Appendix D

Scientific Comments Received on the OIG Scientific Analysis of Perchlorate (External Review Draft)

On December 30, 2008, the OIG provided the environmental risk assessor community with the opportunity to review and provide scientific comment on the *OIG Scientific Analysis of Perchlorate (External Review Draft).* The OIG requested that scientific comments be submitted by March 10, 2009.

The OIG received comments from the following seven federal or State offices:

- EPA Office of Water (OW)
- EPA Office of Research and Development (ORD)
- EPA Children's Health Protection and Environmental Education (OCHPEE)[1]
- U.S. Department of Defense (DOD), Chemical Material Risk Management
- Comments from Dr. Pirkle, Dr. Osterloh, and Dr. Blount of the U.S. Department of Health & Human Services/Centers for Disease Control and Prevention (DHHS/CDC)
- Alabama Department of Environmental Management
- Massachusetts Department of Environmental Protection (Massachusetts DEP)

The OIG received comments from the following five private or public organizations:

- Consultants in Epidemiology and Occupational Health, LLC
- Environmental Working Group
- Human Health Risk Research, Inc.
- Intertox, Inc. (on behalf of the Perchlorate Study Group)
- Opdebeeck Consulting, Sarl

These 12 sets of comments on the *OIG Scientific Analysis of Perchlorate (External Review Draft)* are provided for the record in this appendix. The scientific articles provided or cited in these comments are not provided in this appendix, but are available in the public domain. If you have accessibility issues, contact our Office of Congressional, Public Affairs and Management at (202) 566-2391.

The Massachusetts DEP and the Environment Working Group allege that the OIG's hiring of a "defense industry contractor," ICF International, to perform a technical review of the OIG scientific analysis of perchlorate represents a potential conflict of interest. In response to a

[1] OCHPEE provided comments only on the previous internal discussion draft of the OIG Scientific Analysis of Perchlorate. OCHEE did not comment on external review draft version.

request by the Massachusetts DEP for transparency in the process, the OIG is providing the following two documents for the record:

- ICF International's Technical Review (Dated May 9, 2008) of the OIG Scientific Analysis of Perchlorate (Working Draft)
- OIG Workpaper documenting ICF International's May 21, 2008, presentation on its Technical Review to the OIG

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR 1 3 2009

OFFICE OF
WATER

MEMORANDUM

SUBJECT: Office of Water Comments on the *OIG Scientific Analysis of Perchlorate - External Review Draft*

FROM: Michael H. Shapiro
Acting Assistant Administrator

TO: Bill Roderick
Deputy Inspector General
Office of Inspector General

These comments supplement comments the Offices of Water (OW) and Research and Development (ORD) previously provided on the discussion draft of the *OIG Scientific Analysis of Perchlorate: Cumulative Risk Assessment and Its Implications on Public Health* (i.e., the audit report and supplemental report) in October 2008. My understanding is that ORD will be providing comments on this External Review Draft under separate cover.

OW continues to have concerns about the OIG report. Generally, the statements of fact derived from reports, policy documents, and the scientific literature are accurate. However, much of the interpretation of these data and policy inputs deviates significantly from standard application or extends the data beyond its practical limits. The approach to data analysis and application communicated in the OIG report is creative, but we believe it overreaches the current state of the science. Our specific concerns are outlined below:

1. The early parts of the document lay out those Agency science policy documents that argue in favor of cumulative risk assessment as a more realistic approach to estimating the status of an exposed population. While this approach is directionally correct, it is one that has only received limited application across the Agency because of the highly data intensive nature of the assessments.

2. There appears to be a strong implication that lacking a cumulative risk assessment, incremental risk reduction by considering individual chemicals is not appropriate. This argument is not science-based and does not belong in this document. This discussion reflects interpretation of the Agency's public health goals and should be raised in some other venue.

3. The use of *in vitro* data in support of risk assessment was endorsed as a step forward in the National Academy of Sciences (NAS) report *Toxicity Testing in the 21st Century*. However, that report also noted the very large amount of effort remaining in order to understand how and when to use *in vitro* data in lieu of whole animal or human data, commenting that the workgroup anticipated that the development process was likely to span two decades. The OIG report relies heavily on the relative affinities of anions with the sodium/iodide symporter (NIS) to develop relative potency factors (RPFs) for the anion cluster. While interesting, this approach fails to account for many other physiological factors that may impact toxicity at different life stages and among species.

4. The report discusses the uncertainties underlying the NAS reference dose (RfD) for perchlorate. It then proceeds to lay out the development of an RfD for NIS that contains many counter-conservative assumptions and nonstandard selections of endpoints and uncertainty factors for three compounds. This process is further complicated by the overlay of the RPFs.

5. The review of exposure to various subpopulations and life stages is nicely done. Unfortunately, this section once again appears to focus on making the argument that incremental risk reduction is not appropriate, or put another way, EPA should approach regulation of adverse effects on an "all or nothing" basis.

6. A large portion of the risk characterization focuses on the status of iodide nutrition in the US and its impact on thyroid hormone status. This factor is beyond the purview of EPA regulatory activities. While potentially important, the Agency has no statutory basis to impact it. As such, it is simply the background against which an EPA risk assessment is developed and a constant for all intents and purposes.

7. The report appears to propose iodide supplementation as a means for addressing the effects of goitrogens at large. However, no discussion of the potential toxicity of excess iodide is provided. Toxicity could occur in that portion of the population that is currently receiving sufficient iodide if exposure were significantly increased. Iodide has a classic U-shaped dose-response curve that is often seen for nutrients, expressing toxicity both at low and high concentrations, but having beneficial effects at mid-range doses. Supplementation would be particularly problematic for young children who are currently estimated to receive adequate iodide in their diet and who are highly susceptible to the effects of excess iodide.

If you have any questions about our comments, you can contact me or Elizabeth Doyle, Chief of the Human Health Risk Assessment Branch in the Office of Science and Technology, at 202-566-0056.

cc: Kevin Teichman, ORD Deputy Assistant Administrator for Science

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

March 10, 2009

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Response to OIG draft report *Scientific Analysis of Perchlorate: Cumulative Risk Assessment and Public Health Implications* (2008-0010)

FROM: Kevin Teichman
Deputy Assistant Administrator for Science

TO: Eric Lewis
Director of Special Reviews
Office of Inspector General

Thank you for the opportunity to comment on the draft report *Scientific Analysis of Perchlorate: Cumulative Risk Assessment and Public Health Implications*. EPA has put a great deal of effort into evaluating the health effects associated with perchlorate exposure and has undertaken an analysis of the need for regulation of this chemical in groundwater. EPA has also worked with other federal agencies (e.g., FDA, USDA, etc.) as a part of this effort as a member of the Interagency Working Group for Perchlorate. The potential for cumulative exposure to chemicals other than perchlorate that might affect thyroid hormone function is an issue that should not be taken lightly as your report highlights.

Several major issues were identified following our review of the document and the accompanying material including:

Use of the *in vitro* Tonacchera et al. (2004) study for relative potency factor determination: The Tonacchera et al. (2004) study is an *in vitro* study that estimated the relative potency of instantaneous sodium-iodide symporter (NIS) inhibition for several compounds in Chinese hamster ovary (CHO) cells. The OIG report uses this study to predict relative potency for use in the cumulative risk assessment. The use of *in vitro* data for this purpose has major limitations. It is widely acknowledged that use of *in vitro* data has limitations related to kinetics and dynamics, i.e., isolated tissue preparation does not necessarily behave similarly to intact *in vivo* systems. EPA has published numerous guidance documents (U.S. EPA, 1986, 1997, 1998, 2000, 2002, 2007a), evaluation criteria (U.S. EPA, 2003), and state-of-the-art assessments for the cumulative risk of the organophosphorus, triazine, chloroacetanalide and N-methyl carbamate pesticide classes (U.S. EPA 2006a,b,c; 2007b). To date, no *in vitro* data have been used to estimate relative potency due to these concerns. EPA believes that use of the *in vitro* NIS data is premature and may increase the overall uncertainty in the cumulative risk assessment.

Level of peer review: The technical review by ICF International scientists alone is not a sufficient or sound peer review for this document. Previous perchlorate assessments have been reviewed by international expert panels via EPA's Scientific Advisory Board, or more recently by the National Academy of Sciences. The OIG's report should be subjected to the same level of peer review by experts in the field of perchlorate toxicity, thyroid function and cumulative risk. In addition, the ICF review clearly states that the Tonacchera et al. (2004) study is not sufficient for use in a cumulative risk approach for goitrogens.

Lack of consideration of NHANES data on effects of perchlorate exposure in the population: The conclusions of the report indicate that effects from perchlorate exposure that would be observable in the population would be masked by exposure to thyroid-active compounds in the diet. Consideration has not been given to the results of the Center for Disease Control and Prevention (CDC) epidemiological analysis (Blount et al., 2006) that demonstrated a relationship between low levels of perchlorate exposure and circulating levels of thyroid hormone, even in the presence of other thyroid-active chemicals from the diet.

In summary, in our opinion, the issues we have raised about the scientific analysis in the draft report , i.e., (1) the study relied upon to determine relative potency; (2) the level of peer review, and (3) the lack of consideration of the NHANES data, call into question the supporting basis for the recommendations in the report.

At the same time, we will continue to take into account the potential for cumulative risk from exposure to environmental chemicals when planning our agenda for the upcoming years.

References cited:

Blount, B.C., J.L. Pirkle, J.D. Osterloh, L. Valentin-Blasini, L. Caldwell. 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. *Environmental Health Perspectives*. 114(12):1865-1871

Tonacchera, M., A. Pinchera, A. Dimida, E. Ferrarani, P. Agretti, P. Vitti, F. Santini, K. Crump, J. Gibbs. 2004. Relative Potencies and Additivity of Perchlorate, Thiocyanate, Nitrate, and Iodide on the Inhibition of Radioactive Iodide Uptake by the Human Sodium Iodide Symporter. *Thyroid* 14:1012-1019

U.S. EPA. 1986. Guidelines for the Health Risk Assessment of Chemical Mixtures. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC. September. EPA/630/R-98/002.

U.S. EPA. 1997. Guidance on Cumulative Risk Assessment, Part 1. Planning and Scoping. U.S. Environmental Protection Agency, Science Policy Council, Washington, DC. Attachment to memo dated July 3, 1997 from the Administrator, Carol Browner, and Deputy Administrator, Fred Hansen, titled "Cumulative Risk Assessment Guidance-Phase I Planning and Scoping." Available at http://www.epa.gov/OSA/spc/2cumrisk.htm.

U.S. EPA. 1998. Methodology for Assessing Health Risks Associated with Multiple Pathways of Exposure to Combustor Emissions. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. December. EPA/600/R-98/137.

U.S. EPA. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/R-00/002. Available at http://www.epa.gov/ncea/raf/pdfs/chem_mix/chem_mix_08_2001.pdf.

U.S. EPA. 2002. Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at http://www.epa.gov/oppfead1/trac/science/cumulative_guidance.pdf.

U.S. EPA. 2003. Framework for Cumulative Risk Assessment. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/600/P-02/001F. Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=54944.

U.S. EPA. 2006a. Organophosphorus Cumulative Risk Assessment 2006 Update. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at: http://www.epa.gov/oppsrrd1/cumulative/2006-op/op_cra_main.pdf

U.S. EPA. 2006b. Cumulative Risk from Triazine Pesticides. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at: http://www.epa.gov/oppsrrd1/REDs/triazine_cumulative_risk.pdf

U.S. EPA. 2006c. Cumulative Risk from Chloroacetanalide Pesticides. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at: http://www.epa.gov/pesticides/cumulative/chloro_cumulative_risk.pdf

U.S. EPA. 2007a. Concepts, Methods and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-06/013f. Available at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=190187

U.S. EPA. 2007b. Revised N-Methyl Carbamate Cumulative Risk Assessment. U.S. Environmental Protection Agency, Office of Pesticide Programs, Washington, DC. Available at: http://www.epa.gov/oppsrrd1/REDs/nmc_revised_cra.pdf

Review of OIG Scientific Analysis of Perchlorate: Supplemental Report (Discussion Draft) Dated September 2, 2008

Review Dated September 22, 2008 by Michael Firestone, Ph.D., Science Director, OCHPEE, EPA

Based on my overview of not only the OIG risk assessment, but the ICF review of the OIG assessment, I have reached the following conclusions:

1. I am supportive of the concept of applying cumulative risk considerations to questions regarding the risk of perchlorate vis-à-vis exposure to other inhibitors of the Na^+-Iodide symporter (NIS) including thiocyanate and nitrate.

2. That said, to better understand the problem, it would have been more informative to have been able to base the assessment on exposure data that simultaneously examined and directly linked exposure through diet and fluid intake in individuals to biomonitoring levels in the same individuals. Rather, OIG independently considered NHANES biomonitoring study summary results for iodide levels and intake estimates derived from FDA's Total Diet Study to consider exposure through foods. Thus, directly linking iodide deficiency to exposure of all NIS inhibitors in specific individuals to food and fluid intake is not possible. Further, the NHANES data does not appear to reflect biomonitoring in young infants, a key lifestage.

3. OIG's risk assessment relies heavily on using the Tonacchera, et al (2004) paper to model competitive inhibition in humans – their study is based on data derived from a study exposing Chinese hamster ovary cells stably expressing human NIS. This study concluded that the "relative potency of ClO_4^- to inhibit $^{125}I^-$ uptake at the NIS was found to be 15, 30 and 240 times that of SCN^-, I^-, and NO_3^- respectively on a molar concentration basis, with no evidence of synergism, … consistent with a common mode of action by these anions." However, as noted in the ICF review:

   > "… the *in vitro* model used by Tonacchera has a number of limitations that reduce its utility for quantitative risk assessment … (in that their) model does not replicate important features of thyroid physiology that would affect maternal responses to thyroid stressors. …In addition, it does not consider the complex and extensive network of controls in the hypothalamus-pituitary axis and liver … In vitro measurements of NIS inhibition appear to be poor predictors of the key event in perchlorate neurodevelopmental toxicity, which is reduced maternal thyroxine levels during the first trimester of pregnancy."

4. The draft OIG assessment is fundamentally incomplete in that it fails to adequately consider exposures and risk to young infants, a key concern expressed by EPA's Children's Health Protection Advisory Committee (March 8, 2006) → (http://yosemite.epa.gov/ochp/ochpweb.nsf/content/30806_3.htm/$file/30806_3.pdf). While the assessment does model NIS inhibition load, it fails to reach a conclusion

about risk by simply declaring that "a cumulative risk assessment approach is needed …" (p.85). I did note that the OIG drfat does acknowledge that modeling for nursing infants "was not performed due to the lack of sufficient time …"

5. OIG identifies iodide deficiency as a an important stressor – however, the OIG risk assessment fails to consider that high maternal perchlorate levels have been associated with concurrent lowered iodide levels – thus, these two stressors may not be independent factors in leading to perchlorate risk.

6. Just because some stressors may exert a greater effect than others should not be taken as an excuse not to clean-up all problematic environmental contaminants. It seems very unusual to recommend iodide supplementation as a fix for the cumulative problem related to both environmental contamination of perchlorate and nitrate and the public health issues associated with iodide deficiency. Rather, since fetal risk already occurs because many women are already iodide deficient, exposure to contaminants like perchlorate just increases the risk – I don't believe this should be an excuse to dismiss the problem of environmental contamination.

OFFICE OF THE UNDER SECRETARY OF DEFENSE
3000 DEFENSE PENTAGON
WASHINGTON, DC 20301-3000

ACQUISITION
TECHNOLOGY
AND LOGISTICS

MAR 1 1 2009

U.S. Environmental Protection Agency
Attn: Perchlorate Comments for the OIG
c/o: OCPM (Mail code – 2491T) Room 3106
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Dear Mr. Roderick:

Enclosed please find the Department of Defense Consolidated Comments on the Office of External Review Draft Inspector General Scientific Analysis of Perchlorate, Assignment No. 2008-0010, December 30, 2008. The Department of Defense appreciates the opportunity to review this document. The enclosed technical comments are provided with the intent of bolstering the scientific credibility.

These comments represent those of the Department of Defense; all reviewing scientists are supportive of this report, agree with its overall conclusions, and the comments offered. We have identified the Service Component that originated each comment to address your request that the scientific/technical credentials of those offering comments be provided. The following individuals contributed to the enclosed executive summary and detailed comments:

Janis E. Hulla, PhD, DABT
US Army Corps of Engineers (USACE), [redacted]
PhD in Pharmacology [redacted]
Medicine, MS in Biochemistry, and BS in Microbiology [redacted]
[redacted] Diplomat of the American Board of Toxicology (DABT)

Ms. Vera Wang
Navy and Marine Corp Public Health Center (NMCPHC), [redacted]
Division Head, Toxicology/Human Health Risk Assessment
Environmental Programs Department
M.S. Biochemistry [redacted] (12 years experience in biological/biochemistry research)

B.S. Industrial Chemistry, [redacted]
Certified Industrial Hygienist (CIH)

Ms. Katharine Kurtz,
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B.S., Biology, [redacted] (6 years experience in oncology/bioengineering) [redacted]

Under Contract to the U.S. Air Force:
Dr. Ivan Boyer, Ph.D. DABT
Noblis, Inc., under contract to the U.S. Air Force, [redacted]
Ph.D., Toxicology, [redacted]
M.S., Toxicology, [redacted]
M.S., Biology, [redacted]
B.S., Biology / [redacted]

Mr. Andrew Rak
Noblis, Inc. under contract to U.S. Air Force, [redacted]
M.S., Environmental Toxicology, [redacted]
B.A., Environmental Science / [redacted]

We believe it is especially important that the public have understanding of and confidence in EPA's decision making regarding perchlorate and expect that the EPA program offices will consider, reflect, and to the extent practicable, harmonize the alternative analytical approaches offered by the OIG report when conducting their future decision making.

