Appendix A

List of Selected Studies Performed Since the 1999 Peer Review

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

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

Argus Research Laboratories, Inc. (2000) Oral (drinking water) developmental toxicity study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-003D.

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

Bekkedal, M. Y. V.; Carpenter, T.; Smith, J.; Ademujohn, C.; Maken, D.; Mattie, D. R. (2000) A neurodevelopmental study of the effects of oral ammonium perchlorate exposure on the motor activity of pre-weaning rat pups. Wright-Patterson Air Force Base, OH: Naval Health Research Center Detachment, Neurobehavioral Effects Laboratory; report no. TOXDET

BRT-Burleson Research Technologies, Inc. (2000a) Ammonium perchlorate: effect on immune function. Quality assurance audit: study no. BRT 19990524 -- plaque-forming cell (PFC) assay; study no. BRT 19990525– local lymph node assay (LLNA) in mice. Raleigh, NC.

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

BRT-Burleson Research Technologies, Inc. (2000c) Ammonium perchlorate: effect on immune function. Raleigh, NC: BRT 19990524 study protocol: plaque-forming cell (PFC) assay; BRT 19990525 study protocol: local lymph node assay (LLNA) in mice.

Clewell, R. A. (2001a) Consultative letter, AFRL-HE-WP-CL-2001-0006, physiologically-based pharmacokinetic model for the kinetics of perchlorate-induced inhibition of iodide in the pregnant rat and fetus [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 10.

Clewell, R. A. (2001b) Consultative letter, AFRL-HE-WP-CL-2001-0007, physiologically-based pharmacokinetic model for the kinetics of perchlorate-induced inhibition of iodide in the lactating and neonatal rat [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 24.

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

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

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

Greer (2000). Does environmental perchlorate exposure alter human thyroid function? Determination of the dose-response for inhibition of radioiodine uptake. In: Abstracts of the 12th International Thyroid Congress; October; Kyoto, Japan. Endocrine J. 47 (suppl.): 146.

Keil, D.; Warren, D. A.; Jenny, M.; EuDaly, J.; Dillard, R. (1999) Effects of ammonium perchlorate on immunotoxicological, hematological, and thyroid parameters in B6C3F1 female mice. Final report. Charleston, SC: Medical University of South Carolina, Department of Medical Laboratory Sciences; report no. DSWA01-97-0008.

Lawrence (2001). Low dose perchlorate (3 mg daily) and thyroid function [letter]. Thyroid 11: 295.

Mahle, D. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0043, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; October 11.

Mahle, D. (2001). Consultative letter, AFRL-HE-WP-CL-2001-0001, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; May 1.

Merrill, E. (2001a) Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit report for the study of perchlorate pharmacokinetics and inhibition of radioactive iodine uptake (RAIU) by the thyroid in humans (CRC protocol #628) [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 10.

Merrill, E. A. (2001c) Consultative letter, AFRL-HE-WP-CL-2001-0005, PBPK model for iodide kinetics and perchlorate-induced inhibition in the male rat [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; May 8.

Merrill, E. A. (2001d) Consultative letter, AFRL-HE-WP-CL-2001-0008, PBPK model for perchlorate-induced inhibition of radioiodide uptake in humans [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; June 5.

Merrill, E. A. (2001e) Consultative letter, AFRL-HE-WP-CL-2001-0010, comparison of internal dosimetrics using PBPK models for perchlorate induced inhibition of thyroid iodide uptake and sensitivity analysis for male rat model [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; December 20.

Parsons Engineering Science, Inc. (2001) Scientific and technical report for perchlorate biotransport investigation: a study of perchlorate occurrence in selected ecosystems. Interim final. Austin, TX; contract no. F41624-95

Yu, K. O. (2000) Consultative letter, AFRL-HE-WP-CL-2000-0038, tissue distribution and inhibition of iodide uptake in the thyroid by perchlorate with corresponding hormonal changes in pregnant and lactating rats (drinking water study) [memorandum with attachment to Annie Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; June 28.

Yu, K. O.; Todd, P. N.; Young, S. M.; Mattie, D. R.; Fisher, J. W.; Narayanan, L.; Godfrey, R. J.; Sterner, T. R.; Goodyear, C. (2000) Effects of perchlorate on thyroidal uptake of iodide with corresponding hormonal changes. Wright-Patterson AFB, OH: Air Force Research Laboratory; report no. AFRL-HE-WP-TR

Yu, K.O. (2001). Consultative letter, AFRL-HE-WP-CL-2002-0001, intravenous kinetics of radiolabeled iodide in tissues of adult male Sprague Dawley rat dosed with $^{125}I^-$ plus carrier [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; December 21.

Yu, K.O. (2002). Consultative letter, AFRL-HE-WP-CL-2002-0002, intravenous kinetics of radiolabled iodide and perchlorate in tissues of pregnant and lactating Sprague Dawley female rats dosed with perchlorate and/or carrier free $^{125}I^-$ [memorandum with attachment to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; January 7.

Other references:

Crump, C.; Allen, B.; Faustman, Elaine. (1995) The use of the benchmark dose approach in health risk assessment; Risk Assessment Forum. U.S. Environmental Protection Agency, Washington, DC.

Honeycutt, M. (2001) Technical justification for a revised interim action level for perchlorate [interoffice memorandum]. Texas Natural Resource Conservation Commission; December 11.

Johnson, S. (2001) Letter to Dr. Bruce Alberts. U.S. Environmental Protection Agency, Washington, DC; December 14.

Merrill, E.A. (2002) Consultative Letter, AFRL-HE-WP-CL-2002-004, Additional information regarding the comparison of pbpk-derived internal dosimetrics for perchlorate-induced inhibition of thyroid iodide uptake and sensitivity analysis for the male rat model [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; February 19.

Narayanan, L.; Goodyear, C.; Mattie, D. (2000) Consultative Letter. AFRL-HE-WP-CL-2000-0034, Thyroid hormone and TSH co-laboratory study report [memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB, OH: Air Force Research Laboratory; June 15.

Smith, P.; Theodorakis, C.; Anderson, T.; Kendall, R. (2001). Preliminary assessment of perchlorate in ecological receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. Ecotoxicology 10: 305 - 313.

Susarla, S.; Collete, T.; Garrison, A.; Wolfe, N.; McCutcheon, S. (1999). Perchlorate identification in fertilizers. Environmental Science & Technology 34.

Susarla, S.; Bacchus, S.; Wolfe, N.; McCutcheon, S (2000a). Phytotransformation of perchlorate contaminated waters. United States Environmental Protection Agency, Athens, GA; National Exposure Research Laboratory.

Susarla, S.; Susarla, S.; Bacchus, S.; Wolfe, N.; McCutcheon, S (1999) Phytotransformation of perchlorate and identification of metabolic products in myriophyllum aquaticum. International Journal of Phytoremediation 1: 97 - 107.

Appendix B

List of Expert Peer Reviewers

United States
Environmental Protection Agency
National Center for Environmental Risk Assessment

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

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

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Appendix C

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

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

Reviewers' Comments

February 2002

Notice

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

Table of Contents

Charge to Reviewers

Peer Reviewer Comments

William Adams

Michael Aschner

Nancy Carrasco

Michael Collins

Thomas F.X. Collins

Anthony Cox

Teresa Fan

David Hoel

David Jacobson-Kram

Michael Kohn

Loren Koller

Kannan Krishnan

Merle Paule

Mehdi Razzaghi

Gary Williams

Ronald Wyzga

Thomas Zoeller

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

March 5–6, 2002

CHARGE TO THE REVIEWERS

Introduction and Background

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

EPA issued a provisional toxicity assessment for perchlorate in 1992 and a revised provisional assessment in 1995 based on the effects of potassium perchlorate in patients with Graves' disease, an autoimmune disease that results in hyperthyroidism. In March 1997, the existing toxicologic database on perchlorate was determined to be inadequate for quantitative human health risk assessment by an independent non-EPA external peer review panel. A lack of data on the ecotoxicological effects was also noted. In May 1997, a perchlorate testing strategy was developed based on the known mode-of-action for perchlorate toxicity (the inhibition of iodide uptake in the thyroid and subsequent perturbations of thyroid hormone homeostasis), and an accelerated research program was initiated to gain a better understanding of the human health effects of perchlorate, examine possible ecological impacts, refine analytical methods, develop treatment technologies, and better characterize the occurrence of perchlorate in groundwater and surface waters.

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

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

Disclaimer

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

Purpose of the Peer Review

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

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

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

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

Instructions to Reviewers

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

- Studies published since 1999 that have not undergone peer review.

- EPA's interpretation of these and other studies in the perchlorate assessment.

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

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

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

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

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 1
Reviewer Assignments

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

Table 1 (Continued)
Reviewer Assignments

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

Table 1 (Continued)
Reviewer Assignments

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

Table 2
Studies Conducted Since 1999 That Require Peer Review

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

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

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

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

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

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

Topic Area	Relevant Studies
	Yu, K.O. (2001). Consultative letter, AFRL-HE-WP-CL-2002-0001, intravenous kinetics of radiolabeled iodide in tissues of adult male Sprague Dawley rat dosed with $^{125}I^-$ plus carrier [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; December 21.
	Yu, K.O. (2002). Consultative letter, AFRL-HE-WP-CL-2002-0002, intravenous kinetics of radiolabled iodide and perchlorate in tissues of pregnant and lactating Sprague Dawley female rats dosed with perchlorate and/or carrier free $^{125}I^-$ [memorandum with attachment to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; January 7.
	Mahle, D. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0043, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; October 11.
	Mahle, D. (2001). Consultative letter, AFRL-HE-WP-CL-2001-0001, hormone and perchlorate data from cross-fostering study [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory; May 1.

Attachment 1

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

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

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

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

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

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

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

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

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

Attachment 2

General Questions for Reviewing the Topic Areas

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

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

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

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

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

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

   - Are the assumptions and uncertainties clearly and adequately expressed?

Attachment 3

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

William Adams

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

Questions:

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

Attachment 1
Questions & Answers (for D.1)

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

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

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

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

   Parson's Engineering Science, Inc.: Interim Final Scientific Technical Report for Perchlorate Biotransport Investigation
   The purpose of this study was to determine the potential for perchlorate to accumulate in various environmental compartments and media at sites where perchlorate was known to be released. The study was intended to be a screening level study and not an in-depth assessment at any one site. The study design was adequate although it would have been helpful to have a more complete data set and consistency in the number of samples collected at each site. This would have required more intensive sampling, but would have improved the data set. The primary limitations to the data set are: (1) lack of co-located water-sediment-tissue samples and soil-tissue samples, (2) small sample sizes and lack of specific types of samples at each site and (3) insufficient sensitivity for

perchlorate in tissue analyses (i.e., MDL = 400 ppb). These limitations limit the ability to correlate tissue levels to soil/sediment of water and a limit the ability to calculate defensible accumulation factors.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Attachment 2
General Questions for Reviewing the Topic Areas

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 1. Perchlorate Acute Toxicity (mg/L)

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

* value estimated from dose-response data.

Table 2. Perchlorate Chronic Toxicity (mg/L)

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

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

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

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

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

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

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

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

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

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

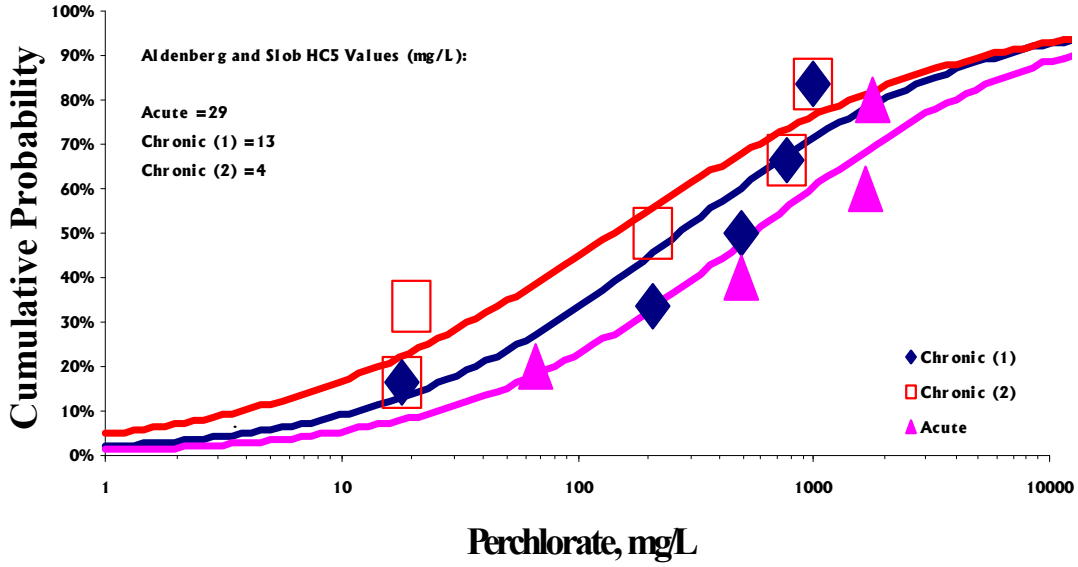
D.5 Do the analyses support the summary and conclusions presented? Are relevant and important aspects of uncertainty addressed sufficiently?

