages. Another reviewer noted that NIS is expressed in placentas of both rats and humans, despite notable physiological differences across these species.

EPA Response(s) and Recommendation(s) for Revision(s): As discussed in Chapter 7 of the 2002 ERD, the Agency evaluated both the AUCB and the peak concentration of perchlorate in blood as the potential dose metric. The AUCB was chosen as the metric that is calculated with better accuracy when based on i.v. data and the one which essentially reflected both the blood and thyroid concentration at steady-state. As discussed earlier, the Agency also chose AUCB due to better validation of that metric, because thyroid concentrations of perchlorate had not been measured in fetuses and neonates. While choosing AUCB due to better validation of the model structures for that metric, the Agency notes, (Table 7-1 of the 2002 ERD) that the HEE based on using the AUC of perchlorate in thyroid tissue (AUCT) as the dose metric is more than two-orders of magnitude less than that based on AUCB. As discussed in Chapter 2, the Agency agrees that uncertainties in the mechanistic interaction of perchlorate with the thyroid are a concern. This decreases confidence in life stage extrapolation and will be reflected in considerations of interspecies and intrahuman variability in Section 7.3. The peer panel also

highlighted that the relationship of circulating serum thyroid hormone levels to concentrations at specific receptors in the brain as the target tissue is not well characterized.

The Agency agrees that the proposed parallelogram is a reasonable approach and that it has been used to predict effective doses for structurally related chemicals (Jarabek et al., 1994). The Agency does not disagree that a BBDR model would be informative. However, the development and validation of such a model structure, especially for different life stages, will represent a significant endeavor of additional experimentation and model iteration likely to take many years.

7.2.1 Revisions to Section 7.1.2 (Dosimetric Adjustment of Exposures Associated with Effect Levels)

Adjustments for interspecies differences in the internal dose delivered to target tissues should be made before an evaluation of the data array for valid comparisons across endpoints (U.S. Environmental Protection Agency, 1994). Because the point-of-departure analysis involved evaluation of effects at different life stages, a decision had to be made with respect to whether the dosimetry adjustment was equally robust for each life stage and, if not, which dosimetry adjustment was best to represent the array of effects. A decision also had to be made with respect to which dose metric to choose. The rationale for the Agency choices will be described in separate sections.

7.2.1.1 Revisions to Section 7.1.2.1 (Choice of dose metric)

Based on the mode of action and the available PBPK model structures, two dose metrics were considered to describe the biologically effective dose for perchlorate: (1) the area under the curve (AUC) for perchlorate in the serum associated with drinking water exposures and (2) the percent of iodide uptake inhibition in the thyroid. These correspond to the different exposure components along the exposure-dose-response continuum in the mode-of-action model (Figure 2-1 in this response and Figure 7-1 in the revised assessment).

As described in Chapter 6 of the 2002 ERD, the area under the curve of perchlorate in blood (AUCB) was developed as the first dose metric. Data used to validate the AUCB were the serum perchlorate measurements made in rats after i.v. exposures and in humans after drinking water exposures. Table 7-1 presents the human equivalent exposures (HEE) estimates calculated using the PBPK models for perchlorate AUCB as the dose metric. Table 7-2 shows the ratios for this same dose metric that can be applied in the parallelogram approach to arrive at estimates for

different life stages used to observe effects in the different experimental endpoints. Fetal rat predictions were based on data developed for GD21. Neonatal rat predictions were based on data for PND10. This approach was taken because PBPK models for human pregnancy and lactation do not exist for perchlorate distribution. The calculation using the ratios approach is described in Chapter 6. The resultant adult HEE values for the different life stages of the rat experiments are shown in Table 7-3.

It can be observed in the tables in Merrill (2001e) that the pregnant and lactating rats have significantly higher average AUCB concentrations at the lowest drinking water dose (0.01 mg/kg-day). Merrill (2001e) suggested that this is likely due to increased binding in the serum (Merrill, 2001e). It has been shown that the estrus cycle affects the concentration of binding proteins within the blood. Thyroxine, which is displaced from plasma proteins by perchlorate, is bound to a greater extent in the pregnant rat (Iino and Greer, 1961). It follows then that perchlorate would also be bound to a greater extent during pregnancy and possibly lactation. Because serum binding affects only the low doses, it is reasonable that the higher doses (1.0 through 100 mg/kg-day) would be similar across the male, pregnant and lactating rats (Merrill, 2001e). However, the higher AUCB values may also be due to inadequate characterization of perchlorate at the NIS. Inaccuracy in that characterization would be exaggerated in predictions for the fetal and neonatal life stages because of dependencies on NIS in placenta and mammary tissues.

The second dose metric considered was percent of iodide uptake inhibition in the thyroid. To predict the "transient" phase of initial iodide inhibition in the rat, i.e., before upregulation of the NIS or increases in TSH, the second dose metric was based on RAIU measurements made in adult male rats dosed with perchlorate by i.v. two hours prior to an i.v. dose of radiolabled iodide. Tables 7A-4 through 7A-7 are a comparable set of tables but are based on using thyroid uptake inhibition as the dose metric. Table 7-5 shows the percent of iodide uptake inhibition predicted at each dose for the various life stages used in the various laboratory rat experiments.

Because developmental effects are of concern, an argument could be made that peak concentrations — and not AUCB — is the appropriate dose metric based on the rationale that any transient dose could be responsible for permanent deficits. However, the Agency chose to use the AUCB values, as opposed to peak concentrations, based on the assumption that these dose metrics would represent an averaging of the serum and thyroid perchlorate concentrations and would be better correlated with the inhibition effect on iodide uptake. The correlation was

shown to be good between the AUCB and the degree of inhibition (see Figures 6-47 through 6-50 in the 2002 ERD).

Further, due to the rapid phase of distribution after an i.v. dose, concentration measurements are very difficult to attain experimentally and is more variable. Using simulated peak concentrations after i.v. injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail-vein volume and the exact time of mixing in the whole blood compartment (Merrill, 2001e). It was also observed by EPA that the ratios for peak perchlorate serum values (Merrill, 2001e: Table 6) were in good agreement with those for the perchlorate AUCB and that the AUCB resulted in slightly more conservative human equivalent exposure estimates if there was any real difference at all.

Merrill (2001e) expressed concern regarding the thyroid values in neonates and fetuses because these values were not validated against experimental data. Fetal and neonatal thyroid were never actually analyzed for perchlorate concentration. In the case of the fetus, kinetic parameters were determined by fitting model simulations of fetal thyroid concentration to available iodide data and assuming that the perchlorate:iodide ratio would be similar to that of the mother. In the case of the neonatal rat, no data were available for thyroid concentrations for either perchlorate or iodide. Thus, model predictions were based on allometrically scaling maternal parameters for thyroid uptake. It was the opinion of the AFRL/HEST authors that while the thyroid parameters in the fetus and neonatal rat were highly informative, they should not be used in the formal risk assessment (Merrill, 2001e). EPA concurs with these considerations and recommendation.

Tables 7A-3 and 7-7A demonstrate good correspondence in the HEE estimates predicted for both dose metrics at the lower doses for the lactating and neonatal rats, but not for the male adult, pregnant, or fetal rats for which there is an order of magnitude difference. The iodide inhibition metric predicts a 10-fold lower HEE in both the adult male and pregnant dam when compared to the HEE estimated based on the serum AUCB. The fetal rat value for iodide inhibition was viewed as unreliable for the reasons stated above. All of the factors influencing this disparity are not fully appreciated at this time but can reasonably be ascribed to uncertainty in the thyroid descriptions that were not validated with experimental data and will require additional studies to characterize accurately. Because there was good agreement between the AUCB and percent iodide inhibition at the dose levels associated with effects near the point of

departure, the AUCB dose metric was chosen as the basis for the adjustment factor to arrive at an HEE estimate.

7.2.1.2 Revisions to Section 7.1.2.2 (Choice of Representative Life Stage)

As described above, there is some uncertainty in the thyroid descriptions in the fetus and neonate due to lack of experimental validation. The uncertainty may also be due to some inaccuracy in the description of perchlorate at the NIS which would also include the placenta and mammary tissue for the fetus and neonate, respectively. Consideration of these uncertainties led the Agency to choose the adult pregnant rat as the representative life stage for dosimetric adjustment of the data in dams, fetuses, and neonates from the developmental neurotoxicity studies. This decision is supported by maternal blood levels likely influencing the fetus *in utero,* and the resultant changes in brain morphometry. Dams on GD21 were shown to be hypothyroid (with statistically-significant decreases in T4 and increases in TSH). Because the temporal windows underlying the neonatal brain morphometry effects are unknown and because the brain morphometry effects may have occurred *in utero* due to the dams' hormone deficiency, the HEE estimate for dams of 0.01 mg/kg-day was chosen for the operational derivation. This choice was not as conservative as using the HEE for iodide inhibition in the dams (0.002 mg/kg-day), but it is viewed as more accurate given concerns for the reliability of the thyroid estimates. Thus, adjustment based on the dosimetry in the pregnant dam for the serum hormone data on GD21 and fetal endpoints is considered appropriate. Dosimetry adjustment based on the adult male rat for the serum hormone changes at the 14-day and 90-day sacrifices would typically be on the order of 2-fold higher and are considered essentially the same as the pregnant rat. Choice of the pregnant rat dosimetry adjustment is thus applied to the point of departure for the entire database.

7.3 CONSIDERATIONS FOR APPLICATIONS OF UNCERTAINTY FACTORS

The Agency received a wide range of comments on the magnitude of the uncertainty factors applied in the operational derivation of the RfD. The suggestions spanned the range from a composite UF of 10 to a composite UF of at least 1000. Responses are provided to comments on each individual UF. The Agency's recommended new values and revision to Section 7.1.4

(Application of Uncertainty Factors) of the 2002 ERD for the revised assessment are provided in Section 7.3.6 of this response document.

7.3.1 Comments on Intrahuman Variability

This section presents comments that were received on the UF of 3 proposed in the 2002 ERD for intrahuman variability.

Comment(s): *The peer reviewers generally supported the Agency's proposed UF of 3 for intraspecies variability. One reviewer noted that the Agency often assigns a factor of 10 for this area, but instead proposed a factor of 3 based on the variability observed in the data and PBPK modeling for the adult humans. He commended the EPA for using the PBPK modeling to derive an appropriate UF, though he noted that the 2002 ERD did not describe exactly how the Agency arrived at the factor of 3. Another reviewer, when reviewing the draft of peer panel report, indicated in post-meeting comments that the EPA could rationalize using an uncertainty factor of 10 for intraspecies variability. Regarding EPA's comment that the data from human subjects "...do not represent kinetic data for potentially susceptible populations of the hypothyroid and hypothyroxinemic pregnant women and their fetuses," another reviewer recommended that the Agency refer to recent publications from the NAS Committee on Reference Dietary Intakes for alternate approaches regarding consideration of medically disadvantaged groups receiving treatment as potentially susceptible populations. The reviewers discussed susceptibility to perchlorate in greater detail when responding to charge question F.4 (see Section 7.4 below). Submitted public comments noted that the typical UF for intrahuman variability is 10 and not 3. Explicit concern was also expressed about uncertainty with respect to fetal and neonatal dosimetry and susceptibility, noting that fetuses and neonates require 7.5 to 15 times the amount of iodide as that required by adults and are particularly and permanently sensitive to iodide deficiency.*

EPA Response(s) and Recommendation(s) for Revision(s): The original rationale for the UF of 3 proposed by the Agency in 2002 for intrahuman variability was the view that the use of the PBPK models had addressed the pharmacokinetic (PK) component to a significant degree. As explained in Section 7.1.4 of the 2002 ERD, the uncertainty factors for intrahuman variability and interspecies extrapolation are thought to each be composed of both a PK component and a pharmacodynamic (PD) component. Often the factors for intrahuman variability and interspecies extrapolation are considered together given this commonality of components. Discussion at the peer review suggested that the combination of these two factors is warranted because the EPA was encouraged to combine these data to identify the point of departure. The Agency agrees that considerations of uncertainty within each of these components for both

laboratory animal data extrapolation and with respect to intrahuman variability need to be revisited based on these comments.