Sincerely,

Shannon E. Cunniff
Director, Chemical Material Risk Management

Enclosures A and B
As Stated

Attachment A
Title: Department of Defense Comments on the Office of Inspector General Scientific Analysis of Perchlorate, dated 30 December 2008

- Executive Summary - The OIG's analysis advances state-of-the-science environment health risk assessment toward meeting the vision outlined in the 2007 NAS report, "Toxicity Testing in the Twenty-First Century". The TIU cumulative risk assessment targets an important point of confluence, thyroid iodide uptake, in the toxicity pathway through which four environmental factors, ClO4-, SCN-, I- and NO3-, might affect neurodevelopment. The analysts merge FDA's dietary survey data and CDC biomonitoring data with a) experimental determinations of relative iodide uptake inhibition potencies, b) hormone determinations from environmentally exposed population and c) linkages between hormone levels, iodine status and neurodevelopment affects. The approach is logical and incorporates the most recent risk assessment tools, advancements and recommendations.

- Major Issues –
  - The report would benefit from an analysis of the steps of a risk assessment to include a quantitative estimate of uncertainty in the approach.
  - The report needs to provide a balanced discussion of the issues stemming from questions about whether the reduction of iodide uptake in the thyroid should be considered to be an adverse (i.e., toxic) effect or precursor to an adverse effect.
  - There are some conclusions in the report that seem to contradict each other.
  - The relevant scientific literature should be cited to support the conclusions in the report.
  - The OIG report does not appear to consider the range of chemical risk assessments performed by the EPA. It needs to clearly define the context and scope of the conclusions regarding the use of "outdated" single chemical risk assessment approaches.
  - The report needs to identify and discuss the limitations of *in vitro* studies.
  - The OIG report should acknowledge or emphasize the substantial technical and legal limitations that often preclude a quantitative cumulative risk-based approach to the evaluation of risks from chemicals.
  - The OIG report should discuss the limitations and uncertainties associated with using the Banerjee et al. (1997) study, as well as the other epidemiological studies summarized in the report, as a basis for concluding that NIS inhibition, in general, will cause

severe hypothyroxinemia in pregnant women and mental deficits in children.

- The OIG report should provide a clear presentation of use of the scientific method in the design and interpretation of the results of epidemiological investigations.
- The treatment of [I-] as the unknown constant, X, in the derivation of the range of serum [I-] through which TIU is unchanged at a given ClO4- exposure level dismisses specific characteristics of thyroid physiology.
- The OIG report should evaluate the adequacy of using RDAs as thresholds for defining iodide deficiency.
- The OIG report should address the range of recommended iodide daily intake values and the potential impact of selecting alternate RDAs on the conclusions of the analysis, present a critical analysis of the most appropriate value for delineating the size of the iodide-deficient populations of concern, and the uncertainties associated with using an RDA for this purpose.
- When discussing percentage of total iodide uptake (%TIU) to be used as the LOAEL, the OIG report does not clearly distinguish between a measurement of effect and a measurement of exposure.

- Substantive Comments – See Table
- Editorial Comments – See Table
- List of Reviewers – with credentials

Janis E. Hulla, PhD, DABT
US Army Corps of Engineers (USACE), [redacted]
PhD in Pharmacology from the [redacted] MS in Biochemistry, and BS in Microbiology from [redacted]
Diplomat of the American Board of Toxicology (DABT)

Ms. Vera Wang
Navy and Marine Corp Public Health Center (NMCPHC), [redacted]
Division Head, Toxicology/Human Health Risk Assessment
Environmental Programs Department
M.S. Biochemistry, [redacted] (12 years experience in biological/biochemistry research)
B.S. Industrial Chemistry, [redacted]
Certified Industrial Hygienist (CIH)

Ms. Katharine Kurtz,
Navy and Marine Corp Public Health Center (NMCPHC),
Environmental Health Scientist
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M.S., Organic Chemistry/Toxicology,
(7 years laboratory research experience; 8 years Navy laboratory Director experience)
B.S. Chemistry,

Mr. David Scarsella
Navy and Marine Corp Public Health Center (NMCPHC),
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M.S., Environmental Policy,
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Noblis, Inc., under contract to the U.S. Air Force,
Ph.D., Toxicology,
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M.S., Biology,
B.S., Biology / Psychology,

Mr. Andrew Rak
Noblis, Inc. under contract to U.S. Air Force,
M.S., Environmental Toxicology,
B.A., Environmental Science

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
1 DoD		Global	We would like to acknowledge the U.S. Environmental Protection Agency (U.S. EPA) Office of Inspector General's (OIG)' logical, scientific, and innovative cumulative risk assessment approach to total iodide uptake (TIU) inhibition as addressed in their External Review Draft Scientific Analysis of Perchlorate, dated December 2008. We appreciate the opportunity to participate in the public review and comment on the scientific nature of this work.		O
2 USACE		Global	The OIG's Analysis advances state-of-the-science environment health risk assessment toward	Include a quantitative estimate of uncertainty in the approach. NAS, "Toxicity Testing in the Twenty-First Century: A Vision and a	S/Major

D-17

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			meeting the vision outlined in the 2007 NAS report, "Toxicity Testing in the Twenty-First Century". The TIU cumulative risk assessment targets an important point of confluence, thyroid iodide uptake, in the toxicity pathway through which four environmental factors, ClO4-, SCN-, I- and NO3-, might affect neurodevelopment. The Analysts merge FDA's dietary survey data and CDC biomonitoring data with a) experimental determinations of relative iodide uptake inhibition potencies, b) hormone determinations from environmentally exposed population and c)	Strategy." Committee on Toxicity Testing and Assessment of Environmental Agents Washington, DC, National Academies Press, 2007. USEPA, "Concepts, Methods, and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures, and Effects: A Resource Document." Environmental Protection Agency Office of Research and Development, National Center for Environmental Assessment, Aug. 2007.	D-18

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			linkages between hormone levels, iodine status and neurodevelopment affects. The approach is logical and incorporates the most recent risk assessment tools, advancements and recommendations. However, absence of quantitative estimates of uncertainty is a missed opportunity to garner confidence in the approach. The discussion of "Approaches to Address this Public Health Issue", acknowledges that environmental health is just one component of the public health challenge and articulates a cogent strategy for the most effective and efficient federal risk		D-19

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			management.		
3 NMCPH		Global	Iodide uptake inhibition (IUI) is the acronym U.S. EPA and other federal agencies have traditionally used to describe perchlorate competitive inhibition of iodide at the sodium iodide symporter (NIS). The U.S. EPA OIG's use of "total iodide uptake (TIU) inhibition" throughout this External Review Draft instead of total IUI may be confusing for some readers, thus requiring that the terminology be further clarified. The lack of iodine is a thyroid stressor, but cannot be described as a NIS	Consider addressing the use of the acronym TIU instead of IUI. Edit the use of the terms "iodine" and "iodide" throughout the document, and explaining the difference between them for the sake of all readers.	E

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Attachment B: Department of Defense Comments on the Office of Inspector General Scientific Analysis of Perchlorate December 30, 2008 EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY		
Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			inhibitor (as can perchlorate, thiocyanate, and nitrate).		
4 NMCPH		Global	Since the draft OIG report was released, U.S. EPA issued an Interim Drinking Water Health Advisory (Interim Health Advisory) for exposure to perchlorate of 15 µg/L in water and OSWER has also issued guidance pertinent to site clean up.	The different reports coming out of EPA – including the OIG report may further confuse the public about EPA's position. After the OIG report is released, it may be necessary for EPA to implement a comprehensive risk communication strategy to explain its decision. We recognize this is not an action the OIG can take	S
5 NMCPH		Global	Differences exist in the "key critical effect" selected for NIS uptake inhibition between (1) U.S. EPA Internal Draft Deliberative "Toxicological Review of Hydrogen Cyanide and Cyanide Salts", December 2008, which was based on	As a matter for EPA, but not necessarily for the OIG, differences may need to be resolved in the "key critical effect" selected and where it resides on the "continuum of change" from NOEL to LOAEL before finalizing the various draft EPA publications.	S

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			an occupational exposure study (El Ghawabi, et al. (1975)) of thiocyanate as a metabolite, (2) U.S. EPA Pre-Decision Draft "Drinking Water: Preliminary Regulatory Determination on Perchlorate", December 2008, and (3) and the U.S. EPA OIG External Review Draft Scientific Analysis of Perchlorate. The OIG Scientific Analysis of Perchlorate developed a Lowest Observed Adverse Effect Level (LOAEL) based on their use of data derived from various occupational studies. This OIG cumulative risk assessment considers the total stress to the thyroid from four named		

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			stressors, perchlorate, thiocyanate, nitrate, and insufficient iodide. The OIG Scientific Analysis of Perchlorate states *"The first adverse effects in the fetus occur before the mother's thyroid performance shows any signs of stress (i.e., changes in thyroid hormone levels). Thus risk assessors should use neither maternal hypothyroidism nor hypothyroxinemia as the key biological event in a risk assessment. By contrast, the first adverse effects in the fetus occur when the maternal TIU is between 25 and 50% of normal,"* (page 6). The NAS used a no		D-23

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			observed effect level (NOEL), and only considered one chemical, perchlorate.		D-24
6 AF	Overall	N/A	The Air Force acknowledges that this document is not intended to be a chemical risk assessment, but understands that it will likely play a role in informing future regulatory actions regarding perchlorate. In general the Air Force agrees with the findings and overall conclusions of the OIG report. Given the importance of the document we would like its findings and conclusions to be well supported, and offer the following technical	None	O

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			comments for consideration and incorporation in the document. In addition to technical comments on the OIG's report, the Air Force is also providing recommendations for further research to address some of the data gaps identified in the report.		
7 AF	Executive Summary	Page 6	The OIG report states: "…analysis of the scientific literature identified that the fetal thyroid is less able to adapt to a low TIU than the mother's thyroid." The text continues: "Therefore, the first adverse effect occurs to the fetus before the mother's thyroid performance shows any signs of stress (i.e.,	The report should cite the relevant scientific literature supporting the conclusion that "… the fetal thyroid is less able to adapt to a low TIU than the mother's thyroid." The OIG report should clarify that the premise for the conclusion that neither maternal hypothyroidism nor hypo-thyroxinemia should be used as the key biological event in a risk assessment is predicated on accepting the statement that "…the fetal thyroid is less able to adapt to a low TIU than the mother's thyroid."	S

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			changes in thyroid hormone levels). Thus, risk assessors should use neither maternal hypothyroidism nor hypothyroxinemia as the key biological event in a risk assessment." Concern: References to the pertinent scientific literature are not provided.		D-26
8 AF	5.1.3	Page 48 - 49	Definitive statements in the OIG report indicating that hypothyroxinemia in the first 20 weeks of pregnancy produces mental deficits and attention deficit and hyperactivity disorders (ADHD) in off-spring are not adequately supported in the OIG report. The report	The report should cite the peer-reviewed and published literature supporting these conclusions. The text should identify and discuss potential confounders and alternative explanations for observations that ADHD or mental deficits are associated with environmental exposures to chemicals. The last sentence of the quoted excerpt should be revised and expanded to explain that the associations observed between hypothyroxinemia and mental deficits or ADHD in epidemiological studies are not proven cause-and-effect relationships.	S

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			states: "….iodide deficiency (i.e., the lack of an adequate uptake of iodide by the thyroid) induces hypothyroxinemia, which is a thyroid condition characterized by a decrease T4 serum level and a normal or slightly elevated T3, without an increase in [thyroid stimulating hormone (TSH)] levels (i.e., TSH levels are normal)…The decrease in T4 in pregnant women with hypothyroxinemia in the first 20 weeks of		D-27

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			pregnancy is associated with mental deficits and an increased frequency of ADHD in their children…Therefore, maternal hypothyroxinemia induces fetal brain damage through the same cause as maternal hypothyroidism – a decreased maternal supply of T4." However, the OIG report does not cite the peer-reviewed, published literature supporting these conclusions or discuss other potential explanations		D-28

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			for the reported associations between environmental exposures to chemicals and mental deficits or ADHD.		D-29
9 AF	5.1.4.1	Page 53	The OIG report identifies maternal hypothyroxinemia as the critical effect to be evaluated in a risk assessment of sodium/iodide symporter (NIS) inhibitors and states : "Maternal hypothyroxinemia (i.e., during pregnancy) is documented to be associated with mental deficits (e.g., ADHD) in the children… the OIG	The report should discuss the limitations and uncertainties associated with using the Banerjee et al. (1997) study, as well as the other epidemiological studies summarized in the report, as a basis for concluding that NIS inhibition, in general, will cause severe hypothyroxinemia in pregnant women and mental deficits in children.	S

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			has identified the observed severe hypothyroxinemia in the Banerjee study [of] exposed workers to be an adverse effect (i.e., if severe hypothyroxinemia were to occur in pregnant woman population) from which an excess NIS inhibition RfD can be calculated from." Although cyanide-exposed electroplating workers examined in the epidemiological study of Banerjee et al. (1997) may have exhibited "severe hypothyroxinemia," this observation does not		D-30

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			provide a sufficient basis for OIG's conclusions that NIS inhibition, in general (including inhibition by thiocyanate, nitrate, and perchlorate), will cause severe hypothyroxinemia in pregnant women and mental deficits in their children. Note, for example, that cyanide has many well-known toxic effects, most of which can be explained by the inhibition of cellular respiration, rather than by the toxic action of its metabolite (i.e., thiocyanate).		D-31
10 AF	8.1	Page 99	The OIG report states: "The IQ [Intelligence Quotient] level of children is associated with the amount	The text should be revised to clarify that the benefits of milk consumption on the mental development of children are not necessarily attributable to the iodide in milk.	S

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			of milk consumed… Milk is known to be a significant source of iodide in the diet." The conclusion inferred by these and other statements in this section, taken together, is not supported by references or citations to the current peer-reviewed literature. The basis for suggesting that the benefits of milk consumption on the mental development of children are solely attributable to the iodide in milk is unclear.		D-32
11 AF	Executive Summary	Page 5	The OIG report states: "…the other common dietary NIS inhibitors, thiocyanate and nitrate, act through the same mechanism of toxicity." This	The text should be revised and elaborated to provide a balanced discussion of the issues stemming from questions about whether the reduction of iodide uptake in the thyroid should be considered to be an adverse (i.e., toxic) effect or precursor to an adverse effect.	S

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			statement may be true only if the reduction of iodide uptake in the thyroid is considered an adverse effect; the NAS concluded that it is not. Critics of NAS's approach (e.g., Ginsburg and Rice, 2005) note that reduced iodide uptake is a "precursor to an adverse effect," on which EPA guidelines indicate an RfD can be based. In contrast, NAS indicated that reduced iodide uptake is a precursor, but not an "immediate precursor," to an adverse effect, and therefore their recommended RfD is based on a no observed effect level (NOEL), as opposed		D-33

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			to a no observed adverse effect level (NOAEL).		
12 AF	Executive Summary	Page 5	The OIG report concludes: "No increase in uncertainty factor value derives a perchlorate RfD that is low enough to prevent mental damage in children, by itself." Elsewhere, the report concludes that controlling exposure to perchlorate will not have a meaningful impact on adverse effects from perchlorate exposure. These conclusions appear to be contradictory.	The OIG report should clarify these conclusions because they appear to contradict each other.	S
13 AF	Entire Report	Global	The OIG report uses the term "lack of iodide," which literally means the total absence of iodide, throughout the report to mean "iodide deficiency."	The report should be revised to use the term "Iodide deficiency", which more accurately describes the condition observed, or other, similar term rather than "lack of iodide."	E

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14 AF	Executive Summary	Page 5	The OIG report does not correctly state the definition of the RfD. The RfD is defined on the EPA's Integrated Risk Information System (IRIS) web site as: "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors	The report should explain how a useful RfD might be developed and applied as a non-static value (i.e., a dose that changes based on exposures to chemicals with the same target organ or mode of action).	S D-35

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			generally applied to reflect limitations of the data used." However, the OIG report (page 5) states: "…the perchlorate RfD is not a static value, but changes depending on the exposure level to the other three NIS stressors (i.e., thiocyanate, nitrate, or lack of iodide)." The idea that a useful perchlorate RfD can be a moving target is not consistent with the current concept of the RfD. No other RfDs have been developed using a non-static approach.		D-36
15 AF	2.3	Page 16	The OIG report does not appear to consider the	The OIG report should be revised to clearly define the context and scope of its conclusions regarding the use of "outdated" single	S