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

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

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

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



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

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

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

Vegetation

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

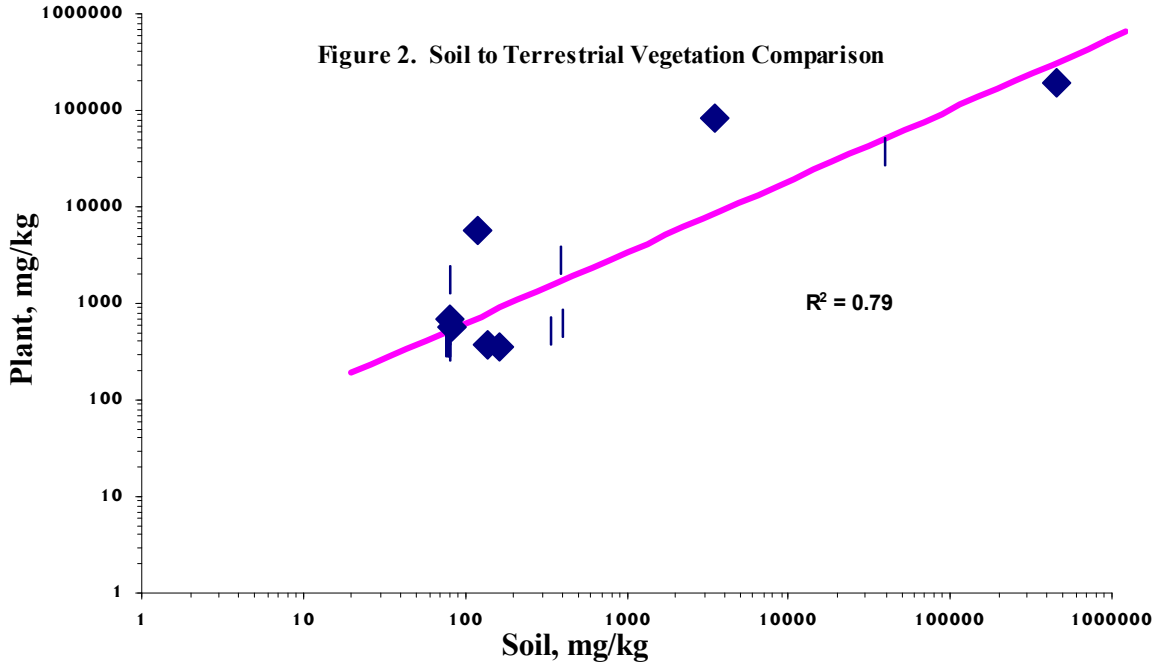
Table 3. Water to terrestrial plant perchlorate concentration ratios with rooted plants

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

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

In comparison with terrestrial plants, the data for aquatic plants indicate that they do not accumulate perchlorate to the same extent as terrestrial plants. BCF values range between 1-2 as depicted in Figure

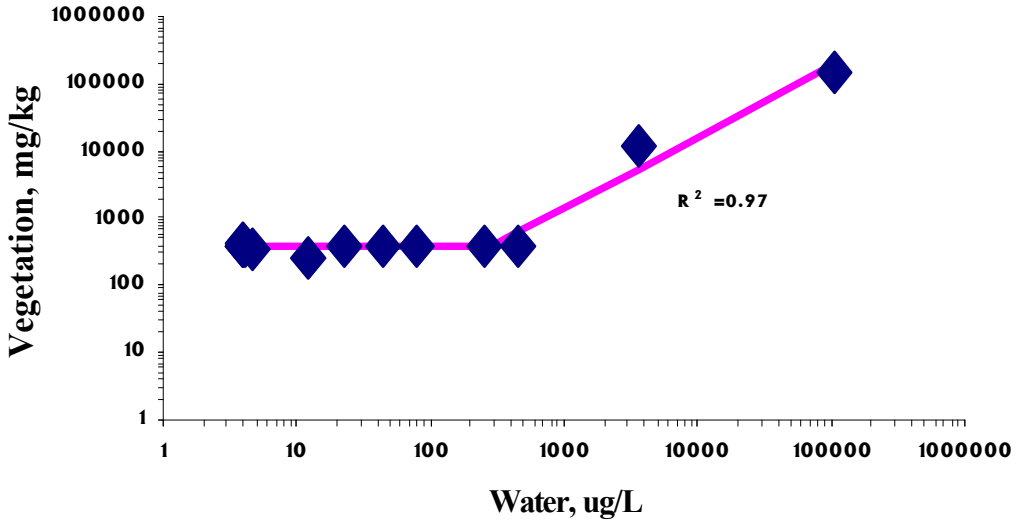
3 (data from Parsons EA report). It should be noted that most of the aquatic plant data is for algae and not rooted macrophytes. However, the lower accumulation by algae is noteworthy in that algal species typically have large BCF values for many inorganic substances due to their high surface area to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



Fish

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

Figure 3. Water to Aquatic Vegetation



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

Figure 4. Water to Fish

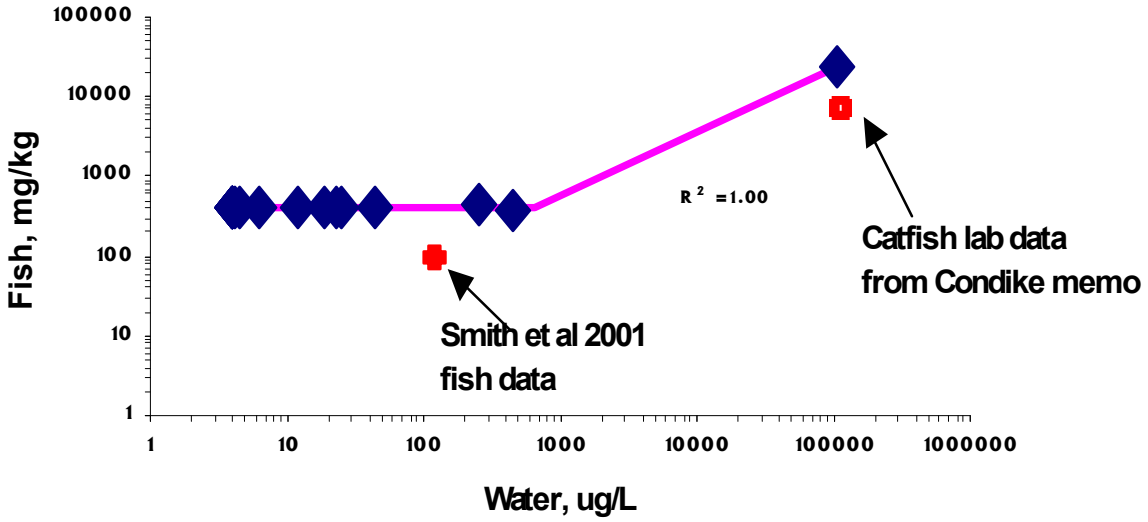
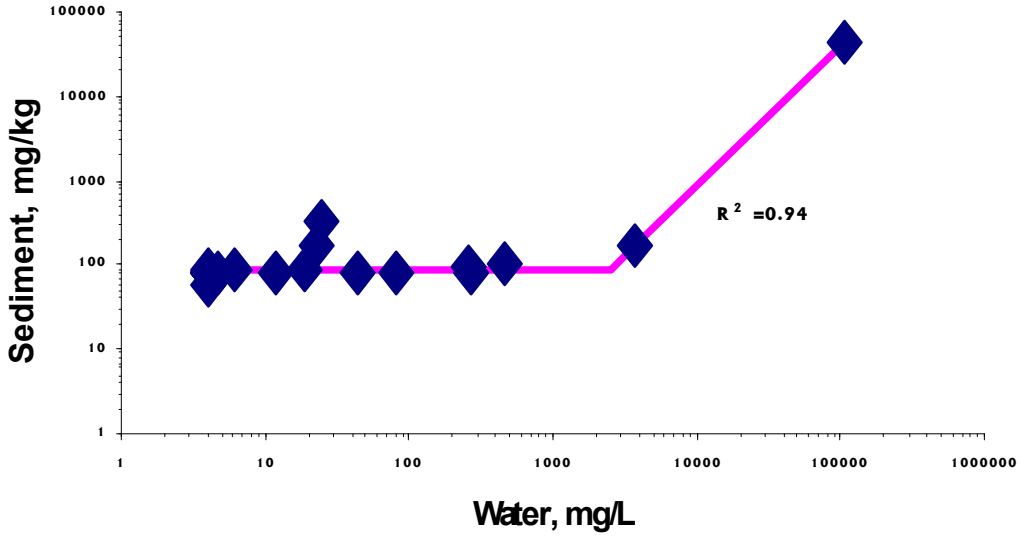


Figure 5. Water to Sediment



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

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

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

References Cited

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

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

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

Michael Aschner

PERCHLORATE ENVIRONMENTAL CONTAMINATION: TOXICOLOGICAL REVIEW AND RISK CHARACTERIZATION

Hazard Characterization and Mode of Action

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

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

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

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

E-9-10
"An administered dose of 0.01 mg/kg/day was supported as a lowest-observed-adverse-effect level (LOAEL) based on the effects on brain morphometry in pups from a PND21 sacrifice in a neurodevelopmental study that repeated similar observations made in a similar 1998 study…" I would have to disagree with this conclusion based on the problems that are inherent to both Argus studies. The studies are deemed inconclusive (see below).

Specific comments for the Introduction (Chapter 1)
The chapter provides background information and historical perspective on the evolution of perchlorate contamination, analytical detection methods, health effects and toxicity, and risk assessment, as well as an exhaustive overview of ecotoxicology screening levels assessment. The layout is logical, and the chapter is easy to follow. It establishes the chronology of events, and provides essential background information to the reader. Overall, this is deemed an excellent introduction to the topic, and there are only a few minor comments or suggestions.

Given that recent publications have reported detection of perchlorate in tap water at levels as low as 0.1 ppb (Handy et al., 2000; Koester et al., 2000), it would be useful to provide additional data (and they likely exist)

on the national occurrence of perchlorate in the drinking water. While Table 1-1 (Mayer, 2001) is helpful, the data seem restricted to the occurrence of perchlorate in suspected contaminated water sites. It should also be indicated what the parenthesis symbolize (presumably, lower detection levels). Information on the level of perchlorate in the general drinking water supply should be conducted and independently confirmed. If this has not yet been considered, an explanation should be provided.

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

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

A.2 The EPA has framed the conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of Perchlorate?

The conceptual basis for perchlorate's effect on the NIS is well articulated. There are, nevertheless, deficiencies in the discussion on neurobehavioral and neurodevelopmental sequelae to hypothyroidism. While the general outcome of hypothyroidism is detailed, it is difficult to gauge similarities between the action of perchlorate and other anti-thyroid chemicals. The magnitude (on a quantitative basis) of hypothyroidism in other conditions (directly- and indirectly-induced thyroid dysfunction) is not well described, making it difficult to compare with the effect of perchlorate. Some of the effects of hypothyroidism are embedded in various chapters, but nowhere is there a concise description in which hypothyroidism (either direct or indirect) is correlated with developmental CNS effects. A section describing these effects would be helpful, especially if it could be documented at the level of hypothyroidism produced by perchlorate.

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

respectively, are deemed very helpful. The other Figures and Tables (Figures 3-4 through 2-12; Tables 3-3 through 3-8) are also extremely useful.

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

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

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

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

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

what magnitude of changes in maternal hormonal levels are associated with aberrant neurodevelopmental outcome in the offspring. Thus, it is difficult to contrast with the perchlorate studies.

A.3 The1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inference can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

This question is beyond my expertise.

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

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

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

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

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

SPECIFIC COMMENTS RE ARGUS STUDY 2001

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

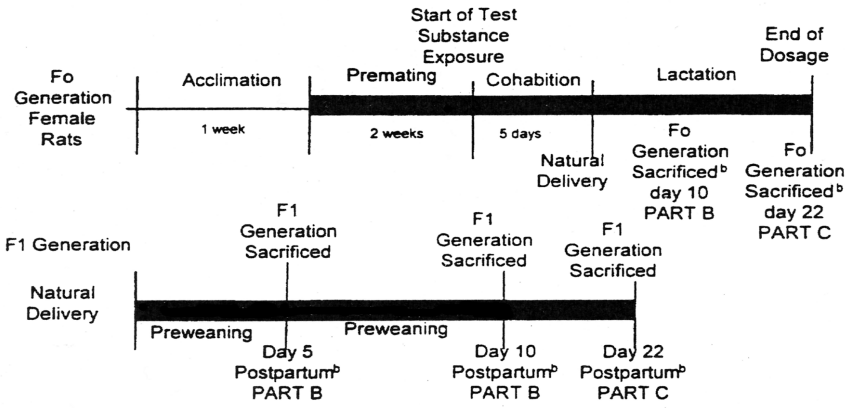
Notwithstanding the above strengths, there is major concern about the study design and methodology, including statistical analysis (multiple t-tests are flawed), raising questions about the validity of the conclusions and inferences, specifically those associated with the brain morphometry measurements.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

Brief summary of the study

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

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

Study Segments B and C[a]



Test Substance Exposure Period through Maternal Milk.
Test Substance Exposure Period.

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.
b. Blood and tissue sample collection.

Methods for Brain Pathology Supplement (page 544)

No problems are identified for tissue collection and fixation [heads removed and placed in Bouin's fixative for >48 hours, rinsed x2 in 50% ethanol (EtOH) and placed in 10% neutral buffered formalin]. To date, brains have been analyzed for the pups sacrificed on LD10 and LD22. The original protocol called for initial studies to be conducted in the control and high dose groups (0 and 30 mg/kg ammonium perchlorate/day, groups I and V, respectively), with additional sectioning and analysis in the other groups should there be any significant changes in the high dose group vs. controls. The sections were cut at 60 micrometers (µm). In the LD10 pups, morphometric linear measurements were conducted bilaterally in 10 brain regions (excluding #9 and 10). In the LD22 pups, linear measurements were conducted bilaterally in 21 brain regions (2 gross and 19 microscopic, the latter representing 9 bilateral measurements plus one). The areas in which measurements were carried out are listed on page 901. All the measurements were carried out with a calibrated ocular micrometer. Full-face images of the coronal sections were also digitally captured and saved for future evaluation, should it be necessary.

Problems/clarifications

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

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

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

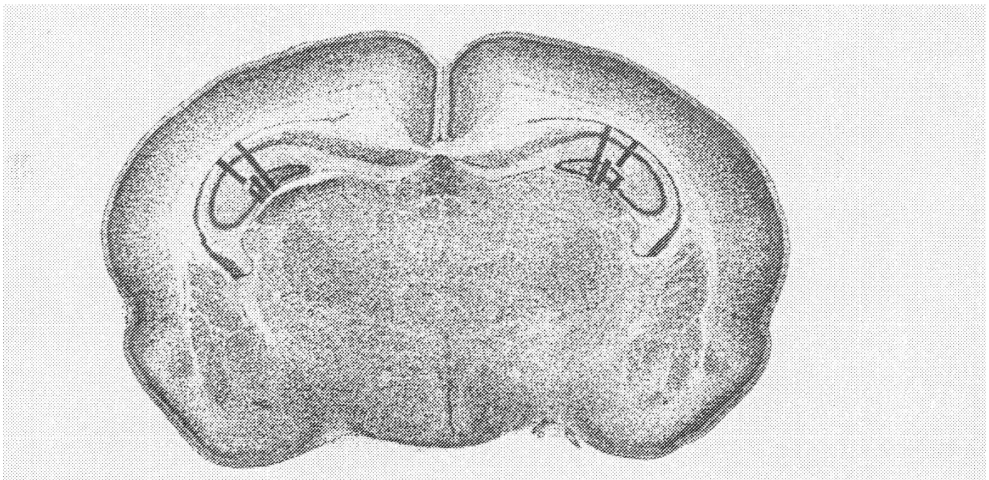


FIGURE 2 – Coronal section, level 2 (level of the infundibulum) showing the four bilateral measurements of the hippocampus (full-hippocampal thickness, dentate, CA1, and CA3).

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

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

Problems/clarifications

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

TABLE 1

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

Maternal Dosage Group 1 (0 mg/kg/day)

Rat Number	Right Striatum (μ)	Left Striatum (μ)	Rt. Corpus Callosum #1 (μ)	Lft. Corpus Callosum #1 (μ)	Right Hippocamp. (μ)	Left Hippocamp. (μ)	Right Dentate (μ)
16731	3024	3024	317	346	1392	1368	792
16732	2688	2736	192	192	1512	1512	888
16734	2736	3072	240	269	1512	1488	864
16735	2688	2784	240	230	1392	1392	816
16736	3120	3072	269	239	1320	1344	744
16737	2784	2736	202	192	1368	1368	792
16738	2880	2976	182	202	1392	1416	768
16740	2832	2832	211	211	1296	1200	720
16741	2928	2976	307	365	1392	1344	720
16742	2592	2784	202	192	1512	1464	888
16745	2784	2880	211	211	1368	1200	792
16746	2736	2640	182	163	1272	1320	720
16747	2832	2736	221	288	1440	1320	816
16749	2688	2736	163	163	1440	1392	768
16750	2784	2784	202	192	1296	1392	744
16753	3024	3024	240	250	1440	1320	840
MEAN	**2820**	**2862**	**224**	**233**	**1397**	**1365**	**792**
SD	**144.0**	**141.2**	**43.5**	**59.9**	**77.0**	**86.7**	**56.8**

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

Lack of dose-response is problematic, as are the trends (sexual dimorphism). Most troubling is the lack of symmetry in the findings between left and right brain hemispheres (see Tables below). The most likely explanation is that these effects are attributable to artifacts associated with the cutting of

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

The sex-related differences in the thickness of corpus callosum are in disagreement with the findings from the developmental neurotoxicity study that was previously conducted by the same contract laboratory (1998). In the previously conducted study, thicker corpus callosum were noted for both sexes, albeit on a different day (PD12) in the high dose group, which was 10 mg/kg/day (identical treatment paradigm), with more pronounced changes in the females.