Regarding the uncertainty in the PD component, the threshold for thyroid hormone disruption necessary for altering gene expression and subsequent changes in brain structure and function is unknown. Evidence from the human literature suggests that the rat may be less sensitive as a model. Data suggest that human neurological development may be sensitive to degrees of hypothyroidism or hypothyroxinemia lower than that established in laboratory animal models. Thus a PD component should be considered.

Regarding pharmacokinetics, the peer panel highlighted that the relationship of circulating serum thyroid hormone levels to concentrations at specific receptors of the brain as the target tissue is not well characterized. Thus, there exists some uncertainty in the use of AUCB as the dose metric and this invoked consideration of application of a factor for the PK component.

The human PK data are limited to euthyroid adult subjects. Thus, consideration of human variability, notably the lack of characterization of fetal and neonatal dosimetry, would also warrant application of an uncertainty factor for PK. Fetal and neonatal sensitivity are particularly important considerations, noting that the lack of characterization of the interaction of perchlorate at the NIS at the thyroid also applies in the placenta and mammary tissue. Because fetuses and neonates are especially dependent on the transfer of iodide by these tissues and because they represent sensitive life stages with respect to tissue response, a factor for uncertainty in both the PK and PD components should be considered. Additional uncertainties applicable to human variability include sensitivities resulting from iodine deficiency or compromised thyroid status, along with genetic variations in the NIS, These have not been addressed or quantified. Uncertainty in the interspecies extrapolation also exists due to the lack of adequate validation with human data for these life stages.

7.3.2 Comments on Interspecies Extrapolation

This section presents comments that were received on the UF of 1 proposed in the 2002 ERD for uncertainty in interspecies extrapolation.

Comment(s): *The reviewers expressed differing opinions on whether an uncertainty factor for interspecies extrapolation is necessary. One reviewer 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 to not use an interspecies uncertainty factor. Another reviewer agreed and supported the Agency's proposed approach for interspecies extrapolation. Two reviewers wondered if rodents are more sensitive to perchlorate exposure than humans and said an interspecies UF of less than one might be warranted. 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 UF of 0.1 or even lower may be defensible. Another reviewer acknowledged that rats and humans have notable differences in thyroid physiology but cautioned that researchers have not clearly established differential sensitivity to perchlorate exposure.

EPA Response(s) and Recommendation(s) for Revision(s): The Agency had originally proposed a factor of 1 for the UF applied to address interspecies extrapolation in the 2002 ERD. However, this engendered considerable debate at the time and has also received a number of comments in the peer review process. While the view was held that the PBPK model adequately described the PK in the rats, concerns included the lack of confidence in the dose metric for the fetal and neonatal lifestages as described in the Section 7.2. These concerns are now augmented by discussions by the peer review panel regarding the description of the interaction of perchlorate at the NIS in the thyroid and in other tissues, notably those relevant to fetal and neonatal dosimetry (placenta and mammary tissue).

The Agency disagrees that the interspecies UF should be based on thyroid hormone rather than iodide inhibition as the key event. The data demonstrate and the PBPK models predict similar degrees of iodide inhibition between adult rats and humans. The inhibition in iodide uptake is the most proximate effect caused by perchlorate exposure and to decrements in T4 that could result in permanent neurodevelopmental damage. Because early biological effects are more prevalent in a population at risk than the late events of historical interest (e.g., morbidity and mortality or, in this case, hypothyroidism) and may be more specific to the exposure than the outcome itself, molecular epidemiology is currently utilizing the precision afforded by the use of this type of mechanistic data to improve the etiologic classifications of environmental disease (Hattis, 1986; Cullen, 1989; Hulka and Wilcosky, 1988).

7.3.3 Comments on LOAEL to NOAEL Extrapolation

This section presents comments that were received on the UF of 10 proposed in the 2002 ERD for uncertainty in extrapolation of the LOAEL used in the operational derivation of the RfD to a NOAEL.

Comment(s): *The peer reviewers had few comments on this element of the composite UF. Three reviewers acknowledged that EPA typically applies a factor of this magnitude when extrapolating a LOAEL to a NOAEL. Aside from expressing concerns about the general practices of assigning exact numerical figures to this type of uncertainty (see general comments in Section 7.3.6 below), none of the reviewers questioned the proposed use of this UF. In the event that EPA would base its operational derivation on human data, one reviewer said that this factor may not be necessary given that the authors of the Greer study (Greer et al.,2000,2002) report identifying a NOAEL.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency does not recommend any changes in the UF used in the operational derivation of the harmonized RfD (see Section 7.5 below) in response to these comments. The application of a 10-fold factor for extrapolation of a LOAEL to NOAEL is standard and warranted when considering effects such as those on brain morphometry, thyroid histopathology, and serum hormones observed in the database.

The Agency also agrees that if the operational derivation were to be based on the human clinical data as discussed in Section 7.1.5.1. (Comparison with Operational Derivation Based on Human Data) of the 2002 ERD, then the use of the lower limit on the benchmark dose (BMDL) of 0.002 mg/kg-day could serve as a surrogate for the NOAEL and obviate this UF. This would represent a change to the UF of 3 for extrapolation of the minimal LOAEL at 0.007 mg/kg-day as proposed in the 2002 ERD.

7.3.4 Subchronic to Chronic Duration

This section presents comments that were received on the UF of 3 proposed in the 2002 ERD for uncertainty in the use of short-term data rather than chronic data as the basis of the derivation.

Comment(s): *Some peer review panel members supported the proposed UF for the lack of chronic exposure study while others did not. The discussion leader indicated that the longest-duration study (90-days) did not provide convincing evidence that the exposure over longer durations will not reveal additional effects. Another reviewer agreed and added that the presence of tumors following 19-weeks of study merited concern. Though he was not concerned that the tumors in rats are relevant to humans, another reviewer indicated that no data have convinced him that in utero programming of the HPT axis does not occur and as a result he indicated that the proposed UF may be justified. Another reviewer 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 UF for the thyroid tumors was 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. This reviewer concluded that the UF for subchronic to chronic exposure duration was not justified.

Three reviewers also commented on whether EPA should consider a UF for exposure duration if the Agency chooses to base its point of departure on the human data. Two reviewers indicated that a 10-fold UF would be warranted if the human data were used, given that the longest duration in a controlled study was 14-days. Another reviewer noted that a lower UF may be appropriate, particularly if epidemiological studies provide perspective on the implications of chronic exposure.

EPA Response(s) and Recommendation(s) for Revision(s): In response to these comments, the Agency first wishes to clarify that the UF of 3 for extrapolation of subchronic to chronic data was applied in the operational derivation of the RfD proposed in the 2002 ERD because the observation of tumors at 19 weeks indicated a potential for *in utero* programming. The Agency wishes to reinforce, as described in responses provided in Chapter 4, that the tumors were demonstrated to be statistically significant and are considered biologically significant. Existing Agency guidance has established their relevance to human health risk assessment (U.S. Environmental Protection Agency, 1998b). The Agency also wishes to clarify a misconception expressed by a panel member that the pups in the F1 generation that exhibited tumors had not incurred additional exposures. The F1 pups had been used to parent the F2 generation and thus were exposed until sacrifice at 19 weeks.

The original strategy sought to obviate the need for a 2-year study by determining a NOAEL in rats for thyroid histopathology as a precursor lesion to tumors in a 90-day study. However, the finding in a multi-generation study of a statistically-significant increased incidence in thyroid tumors in F1-generation rat pups at 19 weeks (P2, second parental generation) with a dramatically reduced latency called this premise into serious question, especially in light of an emerging appreciation of findings suggesting a phenomenon known as *in utero* programming with endocrine disruption (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). The increase in incidence and decrease in latency were statistically significant when compared to all of the archived NTP historical data for this type of tumor in this strain and sex of rats. Thus, *in utero* disruption of thyroid hormones in the developing fetus may predispose the developing neonate and adult to future environmental insults to the thyroid gland by making the fetus more sensitive. Weiss (2000) has noted that changes in brain functions occur throughout life and some consequences of early damage may not even emerge until advanced age. This could be

exacerbated if environmental insults to the thyroid were to be continued throughout life. The 2002 peer panel agreed and recommended that any additional testing consider a womb-to-tomb design (i.e., a 2-year chronic study that starts with exposures *in utero*) at a 10-fold lower dose than what currently exists in the database.

The concern for *in utero* programming is exacerbated by the observation that the NOAEL/LOAEL estimates for hormone perturbations and histopathology decrease between the 14-day and 90-day time points. This is more evidence to suggest that a recalibration of the regulatory feedback system or changes in cellular sensitivity and demand for thyroid hormones with extended exposures may occur. A duration dependence is also evident in the EPA analyses performed on the RAIU and hormone data of the Greer et al. (2002) study. These analyses were pursued to address concerns expressed by the 2002 peer review panel that the duration of the Greer study was insufficient to really test the HPT axis.

The Agency agrees that this subchronic-to-chronic extrapolation factor could also be viewed as part of an uncertainty for database deficiency because there are no long-term bioassays of perchlorate with contemporary design and data quality to address potential neurodevelopmental effects due to chronic perchlorate exposures.

7.3.5 Comments on Database Insufficiency

This section presents comments that were received on the UF of 3 proposed in the 2002 ERD for uncertainty due to data gaps.

Comment(s): *The majority of peer panel members did not support this UF if it was based solely on the lack of information on immunotoxicity. The peer reviewer assigned to immunotoxicity did not support the UF to account for database insufficiencies relevant to potential immunotoxic effects, particularly 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 UF. Moreover he questioned the relevance of 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. He concluded that the UF for database insufficiencies was unwarranted.*

Another reviewer viewed the partial UF of 3 for lack of chronic data and the partial UF of 3 for insufficiency on immunotoxicity 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 endpoints (e.g., reproductive, developmental, neurotoxic) EPA should instead use an overall database UF of 1 if the point of departure was based on laboratory

animal data. Other reviewers felt that the database factor could be justified for insufficiencies other than immunotoxicity.

EPA Response(s) and Recommendation(s) for Revision(s):

Two chronic bioassays in different species, two developmental studies in different species, and a two-generation reproductive study are usually required for derivation of an RfD with high confidence. The testing strategy for perchlorate also placed a high priority on some additional studies based on the mode of action for perchlorate. These included developmental neurotoxicology (DNT) studies, some pharmacokinetic studies, and evaluation of immunotoxicity. The concern over the lack of chronic bioassays has been discussed above under uncertainties in subchronic to chronic extrapolation.

As discussed in Section 4.7, the EPA applied a partial factor of 3 for database deficiency in the 2002 ERD based on the concern that an endpoint that was specifically indicated in the original testing strategy had not been characterized adequately. The relationship of the immunotoxicological endpoint to other endpoints (e.g., thyroid hormone) is not the issue — rather the rationale for application of this particular UF is whether a key endpoint has been adequately characterized, i.e., does an uncertainty remain regarding adequacy of the database on a given endpoint. However, the 2002 peer panel was not convinced of the seriousness of this data gap and the majority recommended that this factor be dropped. As will be discussed below, the Agency has seriously considered this recommendation.

7.3.6 General Comments on Uncertainty Factors

Comment(s): *One final general comment that was made at the peer review regarding uncertainty factors was that some of the peer panel members prefer other approaches rather than applying simple, multiplicative factors to address uncertainty in RfD derivations. One reviewer thought that the use of generic uncertainty factors implies that risk assessors lack an understanding of the toxicity mechanisms. One reviewer suggested the use of Bayesian model averaging or Monte Carlo modeling to derive a probability distribution for the point of departure rather than applying 10-fold or 3-fold factors that do not appear related to any physiological process. Another reviewer suggested establishing confidence intervals for uncertainty factors so that the composite factor can be expressed as a range rather than as a single number.*

EPA Response(s) and Recommendation(s) for Revision(s): The Agency believes that the application of partial factors in this assessment has been based explicitly on an appreciation of the mode of action and the overlap of uncertainties. Bayesian modeling of all of the responses will be precluded due to the differences in study designs and lack of overlap in the domains and endpoints tested. Bayesian modeling was applied to the neurobehavioral studies that utilized similar outcome measures and experimental design. The Agency agrees that Monte Carlo modeling of the pharmacokinetic parameters input to the various model structures could prove to be a useful exercise.

7.3.7 Recommendations for Revised UF and New Section 7.1.4 (Application of Uncertainty Factors)

Based on the consideration of the peer panel and pubic comments on the choice of uncertainty factors, the Agency proposes the following changes to Section 7.1.4 of the 2002 ERD for the final assessment.