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			range of chemical risk assessments performed by the EPA. The report states "…EPA has known that a single chemical risk assessment is an outdated approach to assessing risk. A single chemical risk assessment characterizes the potential adverse effect and quantifies the risk from only a single chemical pollutant. A single chemical risk assessment does not evaluate the combined effects from multiple chemicals acting through the same mechanism of toxicity." These statements are not necessarily applicable, for	chemical risk assessment approaches.	D-37

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			example, to all site-specific risk assessments conducted to inform remedial decisions. "Conventional" site-specific risk assessments under the Superfund-type site remediation programs typically involve assessing the risks from all of the contaminants (including their background concentrations) and complete exposure pathways (including food consumption, if appropriate) at a site. This total site risk assessment approach is achieved by adding together the "hazard quotients" of multiple chemical contaminants, which are calculated using the appropriate RfD for each		

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			chemical, to estimate an overall "hazard index." Further, the "single chemical" approach includes the incorporation of "relative potency factors" or "toxicity equivalency factors" for chemically related contaminants that likely share a common target or mechanism (e.g., organophosphate pesticides and polychlorinated biphenyls). Therefore, the OIG's general approach to "cumulative risk assessment" conceptually is similar to the "single chemical risk assessment" approach used in site-specific risk assessments. In their analysis, OIG incorporates		

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			"lack of iodide" as a stressor that should be considered in the assessment of risks associated with NIS inhibitors or "goitrogens." However, the risk characterization in a "conventional single chemical risk assessment" equally offers risk assessors the opportunity to address the important role that iodide deficiency may play in determining the risks associated with these chemicals. Confidence in the results of any risk characterization, whether "cumulative" or "single chemical," directly depends on confidence in the data and assumptions used.		D-40

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16 AF	2.6.1	Page 27	There is still much to learn about interactions among numerous environmental stressors, including chemical contaminants. The OIG report states: "Numerous inorganic and synthetic chemicals have been documented to interfere with almost every major step in the production, transport, and peripheral tissue metabolism of thyroid hormones." Until our knowledge and confidence about these potential interactions grows substantially, and alternative "cumulative risk assessment" approaches are adequately verified and	The report should be revised to acknowledge or emphasize the substantial technical and legal limitations that often preclude a quantitative cumulative risk-based approach to the evaluation of risks from chemicals.	S /Major D-41

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			validated, it is a mistake to characterize "conventional single chemical risk assessment" as outdated. For this reason, it is also premature to conclude that "…the only serious technical difficulty in implementing cumulative risk assessment is lack of experience and familiarity among the risk assessor community to embrace the use of cumulative risk assessment."		
17 AF	2.6	Page 23	The OIG report states "The *in vitro* modeling is required because as the complexity of the risk assessment increases (i.e., more factors evaluated), animal testing becomes neither practical	The OIG report should identify and discuss the limitations of *in vitro* studies. The report should note that the results of any *in vitro* study must be evaluated, corroborated, or interpreted using data from appropriate whole animal or epidemiological studies (e.g., pharmacokinetic and neurobehavioral toxicology data) before they can be used with an acceptable degree of confidence in a risk assessment. Conversely, it may be appropriate for the report to	S

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			nor sensitive enough to observe and define the relationship between an increasing number of stressors... no scientific techniques are available to measure subtle cognitive deficits in rat offspring." This statement implies that whole animal testing is infeasible and inadequate to produce the information needed to conduct risk assessments. The OIG report does not identify either the limitations of *in vitro* studies or the advantages of whole animal studies.	present conclusions about the inadequacies of the current *in vivo* models to "measure subtle cognitive deficits" without also concluding that the *in vitro* model will be able to overcome these difficulties.	D-43
18 AF	2.6	Page 23	The OIG report sometimes uses the terms "effects" and "risks" incorrectly. For	The whole report should be revised to delineate clearly the difference between terms such as "risks" and "effects."	E

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			example, the report states: "...the Tonaccherra Model combines the risk from multiple chemicals into a single variable, the TIU, which measures the cumulative effect on the thyroid to the simultaneous exposure to all four NIS stressors." However, the Tonaccherra equation models the effect of multiple chemicals on a single target, namely the NIS. The model does not predict the attendant risks.		

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19 AF	6.1	Page 71	The OIG report should provide a clearer presentation of use of the scientific method in the design and interpretation of the results of epidemiological investigations. The report states: " when considered from a total goitrogen load, the experimental design of this epidemiological study is flipped: Taltal is the control group…y becomes the equivalent of comparing three control groups together."	The passage quoted from the OIG report should deleted. Dependent and independent variables and controls are defined during the planning and designing phases of a study, not re-defined after the study is complete. Post-hoc re-analysis of data is generally discouraged.	S

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Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
20 AF	8.1	Page 96	The report states: "The neurodevelopmental hazard from the lack of iodide is a not threshold effect, but is better characterized by a dose-response relationship." In risk assessments, dose response curves are used to estimate threshold exposures for non-cancer effects and slope factors for cancer effects. A cause-and-effect relationship between exposure to a chemical and an adverse effect is determined first in the hazard assessment. If the mechanism of action suggests that there is no threshold (e.g., the one-hit hypothesis of carcinogenicity), then the toxicity value (e.g., the cancer slope factor) used in a risk assessment may be	The report should be revised to clarify the relationship between dose-response curves and threshold dose concepts in risk assessments. The revised text should reflect that the threshold for an effect or response can be (and often is) estimated from a dose-response curve.	S

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
21 AF	9.4.1	Page 167	When discussing percentage of total iodide uptake (%TIU) to be used as the LOAEL, the OIG report does not clearly distinguish between a measurement of effect and a measurement of exposure. Defining %TIU as a measurement (or biomarker) of exposure would require better characterization of the relationships between exposures to the stressors, changes in TIU, and adverse effects of concern in the human population. In addition, appropriate approaches for addressing uncertainties would need to be developed and justified	The report should clearly indicate that the approach taken depends on defining the %TIU as a measure of effect, and discuss and evaluate the uncertainties associated with this approach.	S / Major D-47

Attachment B: Department of Defense Comments on the Office of Inspector General Scientific Analysis of Perchlorate December 30, 2008 EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY		
Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			based specifically on these relationships and other factors, including data-derived expected or predicted variations in the susceptibilities and responses of individuals exposed to the stressors and the nature and expression of the measurements used to characterize exposures and responses.		
22 AF	9.4.2	Page 168	The OIG report appears to define the term No Observed Adverse Effect Level (NOAEL) incorrectly. The report states: "The traditional use of a NOAEL in a single chemical exposure a NOAEL is the lowest chemical exposure	The report should be revised to correct or clarify its usage of the term NOAEL.	E

D-48

Attachment B: Department of Defense Comments on the
Office of Inspector General Scientific Analysis of Perchlorate
December 30, 2008
EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY

Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: March 2009

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Page & Paragraph (enter "Global" if report section-wide)	Comment	Suggested Action, Revision and References (if necessary)	Category*
			level without adverse effects." The NOAEL is actually the highest exposure level with no observed adverse effects, as defined in EPA's online IRIS glossary.		D-49
23 AF	9.4.1	Page 166	In the exposure assessment section, the OIG report estimates the size of the sensitive subpopulation based on the recommended daily allowance (RDA) for iodide. However, the OIG report does not evaluate the RDA, particularly of the adequacy of using the RDA as a threshold for defining iodide deficiency. The recommended iodide intakes for pregnant women listed in the report, range	The report should evaluate the adequacy of using RDAs as thresholds for defining iodide deficiency. The report should address the range of recommended iodide daily intake values and the potential impact of selecting alternate RDAs on the conclusions of the analysis. The report should present a critical analysis of the most appropriate value for delineating the size of the iodide-deficient populations of concern, and the uncertainties associated with using an RDA for this purpose	S / Major

Attachment B: Department of Defense Comments on the Office of Inspector General Scientific Analysis of Perchlorate December 30, 2008 EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY					
Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate			Organization: Department of Defense	Date Submitted: March 2009	
*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.					
Comment No.	**Section**	**Page & Paragraph (enter "Global" if report section-wide)**	**Comment**	**Suggested Action, Revision and References (if necessary)**	**Category***
			from 200 ug/day to 375 ug/day; however, the variability and the uncertainty are not discussed.		
24 AF	Entire report	Global	While it may not be the role of an OIG report to identify additional toxicological studies, we believe there are opportunities to better define the potential for perchlorate toxicity in exposed human populations, especially the proposed relationship between perchlorate and other "goitrogenic" anions. The report could be expanded to identify and recommend additional studies that should be	Future research that could reduce critical uncertainties include: • The observed and proposed associations between thyroidal and neurodevelopmental effects and the combined ("cumulative") intake of I^-, ClO_4^-, SCN^- and NO_3^- should be characterized through additional experimental investigation. • The variability in the capacity of the hypothalamic-pituitary-thyroid axis to compensate for I^- deficiency when the intake of each of the "goitrogenic" anions is varied should be determined experimentally to elucidate the compensatory mechanisms involved (including potentially the up-regulation of the NIS during pregnancy and fetal and neonatal development). • The pharmacokinetics (i.e., relative rates of absorption, distribution, metabolism, and elimination) of I^-, ClO_4^-, SCN^- and NO_3^- during combined exposure to these chemicals should be investigated. These studies are warranted to describe likely pharmacokinetic interactions among these	S

Attachment B: Department of Defense Comments on the Office of Inspector General Scientific Analysis of Perchlorate December 30, 2008 EXTERNAL REVIEW DRAFT – FOR SCIENTIFIC REVIEW AND SCIENTIFIC COMMENT ONLY					
Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate			Organization: Department of Defense	Date Submitted: March 2009	
*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.					
Comment No.	**Section**	**Page & Paragraph (enter "Global" if report section-wide)**	**Comment**	**Suggested Action, Revision and References (if necessary)**	**Category***
			performed to better define the potential for perchlorate to cause adverse effects in exposed human populations, including confirmation and elucidation of the proposed relationships among perchlorate and other "goitrogenic" anions.	ions, in addition to the competitive interactions at the level of the NIS observed *in vitro*, for example, by Tonacherra et al. (2004). Well-designed and executed whole animal studies are needed to support the development of pharmacokinetic models. The kinds of studies listed above would be especially likely to reduce the significant uncertainties in the cumulative risk assessment attributable to inadequate data in these and other areas.	D-51

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30341-3724

February 4, 2009

Bill Roderick
Deputy Inspector General
c/o: OCPM (Mail code - 2491T) Room 3106
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Perchlorate Comments for the Office of
Inspector General (OIG)

Dear Mr. Roderick:

Please find attached scientific comments on the *OIG Scientific Analysis of Perchlorate (External Review Draft)*. These comments are the scientific opinions of the undersigned and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Thank you for the opportunity to comment on this document.

Sincerely,

James L. Pirkle, M.D., Ph.D.
Deputy Director for Science
Division of Laboratory Sciences

Sincerely,

John D. Osterloh, M.D.
Chief Medical Officer
Division of Laboratory Sciences

Sincerely,

Benjamin C. Blount, Ph.D.
Chief, VOC and Perchlorate Laboratory
Division of Laboratory Sciences

Comments on "*OIG Scientific Analysis of Perchlorate (External Review Draft)*"

We feel that some statements found in Appendix A of the "*OIG Scientific Analysis of Perchlorate (External Review Draft)*" warrant comment. Specifically, the findings of Blount et al (2006) and Steinmaus et al (2007) *are* biologically coherent and focused on a large population with additional potential stressors of iodide uptake. The data set studied by Blount et al and Steinmaus et al is the *only set of data* that identifies a susceptible group (women with lower iodide concentrations) and measures (and adjusts for effects of) the other competing goitrogenic anions. That a population with lower iodide excretion showed a more pronounced association between thyroid hormone levels and perchlorate levels is coherent with the known mechanisms of the interactions of iodide and perchlorate. While the Blount analysis indicates a potential effect of perchlorate at levels below those seen in the Greer study, the OIG analysis suggests that some new mechanism should be postulated to account for this difference. A new mechanism is not required; only to examine the differences between the Greer study and the study analyzed by Blount et al and Steinmaus et al. The Greer study did not examine a susceptible population (women with lower iodide excretion), was not sufficiently powered to see small changes, and did not examine subjects under chronic exposure conditions or for an extended period of time after their experimental perchlorate exposure. It is therefore not surprising that the Greer study did not observe changes in thyroid function and the analyses by Blount et al and Steinmaus et al were able to detect these changes. It is also possible that mechanisms in addition to NIS inhibition are at play -- McClanahan et al (2009) recently published evidence that perchlorate can act via a second mechanism to impair thyroid hormone production/secretion.

In general, we are puzzled by the "critical review" of Blount et al and Steinmaus et al; this text is appended to the main body of the report, which lacks similar critical reviews of other recent publications on this topic. The authors of *Appendix A* state that "…repeating the analysis in the next NHANES data set would not represent an independent evaluation…" because the cross-sectional study design is the same as the study design used by Blount et al and Steinmaus et al. Yet the authors of *Appendix A* cite numerous conference abstracts that describe studies of much smaller populations, each utilizing a similar cross-sectional study design. The fact that similar cross-sectional designs are used in these studies is not cited as a lack of independent information. In our opinion, detailed study of perchlorate exposure and thyroid function in ~4000 new U.S. residents from a different NHANES study would provide additional valuable insight in a completely new population of individuals, and would provide additional important evidence concerning the relationship between thyroid function and perchlorate levels at the relatively low levels experienced by the general population.

In addition, the NHANES sample size is huge compared to the aggregate sum of persons in all these cross-sectional studies combined. The statistical power afforded by the large NHANES population size merits special consideration in light of the need for considerable statistical power to detect the effect sizes presented in the Blount et al and Steinmaus et al analyses.

Finally, the Blount analysis did not state or show that "13 μg/day of perchlorate exposure induces toxicity." This citation should be corrected.

References:

Blount, B. C.; Pirkle, J. L.; Osterloh, J.; Valentin-Blasini, L.; Caldwell, K. L. 2006. *Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States.* Environmental Health Perspectives 114, 1867–1871.

Steinmaus C, Miller MD, Howd R, 2007. *Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001-2002 National Health and Nutrition Examination Survey.* Environmental Health Perspectives 115:1333–1338.

McLanahan ED, Andersen ME, Campbell JL and Fisher JW, 2009. *Competitive Inhibition of Thyroidal Uptake of Dietary Iodide by Perchlorate Does Not Describe Perturbations in Rat Serum Total T4 and TSH.* Environmental Health Perspectives in press. doi: 10.1289/ehp.0800111 [Online 5 January 2009]

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DIRECTOR

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March 9, 2009

Attn: Perchlorate Comments for the OIG
c/o: OCPM (Mail code - 2491T) Room 3106
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: **ADEM Review Comments:** *EPA Office of Inspector General Scientific Analysis of Perchlorate (External Review Draft)*, dated December 30, 2008.

Dear Sir or Madam:

The Alabama Department of Environmental Management (ADEM or the Department) has completed its review of the referenced *Office of Inspector General (OIG) Scientific Analysis of Perchlorate.* This document is being distributed to receive scientific comments on the use and application of a cumulative risk assessment approach to characterizing the public health risk from a low Total Iodine Uptake during pregnancy and lactation. ADEM has generated the enclosed comments.

If you have any questions regarding this correspondence, please contact Sarah Gill at (334) 271-7734 or via e-mail at sgill@adem.state.al.us or Mr. Prem Kumar at (334) 394-4377 or via e-mail at KPKumar@adem.state.al.us.

Sincerely,

Wm. Gerald Hardy, Chief
Land Division

Enclosure

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ADEM Review Comments:
Office of Inspector General Scientific Analysis of Perchlorate
Dated December 30, 2008

Reviewer 1 Comments:

1. **Page 141, Section 9.1.4 Perchlorate PBPK and the Greer Perchlorate Exposure Study:** The paper states "*The %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.007 and 0.5 mg/kg-day are in excellent agreement with the %TIU observed in the Greer study (see table above). However, the %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.02 and 0.1 mg/kg-day are not in particularly good agreement with the %TIU observed in the Greer study (see table above). In short, half of the %TIU values results agree, while the other half of %TIU values do not agree so well.*" This appears to be the extent of the data analysis comparing the observed and predicted data. The author(s) should consider plotting the data and performing a regression analysis or some other form of statistical analysis. A simple linear regression, comparing the observed %TIU in the Greer study to the %TIU predicted by the Tonacchera model for the same perclorate dose, yields an R^2 of 0.92, a slope of 0.99, and an intercept of 6.14. These results indicate a high degree of agreement between the predicted and observed data. Given the limited number of data points (four) available for comparison, no definitive conclusion can be made that the model accurately predicts the %TIU at a given perchlorate dose, but, by the same token, no definitive conclusion can be made that the model does not accurately predict the %TIU at a given perchlorate dose.