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

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

Text Table A

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

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

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

Text Table A

Measurements Differing Significantly Between Treatment Groups and Control Group

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

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

SPECIFIC COMMENTS RE ARGUS STUDY 1998

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

Methods for Brain Pathology Supplement, page 22
Subset 1 was sacrificed and analyzed on DP12 (corresponding to PD11 using EPA's nomenclature). Subset 4 was sacrificed and analyzed on DP 82 (corresponding to PD81 using EPA's nomenclature).

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

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

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

Specific comments on Bekkedal et al., 2000

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

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

Problems/considerations regarding brain morphometry

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

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

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

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

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

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

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

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

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

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

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

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

Comparison between the Argus 2001 study and others is limited by differential dosing regimens and sacrifice times, but even with these differences, it is exceedingly difficult to reconcile the differential responses (Note the differential responses in male CA3 hippocampus between LD10 and LD22 pups

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

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

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

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

Minor issues:
Page 5-53-20 and 23 – change “effected” to “affected”.

Specific comments about thyroid hormone measurements

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

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

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

Thyroid hormone analysis
Argus 1998 study, page 23
"All dosages of ammonium perchlorate increased TSH, T3 and T4 serum levels for F0 generation dams at DL 10 over the control group values. Serum TSH was statistically elevated ($p<0.01$ or $p<0.001$) for the 1.0, 3.0, and 10 mg/kg/day groups, however, no dosage-response was evident between the 3.0 and 10.0 mg/kg/day dosage groups. Serum T4 values were greater than the control value in all groups exposed to the test substance, however, only the 0.1 and 10.0 mg/kg/day dosage group values were statistically significant ($p<0.001$ and $p<0.05$, respectively). Serum T3 values were significantly increased ($p<0.05$ to $p<0.001$) over the control group value in the 0.1, 3.0 and 10 mg/kg/day target dosage groups". No hormone measurements were carried out in the pups in the Argus 1998 study.

Argus 2001 study
EPA document page 5-59 refers to maternal hormone changes. "Exposure to perchlorate produced significant decreases in thyroid hormones and an increase in TSH in the dams. For effects on maternal T3, there was no age-by-treatment interaction and the NOAEL at all time points was 1.0 mg/kg-day. There was a significant age-by-treatment interaction for effects on maternal T4. Step down analysis resulted in a LOAEL at 0.01, 1.0, and 30.0 mg/kg-day at GD21, PN9 and PN21. The 0.01 mg/kg-day level is a LOAEL for the dams at GD21. There was also a significant age-by-treatment interaction for the effects on maternal TSH. Step-down analyses resulted in LOAEL at

0.01, 0.01 and 0.1 mg/kg-day at GD21, PND9 and PND21. As for the effect on T4, there was no NOAEL at GD21 for the effects on TSH".

Problems/clarification

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

Behavioral Evaluations pages 5-43 through 5-52

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

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

Yes.

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

The brain morphometric measurements are suggestive of an association between exposure to ammonium perchlorate and an adverse effect in the rat brain (morphometry), but are limited because chance, bias, and confounding cannot be ruled out with confidence. The findings are not consistent in direction across dose and exposure time; they are inconsistent between studies performed by the same laboratory (Argus) and a consistent positive association cannot be ascertained. It cannot be concluded with certainty that the measured effects were within the range expected on the basis of sampling error and selection bias, and, therefore they are deemed inconclusive.

Human Health Dose-Response Assessment

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

Per above, the brain morphometric studies are inconclusive.

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

I feel unqualified to address this issue.

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

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

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

Yes.

Risk Characterization

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

Yes.

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

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

Nancy Carrasco

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

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

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

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

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

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

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

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

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

A.2. The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

See H.1

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

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

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

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

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

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

Minor points.

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

Michael Collins

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

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

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

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

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

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

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

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

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

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

(h) Goleman WL, LJ Urquidi, TA Anderson, EE Smith, RJ Kendall and JA Carr (2002) Environmentally relevant concentrations of ammonium perchlorate inhibit development and metamorphosis in Xenopus laevis. Environ. Toxicol. Chem. 21(2): 424-430.

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I_2) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

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

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

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

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

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

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

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

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

(1) Regarding the interpretation of this study by the EPA document, there appeared to be disagreement between the EPA and Argus with respect to the issue in C.1 (3). In this instance, my interpretation corresponds to the criticism of EPA, but the issue is not well delineated in the EPA document.

(2) There are differences of interpretation between EPA and Argus as indicated on pages 5-81 to 5-83 in the EPA document. It is this reviewer's opinion that the alopecia is not a significant finding (in agreement with Argus), but that the developmental endpoints may have relevance (in agreement with Argus).

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

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

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

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

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

As mentioned previously, with the exception of the data in Section 5.5.3, the data for most of the animal experiments is consistent with the idea that the mechanism of perchlorate toxicity is mediated by the inhibition of NIS. Many different toxicological endpoints can be explained by the inhibition of NIS, which subsequently leads to a depletion of T4 or T3 or an increase in rT3, and then upregulates the TSH release from the pituitary leading to an alteration in the hypothalamic-pituitary-thyroid axis. The point of departure has not been well defined in the document in the section entitled "Point of Departure Analysis", and this should be rectified. The point of departure is an attempt to estimate the threshold for the most sensitive endpoint, and in this case it has been chosen to be a LOAEL of 0.01 mg/kg/day. This is somewhat substantiated by the data, but there is frequently a nagging feeling that the derivation of the LOAEL is not really scientifically-based, but represents more of an art.

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

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

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

In general, the concept of uncertainty factors, namely determining the product of a series of factors which are based on an order of magnitude of uncertainty and then deriving a reference dose is not appealing from a scientific perspective. It is my opinion that the quantity of each of these factors could be debated. Surely the intraspecies variability having a factor of 3 is not consistent with the number of factors which can make human variability so large. This factor should be 10. Also, the lack of any uncertainty factor for interspecies extrapolation seems to over-emphasize the current hypothetical idea of how perchlorate works. Deriving a value of 0.01 mg/kg/day seems equally suspect from my perspective as the LOAEL. It could be argued that there should be modifying factors predicated on the certainty of the data.

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

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

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

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

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

(1) Affinity of perchlorate for the NIS.
(2) Specificity of perchlorate for the NIS
(3) Alternate routes of iodide transport into cells, and quantification of various routes.

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

(1) Page 3-5, line 21. The use of three radiolabelled molecules to determine follicle volume and membrane potential is not clear.
(2) Pages 3-9 to 3-11, description of cellular processing is unclear.
(3) Concept of minimum reporting limit is not clear.
(4) Page 3-4, line 1: Radioiodide accounts for 30 % of thyroid gland volume?
(5) Page 3-4, line 4 and 3-9, line 14: Is there a contradiction regarding salivary glands?
(6) Page 3-13, line 31: Selectivity based on large cation?
(7) Page 3-16, line 12: "...antithyroid-mediated neoplasia...." Accurate wording?

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

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

Thomas F.X. Collins

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

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

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

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

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

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

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

The 6 amendments to the study were either routine (e.g., AniLytics designated for analysis of samples) or added to the robustness of the study (e.g., expansion of the list of tissues to be

analyzed, and blood taken from pups not selected for mating provided for additional evaluation of TSH, T3 and T4 levels). The deviations listed did not affect the results of the study.

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

Yes. Yes. Yes.

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

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

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

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

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

Otherwise, the data are accurately reported. In some cases, the TSH data do not follow the expected results if the thyroid is being inhibited. E.g., there is an increase in T3 values in P1 adults,

where there should be a decrease. There is no effect on T4 values in F1 females, but there is an effect in males. In F1 weanling males, there is no effect on TSH, T3 or T4. In F1 weanling females, TSH was slightly increased, and T3 and T4 were decreased. In F2 weanlings, there were no effects except for a slight increase in T4 in males and females.

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

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

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

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

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

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

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

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

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

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

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

A2.4. Authors of the Toxicological Review and Risk Characterization Document in some cases have performed statistical analyses beyond those in the original study reports. Where these

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

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

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

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

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

Yes.

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

Yes.

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

Yes. There are effects on the NIS. In some cases, increased TSH levels were seen, as well as decreased levels of T3 and T4. After the re-analysis of the thyroid samples, 2 pups (with 3

adenomas) were recorded. The proposed mode of action involves the gradual sequence of colloid depletion, hypertrophy, and hyperplasia of the thyroid.

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

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

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

Questions: Sperm Evaluation:

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

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

Comments (See Table 1 below):

Cauda epididymal weights and sperm density:

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

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

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

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

Testicular Weight (Left) and spermatid density:

Observation1: For both the P and F1 generation animals there was minimal effect on left testicular weights, testicular weight expressed per gram of brain weight or testicular weight expressed per gram of body weight. Additionally, there does not appear to be a significant change in sperm density in the P generation when the control and the high (30 mg/kg) dose groups are compared. However, there does appear to be a dose related non-statistically significant decrease in spermatid density in the F1 generation (see Table 1). A calculation of Daily Sperm Production (DSP; concentration/gram of testis/6.10; Robb et al., 1978) for the P generation did not reveal any dose related effect on DSP. In contrast, similar calculations for the F1 generation revealed a dose related decrease in DSP (See Table 1) ranging from 20.5 (which is comparable to historical values for the rat) in the control group to 16.1 in the 30 mg/kg dose group.

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

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

Table 1

Male Reproductive Organ Weights and Sperm Numbers Data

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

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

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

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

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

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

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

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

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

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

Extra analyses were not done.

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

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

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

Yes.

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

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

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

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

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

No.

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

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

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

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

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

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

No additional statistical analyses were done.

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

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

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

Yes.

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

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

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

No effect.

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

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

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

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

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

Springborn Laboratories (1998): Sprague-Dawley rats were given ammonium perchlorate (0, 0.01, 0.05, 0.2, 1.0, or 10 mg/kg-day) by drinking water for up to 90 days. Rats (10/sex/dose) were sacrificed at 14 , 90, and 120 days (after a 30-day recovery). Clinical observations, body and organ weights, feed and water consumption, hematology, clinical chemistry, and ophthalmology were measured. Liver, kidneys, lungs, thyroid/parathyroid, and gross lesions were examined microscopically. No clinically remarkable findings were noted. Only animals in the 0, 0.05, 1.0, and 10 mg/kg-day groups were continued to 120 days. Absolute thyroid weight and thyroid weight relative to body weight and brain weight were increased significantly in males at 10 mg/kg-day after 14 and 90 days of treatment and in females at the 10 mg/kg-day group, indicating a LOAEL of 10 mg/kg-day. Thyroid weight was normal at the end of 120 days. Male rats showed follicular cell hyperplasia by day 14 [at a dose not identified in the report] which was not fully recovered by day

120. On day 14, females showed decreased colloid and follicular cell hypertrophy at 10 mg/kg. By 90 days, colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia in both sexes were significantly increased at 10 mg/kg-day (LOAEL). By 120 days, thyroid histopathology had been reversed. Upon re-evaluation of the hormone analyses, the LOAEL for T3 effects in males and females was 0.01 at day 90, but there was recovery at day 120 (NOAEL at 10 mg/kg-day). There was a free-standing LOAEL of 0.01 mg/kg-day on day 90 for effects on T4 in both sexes. After 120 days, there was a free-standing LOAEL of 0.05 mg/kg-day in males and a NOAEL of 1.0 mg/kg-day in females for effects on T4.

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

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

No.

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

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

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

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

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

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

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

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

ecotoxicological effects of perchlorate.

Yes. No. No suggestions.

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

Yes.

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

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

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

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

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

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

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

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

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

No.

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

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

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

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

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

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

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

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

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

The section was difficult to understand.

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

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

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

See above.

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

Yes.

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

See above.

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

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

Argus (2000): Presumed pregnant rats (24/group) were given ammonium perchlorate in drinking water at 0., 0.01, 0.1, 1.0, or 30 mg/kg-day for 15 days before cohabitation and continuing to sacrifice. The animals were assigned to groups by random stratified procedure. There were 19, 19, 17, 20, and 20 pregnant rats per group, respectively. All were sacrificed (cesarean section?) at GD 21. Gravid uterine weight were recorded, and resorptions, corpora lutea, implantations, and live and

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

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

No.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Yes.

SPECIFIC CHARGE QUESTIONS ORGANIZED BY TOPIC AREA

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

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

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

No.

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

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

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

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

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

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

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

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

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

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

Yes.

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

Yes.

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

They are appropriate.

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

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

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

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

extrapolation?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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A.2 Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data	The roles of the key event and subsequent neurodevelopmental and neoplastic sequelae *are* clearly articulated (see e.g., Figure 7-2, p. 7-5) and seem consistent with available data in rats. However, the relative importance of the key event and of subsequent neurodevelopmental and neoplastic sequelae are less clearly articulated, especially for purposes of comparing rat responses to human responses. (For example, do rat thyroids grow throughout life, while human thyroids do not, and does this create a potential for subsequent neoplastic development following perchlorate dosing in rats but not in humans?) The key question of why "Acute exposure to ionizing radiation, especially in childhood, remains the only verified cause of thyroid carcinogenesis in humans" (Hard, 1998) while perchlorate and other chemicals cause thyroid carcinogenesis in rats, has not been explained in terms of clearly articulated roles for the key event and subsequent processes.
A.3 What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?	At least for carcinogenesis, the AUC for dose is probably less relevant than the duration and magnitude of precursor responses (e.g., compensating hyperplasia) induced by the dose. Possibly, the AUC for precursor responses such as upregulation of TSH would be more predictive as an indicator of biologically effective dose than the AUC of perchlorate.
A.4 Is the harmonized approach to characterize the potential risk of both noncancer and cancer toxicity protective for both?	Yes. (It seems likely that the excess cancer risk will be zero or undetectable when there is no noncancer toxicity. The noncancer toxicity is addressed using "precursors" that, in fact, may not be associated with any increased risk of harm, i.e., it is not clear that they really are "precursors", in the sense of being on the causal path leading to harm. So, if anything, the harmonized approach may be over-protective for both noncancer and cancer end points.)
B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? (See Table 2 and Attachment 1)	Yes. None of the studies published since 1999 that I have reviewed or am aware of has yet overcome the basic limitations on lack of good human exposure data, well-controlled confounding, adequate power, etc. Moreover, none of them has focused on the quantitative pharmacodynamic processes needed to complete the PBPK front end and form a full biologically-based risk assessment model for perchlorate. The new studies and letter of Greer and Braverman are consistent with other literature in suggesting potential points of departure for human health risks that are much higher than the rat-based value; however, the QA/QC audit by Merrill reveals several potential weaknesses in the data. None of these studies changes the general point that rat-based studies suggest a point-of-departure for RfD calculations that may be much smaller than those from (limited and imperfect) human data. The new studies do suggest some possible numerical values that might be used in sensitivity analyses as possible values for a human-based point-of-departure (recognizing that there are many uncertainties and limitations of the human data.)