The types of uncertainty factors (UF) applied for various extrapolations required to arrive at a reference dose were discussed in Chapter 3 (of the revised assessment). Figure 7-5 illustrates schematically that the interspecies and intraspecies UFs embody attributes of both uncertainty and variability. A factor for variability across humans typically is applied to account for potentially susceptible portions of the population. As shown in Figure 7-6 (Jarabek, 1995b), both of these factors may be broken into components of approximately three each for pharmacokinetics (toxicokinetics) and pharmacodynamic (toxicodynamic) processes. This scheme is consistent with that used by the World Health Organization (WHO) (Jarabek, 1995b).

As in the 2002 derivation, EPA is recommending a total of four (4) uncertainty factors applied in this derivation, resulting in a composite factor of 300. The Agency has, however, revised which factors and the magnitude applied for each as described below. The partial factors of 3 represent "halving" of each UF that is believed to be an upper bound on a lognormal distribution; i.e., $10^{0.5}$ (U.S. Environmental Protection Agency, 1994).

The Agency is now recommending a 10-fold factor combined to account for intrahuman variability and to address concerns regarding uncertainties in interspecies extrapolation. Each of these factors would usually be a 10, and a reduced factor as applied in the 2002 ERD was still considered warranted, but the Agency now recongizes concerns with respect to uncertainties underlying both the pharmacodynamic and pharmacodynamic components of each factor.

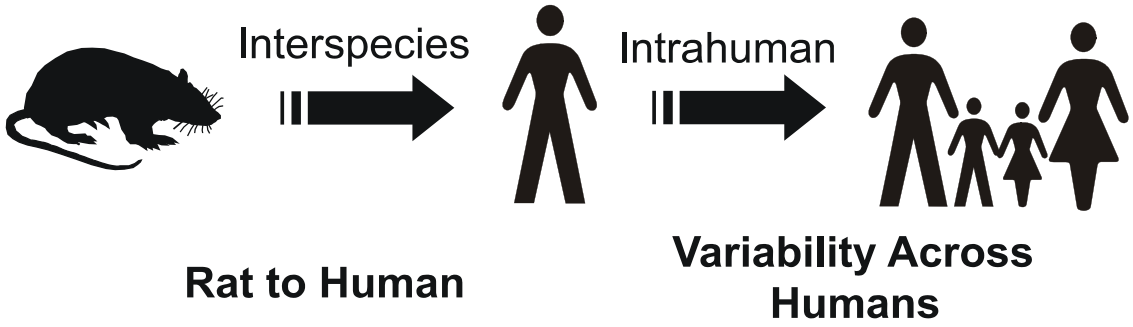

Figure 7-5. Consideration of uncertainty and variability influence interspecies and intrahuman extrapolation.

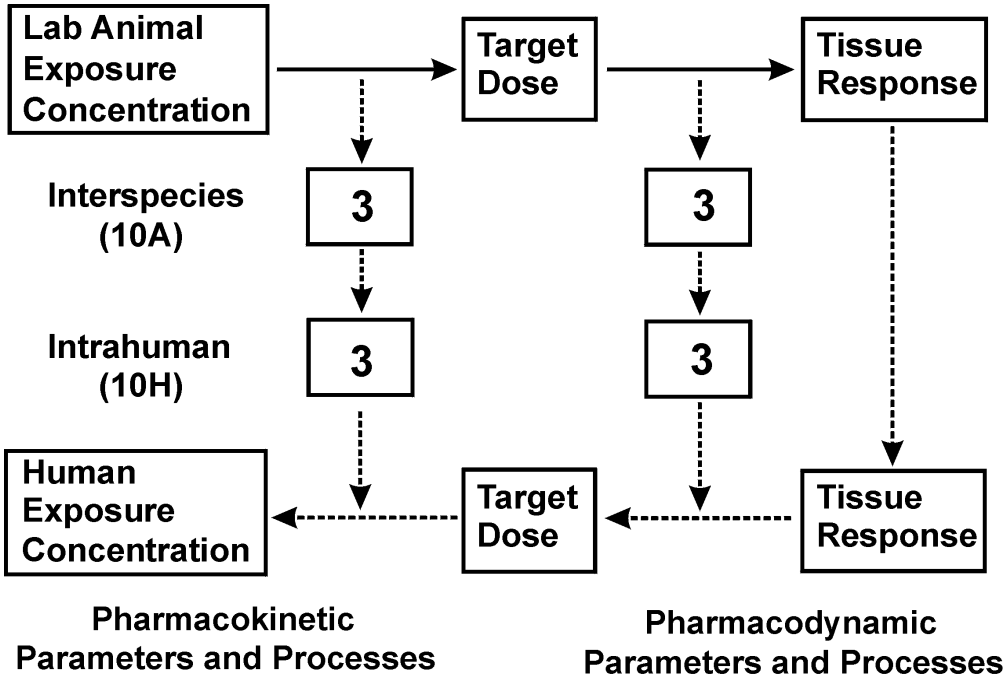

Figure 7-6. Schematic of uncertainty factor components incorporated into exposure-dose-response characterization for interspecies and intrahuman extrapolations (Jarabek, 1995b).

Because laboratory animal and human data are combined to identify the point of departure, uncertainty in the these data to represent potential intrahuman and interspecies variability warrants further analysis. With respect to the laboratory animal data, the threshold for thyroid hormone disruption necessary for altering gene expression and subsequent changes in brain structure and function is unknown. Evidence from the human literature suggests that the rat as a model may be less sensitive. Data suggest that human neurological development may be sensitive to degrees of hypothyroidism or hypothyroxinemia lower than have been established to date in laboratory animal models. The peer panel also highlighted that the relationship of circulating serum thyroid hormone levels to concentrations at specific receptors of the brain as the target tissue is additionally not well characterized. Thus, there exists some uncertainty in the use of AUCB as the dose metric.

The human pharmacokinetic data were limited to euthyroid adult subjects. Thus, consideration of human variability, notably the lack of characterization of fetal and neonatal dosimetry warrants application of an uncertainty factor. As noted previously, uncertainties remain regarding NIS dosimetry. This uncertainty applies to the placenta and mammary tissues as well. Uncertainties also remain regarding adequate characterization of the risks to sensitive populations, due to compromised thyroid status, iodine deficiency, or genetic susceptibility.

A full factor of 10 is again recommended for the extrapolation of the LOAEL values observed at the point of departure to NOAEL values. The LOAEL levels were based on changes in brain morphometry and serum hormones. Designating these changes to be adverse is consistent with the proposed mode of action and existing Agency guidance and procedures. The neurotoxicity assessment guidelines (U.S. Environmental Protection Agency, 1998a) specify any changes in brain structure as adverse. The Office of Pollution Prevention and Toxic Substances has used thyroid hormone changes to designate adverse effect levels. Finally, the shallow slope of the response curve at these lower levels suggests that a full factor of 10 should be applied.

The Agency is applying a 3-fold factor for uncertainties due to extrapolation of studies with less than chronic duration and for database insufficiencies combined. As shown in Table 3-6 of the 2002 ERD, two chronic bioassays in different species, two developmental studies in different species, and a two-generation reproductive study are usually required for derivation of an RfD with high confidence. As shown in Table 3-7 of the 2002 ERD, a ten-fold factor is typically applied for each of these areas. These tables are provided as Tables 7B-1 and 7B-2 in Appendix 7B for ready reference.

The original strategy sought to obviate the need for a 2-year study by determining a NOAEL in rats for thyroid histopathology as a precursor lesion to tumors in a 90-day study. However, the finding in a multi-generation study of a statistically-significant increased incidence in thyroid tumors in F1-generation rat pups at 19 weeks (P2, second parental generation) with a dramatically reduced latency called this premise into serious question, especially in light of an emerging appreciation of findings suggesting a phenomenon known as *in utero* programming with endocrine disruption (Prins et al., 2001; Phillips et al., 1998; Seckl, 1997). The increase in incidence and decrease in latency were statistically significant when compared to all of the archived NTP historical data for this type of tumor in this strain and sex of rats.

The concern regarding the potential for *in utero* programming is exacerbated by the observation that the NOAEL/LOAEL estimates for hormone perturbations and histopathology decrease between the 14-day and 90-day time points. This provides further evidence suggesting that a recalibration of the regulatory feedback system or changes in cellular sensitivity and demand for thyroid hormones with extended exposures may occur. Thus, *in utero* disruption of thyroid hormones in the developing fetus may make the fetus more sensitive, predisposing the developing neonate and adult to impacts from future environmental insults to the thyroid gland. Weiss (2000) has noted that changes in brain functions occur throughout life, and some consequences of early damage may not emerge until advanced age. This could be exacerbated if environmental insults to the thyroid are continued throughout life. The 2002 peer panel agreed and recommended that any additional testing consider a womb-to-tomb design (i.e., a 2-year chronic study that starts with exposures *in utero*) at a 10-fold lower dose than what currently exists in the database. Thus, this factor can also be viewed as part of a database deficiency because there are no long-term bioassays of perchlorate with contemporary design and data quality.

The subchronic-to-chronic extrapolation factor was combined to cover concerns regarding the potential for perchlorate to cause contact hypersensitivity. As discussed in Section 4.7, the Agency considers that full characterization of immunotoxicity remains a data gap contributing to residual uncertainty with respect to the adequacy of the database. However, the 2002 peer panel was not as convinced of the seriousness of this data gap as a stand alone concern. The majority recommended that this factor be dropped and the Agency has therefore subsumed this uncertainty under one factor. The database uncertainty factor now addresses both the lack of

chronic data and the lack of immunotoxicity characterization, thereby eliminating the 3-fold factor applied for the immunotoxicity data gap alone.

In summary, after reconsideration of the data and comments, EPA views a composite UF of 300 as appropriate. Factors are applied to account for: uncertainties in the extrapolation of laboratory animal data together with addressing the potential variability of dosimetry and responses across life stages within humans; extrapolation of a LOAEL; and residual database uncertainty regarding the lack of chronic data and an incomplete immunotoxicological database. Because partial factors are applied in an attempt to address the overlap among the factors, this composite factor is not considered to be unduly conservative.

7.4 FACTORS INFLUENCING SUSCEPTIBLITY

This section provides the disposition of comments and recommendations for revision based on comments received from the 2002 peer panel and from public and stakeholder submissions regarding the topic as posed in Charge Question F.4 — Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Comment(s): *The reviewers had multiple responses to how EPA identified susceptible populations and whether additional ones should be considered. Regarding EPA's approach, two reviewers indicated that they would have preferred identifying susceptibilities based on mechanistic arguments. One reviewer suggested that EPA should identify susceptibilities from insights on the most relevant biochemical events and how these differ among subpopulations. The other reviewer 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). A third reviewer noted that EPA could address potential susceptibility directly in its benchmark dose calculations by using mixture models that explicitly account for susceptibilities in their calculation. Other reviewers identified the following potential susceptibilities for EPA to consider in the human health dose-response assessment: genetic variation in NIS across the population; 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).*

EPA Response(s) and Recommendation(s) for Revision(s): The EPA does not find the data sets rich enough to consider mixture modeling. The Agency will enhance the description of the mechanistic motivation for the susceptibilities listed, and augment those listed in the 2002 ERD

with the additional suggestions provided by the peer panel. The following text will be used to replace Section 7.1.5.3 (Possible Susceptibility) in the revised assessment.

The mode of action for perchlorate toxicity is the inhibition of iodide uptake at the sodium(NA^+)-($Iodide^-$) symporter (NIS) present in various tissues including the thyroid, GI tract, placenta, and mammary tissue. Inhibition of iodide uptake results in perturbations of thyroid hormone homeostasis. Potential toxicity due to this perturbation include both neurodevelopmental effects and neoplasia.

Susceptible populations can be identified based on the mode of action. Individuals with genetic variants of the NIS may be at risk due to its critical role in iodide uptake in these tissues. Pregnant women have an increased demand for iodine, and thyroid hormone homeostasis is critical for them to protect their fetuses. The fetus is especially vulnerable to thyroid hormone deficiencies due to the essential role these hormones play in development. The fetus also has na increased demand for iodine. The thyroids of neonates have been demonstrated to be insufficient to supply thyroid hormone for a single day. Individuals who are iodine deficient are also susceptible, as are people with compromised thyroid function, often including the elderly (especially women), hypothyroxinemic and hypothyroid individuals (either due to primary dysfunction of the gland or decreased hormones secondary to systemic disorders), and people treated with anti-thyroid drugs or with other anti-thyroid exposures. These groups may be more susceptible than the general population to the effects of perchlorate. Patients with cardiac dysfunction or elevated levels of cholesterol may also be at risk.