2. **Page 174, Section 9.1 Corroboration of Tonacchera Model with Effects Observed in Humans:** The final sentence in this section states "*Since a pinch of table salt weighs about 460 mg, 14.3 mg of salt is equivalent to about 1/32nd of a pinch of salt. Since a smidgen of table salt weighs about 230 mg, 14.3 mg of salt is equivalent to about 1/16th a smidgen of salt.*" There is no scientifically accepted standard measurement for a 'pinch' and a 'smidgen'. Therefore, it is suggested that the above statement be removed from page 174 and page 184.

3. **Page 190, Appendix A:** The test states "*The R^2 value of 0.240 reported in the Blount analysis shows that perchlorate accounts for only 3% of the variation seen in the serum tT_4 (Charnely 2008).*" The R^2 value is the proportion of variability in a data set that is accounted for by the statistical model and ranges from 0 to 1. If R^2 is 0.24, then 24%, not 3%, of the variation is predicted by the Blount analysis. Also, the reference quoted is not included in the list of references in Appendix B.

4. While the paper is titled "Scientific Analysis of Perchlorate", it is actually an analysis of the use of cumulative risk assessment to evaluate NIS inhibition. The author(s) provide a good summary of past studies on NIS inhibition and present several valid points regarding the relative impacts of iodide nutritional deficiency, nitrate, thiocyanate, and perchlorate on the thyroid. However, there are still

uncertainties and obstacles to applying a cumulative risk assessment (see Reviewer 2's comments) that are not acknowledged in the paper.

5. The paper contains a large number of grammatical errors, which in a few places are significant enough to make it difficult to understand what the author(s) intended to convey. It is suggested that the author(s) have the paper edited to address grammatical errors.

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Background: Ph.D. Civil Engineering
Area of Expertise: Environmental engineering

Reviewer 2 Comments:

These comments are regarding the US Environmental Protection Agency Office of Inspector General (OIG) Scientific Analysis of Perchlorate (External Review Draft) report, which is being distributed to receive scientific comments on the use and application of a cumulative risk assessment approach to characterizing the public health risk from a low Total Iodine Uptake during pregnancy and lactation. A review of scientific literature was performed to derive these comments.

CUMULATIVE RISK ASSESSMENT APPROACH

The cumulative risk assessment approach using the dose addition method for all four sodium iodide symporter (NIS) stressors to characterize the risk to public health from a low total iodide uptake (TIU) during pregnancy and lactation is a very novel approach, but reviewing the literature pertaining to this area shows that very little quantitative data is available to support this approach, especially for pregnancy and lactation. Differential exposure to mixtures of environmental agents, especially chemical stressors, can contribute to increased vulnerability of human population. Cumulative Risk Assessment is a tool for organizing and analyzing information to evaluate the probability and seriousness to multiple environmental stressors, and is hampered by three interrelated problems:

1. Relatively little is known about magnitude, duration, frequency and timing of cumulative exposure to these important environmental mixtures.
2. Scant evidence is available on whether mixture-related effects are additive at exposure levels during pregnancy and lactation.
3. There is inadequate knowledge and insufficient understanding of the interactive mechanisms of toxicity that occur among mixture constituents.

Cumulative risk assessment will be most useful if the uncertainty factors/safety factors built into the conventional risk assessment process adequately protect the public health from cumulative effects within a sufficient margin of safety. A cumulative risk assessment approach as suggested by the OIG Analysis may provide guidance about which NIS inhibitors that are part of our day-to-day lives constitute a serious health risk that is not adequately accounted for by traditional risk assessment methods (Sexton and Hattis, 2007).

The application of a cumulative risk assessment approach to public health risk is in its infancy because of so many uncertainties. According to Dasgupta, *et al.* (2008), it may be doubtful that thiocyanate and/or nitrate pose a greater risk of low iodide uptake based on the selectivity factors for the NIS and projected dietary intake amounts with special reference to lactation (breastfed infants). Little nitrate is excreted in milk and bears little relationship to dietary nitrate.

For thiocyanate, neither urinary excretion nor ingested amounts may provide a reliable index of the circulating levels of thiocyanate seen by the mammary NIS. Urinary excretion may not reflect circulating levels because, in part, thiocyanate is formed *in vivo* from cyanide, is present in certain foods (Eminedoki, *et al.,* 1994), and is formed during protein metabolism (Himwich and Saunders, 1948). Cigarette smoke is an important source of hydrogen cyanide. The cyanide-to-thiocyanate conversion takes place in the liver and kidney. Ingested thiocyanate is known to be oxidized by a variety of mechanisms (Grisham and Ryan, 1990) and form various adducts (Funderburk and Van Middlesworth, 1967). Of interest is that thiocyanate also has benefits: its *lactoperoxidase*-mediated oxidation products are powerful protection against the human immunodeficiency virus (Wang, *et al.*, 2000).

There is no universal agreement on the extent of the threat posed by perchlorate during lactation (Braverman and Pearce, 2005; Kirk, *et al.*, 2005 and Lamm, *et al.*, 2005). There is no conclusive data as to if, and to what extent, perchlorate inhibits transport to human milk. Extrapolation from mouse NIS experiments to the human mother-infant pair is tenuous, especially when such *in vitro* experiments involve concentrations far removed from reality, and through the NIS in a manner different from that of iodide (Dohan, *et al.*, 2007).

According to Dasgupta, *et al.* (2008), until proven otherwise *in vivo*, the role of thiocyanate as an iodide transport inhibitor in human lactation, especially *vis-à-vis* perchlorate, should not be overemphasized, especially when such analysis is based on urinary thiocyanate content and selectivity factors determined *in vitro* on non-human NIS systems. They also opined that the impact of perchlorate and thiocyanate on inhibiting iodide transport in human milk (lactation) may have been overemphasized.

Though OIG emphasizes the relevance of a cumulative risk approach using all the NIS stressors (perchlorate, thiocyanate, nitrate and the lack of iodide) to better characterize the risk to the public, according to Wilkinson, *et al.* (2000) it is important to emphasize that there remains a great deal of scientific uncertainty about how to proceed with

cumulative risk assessment as described in the Food Quality Protection Act. The serious difficulties associated with defining common toxicity and "concurrent exposure", combined with the current paucity of data and methodology required to conduct cumulative risk assessment, suggest that the procedure is not yet ready for use in public health risk, especially to characterize TIU during pregnancy and lactation.

Because of the complexity of considering so many factors simultaneously, there is a need for simplified risk assessment tools (such as software packages, databases and other modeling resources) that would allow screening level risk assessments and could allow communities and stakeholders to conduct risk assessment and thus increase stakeholder participation. In practice, measuring or estimating concurrent exposure to multiple stressors is not straightforward, even if the toxicologically relevant temporal and spatial aspects are known. There is no established state and federal environmental tracking system that provides systematic collection, integration, analysis, interpretation, and dissemination of information about the environmental hazards, including sources, environmental concentrations, exposures, doses, and potentially related health effects. The creation of linked monitoring systems, databases , and registries offers the prospect of better data on cumulative exposures and improved understanding of the connection between combined exposures and chronic health effects of thyroid inhibitors (Litt, *et al.*, 2004 and McGeehin, *et al.*, 2004).

Considering the lack of sufficient information about the effects of perchlorate in sensitive populations such as pregnant and lactating women, the recommendation of the National Academy of Sciences to define a no-observed-effect-level as a non-classic departure point for perchlorate risk assessment seems to be defensible. Looking at the mode of action and available scientific literature on the four NIS stressors on total iodine uptake during pregnancy and lactation, cumulative risk assessment is a holistic approach of combining the effects of exposure to multiple stressors via all relevant sources, pathways, and routes. The methods used in cumulative risk assessment are still being perfected and it is recommended that EPA and others continue research to make this approach more reliable, realistic and relevant. Since perchlorate has been determined to be the least of four stressors affecting iodine uptake and health effects to pregnant or lactating females, care should be taken by EPA not to rely heavily on limiting perchlorate exposure when effects from the other three stressors cannot be controlled.

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REFERENCES

Braverman, L.E. and E.N. Pearce, 2005. Comment on "Perchlorate and Iodide in Dairy and Breast Milk. *Environ. Sci. Technol.,* **39**: 5498.

Braverman, L.E., X. He, S. Pino, M. Cross, B. Magnani, S.H. Lamm, 2005. The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. *J. Clin. Endocrinol. Metab.*, **90** (2): 700-706.

Dasgupta, P.K., Andrea B. Kirk, J.V. Dyke and S. Ohira, 2008. Intake of Iodine and Perchlorate and Excretion in Human Milk. *Environ. Sci. Technol.,* **42**: 8115-8121.

Dohan, O., C. Portulano, C. Basquin, A.Reyna-Neyra, L.M. Amzel and N. Carrasco, 2007. The Na^+/I^- symporter (NIS) mediates electroneutral active transport of the environmental pollutant perchlorate. *Proc. Natl. Acad. Sci.*, **104**: 20250-20255.

Eminedoki, D.G., M.O. Monanu, and E.O. Anosike, 1994. Thiocyanate levels of mainly dietary origin in serum and urine from a human population same in Port Harcourt, Nigeria. *Plant Foods Hum. Nutri.*, **46**: 277-285.

Fundenburk, C.F. and L. Van Middleworth, 1967. Effect of lactation and perchlorate on thiocyanate metabolism. *Am. J. Physiol.,***213**: 1371-1377.

Grisham, M.B. and E.M. Ryan, 1990. Cytotoxic properties of salivary oxidants. *Am. J. Physiol.*, **258**: C115-C121.

Himwich, W.A. and J.P. Saunders, 1948. Enzymatic conversion of cyanide to thiocyanate. *Am. J. Physiol.*, **153**: 348-354.

Kirk. A.B., P.K. Martinelango, K. Tian, A. Dutta, E.E. Smith and P.K. Dasgupta, 2005. Response to Comment on "Perchlorate and Iodide in Dairy and Breast Milk". *Environ. Sci. Technol.*, **39**: 5902-5903.

Lamm, S.H., M. Feinleib, A. Engel and J. Gibbs, 2005. Comment on "Perchlorate and Iodide in Dairy and Breast Milk". *Environ. Sci. Technol.*, **39**: 5900-5901.

Litt, J., N. Tran, K.C. Malecki, R. Neff, B. Resnick, T. Burka, 2004. Identifying priority health conditions, environmental data, and infrastructure needs: a synopsis of the Pew environmental health tracking project. *Environ. Health Perspect.,* **112**:1414–1418.

McGeehin, A.M., J.R. Qualters and A.S. Niskar, 2004. National Environmental Public Health Tracking Program: Bridging the Information Gap. *Environ. Health Perspect.,* **112**(14): 1409-1413.

Sexton, K. and D. Hattis, 2007. Assessing cumulative health risks from exposure to environmental mixtures – three fundamental questions. *Environ. Health Perspect.*, **115** (5): 825-832.

Wang, H., X. Ye and N.B. Ng, 2000. First demonstration of an inhibitory activity of milk proteins against human immunodeficiency virus-1 reverse transcriptase and the effect of succinylation. *Life Sci.*, **67**: 2745-2752.

Wilkinson, C.F., G. R. Christoph, E. Julien, J.M. Kelley, J. Kronenberg, J. McCarthy and R. Reiss, 2000. Assessing the risk exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? *Regulatory Toxicol. Pharmacol.*, **31** (1): 30-43.

ACRONYMS AND DEFINITIONS

Lowest-observed-effect-level (LOEL): Lowest concentration or amount of a substance, found by experiment or observation, that causes any alteration in morphology, functional capacity, growth, development, or life span of target organisms distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

No-observed-effect-level: Exposure level at which there are no statistically or biological significant differences in the frequency or severity of any effect in the exposed or control populations.

Point of Departure (POD): The dose-response point that marks the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose-response model.

Reference Dose (RfD): An estimate of a daily oral exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime.

Sodium Iodide Symporter (NIS): An integral membrane protein that resides in the basolateral membrane of thyroid epithelial cells, that actively transports iodide (I^-) across the basolateral membrane into thyroid epithelial cells.

Uncertainty Factor (UF): A number (equal or greater than 1) used to divide NOAEL or LOAEL values derived from measurements in animals or small groups of humans, in order to estimate a NOAEL or LOAEL value for the whole human population; also called margin-of-safety, safety factor.Uncertainty factors are used to compensate for a deficiency in knowledge concerning the accuracy of test results and the difficulty in estimating the health effects in a different species and/or in different exposure conditions. As such, the value of the uncertainty factor depends on the nature of the toxic effect, the size and type of population to be protected, and the quality of the toxicological information, and includes scientific judgments.

COMMONWEALTH OF MASSACHUSETTS
EXECUTIVE OFFICE OF ENERGY & ENVIRONMENTAL AFFAIRS
DEPARTMENT OF ENVIRONMENTAL PROTECTION
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DEVAL L. PATRICK
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TIMOTHY P. MURRAY
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IAN A. BOWLES
Secretary

LAURIE BURT
Commissioner

March 9, 2009

Office of the Inspector General
United State Environmental Protection Agency
Washington, D.C. 20460
Perchlorate_comments_for_OIG@EPA.gov

Dear Inspector General:

We are submitting the following comments on the USEPA Office of the Inspector General's *Scientific Analysis of Perchlorate (External Review Draft)*, dated December 30, 2008. Respondents include toxicologists and public health scientists at the Massachusetts Department of Environmental Protection (MassDEP), who participated in the development of the first state drinking water standard for perchlorate.

Overall comments relating to the draft report's preparation, release and conclusions are presented first, followed by a more specific discussion highlighting some of the technical limitations and uncertainties in the assessment. We believe these issues seriously undermine the draft report's analysis and conclusions.

Overall Comments. Although several of the technical aspects of the OIG assessment have merit, the overall document is, in several aspects, seriously flawed. In addition we are troubled by elements of the process followed in the preparation and release of this draft report.

Comment 1: The use of ICF Incorporated (Inc.) to provide technical review of this document prior to its release is troubling. ICF Inc. has provided considerable consulting services on perchlorate to at least one organization, the National Aeronautics and Space Administration, potentially subject to any USEPA regulatory determinations regarding this drinking water contaminant. This raises the appearance of a potential conflict of interest. In light of allegations of regulated industries' influence on certain USEPA policy and regulatory deliberations and decisions (e.g. as reported with respect to the Clean Air Mercury Rule), and in order to maximize process transparency, the USEPA should make the draft documents submitted to ICF Inc., as well as all comments and input provided by ICF Inc., available to the public.

Comment 2: It also appears that this document was rushed "out the door", as appropriate editing and review were clearly not completed. The report is often inconsistent and self contradictory; contains numerous distracting grammatical errors and several technical misstatements; and is incomplete with respect to discussion of scientific uncertainties. For example:

- The document incorrectly states that a reference dose (RfD) is "derived from a dose associated with an adverse effect" (pg. 43). In fact many RfD values are derived from "no adverse effect level" doses.
- The document also states that the USEPA perchlorate RfD was derived from a biological response (iodide uptake inhibition (IUI)) "several steps before the adverse effect (hypothyroidism) (pg. 45) but then subsequently demonstrates, compellingly, that adverse neurodevelopment outcomes in children are in fact associated with thyroid effects well prior to overt hypothyroidism. Indeed the OIG document makes a strong case that IUI is penultimate, rather than several steps upstream, to effects directly associated with adverse neurodevelopmental outcomes.
- Grammatical and typographical errors exist throughout the report.

Comment 3: While cumulative effects on the thyroid gland are a valid issue to address, applying a new cumulative assessment methodology for determining an appropriate drinking water standard for perchlorate significantly deviates from longstanding USEPA protocols. Other drinking water standards and guidelines do not consider cumulative impacts nor has USEPA established a specific protocol for doing so. Is perchlorate an exception to the rule for standard setting or will the OIG recommend that USEPA consider cumulative effects for all other drinking water standards and health advisories? As a result of application of the cumulative effect approach, OIG appears to discount the need for a low perchlorate standard. Instead, OIG considers reassessing the nitrate drinking water standard, but favors adding iodine to prenatal vitamin supplements as the better alternative. However, protocols for these types of exposure standard tradeoffs and risk mitigation measures through dietary treatments do not exist, have never been vetted publically and have not been used with other chemicals. For example, calcium and iron have a protective effect with respect to lead exposures. Will OIG argue for a higher drinking water standard for lead with dietary supplementation with calcium and iron as an alternative? Due to these issues we believe such new protocols should be developed through a public process prior to their application on a chemical with such nationwide significance to public health. We recommend that USEPA's protocol for assessing mixtures to evaluate risks of multiple chemicals acting via the same mechanism of action be used to address perchlorate within a mixture of thyroid toxicants in water supplies, which would support a lower, more protective standard for perchlorate as discussed in comment 4.

Comment 4: Although OIG's use of a cumulative effect approach may have merit, it is inappropriately used to argue against a protective drinking water value for perchlorate. In our view, from a public health perspective and a desire to protect children's health, exposures to multiple thyroid toxicants should lower the acceptable exposure value for any single toxicant not the other way around. OIG's conclusion that reliance on iodide supplementation through vitamins is an adequate public health response to contaminated drinking water supplies inappropriately shifts the responsibility for protecting public health from the polluter to the individual. It also affords those most at risk, the fetus and neonate, with no ability to protect their own health. Under this intervention approach, protection of infants from adverse health effects

attributable to contaminated water supplies is completely dependent on the mother's ability to obtain necessary iodide supplementation and then to follow the recommended supplementation regimen, which may not always be possible due to individual circumstances and variability in the actual iodide content of vitamins, as has recently been reported (Boston Globe, 03/02/09)

Comment 5: OIG's conclusion that "the most effective and efficient approach for reducing health risks of permanent mental deficits in children from low maternal thyroid iodide uptake during pregnancy and nursing is to add iodide to all prenatal vitamins". One water supply in MA had perchlorate at a concentration of 1300 ppb. At this level, iodide supplementation is not likely to protect public health.