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B.2 How well has EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment? (See Attachment 2)	The discussion for the most part seems fair and balanced. However, the contrary view of Soldin et al. (2001), see p. 4-30, deserves more discussion. The unexpected findings of Crump et al. (2000) also deserve more discussion and perhaps more weight in the quantitative risk assessment. Simply noting that there are unexplained contradictions and indications that risk is much lower in humans than might be expected based on extrapolation from rates does not fully interpret the significance of these results for quantitative risk assessment. As discussed later, the epidemiological studies might perhaps be useful, despite their strong limitations, to help develop a sharper plausible upper bound on human risks if Chapter 6 can be revised to include dose-response information about the predicted levels of exposures for which human health responses *are* predicted to occur.
B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?	Yes. The summary of ecological studies (Section 4.1.1) seems generally well done. The fact that other reviewers find little cause for concern is acknowledged (p. 4-30). However, although the epidemiological data are too weak to serve as the sole basis for an adequate hazard characterization, this does not mean that they should not be used at all. In general, the epidemiological studies do seem to be quite reassuring, showing that cancers and other adverse effects do not seem to occur at detectably elevated rates even at relatively high exposure concentrations. This suggests that the assumption that people are as sensitive as rates should probably be modified. It is consistent with the (animal?) evidence referred to (but not specifically cited) on p. 3-19.
B.4 Are the exposure measures constructed from data in the epidemiological studies sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies?	Perhaps some meaningful bounding could be done if the predicted dose-response estimates were more explicitly stated. While the limitations of ecological studies and lack of individual-level exposure data in normal human populations cannot easily be overcome, it does seem that humans are not developing thyroid tumors at the rates that would be expected if 0.00003 mg/kg-day (p. 10-3) were close to (i.e., within a couple of orders of magnitude of) exposure levels at which significant excess cancers occurred. Despite their severe limitations, the data in the epidemiological studies might indeed be sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies *if* these predictions were clear and testable. But the numerical value of 0.00003 mg/kg-day given on p. 10-3 is a level at which appreciable risk is expected to *not* occur. It is notoriously difficult to test such a negative, even if epidemiological data were much stronger than they are. To use the epidemiological data to provide a sanity-check on the risks extrapolated from animal models, it would be valuable to add to Section 10.1.2 some predictions about the lowest exposure levels for which detectable risks *are* expected to be seen in human populations. Then, these can be compared to realistic exposure levels (e.g., in California) to

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	see whether the predictions are consistent with epidemiological observations. In general, the proposed 0.00003 mg/kg-day level (p. 10-3) is much less than the estimated level of 35 to 100 mg/kg day "at which thyroid hormone levels may [begin to be] be reduced or thyrotropin levels increased" suggested by Soldin et al. (2001). Industry vs. regulatory perspectives aside, this gap is large enough to deserve some additional discussion.
B.5 Are the associations observed in the epidemiological data consistent with the proposed mode of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?	The absence of positive associations observed in various (mainly ecological) epidemiological studies *is* consistent with the proposed (non-linear, indirect) model of action. In general, power has *not* been sufficient to detect small effects (e.g., relative risks < 2). Confounders have *not* been controlled.
C.2 How well has EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment? (See Attachment 2.)	The correlation analyses in Appendix 7A are a very blunt tool for analyzing these interesting data. The problem with correlations (ordinal or Pearson's) is that they consider only pair-wise associations. Multivariate interaction and response surface modeling techniques, such as classification trees (CART), MARS – or, better, dynamic models of the relations among T3, T4, and TSH over time – could add much more information and insight than correlation analyses. For an analysis that has been worked on so hard in many parts, it seems important to probe beyond mere correlations, which are necessarily at best quite superficial indicators of multivariate and dynamic relations.
C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?	Yes, for toxicokinetics. Pharmacodynamics have not been modeled.
C.4 Are the NOAELs/LOAELs appropriate? Please explain.	The proposed 0.00003 mg/kg-day level (p. 10-3) is much less than the estimated level of 35 to 100 mg/kg day "at which thyroid hormone levels may [begin to be] be reduced or thyrotropin levels increased" suggested by Soldin et al. (2001). This gap is large enough to deserve additional discussion. It is not clear that the NOAELs/LOAELs are appropriate *for human* risk assessment. Determining whether they are appropriate requires better quantitative modeling of relevant (or at least proposed) *pharmacodynamic* processes in rat and human thyroid glands. This is perhaps the most important gap in the current risk assessment. The relevance of the 0.01 mg/kg-day LOAEL for *humans* is unclear.

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D.7 Comment on the strengths and limitations of the available data to suggest sources of perchlorate exposure other than drinking water.	Data are very limited. The current report does a good job of documenting the limitations in the available ecotoxicity data and exposure data for non-drinking-water paths (e.g., via inhalation).
F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure? Should any other data be considered in arriving at a point of departure?	The proposed point of departure of 0.01 mg/kg-day (p. 10-3) is of uncertain relevance for *humans.* It may be too high by at least several orders of magnitude. It might be valuable to consider several other points of departure, including those suggested based on human data (while acknowledging their limitations). Presented as a form of sensitivity analysis and a guide to relevant uncertainties, a multiple starting-point analysis might be quite effective.
F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.	Although the PBPK model in Chapter 6 seems to be well thought out and well developed, PBPK considerations alone do *not* justify the selected AUC dose metric, nor do they provide an adequate basis for interspecies extrapolation of risk (of RfD vales). The reason is that the toxico*dynamics* are also likely to be very important – enough so that they might dominate the analysis. Rather than using a PBPK-based AUC to accomplish interspecies dose conversion, it is worth asking first (in the hazard identification section) whether *any* HEE truly exists (e.g., do people develop thyroid tumors in response to perchlorate tumors at any level?) If it is assumed that human responses are similar to rat responses, then it may still worth using intermediate responses such as the AUC under the time course of compensating hyperplasia (plotting excess mitoses per unit time over time) as a more predictive dose metric than dose AUC. In summary, the current quantitative modeling and interspecies extrapolation of doses essentially stops with PBPK results, but pharmacodynamics are likely to be crucial and should be considered in the quantitative modeling in order to better understand and appropriately represent interspecies differences.

F.3 Are there other data which should be considered in developing the uncertainty factors?	The epidemiological data suggest that uncertainty about pharmacodynamics may be important (i.e., people may be much less susceptible to various adverse health effects) than extrapolation form the rat data indicates. Whether or not there are other *data* that should be considered in developing the uncertainty factors, other *modeling* techniques should definitely be considered. Model uncertainty seems very important (see e.g., p. 7B-6), and approaches such as model cross-validation and Bayesian Model Averaging (BMA) that account for it should be used in the uncertainty analysis.

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

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

Teresa Fan

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

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

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

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

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

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

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

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

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

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

stomach content may provide some clue.

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

IV. Susarla et al, 2000:

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

V. Susarla et al, 1999:

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

indicated that 54-60% of perchlorate in solution was adsorbed to the sand in a separate experiment.

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

VI. Nzengung, n.d.:

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

VII. Smith et al., 2001:

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

D.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment

2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

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

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

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

water, sediment or soil, and diet. Again, how this difference in exposure route affect perchlorate uptake and metabolism is unclear.

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

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

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

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

- This is a well-written document with the analyses, summary, and conclusion clearly described. Many of the relevant and important aspects of uncertainty have been addressed sufficiently. Some of the limitations that have not been addressed in the document are stated in D.3 and D.4.
- The potential route of exposure through citrus (e.g. Sourthwestern region) and imported fruit and vegetables was discussed but not indicated as an aspect needed to be investigated. Since little information on the perchlorate occurrence in these food items, they should not be discounted as a

potential route of exposure to human.

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

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

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

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

- The report on a wide occurrence of perchlorate in various ecological receptors (e.g. Parsons Engineering report, 2000; Smith et al., 2001; Condike, 2001) in perchlorate contaminated sites indicate that there are exposure routes other than drinking water for wildlife.

- There is indication that vegetation is an important mediator of soil exposure.
- However, it is unclear how and to what extent these exposure routes affect human exposure, especially via citrus and imported fruits and vegetables.
- It is unclear regarding the spatial and temporal influence on these exposure pathways, nor is it clear on the interaction among individual components of the exposure pathway (e.g. diet-related transfer and transformation of perchlorate through different trophic levels).
- It is unclear on the effect and extent of exposure to perchlorate transformation products such as chlorate, chlorite, and hypochlorite.

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

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

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

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

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

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

David Hoel

Topic Area A: Hazard Characterization and Mode of Action

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

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

Topic Area B: Human Health Effects Data

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Topic Area F: Human Health Dose-Response Assessment

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

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

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

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

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

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

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

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

Topic Area G: Risk Characterization

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

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

Topic Area H: General Comments, Conclusions, and Recommendations

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

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

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

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

David Jacobson-Kram

Topic Area A: Hazard Characterization and Mode of Action

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

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

Topic Area C: Laboratory Animal Studies

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

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

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

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

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

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

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

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

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

Topic Area F: Human Health Dose-Response Assessment

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

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

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

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

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

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

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

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

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

Topic Area G: Risk Characterization

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

I believe it does.

Topic Area H: General Comments, Conclusions, and Recommendations

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

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

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

Michael Kohn

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

March 5–6, 2002

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Specific Questions for Area A

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

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

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

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

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

- The problem is that different endpoints probably show very different sensitivities to over-stimulation by chronically depressed T_4 or chronically evaluated TSH. If we knew the expression of which genes were being altered and by how much, we could avoid the “one

size fits all" drawback of simply using serum TSH (worse yet is thyroid perchlorate) as the index of risk. Otherwise, we only have empirical correlations and limited predictive capability.

Specific Questions for Area E

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

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

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

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

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

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

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

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

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

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

Specific Comments for Area F

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

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

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

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

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

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

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

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

Specific Comments for Area G

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

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

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

Specific Comments for Area H

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

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

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

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

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

Dear Kate,

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

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

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

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

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

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Loren Koller

Loren D Koller

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization

Topic Area A: Hazard Characterization on Mode of Action

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Loren D Koller

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

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

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

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

Quality Assurance Audit

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

Loren D Koller

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

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

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

Kannan Krishnan

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

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

This reviewers' response to specific questions follow.

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

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

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

The assessment in the present stage precludes the consideration of multiple mechanisms of action. The single, unifying mode of action used as the basis of this assessment, however, is convincing, consistent with the available data, clearly articulated and scientifically-sound.

A.3 The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-

action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

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

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

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

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

1. *Structure.*

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

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

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

The use of a mathematical description based on saturable uptake is consistent with the proposed mechanism of uptake and it allows to account for competitive inhibition by perchlorate. The NIS, localized in the basolateral membrane of the thyroid gland follicular cells, transports ions from extracellular fluid into the thyroid epithelial cell. As perchlorate is not organified, what is the basis/mechanism involved in moving it across the cells through to the colloids ?

Active transport systems are saturable and susceptible to competitive inhibition, and typically move chemicals against concentration gradients. In the present case, it seems that the effect of the active transport of perchlorate or iodide into the follicular cells is offset by the simultaneous presence of the passive diffusion process which allows the re-establishment of

equilibrium, on the basis of concentration differences and partition coefficients. What is the relative importance of passive diffusion and active transport for iodide and perchlorate ? The active uptake should be the key process here.

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

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

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

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

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

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

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

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

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

Chemical-specific parameters

Affinity constants for active transport

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

Urinary clearance

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

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

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

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

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

Partition coefficients

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

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

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

Other parameters

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

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

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

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

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

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

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

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

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

5. Variability and Uncertainty.

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

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

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

See General comments above.

F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure?

Yes

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

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

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

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

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

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

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

Sufficient well.

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

The profile of effects, the relevant mechanisms are considered in this assessment.

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

Yes, for a screening level assessment.

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

-

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

I have noted a number of minor comments (typos in the text and equations, lack of clarity in text, tables and figures) and will provide them at the workshop.

Merle Paule

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

Comments by Merle G. Paule, Ph.D.

Topic Area A: Hazard Characterization and Mode of Action

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na+)-Iodide (I-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

The importance of the key event is plausibly and logically presented and it is adequately related to the neurodevelopmental and neoplastic sequelae shown to occur with exposure to perchlorate. The clear dose-response relationships between perchlorate exposure and inhibition of iodide uptake, increases in TSH, decreases in T3 and T4, colloid depletion, hypertrophy and hyperplasia of the thyroid are compelling. The U-shaped dose response curves for some metrics including rat pup brain morphometry are not atypical phenomena in biological systems, especially those that by their very nature are adaptive and plastic.

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

All noted effects of exposure correlate with the ability of perchlorate to alter thyroid hormone profiles and this effect is related to amount of compound at the target site (NIS). The use of AUC (area-under-the-curve) data as the dose metric is justified because it plausibly provides a good measure of average exposures likely to affect the NIS.

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

The approach would appear to be protective for both toxicities since it seems clear that the elicitation of both the precancerous effects by perchlorate (colloid depletion, thyroid hypertrophy, hyperplasia) and the alterations in the concentrations/availability of TSH, T3 and T4 and the presumptive effects of these perturbations on neurodevelopment all show similar dose-responses and sensitivities.

Topic Area C: Laboratory Animal Studies

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer

every question in Attachment 1, rather use your professional judgement to address those that are most appropriate to the study in question.

Bekkedal et al., 2000.

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

The strengths of this study lie in the use of the litter as the experimental unit; the use of automated and, therefore, objective measurements of motor activity. While this approach to monitoring motor activity (Opto-Varimex) can be thought of as state-of-the science, the very large standard deviations associated with many of the measurements (often near or greater than 100% of the mean data) reported here, indicate an inherent lack of sensitivity of those measures. This finding suggests problems in the way the assessments are being carried out, the way they are being recorded, with the apparatus itself or some combination of the above. It is also not clear from this report, how the timing of the behavioral assessments were performed. While it is stated that the motor assessments occurred between the hours of 0830 and 1430, it was not stated whether any stratification was employed to distribute time of day assessments equally across treatment groups or if time of day was considered at all. It seems possible that the large variability noted in most of the assessment measures presented in this report might be due to changes in activity levels as a function of time of day.

It is unclear from the text (e.g., page 7) how the doses were calculated, but it appears from Table 1 that the doses were expressed as the ammonium salt and not the anion equivalent? This point needs to be clearly addressed.

It is somewhat confusing that the authors refer to ‘habituation’ (see page 8) both to describe aspects of the measured behaviors in the Opto-Varimex apparatus and processes that occur prior to beginning the Opto-Varimex test session. For purposes of clarity, perhaps ‘acclimation’ would better describe pre-test conditioning of subjects.

There is no reference for the Greenhouse-Geisser statistical test employed in the analyses of the data (page 9).

The Tables would all benefit from having a short legend/descriptive title.

The symbols used in the figures did not reproduce well and were often hard to read.

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

As mentioned above, it is not clear how the timing of the behavioral assessments for each subject were determined. While it is stated that the motor assessments occurred between the hours of 0830 and 1430, it was not stated whether any stratification was employed to distribute time of day assessments equally across treatment groups or if time of day was considered at all. It seems possible that the large variability noted in most of the assessment measures presented in this report might be due to changes in activity levels as a function of time of day.

It would appear that appropriate QA/QC procedures were effected and that no significant deviations from the protocol occurred: however, the document attesting to this presumption is not signed.