7.5 RECOMMENDATIONS FOR REVISION TO SECTION 7.1.5. (OPERATIONAL DERIVATION OF HARMONIZED RFD)

As discussed in Section 7.1.2, the pregnant rat was used as the life stage for the dosimetric adjustment to a human equivalent concentration (HEE) of the various effects used to designate the point of departure at 0.01 mg/kg-day. The resultant HEE is 0.01 mg/kg-day.

According to Dollarhide (1998), who spoke with Argus laboratory on behalf of the sponsor (PSG), the reported doses were of ammonium perchlorate and not the anion itself. Thus, an adjustment for percent of the molecular weight of the salt from ammonium (15.35%) must also be made. Further, because the analytical methods measure the anion concentration in

environmental samples, this is the appropriate expression of the RfD for risk characterization. The derivation of an RfD for the perchlorate anion as itself is as follows:

$$0.01 \text{ mg/kg-day} \times 0.85 / 300 = 0.00003 \text{ mg/kg-day}. \quad (7\text{-}1)$$

Note that the appropriate adjustment for any salt of perchlorate (e.g., adjustment by a factor of 0.72 for potassium perchlorate) should be made when evaluating toxicity data for similar assessment activities.

It is critically important to distinguish the proposed RfD from any guidance value that may result. An RfD would be only one step in the future regulatory process. The RfD can assist in determining, based on a variety of elements, whether a drinking water standard for perchlorate is appropriate. As with any draft EPA assessment containing a quantitative risk value, that risk estimate is also draft and should not be construed at this stage to represent Agency policy. The units for an RfD are mg/kg-day. Conversion of an RfD to a drinking water equivalent level (DWEL) is based on adjusting by body weight (kg) and drinking water consumption (L) to arrive at a level expressed in units of mg/L (ppb). Derivation of a maximum contaminant level goal (MCLG) from a DWEL by the EPA Office of Water typically involves the use of a relative source contribution (RSC) factor to account for non-water sources of exposures such as those discussed in Chapters 8 and 9 (of the revised assessment).

Because the critical effect is considered to be the result of neurodevelopmental deficits resulting from the hypothyroid or hypothyroxinemic state induced by the mother's exposure and because developmental neurotoxicity may emerge later in the life or be exacerbated later in life, conversion factors for the adult of 70 kg body weight and 2 L of water per day are considered appropriate.

Recent guidance from the OW in its Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (U.S. Environmental Protection Agency, 2000) provides a decision flow chart for derivation of the RSC and recommends 80% as a ceiling and 20% as the floor for this factor when data are adequate to estimate sources of exposure. When data are not adequate to estimate other anticipated exposures, OW recommends a default RSC of 20%. (U.S. Environmental Protection Agency, 2000: Chapter 4, Section 4.2.2.4 on apportionment decisions). EPA does not recommend that high-end intakes be assumed for every

exposure source because the combination may not be representative of any actually exposed population or individual.

A hypothetical adjustment of the 0.00003 mg/kg-day RfD by 70 kg and 2 L would thereby result in a DWEL of 1 ug/L (ppb) and application of an RSC between 0.2 to 0.8 would thereby result in an MCLG in the range of 0.2 to 0.8 ug/L (ppb). These values are in the range of current analytical capabilities. As discussed in Chapter 1, improvements to the analytical methods on the near horizon or expected to be published this spring could result in minimum reporting limits in this range and lower (Hedrick, 2003).

Concern is often expressed in the regulatory arena for the potential added susceptibility of children in developing DWEL estimates based on different conversion factors (15 kg and 1 L). Consequently, the EPA asked for additional PBPK simulations to help inform this dialogue. As shown in Table 7A-1, the HEE estimates for a 15 kg human for perchlorate AUCB can be as great as two-fold higher than those predicted for the 70 kg human due to differences in distribution volumes and excretion. Thus, if the 15 kg and 1 L values are used to convert this 2-fold higher HEE value in an analogous derivation to the adult RfD derivation and DWEL calculation above, an estimate of 1 ppb results, equivalent to the adult conversion.

7.5.1 Revision to Section 7.1.5.1 (Comparison with Derivation Considering Human Data)

The point-of-departure analysis presented in Section 7.1 integrated considerations of both the available laboratory animal and human data together. As described in that section and in more detailed analyses presented in Chapter 3 (Chapter 4 of the revised assessment), the EPA felt that both the observational epidemiological and the human clinical studies have significant scientific and technical limitations that preclude their use as the basis for a quantitative dose-response assessment. The clinical study subject attributes (euthyroid adults) and study design issues (sample size, RAIU time points, etc.) made these data less reliable than the laboratory animal toxicological data to ascertain effect levels pertinent to an RfD derivation.

These issues not withstanding, a dose of 0.002 mg/kg-day has been estimated by the EPA based on the data of Greer et al. (2002) and Merrill (2001a). If this study were to be considered for operational derivation of the RfD in lieu of dosimetric adjustment of the effect levels observed in the laboratory animal data using PBPK modeling, the following would need to be considered.

This is a relatively limited data set of small sample size in euthyroid adult subjects. Variability in the data were noted and effects of the covariates of age and duration of exposure were demonstrated but not addressed. As discussed in Section 7.1, the same level of iodide inhibition estimated as the BMDL in based on Greer et al. (2002) was associated with brain morphometry changes and effects on serum hormones in the laboratory animals. The human evidence suggests that the rat model may not be as sensitive human neuropsychological studies for evaluating adverse neurodevelopmental outcomes. The fetus and neonate have different dosimetry than adults because they are dependent on iodide delivery via uptake by the NIS in the placenta and mammary tissues. These dosimetric differences exacerbate enhanced tissue sensitivity in these same life stages. Because the human dosimetry for these life stages has not been characterized well and because laboratory animal models suggest effects at the same level of inhibition, a full factor for intrahuman variability to address uncertainty in both PK and PD is warranted.

Another major concern with the use of these human data is the short exposure duration. The EPA analyses indicate that duration is an important covariate for effects on both the RAIU and the serum hormones. This concern is exacerbated by the lack of chronic studies in the entire database and the suggestion in laboratory animal models that in utero programming occurs. Further, the suggestion of biologically significant effects on the serum hormones when the pattern of dosing and diurnal variation are used as covariates (Marcus, 2003c) can not be completely ignored. Based on these considerations, a full factor of 10 for database deficiencies and duration extrapolation is appropriate.

Thus, a derivation based on the available human data would estimate the RfD at a maximum of 0.00002 mg/kg-day, an estimate in rather good agreement with that proposed based on the laboratory animal data (0.00003 mg/kg-day).

The consistency between the estimates based on the laboratory animal versus the human data is likely due, at least in apart, to the use of AFRL/HEST PBPK modeling (Merrill, 2001c,d; Clewell, 2001a,b) to perform the interspecies extrapolation rather than the use of default factors. It should be noted that the original motivation for performing these human studies (as discussed in Chapter 3 of the 2002 ERD) in the perchlorate testing strategy was to support such interspecies pharmacokinetic extrapolation and not to derive NOAEL estimates for thyroid effects in the human population.

In conclusion, the fact that these two RfD estimates are essentially the same regardless of whether they are based on the human or laboratory animal data provide greater confidence than would the laboratory animal data alone that the RfD proposed by the Agency will be protective of human health.

7.5.2 Revisions to Section 7.1.5.2 (Comparison with Derivation Based on Tumor Data)

To address neoplasia as the other potential adverse endpoint, this section will discuss how an estimate could be derived based on consideration of the thyroid tumor and histopathology data.

7.5.2.1 Revisions to Section 7.1.5.2.1 (Choice of Dose-Response Procedure)

As discussed in Chapter 5, the genotoxicity assays included in the testing strategy determined that perchlorate was not likely to be mutagenic. This was one of the critical determinants in deciding on a dose-response approach for a cancer derivation. The EPA guidance on assessment of thyroid follicular cell tumors (U.S. Environmental Protection Agency, 1998a) sets forth data needs to establish the default dose-response procedure that should be used to establish that a chemical has antithyroid activity (i.e., that it is disrupting the thyroid-pituitary hormone status). Table 7-8 lists the default procedures for thyroid carcinogens that would be used. The thyroid lesions observed (colloid depletion, hypertrophy, and hyperplasia) are among the required lesions to demonstrate antithyroid activity. Table 7-9 shows the types of data required.

Table 7-2. Table 7-8 in Revised Document.
Default Dose-response Procedures for Thyroid Carcinogens
(U.S. Environmental Protection Agency, 1998a)

	Array of Effects		
Example	Mutagenic	Antithyroid	Dose-Response Methodology
1	Either or both unknown		Linear
2	Yes	No	Linear
3	No	Yes	Margin of exposure
4	Yes	Yes	Linear and margin of exposure

Table 7-3. Table 7-9 in Revised Document. Data Demonstrating Antithyroid Activity U.S. Environmental Protection Agency (1998a)

Required	Desirable
1. Increases in cellular growth	6. Lesion progression
2. Hormone changes	7. Structure-activity relationships
3. Site of action	8. Other studies
4. Dose correlations	
5. Reversibility	

What has been proposed in this assessment is the harmonization of the "noncancer" and "cancer" assessment approaches because the target tissue is the thyroid and the mode of action is the same for both the neurodevelopmental and neoplastic sequelae. The proposed RfD based on precursor lesions is analogous to a nonlinear approach and viewed as a protective for thyroid tumors.

Perchlorate has clearly demonstrated an effect on thyroid histopathology in adult, fetal, and neonatal stages, as well as a decrease in lumen size in a dose-dependent fashion. Thyroid and pituitary hormone changes and expected correlations have been demonstrated for T3, T4, and TSH across an array of studies at different time points. The site of action has been established as competitive inhibition of the iodide symporter although there remains some uncertainty as to whether this is the only locus for the effect (e.g., evidence for intrathyroidal activity) because of the efflux (discharge) phenomenon. Dose-correlations in this case were not with tumors, but rather for precursor lesions (colloid depletion, hypertrophy, hyperplasia, and decreased follicular lumen size). Reversibility has been demonstrated in thyroid weight, colloid depletion, hypertrophy, hyperplasia, and thyroid and pituitary hormones in the 30-day recovery period after the 90-day study in rats and in T4 levels of the various immunotoxicity experiments in mice.

Lesion progression was difficult to determine because of dose-spacing and differences in sample size and histological methods among the studies. However, there was a progression within the 90-day study between the 14- and 90-day time points.

Analyses of other anions have established that the mode of action of perchlorate arises from it being an anion that is recognized by the NIS (see Chapter 2 of this response and Chapter 3 of the 2002 ERD).

Thus, the appropriate dose-response procedure for risk assessment of potential neoplasia due to perchlorate exposures would be a nonlinear margin-of-exposure approach. This is based on the demonstration that it is not genotoxic and that its anti-thyroid effects are consistent with a mode of action resulting from inhibition of iodide uptake at the NIS through precursor lesions of perturbation of thyroid hormone economy and resultant histopathological changes in the thyroid gland.

7.5.2.2 Revisions to Section 7.1.5.2.2 (Dose-response Assessment for Thyroid Neoplasia)

Thyroid adenomas were statistically increased in the high dose (30 mg/kg-day) group of F1-generation animals sacrificed as adults (P2-generation) at 19 weeks in the Argus Research Laboratories, Inc. (1999) two-generation reproductive study. Both the latency and incidence of these tumors were remarkable relative to the entirety of the NTP database for this type of tumor in this strain of rat (Dunson, 2001b). Colloid depletion, hypertrophy, and hyperplasia were all observed at dosages of 0.3 mg/kg-day and above with BMDL estimates of 0.9, 0.15, and 0.0004 mg/kg-day. This last estimate is outside the range of possible dosimetric adjustment so it will not be carried forward; but consideration of the overlap among colloid depletion, hypertrophy, and hyperplasia should be superimposed on the derivation. The HEE values for adult versus neonatal rats are comparable at these dosages. Using the adult male rat dosimetric adjustment factor to correspond to sacrifice date, results in HEE estimates of 0.45 and 0.02 for colloid depletion and hypertrophy.