Additional Technical Comments.

Comment 6: The OIG assessment relies upon the Clewell *et al.* physiologically based pharmacokinetic (PBPK) model to predict iodide uptake. The uncertainties and limitations of this model were not considered despite the fact that questions have been raised regarding aspects of the model, in particular its applicability to the fetus and neonate, the groups of most concern.

Comment 7: The OIG assessment assumes a constant proportionality between thyroidal iodide uptake and concentrations in the serum/urine as advanced by Tonacchera *et al.* (2004). Although this assumption may be appropriate for the *in vitro*, petri dish experiments performed by Tonacchera *et al.*, this is an oversimplification that ignores adaptive responses which occur *in vivo*, as well as uncertainties regarding the cumulative impacts of exposures to sodium iodide symporter (NIS) inhibitors on the thyroid and other tissues expressing this protein. Specifically, the OIG document used the *in vitro* study of Tonacchera *et al.* (2004) to estimate the interaction and total amount of iodide uptake inhibition in the thyroid caused by perchlorate, thiocyanate, and nitrate. This analysis was described as a dose addition method. However, the simple kinetic equations used in the document (pg. 39, 72, 132, etc.) which were derived from the Tonacchera *et al. in vitro* lab study on Chinese hamster ovary cells expressing human NIS, do not adequately represent the *in vivo* workings of the hypothalamic-pituitary-thyroid axis. This approach does not account for the complex regulatory mechanisms involved in the modulation of iodide absorption, thyroid uptake, use and disposition. None-the-less, OIG based their analysis and conclusions on this *in vitro* approach with little discussion of the model's limitations and uncertainties. Reliance on such a simplistic approach to predict responses of such a complex system is fraught with uncertainty. Furthermore, even assuming that the Tonacchera model is accurate in predicting serum perchlorate equivalent concentrations (SPECs) in adults, the risk numbers derived by OIG based on the various studies and the derived SPECs are not themselves protective of the most sensitive subgroup, the neonate and the fetus.

The limitations of simple modeling approaches are further evidenced by the National Research Council (NRC) Perchlorate Committee (2005) use of Michaelis-Menton competitive inhibition equations to estimate the iodide uptake inhibition induced by perchlorate at various concentrations of perchlorate and iodide. They concluded that humans who have serum iodide concentrations of 0-1000 ug/L would be equally sensitive to perchlorate's effects on thyroid iodide uptake. However, studies conducted by Blount *et al.* (2006) and Stienmous *et al.* (2007) are inconsistent with this conclusion, as their results indicate that people with urine iodide levels

less than 100 ug/L (assuming urine levels represent serum levels at steady state) are more sensitive to perchlorate's effect than people who have urine levels of iodine greater that 100 ug/L.

Comment 8: OIG also downplayed important results by Steinmaus (2007). This well designed human study, which was conducted in the US, received only cursory review in Appendix A of the document while other human studies conducted in Chile and elsewhere were extensively reviewed in the body of the document. Stienmaus *et al.* concluded that thiocyanate and perchlorate, at a relatively low level, interact in affecting thyroid function in women with low urinary iodine. Thiocyanate alone at urine concentrations about 2000 times that of perchlorate was not associated with altered thyroid hormone levels in women with low urinary iodine levels, but significantly altered hormone levels were observed when perchlorate exposures were also considered. This interactive effect was observed at perchlorate and thiocyanate exposure levels documented to be occurring in the US.

Comment 9: The OIG document also relied on the Tonacchera *et al.* model (pg 133) to justify the NRC (2005) statement that "To cause declines in thyroid hormone production that would have adverse health effects iodide uptake must likely be reduced by at least 75% for months or longer". In the OIG assessment the thyroid iodide uptake (TIU) that is associated with hypothyroidism ($TIU_{hypothyroidism}$) is calculated as the ratio of the urinary iodide concentration (UIC) associated with severe iodine deficiency (20 ug/L urine iodide) to the median UIC in healthy adults (150 ug/L urine iodide). On this basis OIG argues that the $TIU_{hypothyroidism}$ = 20 ug/L/150 ug/L x 100% = 13.3% of the "normal" uptake, which is equated to an 86.7% inhibition of iodide uptake. However, this calculation is overly simplistic as thyroid function is a complex process involving the up and down regulation of iodide uptake. The amount of iodide excreted in the urine in iodine deficient diets is relatively less than that in iodine sufficient diets, indicating that urinary iodide levels in iodine deficient individuals are not representative of ingested iodine levels or iodide uptake as suggested by the OIG document. It is also important to note that, although NIS up-regulation increases iodide uptake in iodine deficient animals, it does not necessarily prevent hormone alterations (Schroder-Van Der Elst *et al.* 2005), especially in fetuses. Therefore, the ratio determined in the previous paragraph is not a good measure of iodide uptake inhibition or its potential to cause adverse neurodevelopment effects in children.

Comment 10: Although the OIG's use of thyroid effect data attributable to other NIS inhibitors is with merit and could provide useful information regarding effect levels, the assessment appears biased. The OIG report provides little evaluation of the limitations of the various epidemiological studies addressing other NIS inhibitors, which compromise their ability to accurately detect and estimate effects. In addition the RfD derivation using data from nitrate exposed populations inappropriately considered the enlarged thyroid effects observed to be non-adverse. This outcome should be considered an adverse health effect, which would lower the associated RfD for perchlorate to a value well below that derived by NRC/EPA.

Comment 11: The OIG interpretation of the data in Braverman *et al.* (2005) is also incomplete. OIG assumed that the increased urinary iodide observed during perchlorate exposure compared to pre-exposure levels was due to increased ingestion of iodide during exposure and adjusted the calculations accordingly. Braverman *et al.* noted that the urinary iodine excretion among

employees during perchlorate exposure was approximately 55% higher than in the pre-exposed state and stated that they found it unlikely that this was attributable to a short-term dietary change. Rather the authors suggested that the thyroid may be concentrating less of the dietary iodide during perchlorate exposure. Schroder-Van Der Elst *et al.* (2005) have also reported an increase in serum levels of iodide in perchlorate exposed rats.

Conclusion. In conclusion, the OIG assessment contains several technical limitations and inadequately considers the many scientific uncertainties involved in predicting thyroid iodide uptake and inhibition and risks of adverse neurodevelopmental effects in children. Due to these deficiencies, as well as the issues previously noted in the first section of these comments, the OIG's conclusions are questionable. Given widespread contamination of drinking water supplies and food items with perchlorate and other thyroid toxicants, MassDEP continues to believe that perchlorate levels in drinking water should not exceed 2 parts per billion in order to protect the fetus and neonate.

Sincerely,

Tsedash Zewdie, PhD
Toxicologist

Carol Rowan West

Carol Rowan-West, MSPH
Director Office of Research and Standards

C. Mark Smith PhD, SM
Deputy Director Office of Research and Standards and Toxicologist

Citations:

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell L 2006b. *Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States*. Environmental Health Perspectives 114(12): 1865-1871.

Braverman LE, XueMei H, Pino S, Cross M, Magnani B, Lamm SH, Kruse MB, Engel A, Crump KS, Gibbs JP 2005. *The Effect of Perchlorate, Thiocyanate, and Nitrate on Thyroid Function in Workers Exposed to Perchlorate Long-Term.* Journal of Clinical Endocrinology & Metabolism 90(2):700-706.

NRC 2005. *Health Implications of Perchlorate Ingestion.* The National Academies Press, Washington, DC, 2005, (ISBN 0-309-09568-9).

Schroder-van Der Elst JPN, Van Der Heide D, Kastelijn J, Rousset B, *et al.* 2005. The expression of the sodium/iodide symporter is up-regulated in the thyroid of fetuses of iodine-deficient rats Endocrinology 142:3736–3741.

Steinmaus C, Miller MD, Howd R 2007. *Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001-2002 National Health and Nutrition Examination Survey.* Environmental Health Perspectives 115(9):1333-1338.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J, 2004. *Relative Potencies and Additivity of Perchlorate, Thiocyanate, Nitrate, and Iodide on the Inhibition of Radioactive Iodide Uptake by the Human Sodium Iodide Symporter.* Thyroid 14:1012-1019.

Attn: Perchlorate_Comments_for OIG@epa.gov

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+ Johns Hopkins University School of Medicine

Date: March 10, 2009

Submission of Vanderver, et al. (2007)

Greetings,

I am submitting to the record the attached published paper by Vanderver et al. (2007) entitled "Cigarette Smoking and iodine as Hypothyroxinemic Stressors in U.S. Women of Childbearing Age: A NHANES III Analysis (Thyroid, August 2007; 17(8):741-746). EPA-OIG has identified thiocyanate exposure as the predominant NIS inhibitor exposure

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From: Ryan Monroe BA[+] and Steven H. Lamm, MD, DTPH*

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+ Johns Hopkins University School of Medicine

Date: March 10, 2009

Submission of Lamm, et al. (under review and 2007)

Greetings,

We are submitting to the record the paper by Lamm et al. (Thyroid, under review) entitled "Perchlorate, Thyroxine, and Low Iodine Association is not seen among Women of Child-bearing Age when Urine Iodine is Adjusted for Creatinine Concentration" (currently under review at Thyroid) and its published abstract (Thyroid, 2007 Sept; 17 (sup 1) S-51) .

This analysis of the NHANES 2001-2002 data is analogous to that of Blount et al., 2006 with the following three differences:

1. The study population is limited to women of child-bearing age (15-44 y/o) - This is the age group of interest;
2. The metric of iodine nutrition is the creatinine-adjusted urinary iodine level (UICr) – This eliminates the problems of dilution, the variability in the clearance of free water, and provides a metric that is reasonably constant over the 24-hour cycle;
3. The spectrum of iodine is presented as terciles - This allows the full range to be observed and for the cut-points to be data driven. The Blount (2006) use of 100 ug/L used a population measure as a critical value for individualized data, which is not appropriate. Our lower tercile cut-point is near that of Blount, but for different reasons. The use of terciles produced sub-sets of about 215 people each, the number that the Blount article suggested was sufficient for analytic stability.

Tables 1- 4 in the resultant analyses demonstrate quantitatively the effects of the specific iodine-uptake inhibitors at both low and high iodine levels:

1. Urinary thiocyanate levels were consistently found to have a significant negative association with serum thyroxine level at high iodine level, whether creatinine-adjusted or not, whether population-adjusted or not.
2. Urinary perchlorate levels show an effect on serum thyroxine at low iodine level in the absence of population and creatinine adjustment and show no effect on thyroxine level at low iodine level under the circumstance of adjustment for population and/or creatinine.
3. Urinary perchlorate levels show a negative effect on serum thyroxine at high iodine level using creatinine-adjusted urinary perchlorate data and whether population adjusted or not.
4. Urinary nitrate levels show a negative association with serum thyroxine only with mid-range iodine levels and in the population-adjusted analyse. The modeled associations for nitrate show no consistent pattern at low or high iodine level.
5. The sizes of the beta-coefficient for perchlorate and thiocyanate are similar at high iodine levels in the creatinine-adjusted analyses.

These data are publically available at the CDC website, and their analyses can be confirmed, validated, and extended by EPA staff scientists.

Cordially,

Attn: Perchlorate_Comments_for_OIG@epa.gov

From: Steven H. Lamm, MD, DTPH* and Joseph G. Hollowell, MD, MPH+

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+ Clinical Professor of Pediatrics, University of Kansas School of Medicine

Date: March 10, 2009

Submission of Lamm, et al. (2005)

Greetings,

We are submitting to the record the abstract "The effect of iodine supplementation on urine iodine in an iodine-sufficient population of pregnant US women". This was published in Thyroid 2005 (S 177). This is responsive to your interest of the effect of iodine supplementation on pregnant women. This shows the effect on urine iodine levels (creatinine-adjusted) but does not examine for an effect on serum thyroxine levels. It is noteworthy that this analysis of the NHANES III data was presented in terms of creatinine-adjusted urinary iodine levels in 2005, prior to the Blount paper.

Cordially,

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March 10th, 2009

Bill A. Roderick
Deputy Inspector
Office of the Inspector General
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW Room 3106
Washington, DC 20460

Subject: **Comments on Office of Inspector General Scientific Analysis of Perchlorate**

Dear Mr. Roderick:

Environmental Working Group (EWG) is a non-profit health and environmental research and advocacy organization based in Washington, DC. We focus much of our research on potential health risks from exposures to hazardous chemicals that contaminate food, water and the environment, or that may be found in consumer products. This letter provides our comments on a draft Office of Inspector General (OIG) report entitled OIG Scientific Analysis of Perchlorate (External Review Draft) (OIG 2008) regarding the use of a cumulative risk assessment model to characterize the public health risk from a low total iodide uptake (TIU) during pregnancy and lactation.

In their perchlorate analysis, OIG has used a cumulative risk assessment to justify their endorsement of the Bush Administration's failure to set a drinking water standard for the rocket fuel ingredient and thyroid toxin perchlorate that pollutes the drinking water of at least 20 to 40 million people nationwide. Perchlorate inhibits the update of the nutrient iodide by the thyroid gland, posing a particular risk for pregnant women and young children by reducing the iodide-dependent production of thyroid hormones that are critical to proper brain development. We look forward to working with the EPA to develop a national drinking water standard for perchlorate that protects pregnant women and their developing babies, infants, small children and other vulnerable populations from this potent thyroid toxin.

EWG supports the use of cumulative risk assessment in setting public health standards. These methods allow public health scientists and policymakers to set safety standards based on a more complete understanding of risk, more than is provided with single-chemical risk models that underestimate human health risks and result in health standards that fail to fully protect the public. But OIG's application of a cumulative health risk model is fundamentally flawed for several key reasons:

1. The OIG uses results from a cumulative risk assessment to conclude that "Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk." OIG ignores a major, peer–reviewed study from the CDC and instead relies on a single thyroid toxicity study in Chinese hamster ovary cells to support this conclusion. In contrast to the study in Chinese hamsters, the CDC studied

1,100 American women and found significant decreases in thyroid hormone levels linked to their consumption of perchlorate-contaminated drinking water. The CDC analysis demonstrates that for women, not hamster cells, reducing perchlorate levels in drinking water would indeed provide a significant opportunity to lower the public's risk.

2. The OIG uses cumulative risk assessment methods to conclude that "The most effective and efficient approach for reducing the health risk of permanent mental deficits in children from low maternal TIU during pregnancy and nursing is to add iodide to all prenatal vitamins and use them before and during pregnancy and nursing," again while endorsing EPA's failure to set a drinking water standard for perchlorate. The OIG also notes that other thyroid toxins can pollute the food supply. It is indisputably important that doctors ensure that their patients are getting proper nutrition during pregnancy, including sufficient iodine. EWG would also certainly support the Food and Drug Administration developing a consumption advisory for pregnancy women and young children for these foods, to ensure that they are sufficiently protected. None of these actions, however, remove EPA's responsibility under the law to protect public health from drinking water pollutants. None of these actions would justify EPA continuing to allow millions of pregnant women each year to drink tap water polluted with a thyroid toxin which, according to CDC studies, impairs the brain development of their children.

3. The OIG not only draws faulty conclusions based on its cumulative risk assessment, but also hired a defense industry contractor to conduct a technical review of its study. The contractor, ICF, has consulted for federal agencies, military contractors and other entities responsible for perchlorate pollution in drinking water supplies, including the Department of Defense, NASA, and perchlorate polluter Kerr-McGee; all of these entities have vigorously opposed strong public health standards for perchlorate. This is an obvious conflict of interest for ICF and should have immediately disqualified the contractor from consideration.

Details on other faulty assumptions and gaps in OIG's assessment are described in sections below.

Given the fundamental flaws in the OIG assessment, and their hiring of a defense industry contractor with an inherent conflict of interest to review the document, we recommend that EPA ignore this external review draft. We recommend that EPA move forward with a new assessment based on sound science, including CDC's studies demonstrating perchlorate's impacts on thyroid hormone levels in women.

Background: Over the last several years, the EPA has been in the process of determining the need to set a national drinking water standard for perchlorate. In October 2008, EPA issued a preliminary decision not to set a drinking water standard

for perchlorate. The EPA Office of Inspector General (OIG) conducted a science review of perchlorate and released its findings in a comprehensive report in December 2008. In its review, the OIG criticizes the EPA's failure to perform a cumulative risk assessment in its 2008 evaluation of perchlorate toxicity. However, the OIG concluded that EPA's current Reference Dose (RfD) is "conservative and protective of public health", despite EPA's faulty approach to the assessment. OIG also agreed with EPA that there is no need to set a national drinking water limit for perchlorate.