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

Again, it is unclear from the text how the doses were calculated, but it appears from Table 1 that the doses were expressed as the ammonium salt and not the anion equivalent. The approach taken should be should be made very clear. Given the huge variation associated with the endpoints monitored, not enough subjects were used to provide adequate power to detect effects using the statistical approach employed by these authors. By re-analyzing the data using a different approach, the EPA was able to demonstrate significant findings in some endpoints.

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

See number 3.

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

See also number 3.

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

Given the re-analysis of the Bekkedal et al., 2000 data by the EPA, the study does provide information useful for hazard identification. This conclusion is afforded further confidence since a similar re-analysis of data from an earlier study of motor activity (Argus Laboratories, Inc, 1998a) showed similar findings. It should be noted that the discussion of the analyses of the Bekkedal data (page 5-49of the EPA Toxicological Review and Risk Characterization Document) is unclear in that it is difficult to know what the authors mean by ‘first’ and ‘final’ habituation intervals. Do these refer to intervals within a single test session or to the entire first session (i.e., on PND 14) versus the entire last session on PND 22?

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

Yes. The subjects in this study would serve as a model of developmental exposure. Even though dams were exposed before, during and after pregnancy, no data were obtained for these subjects, thus data pertinent to pregnant subjects or adult females were not modeled.

Argus Laboratories, Inc., 2001:

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

The strengths of this study lie in the comprehensive period of exposure (2 weeks prior to mating up to lactational day 22 in some groups), use of the litter as the experimental unit; the use of targeted and comprehensive endpoints for elucidation of specific questions (clarity of objectives), objective measurements and adequate sample sizes. In addition, the analyses were comprehensive and thorough.

Data in the table on page 20 are inaccurate in several places: for Group III, rows 2 and 7 and for Group IV row 7 the data do not match that provided in Tables C1 and C3, pages 177 and 179, respectively.

Some text is unclear. For example, on page 23, 4th paragraph, see the first sentence and others. It is unclear just what this sentence means: "Numerous brain regions were slightly attenuated in comparison to DL 10 female pup control group values."

While maternal behavior was mentioned as a dependent variable (page 37), little presentation of those data was found in the report.

It is unclear how the perchlorate doses were administered/calculated: were they expressed as the ammonium salt or as the amount of anion available? This should be very clearly stated.

There appears to be a typographical error on page 40; first sentence under G.7.a.2 Scheduled Sacrifice – Part A. Should this not read "On DG 21" not "DL 21"?

In parts of the report (e.g., page 497) a study Part D is mentioned but it is never discussed in any meaningful fashion nor are data from it presented. Thus, reference to study Part D should be deleted.

There is a potential point of discord between the EPA's interpretation of some clinical observations (e.g., localized alopecia) and that of the study directors (Argus 2001). On this issue it would appear that the view of those who conducted might be the more informed in that they are likely to be more familiar with the occurrence of such findings in untreated subjects at their facility.

2. Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices of specific

testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

It would appear that appropriate QA/QC procedures were effected and that no significant deviations from the protocol occurred. While certain procedures were changed (cardiac puncture to obtain blood samples rather than collection via the inferior vena cava) there is no indication that any would have impacted the outcome of the study.

As a point of clarification of the statement made on page 559, item 2, it would be helpful to know just how the dams and fetuses/pups were selected if it was not done randomly.

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

Again, it was not made at all clear just how the doses were calculated: were they expressed as the anion (perchlorate) equivalent or as the salt? The approach taken should be made very clear to the reader. Given the ability of the endpoints employed to detect significant effects, enough subjects were used to provide adequate power for the statistical approaches utilized. Additional statistical analyses were performed by EPA staff who correctly realized that simple t-tests were not adequate for the analyses of the brain morphometry data, particularly given the critical importance of that data set.

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

See number 3.

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

One interesting statement can be found on the bottom of page 61 and the top of page 62 where the authors state " These significant increases" (in body weight) "were not considered treatment-related because the expected effect of a toxicant would be a decrease, rather than an increase, in the body weights." There is no justification or support for this statement, particularly when increases in the weight of specific organs, such as the brain and the thyroid, are clearly taken as adverse events.

Another interesting observation surrounds the omission in the Summary of Results (pages 69-71), of the findings of significant increases in cerebellar and corpus callosal measurements seen in the males at the 0.01 mg/kg doses on DL22. Likewise for the significant findings at 0.01 and 0.10 mg/kg for female cerebellar (decreases), hippocampal (decreases) and striatal (increases) measures.

There seems to be an error in a standard deviation in Table B14 (PAGE 1) on page 94 where a value of 39.9 is reported under Group IV, days 1-14b. There also appears to be an error in Table C37 (PAGE 1) on page 278 where a value of "4." shows up in the last column for rat #16635.

This report was comprehensive in nature and provided adequate and sufficient data on a variety of measures likely to be affected by the mode of action of perchlorate. These included but were not limited to T3, T4 and TSH plasma levels; weights, pathology and morphometric measurements taken from a variety of brain areas; thyroid measurements that included weights and histopathological analyses. The data presented support the critical conclusions of the authors that "there is no clear-cut evidence of a no-effect level", thus, identifying the 0.01 mg/kg/day dose as a LOAEL.

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

It was curious to see that the dose range used in this study included a 30 mg/kg/day dose given that previous studies had apparently shown 10 mg/kg/day to be a clear effect level.

This study was extremely comprehensive in a very targeted way and focussed on effects likely to manifest as consequences of the suspected mode of action of the perchlorate anion. Given the known trophic effect of thyroid hormones on brain development and the demonstrated ability of perchlorate to alter circulating levels of those hormones, it was logical to expend great effort on examining the effects of perchlorate exposure on a variety of measure of brain integrity, as well as on thyroid hormone levels and thyroid morphology and histology. The data obtained in this study replicate important earlier findings and provide clear evidence that, while generating a U-shaped dose-response curve, significant effects on important biological processes can be detected at doses as low as 0.01 mg/kg/day. Given that the rat appears to be less sensitive to the lethal effects of perchlorate than are other species, the data obtained in this study should be taken as reasonable evidence of likely effect at similar doses in humans.

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

Yes. The subjects in this study serve to model exposures in nonpregnant, pregnant and lactating females, and developmental exposures throughout gestation in the fetus (via maternal exposure) and postnatally to offspring (via maternal milk and possibly via drinking water.). Exposures began prior to mating and continued throughout gestation and lactation to postnatal day 21.

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

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

No.

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

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

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

In the case of the re-analyses of the motor activity data for the Bekkedal et al., 2000 and Argus Research Laboratories, Inc 1998a studies, the efforts appear justified and valid, given the extreme variability associated with the motor activity measurements as obtained in those studies. The fact that EPA's analyses showed the same thing in both studies demonstrated replication of a perchlorate effect. The significant effects found in these data are all, however, above the doses shown to be 'active' in the Argus 2001 study, and therefore, do not directly impact the risk assessment. They do tend to show support for a functional effect of perchlorate exposure on the cerebellum, an association that would also tend to corroborate the morphometric findings reported in the Argus 2001 study.

For the re-analysis of the Argus 2001 brain morphometry data, EPA was clearly justified in the application of their approach, since earlier analyses employed only t-tests. The EPA's findings provided clear confidence in the significance of the effects reported in Argus 2001.

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

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

There are only minor examples of text that were difficult to follow:

On page 5-48, lines 6 and 7 should read "…dependent changes in the later portions (or 5-min blocks) of the 90-min sessions.."

Page 5-49, line 5. What is 'habituation interval'? Likely this refers to the first 5-min block of the 90-minute test session and the 'final interval' refers to the last 5-min block of the 90-min session. This should be clearly stated.

Page 5-75. Lines 8-19 are very unclear…it should be rewritten so that the reader can easily follow what is being described.

Page 5-82, lines 12-16. It is unclear why EPA would elect to consider the observation of transient, localized alopecia as a biologically significant effect of exposure when the finding is apparently relatively normal for animals in the Argus testing facility.

Page 5-84, line 17. What is the 'maternal behavior' that was observed?

Page 5-51, lines 7-9. These are unintelligible.

Page 7-14, Table 7-6. Legend indicates that human data are in the table, but there are none.

Page 7-17, line 8-9. This sentence seems out of context.

Page 7-17, line 26. There is reference to 'the battery' but there is no description or naming of it.
Page 7-18, line 24. There is reference to 'this effect' but it is unclear which effect is being referenced.

Page 10-3; line 9. Should not the word 'deviation' be 'derivation?'

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

Yes and in appropriate detail; nicely summarized in Chapter 10.

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

Yes, clearly.

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

The EPA has adequately explained their description and identification of the NOAELs and LOAELs in these studies. In most cases, these are straightforward derivations.

Topic Area F; Human Health Dose-Response Assessment

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

The review document does a commendable job of addressing all of these issues and in sufficient manner to appropriately describe the approaches utilized and the findings and conclusions presented. It also appears that all of the appropriate data available to date has been considered during the process.

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

The use of AUC instead of peak values seems well justified and it is likely the better of the two metrics in this case. The application of the proposed PBPK models is reasonable and justifiable.

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

Clearly, additional data on the potential effects of perchlorate exposure on immune function would serve to decrease the uncertainty associated with database insufficiency. Additional studies could also serve to effectively establish a NOAEL and thus address the uncertainty associated with having to extrapolate using the LOAEL.

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

The potential increased susceptibility of subjects at greater risk, such as those who are hypothyroid or hypothyroxinemic, was clearly considered in applying the intraspecies uncertainty factor. In addition, it was acknowledged that, for this factor, there was uncertainty in the use of the parallelogram approach in extending the adult structure of the PBPK modeling to address different life stages.

Topic Area G: Risk Characterization

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

Yes, the known potential effects are brought together well in a concisely informed manner.

Topic Area H: General Comments, Conclusions, and Recommendations

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

The obvious occurrence of a U-shaped dose-response curve in a variety of endpoints associated with the effects of perchlorate exposure is intriguing. While such phenomena are not unusual, this issue could be a point of concern for the non-science public, in that the data would seem to suggest that it would be better to be exposed to the higher doses of perchlorate and avoid any of the problems with lower dose exposures. In anticipation of such concern, some discussion of this issue seems warranted.

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

These were listed earlier.

Mehdi Razzaghi

Reviewer Comments

A.Studies

1. Greer, M.A. et al (2000). Does Environmental Perchlorate Exposure Alter Human Thyroid Function? Determination of the Dose-Response for Inhibition of Radoiodine Uptake.

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

Answer: No. The study is based on contaminated drinking water and is performed on 24 volunteers.

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

Answer: No, only iodide thyroid uptake in adult humans is considered. No neurodevelopmental or neoplastic consequences are studied or discussed in the paper.

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

Answer: N/A

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

Answer: The study is not concerned with cancer or noncancer effects, but only the iodide uptake.

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies

relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: There is insufficient information in this study to enable one to assess the reliability of the results. First, it is the design. No information is provided about the subjects selected for the study other than gender and having a normal thyroid. Information regarding their health status, smoking habit, weight, ethnicity, etc. are missing. This information could be crucial in the evaluation of the effect. Although, it is mentioned that the subjects were all in the 18-57 yr old age group, no further information is given about the distribution of age for each gender and in each dose group. Probably a randomized block design would have been more appropriate in this regard. No information regarding the choice of the dosage levels is provided. The experiment is conducted at three dosage levels, and therefore the results could have important impact in the understanding of the mechanism of perchlorate, but unfortunately with such limited information, it is hard to evaluate the study. Another problem is the way the paper analyzes the data. They use a 3-dose linear regression model on log-dose to compare effects. This is a very simplistic approach and consequently raises several questions about the results. How was the goodness-of-fit assessed? Were other models considered? The investigators conclude that a dose of 0.5 mg/day has no-effect and therefore water supplies containing less than this amount should have no effect on human thyroid function. In my opinion this is a very strong conclusion from this study and because of the above problems in design and analysis, such conclusion is not completely reliable.

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

Answer: N/A

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

Answer: This is not an epidemiological study.

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

Answer: N/A

B.5 Are the associations observed in the epidemiological data consistent with the proposed mode

of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?

Answer: A regression model with three points in a 1-way lay out is probably not an adequate way to analyze the data. Although there appears to be a clear trend in the data in most cases, this trend does not seem to hold for the post exposure day 15. And to control confounding factors such as age, health status, etc., a randomized block design would be preferred.

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

Answer: N/A

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

Answer: N/A

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

Answer: N/A

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

Answer: No. Human susceptibility has not been addressed.

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

Answer: N/A

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Answer: No more comments

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Answer: N/A

2. Lawrence, J. et al (2001). Ltr to editor – low dose perchlorate (3 mg daily) & thyroid function (Thyroid, v11, #3, 295).

A.1 Have all relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized? Have the similarities and differences in the toxicity profile across species been adequately characterized?

Answer: No. The experiment is on a group of men and thyroid functions (TSH,T3 and T4) as well as free thyroxine index, urinary iodide, creatinine measurements, and thyroid iodine uptakes are measured.

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

Answer: No. Only iodide thyroid uptake is considered. No neurodevelopmental or neoplastic effects are considered.

A.3 The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

Answer: N/A

A.4 A harmonized approach to characterize the potential risk of both noncancer and cancer toxicity has been proposed based on the key event of iodide uptake inhibition. Comment on whether the approach is protective for both.

Answer: N/A

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: This study is conducted on only one dose, arbitrarily determined, on eight volunteer male subjects. Although no information about the method of data analysis is provided, apparently one-way ANOVA is used to compare the effects. Since no information about the background of subjects is given, it is hard to determine if the correct methodology has been used. Moreover, with only eight subjects in the experiment, there is probably not sufficient degrees of freedom to detect an effect. No analysis of the power of the test is provided.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: N/A

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

Answer: N/A

B.4 Are the exposure measures constructed from data in the epidemiological studies sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies?

Answer: N/A

B.5 Are the associations observed in the epidemiological data consistent with the proposed mode of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?

Answer: The confounding factors are not even addressed. No information about the subjects (age, weight, health status, smoking habits, etc…) is provided. Although no significant effect is found at the dose examined, there are other factors that one should consider before one can conclude that environmental exposure to perchlorate would not affect thyroid function.

F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information

on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure? Should any other data be considered in arriving at a point of departure?

Answer: There is no analysis of point of departure in this study.

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

Answer: N/A

F.3 Are there other data which should be considered in developing the uncertainty factors? Do you consider that the data support the values proposed or different values for each? Do the confidence statements accurately reflect the relevancy of the critical effects to humans and the comprehensiveness of the database? Do these statements make all the underlying assumptions and limitations of the assessment apparent? If not, what needs to be added?

Answer: N/A

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Answer: Susceptibility is not an issue in this study.

G.1 Does the risk characterization chapter adequately and clearly summarize the salient aspects of the human health risk posed by potential perchlorate exposures?

Answer: N/A

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

Answer: None

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Answer: None. The document is clear.

3. Miller, E. (2001a).Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit report for the study of perchlorate pharmacokinetics and inhibition of radioactive iodine uptake(RAIU) by the thyroid in humans (CRC protocol #628) [Memorandum with attachments to Annie Jarabek]. Wright-Patterson AFB,OH:Air Force Research Laboratory; May 10.

A.1 Have all relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized? Have the similarities and differences in the toxicity profile across species been adequately characterized?

Answer:

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

A.3 The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

A.4 A harmonized approach to characterize the potential risk of both noncancer and cancer toxicity has been proposed based on the key event of iodide uptake inhibition. Comment on whether the approach is protective for both.