Using the nonlinear approach and applying a composite factor of 100 to the HEE estimates to account for uncertainty in intrahuman variability, duration, and database deficiencies and using a factor for a minimal LOAEL of 3 to account for the fact that hyperplasia occurred at over an order of magnitude lower than these other two thyroid histopathology indices results in an RfD derivation in the range of 0.005 to 0.0002 mg/kg-day. Applying a larger uncertainty factor for intrahuman variability would result in a range of 0.002 to 0.00007 mg/kg-day. Thus, the derivation based on tumor outcome data supports the mode-of-action concept and corroborates that the proposed RfD as being protective of both neurodevelopmental and neoplastic sequelae.

7.6 ADDITIONAL RESPONSES AND RECOMMENDATIONS

The Agency is including these additional revisions based on the revised assessment and on inquiries it has received regarding specific topics.

7.6.1 Revisions to Section 7.1.6 (Designation of Confidence Levels)

Because a discussion of the proposed confidence levels did not occur at the 2002 peer review, the Agency wishes to revise this section and proposes the following.

Confidence in the set of studies used to designate the point of departure is medium. The dose level of 0.01 mg/kg-day was the lowest dose level tested in the database, and it was determined to be a LOAEL for a number of endpoints. A limited sample size for some of these endpoints further reduces confidence in the characterization. Although some of the key studies such as the developmental neurotoxicity evidenced by brain morphometry and motor activity studies were confirmed with repeat testing, confidence in the database remains medium because the sensitivity of these laboratory animal assays versus evaluation of neuropsychological development in human populations studies is not known. Concern over the lack of a refined characterization of certain endpoints such as immunotoxicity also remains. Based on confidence in the studies used to determine the point of departure and on the database together in setting the overall confidence in the RfD, the confidence in the RfD is medium.

7.6.2 New Section 7.2 (Cancer Hazard Characterization)

As stated in the Executive Summary of the 2002 peer panel report (U.S. Environmental Protection Agency, 2002b), the panel generally supported the proposed key event, mode of action, harmonized approach for characterizing cancer and noncancer toxicity, and approach for low-dose extrapolations. 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 non-linear dose-response model.

This section is being included to provide an explicit summary of the evidence for the potential cancer risk posed by perchlorate exposure and to formalize that the appropriate approach for dose-response assessment of cancer would be non-linear. Thus, the harmonized RfD derived in Section 7.1 is intended to be protective for both noncancer and cancer toxicities.

As described in Section 5.1, perchlorate has been demonstrated to produce thyroid follicular tumors (adenomas or carcinomas) in laboratory animals (male Wistar rats and female BALB/c mice) at high doses. These studies were single high-dose drinking water studies that precluded quantitative determination of a dose-response relationship for thyroid tumor formation. Nevertheless, these data do establish the potential for perchlorate to cause cancer. A battery of genotoxicity assays performed more recently indicate that perchlorate is not likely to be mutagenic or clastogenic so that it is not considered to be directly mutagenic, although the debate on the exact interaction of perchlorate at the NIS and whether reduced forms enter into the cell leaves some concern for additional interaction with the thyroid gland. These findings considered together with the anti-thyroid effects exhibited across the database are consistent with the potential for perchlorate to cause cancer indirectly by perturbation of the HPT axis and this mode of action is considered to be a nonlinear process (U.S. Environmental Protection Agency, 1998a). The perturbation of the HPT axis results from the effects of perchlorate on iodide uptake inhibition by the sodium (Na+)-iodide (I-) symporter (NIS) in various tissues.

Because the NIS controls the levels of iodide transferred to the fetus by the placenta and to neonates by virtue of NIS in mammary tissue that transfer iodide to milk, these lifestages may be especially susceptible to the effects of perchlorate on circulating iodide levels. Thyroid follicular tumors (adenomas) were observed in the F1 generation at 19 weeks during a two-generation study in Sprague-Dawley rats which was evaluating the ability of perchlorate to produce transgenerational effects. The thyroid tumors were demonstrated to occur with a statistically significant increase in incidence and decrease in latency when compared to the incidence of all other studies showing this type of tumor in this strain and sex at terminal sacrifice of 2-year bioassays in the NTP archives (Dunson, 2001b). While all of these studies suggest the potential for thyroid carcinogenicity associated with disruption of thyroid physiology, the early onset of tumorigenesis in the offspring of treated animals is particularly noteworthy, suggesting the potential for fetal impacts.

In summary, under the Draft Revised Guidelines on Carcinogen Risk Assessment (Federal Register, 1999), perchlorate is considered a “likely” human carcinogen at doses sufficient to cause prolonged perturbation of the hypothalamic-pituitary-thyroid (HPT) axis. This conclusion is based on sufficient evidence in laboratory animals, where perchlorate adminstration led to thyroid follicular tumors (adenomas or carcinomas) in laboratory animals (male Wistar rats and female BALB/c mice) at high doses. Thyroid follicular tumors (adenomas) were also observed in

the F1 generation at 19 weeks in a two-generation study in Sprague-Dawley rats, demonstrating unusual early latency and the possibility of increased *in utero* sensitivity. A battery of genotoxicity assays indicate that perchlorate is not likely to be mutagenic or clastogenic. There are no pertinent human epidemiological studies of cancer risk. The mode of action is considered to be through an indirect anti-thyroid effect resulting from iodide uptake inhibition and subsequent disturbance of the HPT axis. This mode of action is established to be operative in various laboratory animal species (rats, mice, and rabbits), and is considered relevant to human hazard identification (U.S. Environmental Protection Agency, 1998a). Human cancer risk will depend on the level of exposure being sufficient to disrupt thyroid function for a prolonged period. The absence of mutagenicity and the established mode of action by HPT disruption indicate that a nonlinear dose-response approach is appropriate for risk assessment of perchlorate. This nonlinear dose-response is formalized through the harmonized RfD proposed in this chapter, which is intended to be protective of both neurodevelopmental effects and thyroid tumors.

7.6.3 Revisions to Section 7.2 (Inhalation Reference Concentration)

The lack of data continues to preclude derivation of an inhalation reference concentration at this time. The 2002 ERD characterized the potential for inhalation exposures from showering to be low due to a low vapor pressure and the large droplet size of shower particles reducing the probability of inhalation. However, the Agency wishes to note that it has received a number of inquiries regarding the potential risk of aerosol or fume inhalation around launch sites of rockets and missiles or near open burn/open detonation operations. Data on the mass median aerodynamic diameter and geometric standard deviation for these processes should be characterized.

APPENDIX 7A

Summary Tables of Human Equivalent Exposure (HEE) Estimates as Calculated with PBPK Models

Table 7A-1. PBPK-Model Calculated Human Equivalent Exposures (HEE) to Various Experimental Doses in the Male Rat for 15 and 70 kg Human-Based on Perchlorate Area Under the Curve (AUC) in Serum or Thyroid as the Dose Metric (Merrill, 2001e)

Adult Male Rat DW[a] Dose (mg/kg-day)	Human 15 kg HEE (mg/kg-day) based on serum[b] AUC	Human 70 kg HEE (mg/kg-day) based on serum[b] AUC	Human 15 kg HEE (mg/kg-day) based thyroid[b] AUC	Human 70 kg HEE (mg/kg-day) based on thyroid[b] AUC
0.010	0.030	0.021	0.0002	0.0001
0.1	0.145	0.100	0.002	0.001
1.0	0.745	0.505	0.008	0.006
3.0	2.05	1.35	0.052	0.035
5.0	3.35	2.25	0.145	0.098
10.0	6.75	4.45	0.725	0.460
30.0	20.3	13.2	163.0	110.0
100.0	65.0	43.8	490.0	330.0

[a]DW = drinking water.
[b]Calculated from PBPK-derived rat AUC(s) at steady state between 240 and 264 hrs during DW exposure, using upregulated Vmaxv_T_P values from (Merrill, 2001e: Table 1).

Table 7A-2. Ratio of PBPK-Derived Perchlorate Area Under the Curve (AUC) Serum Concentrations in Drinking Water for Various Experimental Life Stages (Merrill, 2001e)

Rat DW[a] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	0.63	0.58	1.44	1.16	2.28	1.99
0.1	0.73	0.54	1.06	0.85	1.46	1.56
1.0	0.90	0.84	1.44	1.01	1.61	1.20
3.0	0.94	0.95	1.67	1.71	1.77	1.80
5.0	0.95	0.98	1.74	2.14	1.82	2.18
10.0	0.96	1.01	1.80	2.70	1.87	2.69
30.0	0.97	1.02	1.84	3.33	1.90	3.26
100.0	0.97	1.03	1.85	3.65	1.92	3.55

[a]DW = drinking water.

Table 7A-3. PBPK-Model Calculated Human Equivalent Exposures (HEE) to Various Experimental Life Stages in the Rat Using Serum Perchlorate Area Under the Curve (AUC) as the Dose Metric

Dose (mg/kg-day)	Human Equivalent Exposure[a] (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.02	0.01	0.03	0.01	0.02
0.1	0.10	0.07	0.10	0.05	0.08
1.0	0.51	0.46	0.73	0.43	0.52
3.0	1.35	1.3	2.3	1.3	2.4
5.0	2.25	2.14	3.92	2.20	4.82
10.0	4.4	4.22	7.9	4.4	11.9
30.0	13.2	12.8	24.3	13.5	43.95
100.0	43.8	42.5	81.0	45.11	160.0

[a]Based on predicting the area under the curve in the blood (AUCB) using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-1and 7-2 and Chapter 6 for explanation of calculation.

Table 7A-4. PBPK-Model Calculated Human Equivalent Exposures (HEE) to Various Experimental Doses in the Adult Male Rat for 15 and 70 kg Human Based on % Iodide Uptake Inhibition in the Thyroid

Rat iv Dose (mg/kg)	Adult male rat inhibition at 2-hr post iv dose	Human 15 kg HEE (mg/kg-day)	Human 70 kg HEE (mg/kg-day)
0.01	1.5%	0.006	0.004
0.1	16.3%	0.075	0.048
1.0	74.5%	1.5	0.9
3.0	90.0%	4.8	2.7
5.0	93.5%	8.0	4.9
10.0	96.2%	16.0	9.0
30.0	98.1%	35.0	19.3
100.0	98.7%	50.0	33.0

Table 7A-5. PBPK-Model Predicted % Inhibition of Iodide Uptake in the Thyroid[a]

Rat DW [b] Dose (mg/kg-day)	Adult Male Rat	Pregnant Rat	Fetal Rat[c]	Lactating Rat[d]	Neonate Rat[c,d]	70 kg Human
0.01	1.5%	3.2%	-129.1%	0.5%	0.4%	2.8%
0.1	16.3%	30.1%	27.9%	5.3%	1.3%	23.7%
1.0	74.5%	88.7%	81.2%	62.9%	3.0%	80.2%
3.0	90.0%	93.8%	90.3%	92.8%	3.3%	92.3%
5.0	93.5%	97.0%	90.4%	95.8%	3.1%	95.2%
10.0	96.2%	97.9%	97.9%	97.6%	3.8%	97.4%
30.0	98.1%	98.6%	98.9%	98.5%	6.1%	98.9%
100.0	98.7%	98.8%	99.2%	98.8%	13.4%	99.4%

[a]Based on iv administration to rat and drinking water in human.
[b]DW = drinking water
[c]Values for these tissues not validated versus data.
[d]All calculations are for PND10 in lactating and neonatal rat.

Table 7A-6. Ratios of PBPK-Derived % Iodide Uptake Inhibition in Drinking Water for Various Experimental Life Stages[a]

Rat DW[b] Dose (mg/kg-day)	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat[c]	Male Rat: Neonate Rat[c]	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat[c,d]
0.01	0.48	3.24	−0.01	4.02	−0.02	1.2
0.1	0.54	3.06	0.59	12.75	1.08	4.2
1.0	0.84	1.18	0.92	24.53	1.09	20.7
3.0	0.96	0.97	1.00	27.49	1.04	28.4
5.0	0.96	0.98	1.03	30.45	1.07	31.2
10.0	0.98	0.99	0.98	25.61	1.00	26.0
30.0	0.99	1.00	0.99	16.06	1.00	16.1
100.0	1.00	1.00	1.00	1.37	1.00	7.4

[a]Inhibition in human was PBPK-derived from 2 wks ClO_4^--exposure in drinking water (DW); all rat values simulated from an iv dose.
[b]DW = drinking water
[c]Model predicted in fetal and neonate rats not validated with data.
[d]All calculations are for PND10 in lactating and neonatal rat.