The Environmental Working Group (EWG) has reviewed EPA's 2008 perchlorate assessment and provided written comments to the agency criticizing several different aspects of the assessment (EWG 2008a). EWG has also examined OIG's perchlorate review and we find that although OIG criticizes EPA's approach to the assessment, its agreement with EPA's outdated RfD and its support of EPA's decision not to set a national drinking water limit is not supported by the current science on perchlorate. EWG respectfully requests that OIG revise their findings to strongly urge EPA to amend its RfD and set a health protective national drinking water standard for perchlorate. The OIG's perchlorate review has raised some specific questions that EWG would like to discuss below:

- OIG supports a perchlorate drinking water limit of 24.5 ppb, despite EPA's own finding that a health reference levels of 15 ppb in drinking water is necessary to protect the developing fetus
- OIG supports EPA's outdated RfD, despite criticisms from the agency's own Children's Health Protection Advisory Committee (CHPAC)
- EWG notes a potential conflict of interest for ICF International, the firm that assisted in conducting the OIG review; ICF International has major contracts with the U.S. Department of Defense (DoD), a government agency that has long opposed drinking water regulations for perchlorate
- The OIG review concludes that increasing iodine intake among vulnerable populations is more effective that decreasing perchlorate exposure; a recent study highlights the difficulty in increasing iodine intake among vulnerable populations

Each of these points is discussed in detail below.

Thyroid disorders and perchlorate: Thyroid conditions currently affect more than 20 million Americans. Thyroid insufficiency is especially concerning when it occurs during pregnancy, infancy, and early childhood because research has shown that even subtle decreases in thyroid hormone levels during these critical periods of vulnerability can have permanent adverse effects on brain development in the fetus, infant, and child. Perchlorate, a component of rocket fuel, acts as a thyroid toxin by inhibiting the sodium/iodide symporter (NIS) from transporting iodine into the thyroid gland. This leads to decreased production of thyroid hormones by decreasing iodine availability for hormone synthesis.

Perchlorate has been found in drinking water supplies in at least 26 U.S. states and an estimated 20 to 40 million Americans are exposed to this thyroid toxin through their drinking water. A recent CDC biomonitoring study involving almost 3,000 Americans representative of the U.S. population found perchlorate in the urine of every single participant, indicating widespread exposure to this thyroid toxin (Blount et al 2006). FDA testing of almost 300 types of commonly consumed foods has found perchlorate contamination of almost three quarters of the foods tested. Widespread food contamination by perchlorate necessitates a stringent drinking water standard in order to minimize total exposures for the millions of U.S. residents who have contaminated drinking water in addition to baseline food exposures.

To date, perchlorate remains an unregulated water contaminant, except in Massachusetts and California where state legislators have set maximum contaminant levels (MCL) for the chemical. The EPA has consistently declined to set an MCL, leaving millions of Americans at risk from this thyroid toxin.

OIG supports a perchlorate drinking water limit that fails to protect public health: The OIG review concludes with the following statement: "Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk" (OIG 2008).

OIG's assertion that a perchlorate drinking water limit of 24.5 ppb does not place the public at risk is at odds with the current science and with findings of federal and state public health agencies. In October 2008, EPA released its Preliminary Regulatory Determination on Perchlorate, in which the agency decided against setting an enforceable maximum contaminant level (MCL) for perchlorate. Instead, the agency proposed a health reference level (HRL) of 15 ppb in drinking water, which it determined was needed in order to protect pregnant women from exceeding the current RfD (EPA 2008). Furthermore, the states of Massachusetts and California have both conducted detailed perchlorate assessments and have finalized drinking water standards far below 24.5 ppb; Massachusetts has set an MCL of 2ppb in drinking water and California has set a public health goal of 6ppb.

The 2008 EPA assessment was rife with errors and omissions that are outlined in EWG's public comments to EPA (EWG 2008a) and that resulted in a higher health reference level (HRL) than is supported by current science. However, it should be noted that even EPA's flawed assessment concluded that an (HRL) of 15 ppb in drinking water would be required to protect vulnerable populations. When EPA made this preliminary HRL public, they received hundreds of responses from concerned scientists, advocates, and members of the public who questioned whether this HRL would be protective of public health. EPA was forced to delay finalizing the HRL and instead, asked the National Academy of Sciences to revisit the issue. While this request by the EPA was widely regarded as a delay tactic, it should be noted that the EPA did recognize that a drinking water limit for perchlorate of 24.5 ppb is not protective of vulnerable populations. However, the OIG review concluded the opposite, noting that even a perchlorate drinking water limit of 6 ppb (far lower that EPA's proposed HRL) would not lower the public's risk.

OIG continues to support EPA's outdated RfD: The OIG review also concluded that the EPA's current RfD is "conservative and protective of public health" (OIG 2008). This is in stark contrast with EPA's own Children's Health Protection Advisory Committee (CHPAC), which has strongly criticized the current RfD. This distinguished group of advisors, which includes researchers, physicians, and academicians wrote a strongly worded letter to EPA in November, 2008 criticizing the agency's current RfD, it's preliminary decision not to set an MCL for perchlorate, and its proposed HRL of 15ppb (CHPAC 2008).

It its letter, CHPAC highlights results from a large scale 2006 CDC study that found that women with lower iodine levels had significant decreases in thyroid hormone levels at perchlorate exposures far below the current RfD: "The exposure levels associated with anti-thyroid effects in these women were below the perchlorate RfD. These findings cast doubt on the protectiveness of the RfD, and make it especially important that no group exceed this health benchmark" (CHPAC 2008). They also go on to criticize EPA's proposed HRL of 15 ppb, writing "This benchmark (15 ppb) is clearly too high for infants as the Agency's own calculations show that an HRL of 15 ug/L (ppb) would allow daily exposures for infants that are 2-5 times higher than the Reference Dose (RfD)" (CHPAC 2008).

CHPAC finds that neither EPA's current RfD, nor its proposed HRL, is protective of vulnerable populations, especially infants. Yet, the OIG science review supports this RfD, even noting that it is "conservative". The primary way in which OIG is able to support this outdated RfD is by downplaying the findings from the CDC study. However, other environmental agencies recognize the importance of this study; for instance, California's Environmental Health Hazard Assessment (OEHHA) plans to re-evaluate their perchlorate public health goal of 6ppb because of the new data from the CDC study (The Press Enterprise 2008).

OIG's science review was conducted by ICF, a major DoD contractor: In its review, OIG notes that " ... the OIG contracted with ICF Incorporated, L.L.C. (ICF) under contract number GS-10F-0124J to conduct a 6-week technical review of a working draft of the OIG Scientific Analysis of Perchlorate. The purpose of the technical review was to scientifically critique the essential features of the OIG's application of a cumulative risk assessment to this public health issue" (OIG 2008).

EWG would like to highlight a potential conflict of interest involving ICF, a contractor who has consulted extensively for Federal Agencies, military contractors and other entities responsible for perchlorate contamination. For example, ICF's perchlorate work includes:

- A 2007 contract with DoD's Missile Defense Agency in their Programmatic Environmental Impact Statement. One of the key public concerns for the program is perchlorate releases (FedReg 2007).

- A 2004 contract with NASA to evaluate perchlorate risks to human health.

Perchlorate is used in space shuttle launches and other NASA projects (ICF International 2008). Also a NASA-funded reanalysis of data from the Greer study, a widely disputed industry study purportedly finding no effect of perchlorate on healthy males (ICF Consulting 2004).

- Extensive work with National Defense Laboratories to assess the extent of perchlorate contamination.

- Implementation of a Kerr-McGee-funded study of perchlorate effects in Chilean children with conclusions that are opposite of CDC's findings of significant health impacts from human exposures to perchlorate (Crump 2000). The study is widely touted to exonerate perchlorate with respect to thyroid toxicity.

- A publication funded by the American Water Works Association (whose members would face extensive treatment costs if a rigorous drinking water standard was set) to document the extent of non-water sources of perchlorate exposures (AWWA 2008).

In addition to perchlorate-related consulting, ICF provides critical services to the DoD; in a recent flyer found on ICF's website, the firm notes that "ICF Consulting has provided expert human capital management services to the Department of Defense (DoD) and other Federal agencies for more than 10 years" (ICF 2008a). In addition, the ICF website also mentions that it was one of six companies that was selected to receive a $200 million DoD contract in 2008 to assist the agency in implementing new information technology (ICF 2008b).

The DoD has consistently opposed any federal regulation of perchlorate in drinking water because of concerns that its defense contractors may have to contribute tens of millions of dollars towards clean-up efforts. In a 2005 report, the U.S. Government Accountability Office noted the following: "DoD has used its policy to limit testing for perchlorate that environmental regulators believed was necessary" (GAO 2005). In the conclusion of this report, GAO recommended that EPA should establish a formal system to "track and monitor perchlorate detections and cleanup efforts" (GAO 2005). GAO notes that DoD formally disagreed with the findings and recommendations in this report.

ICF's multimillion dollar contracts with the DoD raise questions about the appropriateness of its involvement in the technical review of the OIG perchlorate analysis. ICF scientists provided technical review of an OIG analysis that in essence supports EPA's preliminary determination not to set an MCL for perchlorate, a decision consistent with DoD's position. EWG is concerned that ICF's technical review may have been influenced by their long-term relationships with perchlorate polluters and cannot be seen as objective or valid for this important assessment.

OIG emphasizes need to increase iodine intake over decreasing perchlorate exposure: OIG concludes it's review by finding that "Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a

meaningful opportunity to lower the public's risk. By contrast, addressing moderate and mild iodide deficiency occurring in about 29% of the U.S. pregnant and nursing population appears to be the most effective approach of increasing TIU to healthy levels during pregnancy and nursing, thereby reducing the frequency and severity of permanent mental deficit in children" (OIG 2008). In essence, OIG discharges EPA from any responsibility for cleaning up perchlorate contaminated drinking water by shifting the focus to iodine intake among vulnerable populations.

Increasing iodine intake among pregnant and nursing populations, which OIG recommends as the ultimate solution to the perchlorate issue, does nothing to address the risks that perchlorate exposure poses to young children. FDA testing of perchlorate in food found widespread contamination; the agency estimated that two-year old children had the highest exposures from food contamination (FDA 2008). EWG analysis of the FDA data found that the average 2 year old could exceed EPA's RfD by drinking water contaminated with as little as 4 ppb of perchlorate. OIG does not address this issue effectively in their review.

In addition, a new study from the New England Journal of Medicine highlights the difficulties in implementing the seemingly simple policy of increasing iodine intake among pregnant and nursing women. In this study, researchers from Boston University found that only half of prenatal vitamin brands they studied contain iodine. Even more disturbing, when the researchers actually tested the iodine content in the brands that contain iodine, they found that in a significant number of samples, the actual iodine content in the vitamin was far lower than the recommended daily dose of 150 ug. Among the vitamins that listed potassium iodide as an ingredient, the average measured iodine concentration was 119 ug, far below the recommended dose of 150 ug. While EWG supports increasing iodine intake among pregnant and nursing women, this could take years as these issues are worked out. In contrast, establishing and enforcing a stringent, health-protective drinking water standard for perchlorate is technologically feasible and could be carried out in a timely manner.

Conclusion: With this science review of perchlorate, OIG continues to support EPA's outdated RfD and a drinking water limit of 24.5 ppb, which even EPA has acknowledged would not protect pregnant women adequately. In this review, OIG conducts a convoluted cumulative risk assessment of NIS stressors and ultimately concludes that no public health benefit would arise from regulating perchlorate in drinking water. This is in stark contrast with important advisory bodies like CHPAC and state environmental agencies such as the Massachusetts Department of Environmental Protection and the California's OEHHA. For too long now, EPA has neglected it's responsibility to objectively address perchlorate contamination of drinking water and this OIG review simply supports EPA's flawed decisions.

In 2006, the state of Massachusetts set a drinking water standard for perchlorate of 2 ppb, the most stringent in the country. On it's website, the MassDEP explains "After intensive study and stakeholder involvement, MassDEP determined that standards were needed. MassDEP's goal was to protect public health, especially pregnant women and children, from a compound which no state or federal water

standard existed" (MassDEP 2006). Perhaps EPA would benefit from adopting this simple but effective approach, rather than continuing to delay a decision that has the potential to benefit millions of U.S. residents.

Thank you for the opportunity to provide comments.

Sincerely,

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References:

AWWA. 2008. Estimating the contribution of drinking water to aggregate perchlorate intake of reproductive age women in the U.S. (Authors: Mendez W, Dedrick E, ICF International). Available online at: http://www.awwa.org/files/GovtPublicAffairs/PDF/PerchlorateAppendixA.pdf

Blount BC, Pirkle JL, Oserloh JD, Valentin-Blasini L, Caldwell KL. 2006. Urinary Perchlorate and thyroid hormone levels in adolescent and adult men and women living in the Unites States. Environmental Health Perspectives 114(12): 1865-71.

CHPAC. 2008. Letter from CHPAC to Director Johnson. Available online at: http://209.85.173.132/search?q=cache:fgJhPl_tIOIJ:yosemite.epa.gov/ochp/ochpweb.nsf/content/perchlorateletter.htm/%24File/PerchlorateLetter.pdf+CHPAC+2008+perchlorate+letter&hl=en&ct=clnk&cd=1&gl=us&client=firefox-a

Crump C, Michaud P, Téllez R, Reyes C, Gonzalez G, Montgomery EL, Crump KS, Lobo G, Becerra C, Gibbs JP. 2000. Does perchlorate in drinking water affect thyroid function in newborns or school-age children? J Occup Environ Med. 2000 Jun;42(6):603-12.

EPA. 2008. Drinking water: Preliminary Regulatory Determination on Perchlorate. Available online at: http://www.epa.gov/safewater/ccl/reg_determine2.html#perchlorate

EWG. 2008a. Letter to EPA re: Preliminary Regulatory Determination on Perchlorate (Docket ID No. EPA-HQ-OW-2008-0692). Available online at: http://www.ewg.org/node/27352

EWG. 2008b. FDA food testing shows widespread rocket fuel contamination of commonly consumed foods and beverages. Available online at: http://www.ewg.org/node/25875

FedReg. 2007. Notice of Availability of the Ballistic Missile Defense System Final Programmatic Environmental Impact Statement. Department of Defense. [DOD-2007-

OS-0008]. February 16, 2007 (Volume 72, Number 32):7917-18. Available online at: http://edocket.access.gpo.gov/2007/E7-2433.htm

GAO. 2005. Perchlorate: a system to track sampling and cleanup results is needed. Available online at: www.gao.gov/new.items/d05462.pdf

Ginsberg GL, Hattis DB, Zoeller RT, Rice DC. 2007. Evaluation of the U.S. EPA/OSWER preliminary remediation goal for Perchlorate in groundwater; focus on exposure to nursing infants. Environmental Health Perspectives 115(3) 361-369.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Fair JD, Klein RZ. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. New England Journal of Medicine 341: 549-555.

ICF Consulting. 2004. Recommendation for an Oral Intake Reference Dose (RfD) for Perchlorate. Prepared for National Aeronautics and Space Administration Environmental Management Division. Fairfax, VA:ICF Consulting.

ICF International. 2008. Derivation of a "Safe Level" for Perchlorate Ingestion, National Aeronautics Space Administration. Available online at: http://www.icfi.com/markets/environment/doc_files/toxicology.pdf

ICF. 2008. ICF International Selected for Multiple Award $200 Million DoD Blanket Purchase Agreement to Help Transform IT Department Wide. Available online at: http://www.icfi.com/newsroom/news.asp?ID=132

ICF. 2009. Human Capital Transformation at the U.S. Department of Defense. Available online at: http://209.85.173.132/search?q=cache:Uq3jASkifV4J:www.icfi.com/Services/Human-Capital/doc_files/human-capital-dod.pdf+ICF+and+human+capital+and+DoD&hl=en&ct=clnk&cd=1&gl=us&client=firefox-a

Leung AM, Pearce EN, Braverman LE. 2009. Iodine content of prenatal multivitamins in the United States. New England Journal of Medicine 360(9): 939-40. MassDEP. 2006. Toxics and Hazards: Emerging Contaminants. Available online at: http://www.mass.gov/dep/toxics/stypes/emercfs.htm

Murray WM, Egan SR, Kim H, Beru N, Bolger PM. 2008. US Food and Drug Administration's Total Diet Study: Dietary Intake of Perchlorate and Iodine. Journal of Exposure Science and Environmental Epidemiology 18(6): 571-80.

OIG (Office of Inspector General). 2008. Scientific Analysis of Perchlorate. Available online at: www.epa.gov/oigearth/reports/2009/20081230-2008-0010.pdf

Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijider JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL. 1999. Low maternal free thryoxine concentrations during early pregnancy are associated with impaired psychomotor development in early infancy. Clinical Endocrinology 50: 149-155.