EPA DOCUMENT

B. Perchlorate Assessment

A.1 Have all relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized? Have the similarities and differences in the toxicity profile across species been adequately characterized?

Answer: Not qualified to answer.

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

Answer: Not qualified to answer.

A.3 The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

Answer: Not qualified to answer.

A.4 A harmonized approach to characterize the potential risk of both noncancer and cancer toxicity has been proposed based on the key event of iodide uptake inhibition. Comment on whether the approach is protective for both.

Answer: Not qualified to answer.

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: Studies were reviewed before.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

Answer: The summary of the eight epidemiological studies performed since the 1999 external peer review clearly reveal that there is an effect due to exposure to perchlorate. However, as noted in the document, most of these studies have limitations due to uncontrolled confounders and hence the utility of these studies for characterization of risk becomes questionable. For example, the only study on the general population (Li et al, 2000) did not control several important confounding effects and risk factors. Other studies also ignored some of the confounding effects. According to the EPA document, of all the studies on children, the study of Schwartz (2001) is by far the most convincing of the neonatal studies. However, there are some ambiguities in the way the results are reported. It is not clear why the thyroid hormone T4 declined at four of the perchlorate exposure levels with age until about 18 hours and then increased over the next 30 hours. Does this mean that there may be a hormetic effect due to exposure to perchlorate? A clarification and a better description would be useful in this case.

B.4 Are the exposure measures constructed from data in the epidemiological studies sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies?

Answer: In general, there appears to be a good analysis of the epidemiological studies. The report summarizes the important results and findings of the studies and points out the weakness of each in some detail. The point-of-departure analysis of chapter 7 is particularly interesting as it emphasizes the use of model approach to risk assessment to determine the benchmark dose and hence reduce the influence of experimental conditions such as dose-spacing, sample size and variability of the NOAEL. Since the ultimate goal is to obtain an acceptable exposure level for humans, it is more reasonable to take a unified approach for cancer and noncancer endpoints. Gaylor, et al (1999) discuss this issue in great depth and propose the use of a statistical lower confidence limit on the dose estimated to produce an excess incidence of adverse health effects in 10% of the animals in bioassay experiments as a point-of-departure.

B.5 Are the associations observed in the epidemiological data consistent with the proposed mode of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?

Answer: The weakness here is mainly in the clinical studies. Although the EPA report provides a good summary of the available studies, unfortunately the studies are not sufficiently informative to warrant reliable conclusions. Generally, they are based on small samples with insufficient power, ignore confounding factors, and therefore one cannot confidently derive a NOAEL for humans from such studies. Although the studies are very informative in

displaying the some mode-of-action aspects of perchlorate, as pointed out in the EPA document, they fail to address some potentially important aspects of mode-of-action for perchlorate.

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Answer: Discussed in the review of studies.

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

Answer: In the review of the Argus Research Laboratories (1998a), which examines the developmental neurotoxicity of ammonium perchlorate (page 5-34 of EPA document) the litter information is not clearly stated. For example at the bottom of page 5-34, it is stated "Other pups (F1-generation) were assigned to four different subsets for additional evaluation." And from then on no information is provided about the litters. Litter information is crucial in all of reproductive and developmental studies because of the existence of intra-litter correlation.

In the evaluation of developmental neurotoxicity of ammonium perchlorate, it is particularly interesting to note the application of Bayesian hierarchical models to assess the weight of evidence of a dose-response trend in motor activity (Dunson, 2001a) applied to the data of Bekkedal et al (2000), a study that evaluates motor activity of rats in both sexes. It would probably be useful to give brief information about the choice of level 1 and level 2 variables in the hierarchical model. Dunson (2001a) also uses a modification of the model to perform a combined analysis of data from Bekkedal et al (2000) and Argus Research Laboratories (1998a) studies (pp. 5-48 to 5-52 of EPA document). This kind of analysis is remarkable, essential and highly useful in better understanding of the dose-response trend. A widely used software package for Bayesian inference, BUGS, is utilized for the analysis. BUGS is a software for full Bayesian inference and it uses the Markov Chain Monte Carlo (MCMC) algorithm to generate posterior samples, i.e. a set of correlated draws from a sequence that converges to the exact posterior distribution. A set of independent draws from the exact posterior distribution (see Everson and Morris, 2000) would probably improve the analysis. Moreover, one should be aware that BUGS does not support some of the prior distributions (e.g. uniform) on the level-2 covariance matrix.

Page 5-90 line 15, 1923 should be 1852.

On page 5-90, it is explained that "To account for the fact that the Argus(1999) study recorded thyroid incidence at 19 weeks and not at the time of natural death or sacrifice at two years, a prior…." . Although the analysis is correct, alternatively one could consider an age-adjusted trend test (see Kodell and Ahn, 1997) for this analysis.

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

Answer: No particular comments.

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure? Should any other data be considered in arriving at a point of departure?

Answer: In Appendix 7B of the EPA document, a very thorough and interesting discussion of BMD for hormone analysis is presented. The Kodell-West procedure is used to derive BMDs for quantitative responses. The fits to the data did not reach statistical significance and lack of fit raised difficulties with interpretation suggesting that the estimates should not be used as the basis for risk assessment. The Kodell-West procedure is based on the assumption of normality of responses with equal variances at all dose levels, and uses a quadratic polynomial as dose response model. Although the model may be very attractive in some cases, the reason why it has not produced satisfactory results may be that the model is not flexible enough to provide adequate fit to the data. Perhaps a more flexible model which allows for bimodality and bitangentiality such as a mixture of two normal models (see Razzaghi and Kodell, 2000) would provide a better description of the data.

According to the EPA document (page 7B-4) The rabbit developmental studies of Caldwell et al (1995) subchronic hormone data were "best fit" by unrestricted power functions. The hormone data from developmental neurotoxicity study and mouse immunotoxicity study were fit by either unrestricted power or polynomial (linear or quadratic) functions. One must be warned that simply because a model provides a good statistical fit to the data does not justify the use of the model in risk assessment. A model should also be biologically interpretable. Surely, if a model is based on the biological mechanism of toxicity, it would lead to more reliable estimates. To this end, the use of biologically based dose response (BBDR) models should be encouraged.

F.2 Comment on the use of the PBPK models for interspecies extrapolation and the choice of the dose metric.

F.3 Are there other data which should be considered in developing the uncertainty factors? Do you consider that the data support the values proposed or different values for each? Do the

confidence statements accurately reflect the relevancy of the critical effects to humans and the comprehensiveness of the database? Do these statements make all the underlying assumptions and limitations of the assessment apparent? If not, what needs to be added?

F.4 Have all the factors influencing susceptibility been clearly described and accounted for in the assessment?

Answer: The issue of susceptibility is of crucial concern. There are several models that incorporate susceptibility in the analysis. Recently, mixture models have been proposed for consideration of susceptible subpopulations (see Razzaghi and Kodell, 2000). It would probably be worthwhile to use such models for identifying factors influencing susceptibility.

Gary Williams

Review of

Perchlorate Environmental Contamination:

Toxicological Review and Risk Characterization

prepared for

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February 22, 2002

C-196

I. REVIEW OF STUDIES PUBLISHED SINCE 1999

1. Argus 2001 Hormone, Thyroid and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate During Gestation or via Maternal Milk

a) Study Objectives

The objectives of this study were:

i) to determine the teratogenicity of perchlorate in the rat;

ii) to measure thyroid stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) changes in developing rats (fetal and neonate) and in adult female rats during pregnancy and lactation;

iii) to measure neurohistological and thyroid effects at the same time points; and

iv) to correlate these effects with hormone concentration changes at different stages in development of the rat.

b) Study Design

A good description of the study design (A.5 p 26) is lacking.

A range of doses of ammonium perchlorate was administered to female Crl: CD® (SD) 1GS BR VAF/Plus® rats during breeding, pregnancy and up to 22 days post partum. After 2 weeks on test, female rats were mated. Inseminated females were designated gestation day (DG) 0. The study consisted of 3 parts:

A. On DG 21, P generation females were killed, uteri were excised and fetuses examined.

B. 4 pups of each sex per litter were continued on study until postnatal day 10 (DL 10) when they and dams were killed. Remaining pups were killed on DL 5.

C. 4 pups of each sex per litter were continued on study until postnatal

day 22 (DL 22) when they and dams were killed. Remaining pups were killed on DL 5.

c) Performance of Study

The study was conducted according to GLPs and no significant deviations occurred.

The criteria for histopathology of the thyroid are not given in the report, but are stated to be those developed for a PWG review of a previous 90 day study (Wolf, 2000). The histopathology was performed at Environmental Pathology Laboratories by K.A. Funk, who is said to have been a member of the PWG (5-53).

The histopathology of brain was performed at Consultants in Veterinary Pathology by R.H. Garman.

d) Dosing of Test Substance

Ammonium perchlorate (99.8% pure) was administered in drinking water at target doses of 0., 0.01, 0.1, 1.0 and 30 mg/kg/day. Actual achieved doses in the precohabitation exposure period were 0.00, 0.01, 0.08, 0.77 and 23.93 mg/kg/day, respectively and in the gestation exposure period, 0.00, 0.01, 0.10, 0.98 and 28.73 mg/kg/day, respectively.

e) Data Presentation

The nomenclature used for days of life differs from that of EPA (5-52).

Text tables are not numbered.

In Part A, fetuses showed no abnormalities (p 46), except thyroid colloid was decreased 1.0 and 30.0 mg/kg/day (p 48). Dams showed dose-related changes in TSH and and T_4 beginning at 0.01 mg/kg/day (p 48) and changes in thyroid weight and histopathology only at 30.0 mg/kg/day (p 47).

In Parts B and C, pup body weights were significantly increased in treated groups (p 61), but this was not considered treatment-related because "the expected effect of a toxicant would be a decrease." Could this effect be due to hypothyroidism?

In Part B, in DL 10 dams, thyroid weights were increased at 30 mg/kg/day (p 51) and decreased colloid was evident at 1.0 and 30.0 mg/kg/day (p 53).

In DL 10 pups, thyroid weights were increased in males at 0.01 mg/kg/day and above and in females at 30 mg/kg/day (p 52). Decreased thyroid colloid was found in high dose males and females and in females at 1.0 mg/kg/day (p 53). Changes in TSH and T_3 were also found (p 55).

In Part C at DL 22, dams showed an increase in hyperplasia at 1.0 mg/kg/day and a decrease in colloid and hypertrophy at 30 mg/kg/day
(p 63). Pups had decreased colloid at 30 mg/kg/day.

f) Statistical Analyses

Appropriate parametric and nonparametric tests were used (p 42).

g) Study Conclusions

The report has a Summary (page 69), but no conclusion.

Neuropathology: no evidence of treatment-related neuropathologic
effects (p 66). Morphometric differences, however, were found even in the pups of dams receiving the lowest exposure.

Thyroid histopathological changes were found in both dams (p 69) and pups (p 70, 71).

h) Overall Assessment

This study is of sufficient quality for hazard identification. The relevance of thyroid effects in rodents to human hazard, however, is questionable because of species differences.

II. REVIEW OF DRAFT RISK ASSESSMENT

1. Chapter 1
No comment

2. Chapter 2
No comment

3. Chapter 3
3.9 line 29 needs authoritative reference for thyroid physiology

4. Chapter 5

5.1.2.3 No studies on genotoxicity in thyroid are available.

5-53 lines 23 and 28, "effected" should be "affected."

5-53, line 29 states that in PND 21 (DL 22) dams there was a clear dose related trend in colloid depletion, hypertrophy and hyperplasia.
This is not evident in the table on p 63 of the Argus report.

5-73 two different analyses of brain morphometry from the 2001 Argus
study yielded significant effects resulting from developmental exposure of rats to perchlorate at doses of 0.001 mg/kg/day and higher.

5. Chapter 7

The toxicity data support the proposed mode of action of perchlorate as an inhibitor of iodide uptake in the thyroid (7-3)

6) Chapter 10

10.1.1 does not mention goiter hazard

III. ANSWERS TO CHARGE QUESTIONS:

A.1 Available data are appropriately utilized. Species differences in toxicity have not been examined.

A.2 Yes. It could be noted that the mode of action is not unique to perchlorate, but is also produced by thiocyanates.

A.3 A non-DNA-reactive (epigenetic) agent should have a threshold at which the no-effect-level for the mode of action would be a nontumorigenic exposure.

A.4 The approach is protective for both.

C.1 The Argus 2001 study does not have a NOAEL.

C.2 see Section II

C.3 Yes

C.4 Yes

F.1 The conclusions are consistent with the mode of action.
However, in applying a point of departure, it should be taken into account that humans regularly consume thiocyanate goitrogens in food at low levels without adverse effects.

F.2 PBPK models are useful for interspecies extrapolation. The key metric is the thyroid exposure.

F.3 Species differences in sensitivity of the NIS to perchlorate should be considered.

F.4 No. Susceptibility of the NIS should be considered as well as species differences in requirement for thyroid hormones.

G.1 No. No discussion of goiter.

H.1 Iodine deficiency is not established as a cause of thyroid cancer in humans.

H.2 see Section II

Ronald Wyzga

Comments on "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization"

Ronald E. Wyzga, Sc. D.

General Comment: It would be helpful to know something about the levels of environmental contamination and of personal exposure. These would help place the contents of this report in perspective and allow us to make some judgment about how important the various uncertainties are. In other words do they really make much of a difference with respect to current exposure levels?

Chapter 4: By and large I found this chapter to be very well written. The various studies are comprehensively described along with their strengths and weaknesses in a most objective way. There could be more discussion about the problems/uncertainties associated with ecological studies at the beginning of the section on page 4-3. .

pp.4-12-4-13: Has the Schwartz study been published in a peer-reviewed journal? Has it been submitted? Are EPA Staff following the disposition of the results of this study? If it will not soon be published, what is the EPA policy about citing/using studies that have not been published in the peer-reviewed literature.

p. 4-15: I worry a bit about the calculation of inhaled "dose" for 2 reasons. I would be surprised if there were not considerable variation in some of the variables in (4-1) over time and across individuals. Has there been a good investigation of this issue? I also am concerned about the reliance on total dose. Alternative measures of exposure (e.g., peak concentrations or exposure to concentrations greater than some fixed level) may be more important. I am struck by the non-linearity of some of the results presented in this chapter. This could suggest that alternative exposure measures are more relevant than total or average exposure.

p. 4-17, ll. 6-7: this is one example of non-linearity.
ll. 23-26: or that there is no effect.

4-21-4-23: The data are interesting and could be used to support some non-linear dose-response functions.

Chapter 5: Extensive toxicological data were available for consideration in the development of the draft RfD for the perchlorate anion described in this document. The testing strategy developed in 1997 and augmented again in 1999 in response to a second external peer review addressed target organs and tissues other than the thyroid, augmented the thyroid data to allow quantitative dose-response assessment, and assessed the effects of perchlorate on reproductive capacity and in potentially susceptible populations. Endpoints considered included cancer and genotoxicity; general toxicity (short-term and subchronic); developmental neurotoxicity; developmental toxicity; two-generation reproductive toxicity; and immunotoxicity. Given perchlorate's mode of action as a competitive inhibitor of iodide uptake in the thyroid, with resultant disruption of the hypothalamic-pituitary-thyroid axis, the endpoints selected for study were appropriate. Furthermore, because

perchlorate's carcinogenic effects are due to its effect on iodide uptake and not due to genotoxic effects, one harmonized risk estimate for both noncancer and cancer sequelae could be derived.