Table 7A-7. PBPK-Model Calculated Human Equivalent Exposures (HEE) to Various Experimental Life Stages in the Rate Using % Iodide Uptake Inhibition in the Thyroid as the Dose Metric

Dose (mg/kg-day)	Human Equivalent Exposure[a] (mg/kg-day)				
	Adult Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	0.004	0.002	—	0.01	0.02
0.1	0.048	0.026	0.03	0.15	0.61
1.0	0.90	0.756	0.83	1.06	22.05
3.0	2.7	0.259	2.70	2.62	74.2
5.0	4.9	4.70	5.05	4.80	149.2
10.0	9.0	8.82	8.82	8.91	230.5
30.0	19.3	19.1	19.1	19.3	309.96
100.0	33.0	33.0	33.0	33.0	33.0

[a]Based on predicting the % iodide uptake in the thyroid using the human PBPK model that achieves an equivalent degree to that simulated for the rat experimental regimen associated at different life stages. See Tables 7-4 and 7-6 and text for explanation of calculation.

APPENDIX 7B

Tables Showing Minimum Database Requirements and Uncertainty Factors Applied for Derivation of an Oral Reference Dose (RfD)

Table 7B-1. Minimum Database for Deriviation of an Oral Reference Dose

Mammalian Database[a]	Confidence	Comments
Two chronic oral bioassays in different species One two-generation reproductive study. Two developmental toxicity studies indifferent species.	High[b]	Minimum database for high confidence
One subchronic oral bioassay	Low	Minimum database for estimation of an RfD

[a]Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate.
[b]Rationale is to address all potentially critical life stages.

Table 7B-2. Factors for Uncertainties in Applied Extrapolations Used to Derive Reference Doses[a]

10_H	–	Human to sensitive human
10_A	–	Experimental animal to human
10_S	–	Subchronic to chronic duration
10_L	–	LOAEL(HEE)[a] to NOAEL(HEE)[a]
10_D	–	Incomplete to complete database
MF	–	Modifying factor. Professional assessment of scientific uncertainties of the study and database not explicitly addressed above. Default for the MF is 1.0 (e.g., applied for small sample size or poor exposure characterization).

[a]HEE = human equivalent exposure.

8. MAJOR RISK CHARACTERIZATION CONCLUSIONS

Topic Area G (Section 8 of the peer panel report) was devoted to a discussion of the overall risk characterization statements found in Chapter 10 of the 2002 ERD. Disposition of some of these comments are already reflected in responses and recommendations throughout the chapters of this document. Disposition of suggestions for future research made during the summary set of comments presented by the panel during discussion of Topic Area H (Section 9 of the peer panel report; General Comments, Conclusions, and Recommendations) are addressed in this section.

Recommendations for revisions to the 2002 ERD based on these comments will be found in Chapter 10 of the revised assessment. The major changes will be in Section 10.1 (Human Health), Section 10.2 (Ecotoxicology), and Section 10.3 (Characterization Progress Summary). These are provided here as separate sections.

Specific recommendations for potential future research were made by the external peer panel. Many of these have been highlighted in the other chapters. Those that were not addressed in EPA responses in the other chapters will be included in the revised document in Section 10.1.4 (Major Uncertainties and Research Needs) for the human health risk characterization and in Section 10.2.5 for the ecotoxicological risk characterization. These sections are provided here as Sections 8.1.2 and 8.2.2.5

8.1 COMMENTS ON HUMAN HEALTH RISK CHARACTERIZATION

In summarizing the relevant premeeting comments, the discussion leader (workshop chair) indicated that the panel generally thought the human health risk characterization adequately summarized the information originally presented in the revised ERD. He noted that the Agency should revise the risk characterization to reflect the findings and comment listed throughout the report. Relatively few specific suggestions were made for improving this section; those that were made are provided herein. The Agency intends to update its discussion in Section 10.1.2

(Dose-Response) and Section 10.1.4 (Major Uncertainties and Research Needs). These are provided here as separate sections.

Comment(s)*: To ensure that the risk characterization reflects the current understanding of perchlorate toxicity, one reviewer recommended that Section 10.1 acknowledge the diversity of opinion regarding how to interpret key toxicity studies, particularly the studies reporting changes in brain morphometry in rats. For a greater perspective on whether perchlorate-related toxicity is believed to occur in humans, two reviewers suggested that Section 10.1 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 suggested that Section 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.*

EPA Response(s) and Recommendation(s) for Revision(s): Specific changes to these peer review comments are provided in two separate sections provided below. The Agency believes that through these changes it has achieved a balance regarding the interpretation of the data in its characterization of the uncertainties in the assessment and explicit list of research needs.

Because the characterization of the extent of perchlorate contamination is constantly evolving the Agency has included the most recent links to perchlorate occurrence and exposure information in Chapter 1. This will ensure that these data are as current as possible for those interested in the topic. There are also links to the Unregulated Contaminants Monitoring Rule (UCMR) data which will provide more accurate exposure estimates as characterization of the exposure ensues as part of determining regulatory readiness.

A discussion of goiter prevalence was provided in Chapter 3.

8.1.1 Revisions to Section 10.1.2 (Dose Response)

The revised harmonized RfD is based on an assessment that reviewed a set of studies were developed to explicitly evaluate these potential toxicities. The quantitative estimate of risk is based on a weight-of-evidence approach to the entire database which included laboratory animal studies, controlled human (clinical) dosing studies, and ecological epidemiological studies.
A harmonized approach was proposed based on the key event of iodide uptake inhibition at the sodium (Na^+)-iodide (I^-) symporter (NIS) and its relationship to disturbances in the hypothalamic-pituitary-thyroid (HPT) axis as evidenced by effects on thyroid and pituitary

hormones, thyroid histopathology, and brain morphometry. Because thyroid hormone is so critical to proper development and function, it is not surprising that the NIS is conserved across species — the data show the perchlorate inhibits thyroid-dependent processes in most mammals and in amphibians and fish. Pharmacokinetic data and physiologically based pharmacokinetic (PBPK) modeling show that humans and rats are similarly sensitive to the effects of perchlorate on iodide uptake inhibition. The inhibition of iodide uptake at the NIS in various tissues (placenta, mammary gland, GI tract, and skin) in addition to the thyroid are likely very important for various life stages that are potentially susceptible such as the fetus and neonate. The inhibition of the NIS for these tissues has not been well characterized with human data.

The database supports a point of departure for operational derivation of the RfD at 0.01 mg/kg-day based on human iodide uptake inhibition studies, changes in maternal thyroid and pituitary hormones on GD21, changes in adult rat thyroid and pituitary hormones in a short-term study, and changes in the brain morphometry and thyroid and pituitary hormones of fetal and neonatal pups. Histopathological changes in the thyroid gland (colloid depletion, hypertrophy, and hyperplasia) are also evident in the data base at levels that support this point of departure.

Using these precursor lesions as the basis for the point of departure is considered to be protective for cancer development as well as for neurodevelopmental sequelae. Tumors are believed to be secondary to the anti-thyroid effects of perchlorate and not due to direct mutagenesis although there is some uncertainty with respect to the exact nature of the interaction of perchlorate in the thyroid tissues. Thus the estimate represents a harmonized approach to potential toxicity of perchlorate due to its anti-thyroid effects and to the inhibition of iodide uptake at the NIS in various tissues.

A composite uncertainty factor of 300 is applied in the derivation. An adjustment also was made for administration of perchlorate as ammonium perchlorate. The RfD is for perchlorate as the anion because that is what is sampled and analyzed in environmental media and because the salts of perchlorate readily dissolve. Uncertainty factors are applied for intrahuman variability and interspecies extrapolation together, for the use of a LOAEL, and for a concern regarding the lack of studies of longer duration and database deficiencies combined. Confidence in the study, the database, and the RfD is rated as medium. Some major uncertainties are the sensitivity that the screening neurodevelopmental studies provide to protect against neuropsychological deficits of exposures that might occur within critical developmental windows or in susceptible human

populations, the nature of the interaction of perchlorate at the NIS in various tissues, and the dosimetry of perchlorate in fetal and neonatal lifestages.

The daily perchlorate exposure to the human population that is likely to be without appreciable risk of either cancer or noncancer toxicity during a lifetime is 0.00003 mg perchlorate/kg-day. It again is noted that this RfD is specific for the anion because that is what is measured in most environmental samples and because most salts of perchlorate readily dissolve. Because of the application of uncertainty factors, this dose is approximately 1/60 of the lower limit on a dose that caused a 5% inhibition of iodide uptake in euthyroid adult human subjects in a study of limited duration (14-days) and 1/300 of the dose that resulted in brain morphometry and thyroid changes in pups and hypothyroid status (decreased T4 and increased TSH) in rat mothers (Argus Research Laboratories Inc, 2001) and in their pups both during gestation (GD21) and in the post-natal period (PND4 through PND21).

8.1.2 Revisions to Section 10.1.4 (Major Uncertainties and Research Needs)

Reliable exposure estimates are required to accurately and comprehensively characterize the risk of perchlorate contamination. This section will briefly summarize research needs associated with aspects of uncertainty about the human health risk dose-response estimate that were highlighted in Chapter 7.

The greatest need for continued improvement in the dose-response assessment is a more accurate characterization of the linkage between the key event of the mode of action (i.e., inhibition of iodide uptake in the thyroid gland), subsequent changes in thyroid hormones, and the correlation to outcome measures in hypothyroxinemic pregnant animals and their pups. Because this need must be addressed in the fetal compartment as well, accurate characterization of toxicokinetics during pregnancy and lactation also is needed. More definitive studies of the degree of change in perturbation of the hypothalamic-pituitary-thyroid axis (i.e., change in hormone levels) that is associated with thyroid histology, with brain changes (e.g., receptors), and with neurobehavioral deficits especially, would improve the confidence in the accuracy of the exposure-dose-response continuum. The current studies may need to be repeated with larger sample sizes and lower doses, and new studies may be needed to evaluate effects on fetal hormone levels and neurodevelopmental measures both in the laboratory and in a survey of the human population. Research on potential factors influencing sensitivity is also critically

requisite. Laboratory animal models of thyroid impairment such as iodide deficiency and "womb- to- tomb" exposure designs should be explored. Finally, mechanistic determinants of these toxicokinetic and toxicodynamic parameters and processes should be further characterized.

The following list was provided by the 2002 external peer review panel members as recommendations for future research.

- Develop validated endpoints of thyroid hormone action on brain development.
- 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.
- Conduct an additional multi-generational developmental study that evaluates a full suite of neurobehavioral, neurodevelopmental, and thyroid histopathological endpoints.
- 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.
- Consider conducting an epidemiological study on the prevalence of goiter among populations exposed to perchlorate.
- Conduct a more extensive chronic exposure (i.e., with a "womb-to-tomb" design) and another study of potential *in utero* programming.
- Characterize the pharmacodynamics by which iodide uptake inhibition leads to neurodevelopmental and neoplastic sequelae.
- Ascertain unequivocally whether active translocation of perchlorate occurs.
- Characterize potential adverse effects resulting from prolonged exposure to perchlorate.

8.2 COMMENTS ON ECOLOGICAL RISK CHARACTERIZATION

The two reviewers assigned to review the ecological effects and evidence for indirect exposure addressed EPA's characterization of ecological risks, drawing mainly from comments they raised earlier in the peer review meeting (see Chapter 5). Although both reviewers initially found EPA's screening-level risk assessment adequate, their views changed upon reviewing an ecotoxicological study (Goleman et al., 2002b) published after the release of the Revised ERD. The two reviewers' comments addressed three general issues. Specific comments will be addressed in Sections 8.2.1. The Agency's substantive revisions to Chapter 10 will be provided in Section 8.2.2.