The Press Enterprise. 2008. State to re-assess perchlorate levels using new data on risks to fetuses. Available online at:
http://www.pe.com/localnews/inland/stories/PE_News_Local_S_perch18.4a460ce.html

Use of a Cumulative Risk Assessment Approach to Characterize Public Health Risks from Low Total Iodide Uptake (TIU):

Comments on the OIG Scientific Analysis of Perchlorate

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General Comments

Rather than define the risk in terms of *low* total iodide uptake, consider defining the risk in terms of *a sustained, inhibitor-related decrease* in iodide uptake when dietary iodine is insufficient to make up the shortfall. Iodide uptake (IU) is measured as the accumulation of radioactive iodine by the thyroid over a fixed time period. Low IU values are associated with abundant iodine nutrition. Thus, low IU values are a normal homeostatic response to abundance and are not a risk factor. High IU values are associated with low or marginal iodine nutrition. As dietary iodine decreases, IU increases. When dietary iodine is low or marginal over a period of time, the concern is that an inhibitor-related decrease in IU from its initial high level to one that is not quite as high might lead to the depletion of thyroidal iodine stores. The cumulative risk assessment process should carefully distinguish between low IU (not a health risk) and a sustained, inhibitor-related decrease in IU from an initial high value, accompanied by dietary iodine insufficiency.

The word "total" does not appear to be especially helpful or informative in the context of iodide uptake. Consider instead identifying the uptake as thyroidal iodide uptake (TIU), iodide uptake by the thyroid (IUT) or just iodide uptake (IU).

Comments and Recommendations Based on New Analyses

New analyses from a perchlorate exposure study in 21 women and 16 men (the Greer study) provide overall support for a cumulative risk assessment approach to characterize the health risks of exposures to perchlorate and other inhibitors of iodide uptake in the most sensitive population subgroups, pregnant and lactating women.

A. Greer Study and Acknowledgements

A perchlorate exposure study was conducted by Dr. Monte Greer (deceased), Dr. Gay Goodman, and Ms. Susan Greer. The radioiodine uptake (RAIU) results were published as Greer et al. 2002, Environ. Health Perspectives, 110:927-937; other contributors to the study are acknowledged therein. Analysis of iodine in urine samples was performed by Mr. Sam Pino and Mr. Michael Previti under the supervision of Dr. Lewis Braverman at Boston Medical Center.

B. Summary of Study Results and Related Recommendations

1. The dose-related inhibitory effect of perchlorate on iodide uptake by the thyroid (IU) varies with the baseline (pre-exposure) IU in both the women and the men.
2. The baseline IU depends on the baseline 24-hr urinary iodine excretion (IE), a surrogate for the daily iodine intake. This dependence is more robust in the women than in the men. Consistent with this sex-related difference, the dose-related inhibitory effect of perchlorate on IU varies with the baseline IE in the women but not the men.
3. Describing the dose-dependence of perchlorate effects in terms of an absolute change in IU (post-exposure minus baseline) rather than as a relative change

(post-exposure/baseline) facilitates correction for the modulating influence of both baseline IU and iodine intake and makes sense for other reasons (see below).

4. The IU decreases with increasing iodine intake. In the Greer study, baseline IE values above 300 μg were associated with 24-hr IU values (measured as a fraction of the radioactive iodine ingested) between 0.10 and 0.23 (average = 0.155). Baseline IE values below 200 μg were associated with 24-hr IU values between 0.13 and 0.34 (average = 0.243). Consider an absolute decrease of 0.031 from the average IU values in the high- and low-IE subpopulations thus defined. This equates to 20% and 13% IU decreases for the subpopulations with high IE and low IE, respectively. Thus, any description of effects in terms of relative rather than absolute change inconveniently weights the region of the IE distribution corresponding to people who are not at risk.
5. The results support quantitative consideration of the influence of both the background IU and the daily iodine intake when assessing the risks of exposures to perchlorate and other inhibitors of iodide uptake in women.

C. Overview of Relevant Results

In Figure 1, the inhibitory effect of perchlorate as the absolute change in the 24-hr radioiodine uptake (RAIU) between baseline and exposure day 14 (E14) in the women is plotted against the baseline RAIU at each of the four doses tested, 0.007, 0.02, 0.1, and 0.5 mg/kg-day. The relationship is statistically significant only at the lowest and highest doses, 0.007 and 0.5 mg/kg-day.

Only one man was assigned to the lowest dose group; therefore regression analysis is limited to the remaining dose groups. In the men the inhibitory effect of perchlorate (as the absolute change in RAIU) is statistically significant at the 0.02 and 0.5 mg/kg-day doses (data not shown).

Figure 1 also shows, in a semi-quantitative fashion, the interaction between the baseline 24-hr iodine excretion (IE-b) and the RAIU-b in the women. It can be seen that the distribution of IE-b levels < 300 μg is dramatically skewed to higher RAIU-b values. The median RAIU-b value is approximately 0.20. All 11 subjects above the RAIU-b median but only 4 of the 10 subjects below the median have IE-b values < 300 μg. The distribution of IE-b values in the men is less skewed: 5 subjects above the RAIU-b median and 3 below the median have IE-b < 300 μg (data not shown).

In the Greer study, the daily iodine intake was estimated as the baseline 24-hr urinary iodine excretion (IE-b). As expected, RAIU-b correlates with IE-b. However, there is substantial variability in this relationship. The correlation is stronger and more robust in the women than in the men (data not shown).

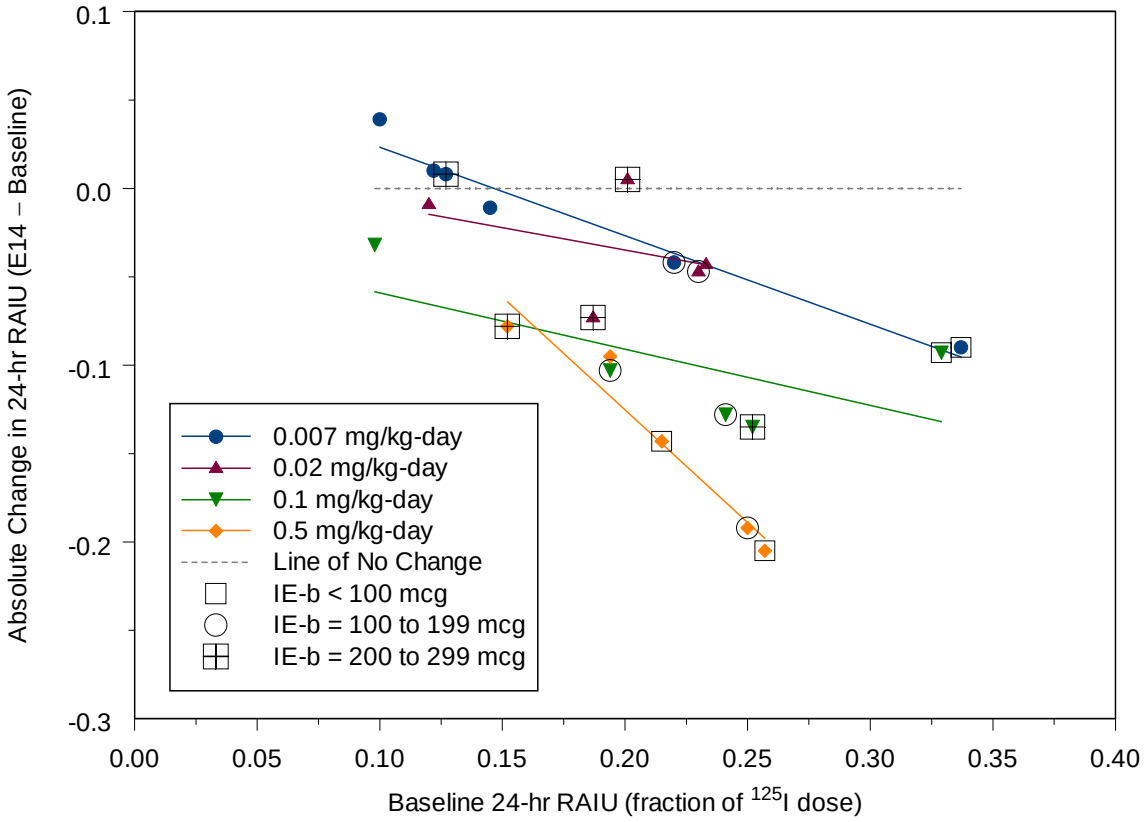

Figure 1. Dependence of the inhibitory effect of perchlorate, measured as the absolute difference between the baseline 24-hr radioiodine uptake (RAIU) and the RAIU on exposure day 14 (E14), on the baseline RAIU, by dose, in women. The dashed line represents zero change in the 24-hr RAIU between baseline and E14. The solid lines are linear regression fits to the data points (solid symbols) in each dose group. Data points with superimposed symbols represent subjects with baseline 24-hr iodine excretion (IE-b) values below 300 µg, subcategorized according to the three IE-b ranges shown. The remaining data points represent subjects with IE-b values above 300 µg.

Consistent with the sex-related difference in the dependence of RAIU-b on IE-b described above, perchlorate inhibition of RAIU is found to vary with IE-b in the women but not the men. The effect of IE-b in the women can be seen in Table 1, where addition of IE-b to the two models in which log dose is the only independent variable improves the fit of both, as indicated by a smaller residual standard error (RSE) and a larger multiple R-squared. As shown in Table 2, for the men there is little or no improvement in RSE or multiple R-squared when IE-b is added to the models in which log dose is the only independent variable.

Tables 1 and 2 also allow comparison of linear regression models in which the change in RAIU between baseline and E14 (the dependent variable) is formulated as either a relative or absolute quantity. In the upper set of models in Tables 1 and 2 the dependent variable is the relative change in 24-hr RAIU. In the lower set of models in Tables 1 and

2, the dependent variable is the absolute change in 24-hr RAIU. For both the women (Table 1) and the men (Table 2), it is clear that the absolute change model yields a more substantial decrease in residual standard error (RSE) and increase in multiple R-squared when RAIU-b, IE-b, or both are added to the regression models in which log dose is the only independent variable.

Independent Variables	Degrees of freedom	Residual Standard Error	Multiple R-Squared	p value
Models of the Relative Change in RAIU (E14/Baseline) in Women				
Log dose	19	0.1657	0.7179	$<2\times10^{-6}$
Log dose + RAIU-b	18	0.1422	0.8031	$<5\times10^{-7}$
Log dose + IE-b	18	0.1279	0.8408	$<7\times10^{-8}$
Log dose + RAIU-b + IE-b	17	0.1310	0.8423	$<5\times10^{-7}$
Models of the Absolute Change in RAIU (E14 −Baseline) in Women				
Log dose	19	0.04261	0.6143	$<3\times10^{-5}$
Log dose + RAIU-b	18	0.02905	0.8301	$<2\times10^{-7}$
Log dose + IE-b	18	0.02997	0.8191	$<3\times10^{-7}$
Log dose + RAIU-b + IE-b	17	0.02777	0.8534	$<3\times10^{-7}$

Table 1. Results of Linear Regression Analysis in Women

Independent Variables	Degrees of freedom	Residual Standard Error	Multiple R-Squared	p value
Models of the Relative Change in RAIU (E14/Baseline) in Men				
Log dose	14	0.1150	0.8091	$<3\times10^{-6}$
Log dose + RAIU-b	13	0.1164	0.8183	$<2\times10^{-5}$
Log dose + IE-b	13	0.1143	0.8248	$<2\times10^{-5}$
Log dose + RAIU-b + IE-b	12	0.1085	0.8542	$<3\times10^{-5}$
Models of the Absolute Change in RAIU (E14 −Baseline) in Men				
Log dose	14	0.04092	0.6177	0.0003
Log dose + RAIU-b	13	0.02069	0.9092	$<2\times10^{-7}$
Log dose + IE-b	13	0.04153	0.6344	0.0014
Log dose + RAIU-b + IE-b	12	0.01948	0.9257	$<5\times10^{-7}$

Table 2. Results of Linear Regression Analysis in Men

RESPONSE TO THE ENVIRONMENTAL PROTECTION AGENCY OFFICE OF INSPECTOR GENERAL SCIENTIFIC ANALYSIS OF PERCHLORATE

FINAL

Prepared for:

PERCHLORATE STUDY GROUP

March 10, 2009

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1.0 INTRODUCTION

Intertox appreciates the opportunity to comment on the United States Environmental Protection Agency (U.S. EPA) Office of Inspector General (OIG) Scientific Analysis of Perchlorate (External Review Draft) released December 30, 2008. We commend the OIG for the seriousness of its approach to the important issues relating to perchlorate remediation and public health protection.

The OIG analysis uses a cumulative risk assessment to estimate the overall risk from all stressors to the sodium-iodide symporter (NIS), the molecular pump that actively transports iodide into the thyroid. Those stressors include ingestion of high levels of perchlorate, ingestion of thiocyanate and nitrate, as well as iodine deficiency.

The OIG undertook this analysis in response to the May 1, 2007 Federal Register notice in which the U.S. EPA noted more information was needed on relative source contribution (RSC) to determine whether to regulate perchlorate under the Safe Drinking Water Act (SDWA).

In its review of the perchlorate reference dose (RfD), OIG questions the basis for the 2005 U.S. EPA RfD which was the National Academy of Sciences (NAS) National Research Council's (NRC) assessment of perchlorate, titled *Health Implications of Perchlorate Ingestion* (2005). The NAS review of the health effects of perchlorate was requested by the U.S. EPA in response to significant criticism of the scientific basis for the U.S. EPA's 2002 draft risk assessment for perchlorate.

The NRC recommended an RfD of 0.0007 mg/kg-d based on iodide uptake inhibition (IUI). In what it characterized as an unconventional move intended to provide a level of safety exceeding customary practice, the NRC chose to base this RfD on a no observed effect level (NOEL), rather than the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), which EPA has traditionally used as the point of departure. The NOEL is the dose at which no effect occurs, adverse or otherwise, and results in a more conservative RfD than the traditional approach of the NOAEL or LOAEL.

The OIG report expresses several possible concerns with the current RfD. The OIG was unsure that the RfD would be protective of human health at all life stages; they express the view that the first adverse effect to occur should be hypothyroxinemia in pregnant women. Most critically, they conclude that the single chemical risk assessment does not, in fact, provide a scientifically accurate assessment of possible human health risk, and suggests a broader examination of all goitrogens. In short, the OIG analysis is a cumulative risk assessment for total NIS stressors using hypothyroxinemia in pregnant women as the first adverse effect.

The OIG has requested "scientific comments on the use and application of a cumulative risk assessment approach to characterizing the public health risk from a low [total iodide uptake] during pregnancy and lactation" (OIG, 2008). We believe the OIG is correct in their assessment of other NIS stressors and in their scientifically based conclusions that the current perchlorate RfD is conservative. We also agree with the OIG in that decreasing the concentration in drinking water from 24.5 ppb to 6 ppb would not offer any meaningful opportunity to lower the public's risk. There are certain aspects of the OIG argument that could benefit from additional information, such as proper/improper interpretation of iodine data based on spot urine samples and the failure to incorporate NIS up-regulation, which we later discuss in this document. As discussed below, we

believe the OIG is scientifically accurate in its assessment and in using an alternative approach. The OIG "confirmed that U.S. EPA's perchlorate RfD is conservative and protective of human health, but limiting perchlorate exposure does not effectively address this public health issue [adverse thyroid effects due to total goitrogen load]."

2.0 THE NRC APPROACH

It is well understood that there are several levels of conservatism inherent in the existing perchlorate RfD, namely the use of a NOEL as a point of departure, the application of uncertainty factors to the NOEL, and the use of a reversible biochemical event (IUI) that is several steps prior to an actual adverse effect as the basis for the NOEL. Furthermore, perchlorate is not bioaccumulative. Rather, it has a relatively short half-life and is eliminated from the body quickly (*i.e.,* the half-life is 6 to 8 hours).

The determination of an RfD was based on the use of a single chemical risk assessment, which was considered the best science available at the time the NRC committee finalized its report in 2005. Between 1997 and 2002, at least 13 toxicological studies of perchlorate were conducted. The most sensitive target organ was determined to be the thyroid gland (U.S. EPA, 2002). There were also several clinical human exposure, epidemiologic, and ecological studies, some in occupational settings and some in populations exposed to perchlorate via the community drinking-water supply.

The U.S. EPA released a draft risk assessments for perchlorate in 1998 and 2002 based on the results of animal studies. In the 2002 draft risk assessment, U.S. EPA interpreted these studies as showing adverse effects in the pups of rats exposed during pregnancy to a perchlorate dose as low as 0.01 mg/kg-day, resulting in a proposed RfD of 0.00003 mg/kg-d (U.S. EPA, 2002). However, serious concerns about the validity of using these animal studies for a human health risk assessment were raised and, as a result, U.S. EPA and other federal agencies requested a review of the underlying science by the NRC (NRC, 2005).

The NRC committee on the perchlorate panel reached a consensus and recommended an RfD of 0.0007 mg/kg-d in the report *Health Implications of Perchlorate Ingestion* (2005). The NRC report recommended an RfD of 0.0007 mg/kg-d based on a NOEL, or the dose at which no effects occur, adverse or otherwise (NRC, 2005). The panel also took care to differentiate between a NOAEL and a NOEL, finding that there was confusion between the two and stating that the NOAEL is based upon an adverse effect, whereas the NOEL is based upon a nonadverse effect (NRC, 2005).