Chapter 7: The introductory material is particularly useful and well-written.

p. 7-6: I'm not sure whether this is an appropriate citation of Weiss. I though his comments applied to IQs and the overall shift in a known distribution function. It is unclear whether such a shift would occur here.

p. 7-15:, ll. 1-4: It would be interesting to consider several alternative dose metrics. E.g., how would the results change if the peak dose level were used?

Point-of-departure analysis showed the exposure dose considered to be a level of concern for the adverse effects of perchlorate to be 0.01 mg/kg-day, based on effects on brain morphometry (increased corpus callosum size), motor activity, thyroid histopathology, and alterations in thyroid hormone levels. However, importantly, this dose level was the lowest tested and is therefore considered the LOAEL. A human exposure equivalent of 0.01 mg/kg-day was derived, which was less conservative than the HEE for iodide inhibition in the dams of 0.002 mg/kg-day. Four uncertainty factors were applied: a factor of 3 for intraspecies variability; a full factor of 10 for LOAEL-to-NOAEL extrapolation due to the shallow slope of the response curve; a factor of 3 to extrapolate to longer duration exposures; and a factor of 3 for database insufficiency, largely in light of uncertainties with respect to immunotoxicity. Notably, the interspecies UF was omitted because the PBPK modeling was deemed to adequately address this extrapolation. The composite UF was thus 300, and the derived RfD estimate is 0.00003 mg/kg-day after adjusting for the percent of the molecular weight of the perchlorate salt from the cation. The RfD that would have been derived from the available human data was estimated at a maximum of 0.00007 mg/kg-day; this estimate is one-half as conservative as the proposed toxicology-based value; if one compares absolute values, there appears to be agreement between the approaches.

p. 7-22: I would have liked to have seen a greater justification for the use of the 10-fold factor associated with extrapolation from the LOAEL.

p. 7-26, ll. 23-26: The presentation of real-world exposure data would help one judge whether the difference in the RfD calculations from animal and human data were significant. There might also have been some consideration of using the Schwartz data to caluculate an RfD.

Dose-response analysis of thyroid neoplasia results in an RfD derivation of 0.005-0.0002 mg/kg-day. It should be noted that the neoplastic effects of thyroid disruption (i.e., elevated TSH levels) are more significant in rodents than in humans as the human thyroid is much less sensitive to this pathogenetic phenomenon than rodents. However, this species difference does not affect the interpretation or regulatory implications of this quantitative risk assessment because the RfD derived for non-cancer effects was more conservative than that derived for cancer effects. Moreover, the similarity in the RfDs derived for cancer and non-cancer outcomes appears to support the mode-of-action concept.

In summary, the draft RfD derived in this quantitative risk assessment appears to be a conservative estimate based on extensive, multi-endpoint toxicological data and incorporating PBPK modeling for inter-species extrapolation. Notably, the fact that the LOAEL was the lowest dose tested (0.01 mg/kg-day) precluded the determination of a NOAEL for perchlorate. However, this uncertainty appears to have been addressed through the application of the full factor of 10 uncertainty factor in the RfD derivation. Additional studies at lower doses would verify the appropriateness of this assumption. In addition, uncertainty remains regarding the immunotoxic effects of perchlorate, particularly with respect to possible contact hypersensitivity, and additional investigation should be conducted to this end.

Responses to Charge Questions
Ronald E. Wyzga, Sc. D.

A.1. Since I am not an expert on this literature, I cannot comment on the first part of this question. The similarities and differences in toxicity profiles across species have been reasonably well-characterized. They are certainly systematically addressed.

A.2. Yes, this is particularly well explained.

A.3. This issue needs to consider experimental data as well as mode-of-action data. Consonance between the two need be assured. I would like to see more discussion here. I note, for example, that some of the experimental data suggest non-linearity of response; this could imply that alternative dose metrics need be considered.

A.4. There are relatively few data on cancer endpoints; hence it is unclear whether the approach taken for non-cancer events is protective. Indeed the lack of genotoxicity data and the absence of malignant tumors (in the studies for which there is documentation) raises the question about whether there is any current evidence for carcinogenicity. What are needed are more studies of the carcinogenic potential of perchlorate. The existing data need be scrutinized to determine whether any malignant tumors were found in studies to date.

B. 1. I would like to see further discussion and analysis of the Schwartz (2001) study and data. I don't understand why this study is not in Table 2. Since it is only a dissertation at present, I don't consider it to be a peer-reviewed study per se. The author should be contacted to determine whether a peer-reviewed publication of this study is likely soon.

B. 2. I am not aware of additional studies. I found the description of the various studies to be clear; in addition, I appreciated the limitations listed for the various studies. By and large, the discussion of the various studies was objective and well-balanced.
I worry a bit about the calculation of inhaled "dose" (p. 4-15) for two reasons. I would be surprised if there were not considerable variation in some of the variables in equation 4-1 over time and across individuals. Has there been a good investigation of this issue? I am also concerned about the reliance on total dose. Alternative measures of exposure (e.g., peak concentrations or exposure to concentrations above some fixed level) may be more important. I am struck by the non-linearity of some of the results presented in Chapter 4. This could suggest that alternative exposure measures are more relevant than total or average exposure. Examples on non-linearity are p. 4-17, ll. 6-7 and p. 4-21-4-23.
I would have liked to have seen more discussion of how consideration of the Schwartz study results would have changed the overall conclusions of the report.

B.3. Yes, but see above comments about consideration of the Schwartz results.

B. 4. This has no straightforward answer – partly because we don't know the correct dose measure. See response to B.2. above.

B. 5. The associations are broadly consistent, but there are results that suggest non-linearity. (See above.) I would have liked to have seen more discussion of these results. Several studies were based on small populations or were ecological studies. The latter always raise confounding issues. They were well-described, but it is difficult to control for confounders definitively in this type of study design.

C. 1. –

C. 2. By and large the studies were well described with limitations clearly stated. What is particularly useful was the framing of the various studies on the context about what we know about mode-of-action.

C. 3. This depends upon the endpoint. The relationship is clearer for some endpoints than for others. For example, the relationship for developmental toxicity endpoints involves considerable conjecture.

C. 4. NOAELS/LOAELS appear to be reasonably defined.

F. 1. Given perchlorate's mode of action as a competitive inhibitor of iodide uptake in the thyroid, with resultant disruption of the hypothalamic-pituitary-thyroid axis, the endpoints selected for study were appropriate. Point-of-departure analysis showed the exposure dose to be considered to be a level of concern for the adverse effects of perchlorate to be 0.01 mg/kg-day, based on effects on brain morphometry (increased corpus callosum size), motor activity, thyroid histopathology, and alterations in thyroid hormone levels. This dose level was the lowest tested and is considered the LOAEL. A human exposure equivalent of 0.01 mg/kg-day was derived which was less conservative than the HEE for iodide inhibition in the dams of 0.002 mg/kg-day. These point of departures appear to be appropriate.

F. 2. I would have liked to have seen consideration of alternative dose metrics. Some kind of sensitivity analysis would have been useful here.

F. 3. I would have liked to have seen a greater justification for the use of the 10-fold uncertainty factor associated with extrapolation from the LOAEL. I also worry about the argument about the need for an uncertainty factor associated with an incomplete database. Databases will never be complete; I personally dislike the use of this safety factor. Presumably the effects most likely expected have been considered. Certainly those associated with the described mode of action have been considered. Hence I would reject that argument.

F.4. No comment here.

G. 1. The presentation of real-world exposure data would help one judge whether the difference in the RfD calculations from animal and human data were significant. I'm also uncomfortable about making any statement about carcinogenicity of perchlorate. The data base does not allow any statement to be made. The data are either negative or not interpretable. In addition, it appears as if perchlorate is not genotoxic.

H. 1. See comments on G. 1 about exposure data.

H. 2. See previous comments on specific sections of document.

Thomas Zoeller

Topic Area A: Hazard Characterization and Mode of Action

A.1 Have all relevant data on toxicokinetics and toxicodynamics been identified and appropriately utilized? Have the similarities and differences in the toxicity profile across species been adequately characterized?

The data on perchlorate toxicokinetics has been largely identified. These issues are discussed in Chapter 3 and in Chapter 6. However, the uptake of iodide and/or perchlorate into milk does not appear to be well described or considered in Chapter 3. Specifically, it seems fundamentally important to highlight the data showing that iodide in breast milk is concentrated 20-30 fold over that in maternal serum (reviewed in). Although measurements of perchlorate in milk do not appear to have been performed, iodide uptake into milk is inhibited by perchlorate and perchlorate is likely to be concentrated in milk as it is in the thyroid gland. This inference is supported by the observation that the sodium/iodide symporter (NIS) that transports iodide (or perchlorate) into the thyroid gland is the same NIS protein expressed in mammary tissue , and its expression is induced and enhanced by prolactin . The residence of perchlorate in milk likewise would be important to identify and evaluate. Considering that milk is the sole, or main, food source for infants, and that the recommended adequate intake of iodine for infants is 110 µg/day for infants 0-6 months and 130 µg/day for infants 7-12 months , it seems important to consider this issue. Finally, perchlorate in milk would both reduce dietary iodine and inhibit iodine uptake into the infant's thyroid gland.

It is somewhat inaccurate to state that rats do not have Thyroid Binding Globulin (TBG). In fact, rats do produce TBG , but its abundance in serum during the life cycle (e.g., pregnancy and lactation) are not well studied.

Aside from these shortcomings, the EPA review of perchlorate toxicokinetics is generally thorough and logical.

The toxicodynamics of perchlorate also is well-characterized in chapter 3. The EPA document clearly identifies that perchlorate inhibition of iodide uptake into the thyroid gland and subsequent inhibition of thyroid hormone synthesis is the mode of action of perchlorate toxicity. In addition, two classes of adverse effects are cited as deriving from this effect. First, the incidence of thyroid cancers may increase as circulating levels of TSH rise in response to reduced thyroid hormone concentrations. Second, reduced circulating levels of thyroid

hormone may impact brain development. These two categories of potential adverse effects are conspicuously absent general physiological effects, but there are no validated measures of adverse physiological consequences of thyroid hormone deficits in animals. Moreover, both cancer and neurobehavioral deficits represent permanent adverse effects.

A.2 The EPA has framed a conceptual model based on the key event for the mode of action of perchlorate as inhibition of iodide uptake at the sodium (Na^+)-iodide (I^-) symporter (NIS). Are the roles and relative importance of the key event and subsequent neurodevelopmental and neoplastic sequelae clearly articulated and consistent with the available data on anti-thyroid agents or conditions and with the physicochemical and biological properties of perchlorate?

The EPA has clearly identified iodide uptake into the thyroid gland as the mode of action of perchlorate toxicity. This concept is fully consistent with the available literature as reviewed by the EPA, and there is little evidence that perchlorate exerts direct actions on physiological systems.

Identification of neurodevelopmental sequelae as an important category of adverse effects is fully consistent with the literature. This literature is appropriately reviewed in the EPA document, but focuses mainly on the clinical literature. The literature on congenital hypothyroidism and gestational hypothyroxinemia is accurately reviewed in the EPA document . However, few details are provided about the role of thyroid hormone in brain development in experimental systems. For example, thyroid hormone exerts a variety of effects on the developing striatum that may, in principle, account for changes in the linear dimension of striatum size in the ARGUS, 2001 study. Likewise with the corpus callosum as well as the other brain areas.

A.3 The 1999 peer review panel agreed with EPA that perchlorate was not likely to directly interact with DNA. What inferences can be made, based on consideration of the mode-of-action data, to inform the choice of dose metric and the approach for low-dose extrapolation?

The EPA document clearly articulates the choice of dose metric and approach for low-dose extrapolation in Chapter 7. It is reasonable to conclude that perchlorate does not exhibit direct genotoxic effects. Moreover, it is reasonable to conclude that the primary mode of action of perchlorate is its interaction with the NIS which reduces iodide uptake into the thyroid gland (and into any other tissue that expresses the NIS and actively takes up iodide). Considering this mode of action, several inferences can be made about characteristics of the most reliable dose metric. First, the choice of dose metric should provide a reliable index of the inhibition of iodide uptake into the thyroid gland, since suppression of thyroid hormone synthesis and

subsequent reduction in circulating levels of thyroid hormone represents the mechanism by which perchlorate would produce adverse effects. The EPA chose the area under the curve (AUC) of perchlorate in serum because it provided the best index of the inhibition of iodide uptake – the direct mechanism of perchlorate toxicity. Moreover, the AUC is an integrated measure of the product of time and concentration, which appears to provide a much more reliable index of toxicity compared to other measures (e.g., peak levels).

The approach for low-dose extrapolation requires either a linear or non-linear model. Considering the mode of action, the relationship between perchlorate exposure and adverse effects must certainly be non-linear. There are several reasons for this. First, the interaction of perchlorate with the NIS certainly follows Michaelis-Menton kinetics. Thus, the mode of action itself is governed by non-linear interaction. The relationship between iodide uptake inhibition and circulating levels of thyroid hormone will also certainly be non-linear. This is a complex issue that includes a variety of compensatory mechanisms, the sum of which will exhibit complex relationships. Finally, the relationship between circulating levels of thyroid hormones and adverse effects – whether they be changes in thyroid histopathology or changes in neuroanatomy – will likewise be complex and non-linear.

The EPA document emphasizes the importance of structural changes in thyroid histopathology whether they are changes in colloid, hypertrophy or hyperplasia. The logic justifying this interpretation does not integrate, or harmonize, cancer and non-cancer endpoints. Specifically, the justification presented is that sustained activation of the thyroid gland by TSH, as evidenced by changes in colloid, can lead to cancer. This reviewer concurs with the conclusion that any change in thyroid structure should be considered adverse, but using an integrated logic. First, because thyroid hormone directly suppresses circulating levels of TSH , TSH is a direct biomarker of thyroid hormone action. In fact, it is the only biomarker of thyroid hormone action identified in the entire database. However, it is not traditional to consider changes in circulating levels of TSH to be considered adverse *per se*. In fact, changes in circulating levels of TSH associated with changes in circulating levels of thyroid hormones are usually considered to be “compensatory” . However, there is no evidence that the developing brain can “compensate” for transient deficits in thyroid hormone; in fact, there is considerable evidence to the contrary . Thus, the critical question, for which we presently have no answer, is whether the hypothalamic-pituitary-thyroid axis is more sensitive to changes in circulating levels of thyroid hormone than are tissues that require thyroid hormone to function properly, such as the developing brain.

Therefore, any change in thyroid histopathology secondary to elevated TSH due to reduced thyroid hormone demonstrates that thyroid hormone levels have been altered enough to affect TSH for a period sustained enough to produce structural changes in the thyroid gland. Likewise, it is reasonably inferred that this degree and persistence of change in thyroid hormone would have consequences in vulnerable tissues such as the developing brain.

Low-dose extrapolation was calculated using the NOAEL for thyroid histopathology and for neurodevelopmental measures and this was compared with the finding of Greer et al. . Interestingly, the two approaches yielded nearly identical results. Given the uncertainties associated with the study by Greer (e.g., variability among subjects, relationship to vulnerable subpopulations), the EPA focus on the experimental literature is warranted.