8.2.1 Specific Comments on Ecological Risk Characterization

Comments on exposure data: *One reviewer indicated that, although the 2002 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 recommended that EPA clarify its statements on chemical transformation of perchlorate, rather than asserting that the contaminant is extremely stable and noted that more detailed information on biological transformation may be necessary, particularly as it applies to potential phytoremediation strategies. Two reviewers 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.*

EPA Response(s) and Recommendation(s) for Revision(s): Because the sources of releases of perchlorate to the environment (i.e., facilities associated with the manufacture, handling, or use of perchlorate in solid rocket propellants) are limited, it is appropriate to focus on contamination associated with such facilities. As discussed in the document, contamination associated with other sources (i.e., fertilizers containing Chilean sodium nitrate) appears to be even more limited than that associated with the manufacturing, handling, and use of solid rocket propellants and would generally pose a *de minimis* risk to human or ecological receptors. As discussed previously, the Agency will clarify the statements about the transformation of perchlorate in the environment.

Comments on aquatic effects assessment: *Two reviewers questioned the adequacy of EPA's screening-level ecological risk assessment for perchlorate, given that a recent study (Goleman et al., 2002b) 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 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 agreed and recommended that future studies also examine rooted macrophytes and detritus, which she indicated may be important for dietary exposures in the aquatic food chain.*

EPA Response(s) and Recommendation(s) for Revision(s)*:* As discussed in Chapter 5, a full discussion of the implications and limitations of the Goleman et al. (2002a, 2002b) studies have been added to the document where appropriate. The Agency also added discussions of additional, more recent studies using *Xenopus* that have addressed the major concerns about the Goleman study.

Comments on terrestrial effects assessment: *Two reviewers indicated that the 2002 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).*

EPA Response(s) and Recommendation(s) for Revision(s)*:* As discussed in Chapter 5, data from an additional study of deer mice (Thuett et al., 2002) are now available as are some data on raccoons and Virginia opossums. Discussions of these results will be added to the revised document where appropriate.

8.2.2 Revisions to Major Risk Characterization Conclusions in Section 10.2 (Ecotoxicology)

Because considerable new data are available that change the assessment of potential ecotoxicological effects of perchlorate from a screening level, this section has changed substantially. The new section in the revised document is as follows.

8.2.2.1 Revisions to Section 10.2.1 (Aquatic Life)

Procedures for deriving acute and chronic aquatic benchmark values were used in Section 8.3.2.1 to jointly characterize the potential effects of the perchlorate ion on the richness and productivity of fish, aquatic invertebrate, and plant communities. Although Tier II values were previously derived, the available data set on perchlorate is now sufficient to derive acute and chronic aquatic benchmark values following the guidance of Stephan et al. (1985). However, the Final Chronic Value obtained using those procedures (Table 8.2) was considered to be insufficiently protective in that the tests used to derive the FCV did not account for thyroid-mediated effects on amphibian development; and therefore a lower chronic benchmark is established. The acute and chronic benchmark values are 22 and 0.12 mg/L (i.e., 22,000 and 120 µg/L), respectively. Perchlorate levels reported for large surface waters (as high as 0.016 mg/L) are below the acute and chronic benchmark values. Thus, at these exposure levels, the likelihood of effects on the richness and productivity of fish, aquatic invertebrate, and plant communities appears to be low.

However, perchlorate levels in public supply wells ranged as high as 0.28 mg/L, and water discharged to the North Branch Potomac River from a CERCLA groundwater pump-and-treat facility at Allegany Ballistics Laboratory (which was not equipped to treat perchlorate) contained 0.250 to 0.280 mg/L perchlorate (Parsons, 2001), which exceeded the chronic aquatic benchmark of 0.12 mg/L. Therefore, effects on aquatic organisms in areas of discharge of contaminated groundwaters, including seeps or irrigation return flows, cannot be precluded. Furthermore, because much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37,000 ms/L) and in groundwater-dominated surface water systems close to sites of contamination (3.5 to 1300 mg/L), sites clearly exist that have perchlorate concentrations high enough to cause toxicity to aquatic life. These sites include springs, such as that sampled along Las Vegas Wash in Nevada (Parsons, 2001) and the INF Pond at Longhorn Army Ammunition Plant in Texas (Parsons, 2001; Smith et al., 2001). On the other hand, concentrations below the acute and chronic benchmark values were detected in larger water bodies immediately adjacent to sites of contamination, such as in Lake Mead immediately adjacent to the mouth of the Las Vegas Wash (less than 0.004 to 0.068 mg/L).

Where high levels of contamination exist, sensitive aquatic organisms such as daphnids or amphibians may be the most likely to experience effects; in the reported tests, effects were seen on both survival and reproduction (neonates per organism) in daphnids and on development in amphibians. Results from algal toxicity tests suggest that even at the higher perchlorate concentrations associated with rocket motor manufacturing, risk of toxicity to aquatic plants is low.

The perchlorate anion can be associated with various cations including sodium, ammonium, and potassium. When sodium perchlorate was tested, the sodium cation was not toxic to daphnids in sodium chloride control tests but did show toxicity to minnows. Ammonium controls were not used in tests with ammonium perchlorate, but ammonium ion is a known toxicant with toxicity that varies according to water temperature and pH. In any aquatic system where perchlorate is present, attention should be given to determining the concentrations of potentially toxic cations that may contribute to ecological effects.

Based on a chronic benchmark value of 0.12 mg/L for perchlorate, the analytical detection methods for perchlorate in water are sufficient. The detection limit achieved for perchlorate in water was 0.004 mg/L (Parsons, 2001; Smith et al., 2001), which is much less than the chronic benchmark value. Thus, the likelihood that adverse ecological effects will occur below detection limits is low.

8.2.2.2 Revisions to Section 10.2.2 (Risks to Consumers of Aquatic Life)

Information from Parsons (2001) and Smith et al. (2001) indicates that perchlorate may occur in tissues of aquatic invertebrates and fish in contaminated waters, but perchlorate concentrations in these organisms are not expected to exceed the surface water concentrations. Therefore, there currently is no indication that consumers of aquatic invertebrates or fish are at increased risk of effects associated with greater concentrations of perchlorate in fish or invertebrate tissues relative to observed perchlorate concentrations in surface water. However, there is some uncertainty about the potential for bioaccumulation of perchlorate at low concentrations (i.e., 0.004 to 0.3 mg/L in water) because of the higher detection limits for perchlorate in animal tissues, which were 0.3 to 0.4 mg/kg in Parsons (2001) and about 0.07 mg/kg in Smith et al. (2001). Furthermore, perchlorate may bioaccumulate to levels exceeding those in water in aquatic plants; therefore, consumers of aquatic plants may be at

greater risk than consumers of aquatic invertebrates or fish, but information is not available concerning effect levels in aquatic herbivores.

8.2.2.3 Revisions to Section 10.2.3 (Terrestrial Life)

The following subsections in this section.

8.2.2.3.1 Revision to Section 10.2.3.1 (Plants)

A benchmark value for effects on plants of 0.1 mg/kg as a wet-weight soil concentration was derived based on a NOAEL of 1 mg/kg for inhibition of lettuce germination, and a 10-fold uncertainty factor to account for interspecies variability (see Section 8.3.2.2.). Terrestrial plants may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentrations in soil at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and can be higher than the benchmark value of 0.1 mg/kg and even higher than the lethal concentrations (≥180 mg/kg; EA Engineering, Science and Technology, Inc., 1998).

In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation-water concentrations (Section 8.3.1.3). Reported surface-water concentrations in the Colorado River, 0.004 to 0.016 mg/L, would translate to 0.004 to 0.016 mg/kg. At the Yuma site, there was a single detection in surface soil of 0.090 mg/kg; all other measurements were below the detection limits of 0.079 to 0.080 mg/kg (Parsons, 2001). This single detected concentration is slightly lower than the benchmark value. The reported groundwater concentration in public wells of 0.28 mg/L would translate to 0.28 mg/kg, which is greater than the benchmark value. Hence, without tests of additional plant species to reduce uncertainty associated with interspecies variability, the possibility of effects in plants irrigated with contaminated groundwater cannot be precluded.

Based on this benchmark value of 0.1 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient for determining whether soils will cause toxicity to plants, and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 0.075-0.080 mg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 0.803 mg/kg. However, most of these limits are less than the benchmark value.

8.2.2.3.2 Revisions to Section 10.2.3.2 (Soil Invertebrates)

A benchmark value for effects on the soil invertebrate community of 1.0 mg/kg as a wet-weight soil concentration was derived based on a 14-day LC_{50} of 4450 mg/kg in earthworms and uncertainty factors to account for the lack of tests of chronic duration and for interspecies variability (see Section 8.3.2.2.). Soil invertebrates may be exposed to perchlorate in soil at disposal sites and at sites irrigated with contaminated surface water or groundwater. Perchlorate concentration measurements at disposal sites range from less than 1 to 1470 mg/kg (Parsons, 2001) and, therefore, can exceed the soil benchmark value of 1.0 mg/kg. In the absence of reliable information concerning the accumulation of perchlorate in irrigated soils, it may be assumed that soil concentrations equal irrigation water concentrations (Section 8.3.1.3). Reported surface water concentrations in the Colorado River, 0.004 to 0.016 mg/L, would translate to 0.004 to 0.016 mg/kg in soils. At the Yuma site, the single detection in surface soil was 0.090 mg/kg with detection limits of 0.079 to 0.080 mg/kg. This detected concentration is a factor of 11 lower than the benchmark value for soil invertebrates (1.0 mg/kg). The reported groundwater concentration in public wells of 0.280 mg/L would translate to 0.28 mg/kg, which is a factor of 4 lower than the benchmark value. Hence, perchlorate does not appear to constitute a hazard to soil invertebrates in soil irrigated with surface water. However, given the large uncertainties concerning exposure, a hazard to soil invertebrates from groundwater irrigation cannot be precluded.

Based on this benchmark value of 1.0 mg/kg for perchlorate, the analytical detection methods for perchlorate in soil are sufficient; and there is little likelihood of adverse ecological effects occurring at levels below detection limits. The detection limit achieved for perchlorate in soils was generally 0.075-0.080 mg/kg (Parsons, 2001), but there was at least one soil sample where the reporting limit was 0.803 mg/kg. However, all of these limits are less than this screening benchmark value.

8.2.2.3.3 Revisions to Section 10.2.3.3 (Herbivores)

Exposures of voles to perchlorate based on measured plant concentrations at rocket motor manufacturing or testing sites (0.11 mg/kg day to a maximum of 49 mg/kg-day) exceed both the LOAEL of 0.01 mg/kg-day and the benchmark value of 0.001 mg/kg-day (see Section 8.3.2.2.). Estimated exposures of voles consuming plants on sites irrigated with surface water (0.18 mg/kg-bw day) and groundwater (3.2 mg/kg-bw day) (see Section 8.3.1.5.) also exceed the

LOAEL and the benchmark value. Hence, there is a potential hazard to all herbivorous wildlife living in areas that may be irrigated with contaminated water. At disposal sites, wildlife would be at risk from the effects of loss of food and habitat from toxic effects on plants, as well as the potential for direct toxic effects via consumption of perchlorate-tainted food or water.

Assuming a water ingestion rate of 0.21 g/g-day (U.S. EPA, 1993a,b), the benchmark value for herbivores is equivalent to a water concentration of 0.0048 mg/L. Perchlorate levels reported for large surface waters (as high as 0.016 mg/L) are greater than this concentration. Much higher perchlorate concentrations have been reported in monitoring wells at rocket motor manufacturing or testing sites (37,000 mg/L) and in groundwater-dominated surface water systems close to sites of contamination (3.5 to 1300 mg/L), and rodent exposures via drinking water at these sites would exceed the rodent NOAEL.

Based on benchmark values for herbivores, the analytical detection methods for perchlorate in plant tissues may not be sufficient for the detection of concentrations potentially toxic to herbivores even though the analytical detection methods for perchlorate in water are sufficient. The detection limits achieved for perchlorate in water and in plant tissues were 0.004 mg/L and 0.4 mg/kg, respectively (Parsons, 2001; Smith et al., 2001).

8.2.2.3.4 Revisions to Section 10.2.3.4 (Carnivores)

Available evidence indicates that concentrations in terrestrial invertebrates are less than the concentrations in plants and similar to that in soils. As a result, there currently is no indication that terrestrial carnivores are at additional risk from perchlorate. Risks of direct toxic effects are therefore lower for carnivores than herbivores. In locations where perchlorate levels are sufficient to significantly affect herbivores, carnivores are more likely to be affected by loss of prey than by perchlorate toxicity. Therefore toxic effects are not quantified.