A.4 A harmonized approach to characterize the potential risk of both noncancer and cancer toxicity has been proposed based on the key event of iodide uptake inhibition. Comment on whether the approach is protective for both.

The EPA document clearly considers both cancer and non cancer toxicity and articulates how both of these categories of effects are caused by the same mode of action of perchlorate. This harmonized approach is protective for both.

Topic Area B: Human Health Effects Data

B.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Greer 2000

This study is reasonably well designed, and is clearly focused on attempting to estimate the risk of thyroid hypofunction resulting from environmental perchlorate exposure. Twenty-four healthy adult volunteers were recruited for this study in which they were given one of three doses of perchlorate in water consumed at 4 set times each day. Measurement of 8- and 24-hr iodide uptake was then performed both prior to perchlorate exposure for baseline estimates and on exposure days 2, 14, and also at 15 days after exposure was terminated. A linear log-dose relationship was observed between perchlorate exposure and iodide uptake inhibition. Extrapolation toward 0

using this linear function indicated that a no-effect level would be achieved at 7 µg/kg (assuming a 70 kg healthy adult). This was confirmed in a separate study.

The authors estimate that the no-effect level of 0.5 mg/day would be consumed in drinking water containing perchlorate at 250µg/L and that, therefore, "...water supplies containing less than this should not affect human thyroid function". This conclusion, without qualification, is clearly not supported by the study for two types of reasons. First, the authors do not report on the post-exposure iodide uptake in the 4 volunteers provided with the putative no-effect level of perchlorate at 7 µg/kg. Others (Lawrence 2001) have shown that a dose of perchlorate that does not produce statistically significant effects on iodide uptake nonetheless produce a very significant rebound following the exposure period. This rebound is a clear and sensitive indication of a biological effect of perchlorate on thyroid function, but the Greer study does not report on this in the population tested with the "no-effect" level of perchlorate. Second, the authors fail to consider the variability in the human population when they refer to the "human thyroid function". Specifically, people with the variety of thyroid disorders, infants, pregnant women and their fetus.

Overall, the study appears to provide reliable information within the main study. The QA/QC audit did not appear to reveal problems that would further limit the reliability of those data. However, the anecdotal information provided on the group of 4 volunteers exposed to 7µg/kg perchlorate are not reliable.

Lawrence 2001

This study, like that of Greer *et al.*, was conducted to further study the effects of perchlorate on thyroid function in an attempt to find a NOEL. Eight healthy male volunteers with normal thyroid function were provided with 3 mg perchlorate in 1L of spring water daily for 14 days. Thyroid function tests, 24-hour urinary diodide, and 8- and 24-hour iodide uptake measurements were taken at baseline, on day 14 of exposure, and 14 days after perchlorate exposure was discontinued. No statistically significant effects were observed in any of these measures after 14 days of exposure. However, 14 days after exposure was terminated, the RAIU was significantly elevated. No mention was made of the measures of thyroid function in the post-treatment samples.

The conclusion that 14 days exposure to 3mg perchlorate did not significantly affect thyroid function is mostly supported by the data, though the post-exposure rebound demonstrates that there was a biological effect. The data appear to be reliable and justify the conclusion that perchlorate given to healthy adult males for two weeks at 3mg/day does not significantly alter circulating levels of thyroid hormone. Given that the half-life of T_4 in humans is nearly a week, and that the majority of circulating T_3 comes from peripheral deiodination of T_4, the duration of the experiment does not appear to warrant extrapolation to life-time exposures.

B.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

The EPA treatment of the new studies by Lawrence, Greer and Merrill appears thorough and reasonable. The interpretation is clear and conclusions warranted.

B.3 Have the epidemiological studies been adequately summarized as a basis for the hazard characterization?

B.4 Are the exposure measures constructed from data in the epidemiological studies sufficient to permit meaningful bounding of the predicted dose-response estimates derived from extrapolation of the laboratory animal studies?

B.5 Are the associations observed in the epidemiological data consistent with the proposed mode of action? Did the experimental design have sufficient power to accurately ascertain the association between perchlorate exposure and the specific outcome(s)? Were confounding factors appropriately controlled?

The epidemiological studies appear to be thoroughly summarized in the EPA document, and their inclusion as a basis for hazard characterization is clear and logical. A severe weakness in all of the epidemiological studies is that measures of exposure are not adequate to identify specific dose-effect relationships. This point is perhaps made best by Li et al. when they state that, “This study was sufficiently sensitive to detect the effects of gender, birth weight, and the day of life on which the blood sample was taken on the neonatal T_4 level, but it detected no effect from environmental exposures to perchlorate that ranged up to 15 µg/L (ppb).” Specifically, if population measures of gender (proportion), birth weight (average), and day of life (average) had been used instead of individual values, no relationship with T_4 levels would have been observed. Thus, the failure to observe a significant shift in average monthly T_4 levels in a population of newborns living in a geographic location in which perchlorate has been reported in the water supply is not, in itself, convincing.

The study by Schwartz, as reviewed in the EPA document, appears to be somewhat more sophisticated in its estimate of exposure, but still lacks the power of individual measurements of perchlorate and its relationship to thyroid function.

Finally, the studies by Crump *et al.* in which the reference population exhibited goiter in 30% of the population is also difficult to make or defend conclusions.

The EPA document evaluated each of epidemiological studies in detail; this reviewer has nothing additional to add.

Topic Area C: Laboratory Animal Studies

C.1 Do any of the studies published since 1999 that have not undergone peer review have any notable limitations and deficiencies? Refer to Table 2 for a listing of the specific studies relevant to this topic area. Please consider the questions in Attachment 1 when formulating your response. You do not need to answer every question in Attachment 1, rather use your professional judgment to address those that are most appropriate to the study in question.

Argus 2001

Please review the strengths and limitations of the experimental protocol of the study. Are the objectives being investigated in each study clearly identified? Is the study design appropriate to address these objectives? Does the study design represent the state-of-the science? Discuss all limitations in experimental design that would affect the ability to interpret significance of the study results. Also indicate where insufficient information has been provided on the experimental design.

This is a very large study that appears to be well designed and clearly presented. The objectives are clearly articulated and the methods employed are clearly described. A logical weakness in the study design appears to be the following. A great many linear measurements of brain areas were incorporated as endpoints of perchlorate toxicity. However, when changes were observed, the interpretation was complicated because it was not known whether these were specific endpoints of thyroid hormone action. Thus, it would appear to have been more appropriate to build valid endpoints of thyroid hormone action into the developmental neurotoxicity endpoints.

Please note any limitations in performance of the study that could decrease the relevance of the study findings. For example, were the studies conducted in accordance with Good Laboratory Practices or specific testing guidance? Did the study include QA/QC? Were there occurrences that necessitated a change to the protocol during the course of the study? If so, what impact did these changes have on the findings?

The study included pathological observations of brain sections, but the conclusion was that processes such as cell proliferation and apoptosis were not affected by treatment. For example, on page 815, the authors write, “As would be expected, the brains from the day 10 postpartum rats were characterized by active cellular migration and cell death (i.e., physiologic cell death or “apoptosis”), as well as by ventricular remodeling. However, no differences were found between the teest substance-treated and control group rats in the degrees of cell death or in the overall pattern of brain morphology.” These conclusions are simply without foundation. No formal measures of these processes were taken. Biologically important treatment effects on the rate of cell proliferation, migration, or apoptosis are simply not observable without formal quantitative analysis and, ideally, with the use of specific markers (e.g., BrdU for proliferation, TUNNEL or activated caspase-3 immunocytochemistry) . Considering that thyroid hormone can affect rates of proliferation in some but not all neuronal populations , and that thyroid hormone can affect the rate of apoptisis in some, but not all, populations of neurons , these measures would have been important to include in the study.

The radioimmunoassay for T_4 is of also of some concern in this study. This assay uses standards that range from 1 µg/dL on the low end, but levels in some of the animals was clearly below this as evidenced by the mean ± SEM (see page 782, Table 1). Thus, it appears that the standard curves may have been generated using the “0-tube”. This would not be a valid approach . Alternatively, they may have generated the standard curve properly, but simply extrapolated between the low standard and 0, which is also invalid. These values draw into question whether these assays were properly conducted, but sufficient information was not available to conclude this.

Were dosing or exposure measures appropriately formulated or controlled? Were appropriate endpoints and time points utilized? Were sufficient numbers employed to observe an effect?

Dosing appeared well formulated and controlled. Sufficient numbers of animals were included. Endpoints were discussed above.

Please comment on the strengths and limitations of the inferences made and presentation of the results in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented therein? Are the conclusions of the report supported by the data? Please explain.

As discussed above, there is no evidence that perchlorate treatment did or did not affect processes such as cell proliferation or apoptosis. These processes simply were not evaluated. Likewise, the conclusion that perchlorate did not produce adverse effects on neural endpoints

appears to be unsupported by the data. A good example of a common flaw in logic is illustrated on page 817. Specifically, the comment is that, "Although the mean thicknesses of the external germinal (granular) layer of the cerebellum were significantly thinner for the Group II and III females in comparison to the female control group, this layer is highly variable in thickness and, therefore, difficult to assess accurately with only six linear measurements. The inter-group differences in thickness of the external granular layer are, therefore, considered to be of no biologic significance." Clearly, the variability in this measure (i.e., thickness of external granular layer) would serve to make it more difficult to obtain statistical differences among treatment groups. Thus, if the treatment produced effects great enough to overcome this variability, it is more likely that these observed effects are not spurious. There is no discussion of the effects of thyroid hormone on these measurements in the brain.

Overall, was the study as designed, performed and reported of sufficient quality for use in hazard identification purposes? Is it important to enhancing the toxicological / ecotoxicological risk characterization of perchlorate exposures? If so, indicate the extent to which it can be used for characterizing adverse effects.

Overall, the study is of sufficient quality, given the qualifications listed above, for use in hazard identification purposes. This study represents the only analysis of the effects of perchlorate in the neonatal brain; thus, it provides important information for evaluating adverse effects.

Do the finding provide information relevant to the evaluating the sensitivities of specific subpopulations (e.g., infants, children, hypothyroxinemic or hypothyroid individuals, pregnant women) of exposed individuals and potential effects?

See above.

C.2 Please consider the questions in Attachment 2 when preparing written comments on how EPA analyzed, interpreted, and presented results of these studies in the perchlorate assessment. You do not need to answer every question in Attachment 2, rather use your professional judgment to address those that are most appropriate to the chapter in question.

The EPA document is very thorough in its description, review, analysis and interpretation of the experimental studies on perchlorate. The EPA has extensively reevaluated data generated by other parties and, generally, these reevaluations have been clearly described and reported. However, reanalysis of the RIA for thyroid hormones (T_4, T_3, TSH) was confusing. It appeared that raw data (i.e., cpm) generated from three different locations (i.e., scintillation counters) were pooled. However, this is not at all clear. Moreover, the statistical reanalysis of the RIA data was not clear.

Aside from this weakness, the EPA document appears to very clearly describe the various analytical strategies employed to evaluate all data it reviews. This is true for published data (e.g., epidemiological data) as well as unpublished data (e.g., Argus, 2001). The analysis and reanalysis of data – whether published or unpublished – appeared warranted and well justified. Conclusions, in general, appear to be logical and to be supported by the data.

C.3 Are the toxicity data consistent with the proposed mode of action for perchlorate?

C.4 The Toxicological Review and Risk Characterization Document assigned no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) in most of the studies discussed in the document. Are the NOAELs/LOAELs appropriate? Please explain.

The toxicity data are largely consistent with the proposed mode of action for perchlorate. The only departure from this are the linear measurements of brain region in the Argus, 2001 study (see above). These measures are not well known to be sensitive to thyroid hormone. It may be that they are, and if one were going to test this hypothesis, perchlorate would be a good drug to use to control thyroid function. However, as a set of endpoints with known relationship to the mode of action of perchlorate, the ones chosen are weak. This is not reviewed in the EPA document. That is, the relationship between mode of action and neurotoxicity endpoints, is not evaluated. It may be that the endpoints used are, in fact, endpoints, but this is not known

An important weakness in the database on perchlorate that the EPA reviews in the document is that of the effect of perchlorate on human infants. There are simply no studies that focus on the infant. Iodide (and perchlorate) is likely to be somewhat concentrated in milk (20-30 fold over maternal serum) , and infant milk consumption is very high on a body weight basis compared to adult water consumption. Therefore, this issue might be more thoroughly developed in EPA document. For example, in developing the implications of the Greer study on establishing an RfD, the EPA might have articulated that perchlorate levels in breast milk taken from lactating women consuming 7µg/kg perchlorate (0.5 mg/day) might be expected to be higher than her serum levels. Perchlorate may not be 20-30 times higher in milk than in serum, but there are no data to refute this possibility. Therefore, a 10 kg infant drinking 1L/day of milk containing perchlorate that may be 10-fold or more higher in concentration than the NOEL in maternal serum, could be receiving perchlorate at a concentration considerably higher than the NOEL in a healthy adult male.

Despite this arguable weakness in the EPA document, the description of how the EPA established NOAEL's and LOAEL's was very clear and thorough. Also, the relative importance of various endpoints used to identify these characteristics was clear and thorough.

Topic Area F: Human Health Dose-Response Assessment

F.1 Are the conclusions and conditions regarding the key event and the weight of the evidence for effects after oral exposure to perchlorate appropriate and consistent with the information on mode of action? Have the diverse data been integrated appropriately and do they support the proposed point of departure? Should any other data be considered in arriving at a point of departure?

It is clear that perchlorate inhibits the Na/I-symporter and that this event is the key mechanism by which perchlorate potentially produces adverse effects on human health. The observation that perchlorate causes iodide release from the thyroid gland is similar to the observation that iodine itself causes iodide release from the thyroid gland – the so-called Wolff-Chaikoff effect . Thus, perchlorate-induced iodide release does not conflict with the conclusion that perchlorate inhibits the NIS.

The mode of action of perchlorate indicates that potential perchlorate-induced adverse effects on human health would be mediated by a reduction in circulating level of thyroid hormone. Therefore, key effects of perchlorate exposure to consider as the point of departure would be measures of thyroid function and measures of thyroid hormone action. The EPA document reviews the LOAELs, including circulating levels of thyroid hormone, changes in thyroid histopathology, and various linear measures of neuroanatomy reported in Argus, 2001. These are important endpoints that reflect changes in circulating levels of thyroid hormone subsequent to perchlorate action.

The EPA document reviews a number of key events considered in identifying the point of departure. However, their arguments for choosing those described above are compelling. It is this reviewer's opinion that it is also essential to consider endpoints affected by perchlorate during postnatal development; therefore, the chosen endpoints around which to build their assessment is logical and important. The assumptions and limitations considered in the assessment appear reasonable. However, it may be reasonable to consider the prevalence of thyroid disorders in the human populations in perchlorate contaminated regions. Some studies indicate that the prevalence of various disorders is quite high, even among young pregnant women in California and Nevada .

Topic Area H: General Comments, Conclusions, and Recommendations

H.1 Please provide comments on additional topics relevant to the perchlorate assessment, but not explicitly addressed in the previous charge questions.

H.2 Please identify specific sections of the document you find unclear or difficult to understand and explain why.

Overall, the EPA document is clear, thorough and logical. Conclusions are based on clear reasoning. Perhaps most importantly, measures of thyroid hormone action are emphasized in establishing the point of departure. Perhaps the least clear issue is the description of the reanalysis of RIA data reported in the Argus 2001 study.