8.2.2.4 Revisions to Section 10.2.4 (Uncertainties)

This discussion of uncertainties is limited to qualitative uncertainties associated with major gaps in the data available for ecological risk assessment of perchlorate. This is because quantitative uncertainties are treated through the use of conservative assumptions. It is also because data gaps are the major sources of uncertainty, not imprecision or inaccuracy of the available data.

8.2.2.4.1 Revisions to Section 10.2.4.1 (Uncertainties Concerning Aquatic Risks)

Aquatic Exposures The primary uncertainty associated with this assessment of aquatic risks is the paucity of data on perchlorate occurrence in surface waters. Due to a lack of systematic sampling and analysis, the spatial and temporal distribution of perchlorate in water is unknown. It is not certain whether the reported concentrations in water represent the highest existing levels. Because the sites sampled by Parsons (2001) and Smith et al. (2001) were associated with facilities involved in the manufacture, use, or disposal of perchlorate salts related to the handling of solid rocket propellents, it is assumed that sampling has been biased to areas of highest likely contamination. However, these studies did not sample all sites with known releases of perchlorate. Moreover, more general monitoring would be needed to ascertain the spatial and temporal distribution of perchlorate not associated with such facilities.

Aquatic Effects While the effects of perchlorate on some species of algae are known, the effects on aquatic macrophytes are largely unknown. As a result, risks to aquatic primary producers are estimated using only the chronic toxicity test results for the alga *Selenastrum*. Because of physiological differences between algae and vascular plants, effects on aquatic primary producers are not adequately assessed. In addition, it is unknown how or if physiological variations among various species of algae or plants may affect their susceptibility to perchlorate.

Algae, aquatic macrophytes, and terrestrial leaf litter are the bases of food chains in many aquatic ecosystems. Because perchlorate has been shown to concentrate in leaves of terrestrial plants and aquatic plants, the potential for direct impacts to primary consumers (i.e., planktonic and benthic invertebrate communities) is a concern that could not be addressed in this assessment.

A 35-day, early-life stage (ELS) test with *Pimephales*, generally regarded as a chronic test but short of a full-life-cycle test, showed no significant effects on any standard endpoint (survival, growth or biomass) at the highest concentration tested (490 mg/L). However, all larvae exposed to perchlorate concentrations, including the lowest concentration of 28 mg/L, exhibited redness and swelling, which was not observed in the larvae exposed to the control water. This finding suggests the presence of subtle effects that could be ecologically significant and raises doubt about whether a chronic NOEC has been adequately determined for this species. For this reason, and because of the potential for chronic effects caused by thyroid dysfunction, chronic effects should be investigated in a full life cycle test.

In addition, the tests with amphibians suggest there are potentially other chronic effects associated with thyroid dysfunction. However, these effects may be ameliorated by the availability of iodine. Although the iodine concentrations found in contaminated natural waters at the Longhorn Army Ammunition Plant site were apparently sufficient to ameliorate the effects observed by Goleman et al. (2002a), the concentration of iodine measured by Carr et al. (2002c) in surface water from the Longhorn Army Ammuntion Plant was relatively high compared with the rivers recently surveyed by Moran et al. (2002). Therefore, the interaction between perchlorate and lower iodine concentrations should be investigated. Also, further well-designed laboratory studies are needed to fully elucidate the threshold concentrations above which chronic effects associated with thyroid dysfunction may be occurring.

The uncertainty associated with the chronic benchmark value for aquatic toxicity is high because standard bioassays are not designed to detect the potential developmental effects associated with perchlorate.

8.2.2.4.2 Revisions to Section 10.2.4.2 (Uncertainties Concerning Terrestrial Risks)

Terrestrial Exposure The available data concerning aqueous perchlorate levels is sparse and has not been collected systematically. As a result, the spatial and temporal distribution of perchlorate in irrigation water is unknown. It is not clear that the reported concentrations in water represent the highest existing levels. Because the sites sampled by Parsons (2001) and Smith et al. (2001) were associated with facilities involved in the manufacture, use, or disposal of perchlorate salts related to the handling of solid rocket propellents, it is assumed that sampling has been biased to areas of highest likely contamination. However, these studies did not sample all sites with known releases of perchlorate. Moreover, more general monitoring would be needed to ascertain the spatial and temporal distribution of perchlorate not associated with such facilities.

The fate of perchlorate in soil, including its tendency for evaporative concentration, is not well characterized. As a result, soil concentrations were assumed to be equal to irrigation water concentrations. This assumption could be low by multiple orders of magnitude if evaporative concentration occurs with perchlorate as it does with metals. The limited data for irrigated soils near Yuma (Parsons, 2001) do not support the occurrence of such a high degree of evaporative concentration, but neither are they sufficient to rule out concentration by up to a factor of 10 or so. More information on the fate of perchlorate in irrigated soils is needed.

The bioconcentration of perchlorate by plants suggests that perchlorate may be elevated in leaves and leaf litter to levels that may affect invertebrate herbivores and soil invertebrate communities. Due to a lack of data concerning dietary toxicity, risks to invertebrates by this route were not assessed.

Available toxicity data for rodents suggest that vertebrate herbivores may be sensitive to low levels of perchlorate in plant tissues. Concentrations potentially causing toxicity are calculated to be lower than those currently detectable by chemical analyses of plants. In Parsons (2001), detection limits for plants were generally about 0.4 mg/kg wet weight; similar detection limits were achieved by Ellington and Evans (2000) and Ellington et al. (2001), as compared to an exposure benchmark of 0.01 mg/kg in plant tissue for a representative herbivore (see Section 8.3.2.2). Therefore, lower detection limits for perchlorate in plant tissues may be needed to completely assess the risks to vertebrate herbivores.

Terrestrial Effects The toxicity of perchlorate to nonmammalian vertebrate wildlife is largely unknown. As a result, risks to birds and reptiles could not be assessed.

The toxicity of perchlorate to terrestrial invertebrates, other than acute lethality to earthworms, is unknown. As a result, risks to other terrestrial invertebrates were inadequately assessed.

8.2.2.5 Revisions to Section 10.2.5 (Research Needs)

Three questions were asked of the ecological risk assessment for perchlorate:
Are ecological risks best characterized as *de minimis* (exposures clearly are below levels of concern), *de manifestis* (risks are clearly significant and require management action to reduce exposures) or somewhere in between and requiring further characterization?

Are analytical detection methods for determining levels of perchlorate in the environment sufficient, or is there a likelihood of adverse ecological effects occurring at levels below current detection limits?

- Is the available ecotoxicological information on perchlorate sufficient, or are additional studies needed?

In the immediate vicinity of facilities that were involved in the manufacture, use, or disposal of perchlorate salts, particularly facilities involved in handling of solid rocket propellents, ecological exposure can exceed levels of concern; and management actions may be

needed to reduce these exposures. Site-specific risk assessments would be needed to guide remediation of such locally contaminated sites. Farther from such facilities, effects on either terrestrial herbivores or sensitive aquatic vertebrates such as amphibians to contaminated groundwaters cannot be precluded; whereas other kinds of ecological exposures appear to be below levels of concern.

The analytical detection methods for perchlorate are generally sufficient; and there appears to be no indication of adverse ecological effects occurring at levels below detection limits, except that detection limits in plant tissues are not low enough to ensure that risks to herbivores are detected. Additionally, there is some uncertainty about the potential for bioaccumulation at low concentrations of perchlorate in surface water because of differences in the analytical detection limits between water and animal tissues.

The available ecotoxicological information on perchlorate is sufficient for this ecological risk assessment. However, additional ecotoxicological studies could reduce the uncertainties about the toxicity of perchlorate to other potential ecological receptors.

While the available information may yield an adequate ecological risk assessment, the following research needs for exposure and effects analysis deserve mention.

8.2.2.5.1 Revisions to Section 10.2.5.1 (Exposure)

Concerning exposure, at least three important issues remain unresolved.

- Because of the potential for evaporative concentration, the fate of perchlorate in irrigated soils should be investigated.
- Because the concentrations that have potential for dietary toxicity to vertebrate herbivores are less than the limits of detection currently achievable by chemical analysis of plants, analytical methods for plant tissues that could lower the limits of detection should be investigated.

8.2.2.5.2 Revisions to Section 10.2.5.2 (Effects)

Also requiring further attention are issues related to the effects of potential perchlorate exposure as follows.

- The effects of exposure of aquatic plants should be determined.

- The effects of dietary exposure to perchlorate should be determined in birds and in herbivorous or litter-feeding invertebrates.
- How the availability of iodine may ameliorate the thyroid-mediated effects of perchlorate on aquatic organisms should be determined, particularly on the metamorphosis of amphibian larvae, such as tadpoles, and how this may affect threshold concentrations associated with chronic effects.
- The effects of dietary and cutaneous exposure to perchlorate should be determined for adult amphibians and aquatic reptiles.
- If perchlorate occurs at significant levels in estuarine systems, its toxicity in saline waters should be determined.

8.2.2.5.3 Revisions to Section 10.2.5.3 (Site-Specific Investigations)

Some of the research needs that were listed in the 2002 ERD have been met by the research conducted by the US Air Force IERA (Parsons, 2001) in which perchlorate concentrations in environmental media (i.e., surface soils, surface water, sediments, and porewater) and biological tissues (i.e., terrestrial plants, invertebrates, reptiles, birds, and mammals and aquatic vegetation, invertebrates, fish, amphibians, reptiles, and birds) were surveyed at six sites with known perchlorate contamination. These data are supplemented by additional sampling at one of the sites, Longhorn Army Ammunition Plant in Texas (Smith et al., 2001). These studies address some questions about exposure that were expressed in the previous drafts of the ERD (U.S. Environmental Protection Agency, 1998d; 2002a), notably the following.

- Because concentrations of perchlorate in water are poorly known and because concentrations in soil and biota are unknown, a survey of perchlorate contamination should be conducted.
- Because, contrary to expectations, perchlorate accumulates to high concentrations in terrestrial vascular plants, the accumulation of perchlorate in aquatic plants and in animals should be investigated.

However, these studies were screening-level surveys that took small numbers of samples during limited periods of time. In addition, the studies were not generally designed to address questions about the effects of exposure. In some locations, concentrations in environmental media were high enough that toxicity to ecological receptors was highly likely (i.e., the risks

were *de manifestis*); and, in other locations, toxicity could not be ruled out (i.e., the risks could not be termed *de minimus*). Therefore, more systematic sampling is needed in these locations to definitively quantify exposures and effects so that the likelihood, nature, and extent of ecological risks may be quantified; appropriate remedial alternatives may be designed; and effectiveness of site cleanup may be judged. In addition, site surveys may be required in other locations where perchlorate contamination is suspected.

8.3 REVISIONS TO SECTION 10.3 (CHARACTERIZATION PROGRESS SUMMARY)

The Agency is recommending the following text to update the revised assessment. The changes principally reflect the additional data that supported improvement of the ecotoxicological screening to a full characterization.

Despite the fact that the appreciation of widespread perchlorate contamination emerged only over 6 years ago, considerable progress has been made in hazard identification and quantitative dose-response characterization for both the human health and ecotoxicological risks of potential perchlorate exposures.

The thyroid has been confirmed as the target tissue in humans, laboratory animals, and wildlife. The key event of the mode of action for perchlorate is iodide uptake inhibition at the NIS with the potential for both subsequent neurodevelopmental and neoplastic sequelae. A harmonized human health reference dose has been proposed to be protective for both sequelae based on a mode-of-action model.

Data are now sufficient to arrive at acute and chronic benchmarks for ecotoxicological risk assessment. Additional work to link the effects on the thyroid and brain from changes in hormones due to the iodide inhibition in various species may refine this assessment in the future.

Additional research is needed to determine the contribution of exposure sources other than drinking water. This requires more progress in the area of analytical methods to extend current approaches to other media, development and validation of models to address transport and transformation in the environment, and understanding the physiological processes relevant to perchlorate uptake in various biological receptors.

As with any risk assessment, additional insights and new research will continue to change our understanding as the knowledge base is informed with new data and as the scientific and

technical areas relevant to the particular risk characterization mature and evolve. Work dedicated to the areas defined in this chapter should allow continued improvement of the risk characterizations for perchlorate in the future.

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