One of the critical studies that served as the basis for this RfD was the study by Greer *et al.* (2002), which demonstrated there was no inhibition of iodide uptake by the thyroid at a dose of 7 µg/kg-d. Importantly, the NRC did not examine Greer *et al.* in isolation. According to the NRC report, the findings in Greer *et al.* are supported by other clinical, occupational, and environmental epidemiologic studies and studies of long-term perchlorate administrated to patients with hyperthyroidism.

Furthermore, the NRC emphasizes, "Inhibition of iodide uptake by the thyroid clearly is not an adverse effect; however if it does not occur, there is no progression to adverse health effects." The committee views its recommendation to use IUI by the thyroid as the basis of the perchlorate risk assessment to be the most health-protective and scientifically valid approach (NRC, 2005).

In using a NOEL, the NRC committee deemed its approach as conservative and health protective as this dose is already lower than any dose in which adverse effects occur. Furthermore, a safety factor of 10 is applied to the NOEL to account for the most sensitive individuals in a population, in this case, hypothyroid or iodine-deficient pregnant women and their developing fetuses. The NRC panel stated that "the NOEL value from Greer *et al.* (2002) is a health-protective and conservative point of departure is supported by…extensive human and animal data that demonstrate that there will be no progression to adverse effects if no inhibition of iodide uptake occurs" (NRC, 2005).

Some have argued that Greer *et al.* was of short duration and have suggested a subchronic to chronic extrapolation. On that issue, the NRC concluded that since the point of departure is based upon IUI—a short-term event—any chronic effects "would have no greater effect" than any short term effects that may occur (NRC, 2005). Thus, the use of IUI as a point of departure is a more cautious and health protective approach than using changes in thyroid hormones (a precursor to possible adverse effects) or to some adverse effect such as hypothyroidism.

The U.S. EPA has based its current RfD for perchlorate and Integrated Risk Information System (IRIS) summary on the NRC report (U.S. EPA, 2005).

3.0 THE OIG APPROACH

The OIG approach is scientifically-based and appropriately incorporates most of the major thyroid related considerations needed in a risk assessment. It focuses on IUI, which is the mechanism of action for perchlorate and numerous other chemicals. As a method to cross check the human clinical data, it sufficiently supports the body of human *in vivo* literature using an *in vitro* approach. Other chemicals inhibit IUI; some of those chemicals, including perchlorate, are naturally found in food and also inhibit iodide uptake as was assessed and reported by Belzer *et al*., (2004).

The OIG undertook this analysis in response to the May 1, 2007 Federal Register notice in which U.S. EPA noted that more information was needed on RSC to determine whether to regulate perchlorate under the SDWA. The OIG felt that a cumulative risk assessment was an appropriate measure of total stress to the NIS. While uncommon, the cumulative risk assessment has been recognized by the U.S. EPA as a method to assess total risk from multiple chemicals with the same mechanism of action (U.S. EPA, 2007).

As noted in the OIG report, perchlorate, thiocyanate, nitrate and low iodine intake fit the criteria of a cumulative risk assessment. OIG reports that thiocyanate, nitrate, and iodine are found naturally in food and water sources, assuming that people are not exposed to any one of these chemicals alone, but in combination. Thus, the focus on one chemical does not in fact provide a scientifically accurate assessment of possible human health risk. A cumulative risk assessment is defined as "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors" (U.S. EPA, 2003).

The OIG uses the results from Tonacchera *et al.* (2004) to model and weigh each "stressor" [perchlorate, thiocyanate, nitrate, and iodine] by the strength of each chemical's ability to inhibit iodide uptake. When each of these factors is applied in the model, a total effect can be estimated. The U.S. EPA and the NRC have both supported use of physiologically-based pharmacokinetic (PBPK) models for risk assessment purposes. Once the OIG had its complete model with all four

NIS stressors accounted for, they were able to estimate the overall effect of IUI on thyroid hormones. Consistent with the NRC, the OIG believes the first adverse effect is a change in thyroid hormones. The OIG was conservative in the interpretation of the iodine data, NIS up-regulation, and protein binding. The OIG determined that perchlorate alone had very little impact on the overall effect on the thyroid when the other three stressors were present.

The two major conclusions in OIG's report:

1. "EPA's perchlorate RfD is conservative and protects human health"
2. "…limiting perchlorate exposure does not effectively address this public health issue… lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk."

The OIG assessment supports the weight of evidence from peer-reviewed science. The current body of literature has demonstrated that other chemicals do affect IUI and that pharmacological-dosing studies demonstrate levels at which side effects and adverse effects may occur. These pharmacological doses are 1000-fold or more greater than the RfD.

However, concerns regarding exposure to low levels of perchlorate continue to be an issue, which stresses the importance that in this alternative approach to risk assessment, the OIG came to the same conclusion as the NRC (2005), the U.S. EPA (2005), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2008): the current RfD of 0.0007 mg/kg-d is health protective and also conservative by a factor of 6.6 times. Regardless of the method used, the current RfD is not expected to cause any adverse health effects, even in the most sensitive population (pregnant women and their fetuses).

There are several scientific points to highlight in the OIG report. First, Tonacchera *et al.* (2004) provided the relative potencies of the various inhibitors of iodide uptake at the NIS using Chinese Hamster Ovary (CHO) cells which were transfected with human NIS.[1] This is a common and accepted cell culture methodology. The OIG reports that Total Iodine Uptake (TIU) is the key biochemical event that, if perturbed sufficiently by the NIS stressors, should be used as the basis for the risk assessment of this public health issue. There is a large body of literature that supports this scientific approach.[2]

[1] The potencies scientifically obtained in Tonacchera *et al.* (2004) where essentially the same as those derived by Belzer *et al.* (2004) based on a review of the literature at the time.

[2] For over half a century, the relative NIS inhibition potencies of prevalent nutritional and environmental NIS inhibitors (*i.e.*, perchlorate, thiocyanate, and nitrate) have been studied and quantified repeatedly with excellent agreement in the results. These key studies are referenced in the OIG report as well as in Tonacchera *et al.* (2004) and can be found there. Of these studies, Tonacchera *et al.* (2004) is the most robust and provides the most suitable starting point for a cumulative risk assessment.

The sole exception, to our knowledge, is a recent paper which, using an atypical modeling approach, agrees with IUI, but suggests a different level of potency for these stressors.[3]

Second, the OIG provides an effective and scientifically-supported demonstration of the overall effect on inhibition of iodide uptake that would occur by reducing the drinking water equivalent level (DWEL) based on the RfD from 24.5 ppb[4] to 6 ppb or lower. The OIG estimates that "potentially regulating perchlorate at a DWEL of 6.0 ppb instead of 24.5 ppb prevents about a 1% change in TIU in pregnant women; a 1% TIU change is only a small fraction of the ± 55% normal variation, observed in the % TIU at baseline in Greer *et al.* population. Therefore, decreasing the perchlorate drinking water concentration from 24.5 ppb to 6.0 ppb will not have a significant effect on the % TIU observed in people." The OIG also found that perchlorate was the least significant of the four NIS stressors (thiocyanates, nitrates, perchlorate, and iodine deficiency) on TIU. The OIG further states that perchlorate "does not provide a meaningful opportunity to lower the public's risk" (OIG, 2008). The OIG draft report concluded that "lowering the public's nitrate exposure provides a more meaningful opportunity to lower the public's NIS inhibition load (*i.e.*, a more meaningful opportunity to lower public risk) than to lower the public's perchlorate exposure below the perchlorate RfD" (OIG, 2008).

Third, the OIG agrees with the NRC in stating that IUI is not an adverse effect. The OIG reiterates the NRC statement that identifies "NIS inhibition as a nonadverse effect that precedes all adverse effects from perchlorate exposure" and further contends that "hypothyroxinemia is the first adverse effect from a low uptake of iodide by the thyroid" (OIG, 2008).

4.0 COMMENTS FOR OIG CONSIDERATION

We have several comments to offer the OIG, relating to their analysis.

4.1 Conducting a Cumulative Risk Assessment

From a scientific standpoint, if there is a clearly defined pathway that leads to an adverse effect and several chemicals have a mechanism of action that affects that pathway, then it becomes less important what those chemicals are and more important that the pathway has been affected. A benefit of the cumulative risk assessment approach is that it allows for the assessment of possible neurological effects such as decreased intelligence quotients (IQ) and decreased motor performance for chemicals that all have the same mechanism of action. The OIG cumulative risk assessment considers the most environmentally-relevant chemicals that cause NIS inhibition. The OIG report

[3] A recent study by Dasgupta *et al.* (2008) found "transport selectivities are an order of magnitude lower than those indicated by in vitro studies, suggesting that the impact of both these anions on inhibiting iodide transport in milk may have been overestimated in the extant literature." The study also states that "the very low fSCN,m [fraction of thiocyanate in breast milk] despite high SCNT,in [total thiocyanate in the infant] suggests that the effective selectivity factor for thiocyanate over iodide transport must be considerably less than that suggested in ref 5 [Tonacchera *et al*., 2004]." However, the conclusions of Dasgupta *et al.* (2008) are based on the amounts of each anion found in breast milk which may reflect a difference in transport of each rather than their ability to block iodide uptake by the NIS. The authors compare their results to that of Tonacchera *et al.* (2004) who found that perchlorate was 30 times more potent than iodide at blocking uptake of radioactive iodide. The two studies are not measuring the same thing and the quantitative results are not comparable. The Dasgupta *et al.* (2008) study was not designed to determine how each anion affects the transport of others, but rather, to evaluate the relative concentrations of each in breast milk. A further analysis of the conclusions drawn from Dasgupta *et al.* (2008) is available (Gibbs, 2009).

[4] 24.5 ppb is the drinking water equivalent level of the RfD of 0.0007 mg/kg-d assuming a 70 kg adult drinks 2 L of water per day.

stands on sound toxicological principles from this standpoint (*e.g.,* dose-response assessment of IUI).

At the same time, there are a number of considerations that this cumulative risk assessment (specifically and in general) should consider.

First, the OIG analysis is based on an *in vitro* study which, in isolation, should not be used as the basis of an RfD when there are human data to derive such values. Since there are quality human clinical and epidemiological studies available for perchlorate, those are the most appropriate to base a human health risk assessment upon for regulatory purposes. The OIG assessment, based on the model used in Tonacchera *et al.* (2004), is an excellent and valuable tool for verifying the results of several other studies and risk assessments. And, using a cumulative risk assessment allows the use of important epidemiological studies on iodine deficiency which would otherwise be unusable in a single chemical risk assessment.

Second, the OIG draft report defines the "total goitrogen load" as the combined NIS inhibition from perchlorate, thiocyanate, nitrate, and iodine deficiency acting on the thyroid. This definition is somewhat misleading because other substances inhibit IUI (*e.g*., pertechnetate, perrhenate, boron tetrafluoride, iodide, bromide, chloride, chlorate, selenocyanate, periodate, and bromate among others (OIG, 2008)). IUI is only one mechanism of action that may lead to adverse effects in the thyroid. Other goitrogens affect other biochemical pathways, such as iodination of thyroglobulin, deiodination of thyroxine, glucuronidation of thyroid moieties, *etc*.[5] Another example is the antithyroid interaction of soy (which does not effect IUI) combined with iodine deficiency has been further elucidated by Ikeda *et al*. (2000, 2001).[6] Thus, being able to define all the parameters that would affect thyroidal function is important in a cumulative risk assessment. At a minimum, we suggest using a more accurate term, such as "total NIS inhibition load" or something similar as well as defining and supporting what is being evaluated in a cumulative risk assessment.

Third, there are several general considerations to be considered when proposing a cumulative risk assessment. While OIG presents numerous U.S. EPA guidance documents to support the use of a cumulative risk assessment, it has not been widely used. The traditional risk assessment evaluates one chemical in a systematic manner with accepted methodologies, while also accounting for uncertainty. The result is the development of a dose that is not considered to cause any adverse effect in the most sensitive population even when exposed daily over a lifetime. Traditional risk assessments have a history with demonstrated validity.

In a cumulative risk assessment, some questions arise that require some thoughtful consideration.

- Would evaluating multiple chemicals mean having to account for multiple sources of uncertainty?
- What chemicals should be included or excluded from a cumulative risk assessment? For

[5] The OIG report lists lindane, malathion, and mancozeb as chemicals that inhibit iodide oxidation by thyroid peroxidase (TPO). The draft report does not mention soy protein in the context of other goitrogens anywhere in their report, although soy protein is a well documented TPO inhibitor and goitrogen. Two recent reviews of the TPO inactivation / goitrogenic action of soy isoflavones are Doerge and Sheehan (2002) and Doerge and Chang (2002).

[6] Ikeda *et al.* (2000, 2001) showed that rats fed an iodine-deficient diet containing soy bean developed a severe hypothyroid state characterized by decreased T4, increased TSH, increased thyroid weight, increased cell proliferation and marked histopathological changes. Another report from Son *et al.* (2001) tested the ability of isolated soy isoflavones to act synergistically with iodine deficiency to produce a hypothyroid state. These results suggested that only whole soy, but not soy isoflavones alone, is sufficient to produce a hypothyroid condition in rats under conditions of iodine deficiency.

example, other chemicals, such as thiocyanate, bromide, chloride, chlorate, and bromate (OIG, 2008) are known to inhibit IUI.

- How does one rank the importance of a particular mechanism of action? Is IUI more important in thyroid physiology than reduction of detoxification mechanisms?
- How does one evaluate multiple chemicals that have different qualitative (animal *versus* human data) and quantitative data in scientific literature?
- How does this cumulative risk assessment account for high doses of iodine which may adversely affect the thyroid? Although it is counterintuitive, iodine at high doses can also cause IUI.[7]
- How does one evaluate other possible confounders in a cumulative risk assessment such as smoking and diet?

The OIG does address and discuss some of these issues. It is also important to stress that this is not a chemical-specific risk assessment; it is an IUI risk assessment. Nonetheless, the science of a risk assessment is continually improving and, based on U.S. EPA guidance (U.S. EPA, 2007), a cumulative risk assessment does have scientific validity.

4.2 Use of Animal Studies

The draft OIG report implies that the NRC committee simply had a preference for human studies over rodent studies, and that the Argus rodent studies on which the 2002 U.S. EPA draft risk assessment is based continue to be relevant to perchlorate risk assessment. The OIG states "due to the scientific controversy surrounding the concentration at which perchlorate should be regulated, the National Academy of Science *Committee to Assess the Health Implications of Perchlorate Ingestion* was charged to assess the current state of the science regarding potential adverse effects of disruption of thyroid function by perchlorate in humans and laboratory animals at various stages of life."

First, it should be recalled that U.S. EPA's own guidelines state that human studies, when available and conducted properly, are preferred to evaluate chemical agents for the deriving the RfD. "If adequate human studies (confirmed for validity and applicability) exist, these studies are given first priority in the dose-response assessment, and animal toxicity studies are used as supportive evidence" (U.S. EPA, 1989).

Second, the NRC's 2005 report clearly stated that "the committee considered several of the animal studies on which U.S. EPA based its point of departure to be flawed in their design and execution. Conclusions based on those studies, particularly the neurodevelopmental studies, were not supported by the results of the studies …"

Lastly, the NRC further stated "although studies in rats provide useful qualitative information on potential adverse effects of perchlorate exposure, they are limited in their utility for quantitatively

[7] "The main factor regulating the accumulation of iodide in the thyroid (*i.e.,* NIS activity), other than TSH, has long been considered to be iodide itself. Stated simply, high doses of iodide cause diminished thyroid function. Acute inhibition of organic iodide binding depends on the intrathyroidal rather than the plasma concentration of iodide. The Wolff-Chaikoff effect and the ensuing escape constitute a highly specialized intrinsic autoregulatory system that protects the thyroid from the deleterious effects of I^- overload but at the same time ensures adequate iodide uptake for hormone biosynthesis. The level of iodide capable of inhibiting iodide organification and concomitantly stopping thyroid hormone synthesis depends on the previous iodide supply status of the animal" (Dohán *et al*., 2003).

assessing human health risk associated with perchlorate exposure."

We recommend the OIG consider revising their comments to accurately reflect these facts.

4.3 Hypothyroxinemia

The OIG method is in agreement with the NRC analysis (2005) that the most sensitive subpopulations are pregnant women and their fetuses, particularly if they are iodine deficient; although they differ from the NRC conclusions and methods by assuming that the first adverse effect is not maternal hypothyroidism, but hypothyroxinemia.

Hypothyroidism is defined as low free T4 and increased TSH and hypothyroxinemia is characterized by low T4 levels with normal levels of TSH. The OIG reports that although hypothyroxinemia in the mother is a "less stressful thyroid condition," they opine that effect may be frank hypothyroidism in the fetus as the mother would have "difficulty meeting her own T4 needs and [would be] unable to meet the fetal demand for T4 for proper brain development" (OIG, 2008).

The draft OIG report clearly states that hypothyroidism is an adverse effect from an extremely low uptake of iodide by the thyroid; however, in asserting that hypothyroxinemia and not hypothyroidism is the first adverse effect from perchlorate exposure or low iodide by the thyroid, they appear to be in conflict with the NAS committee conclusions. However, as both assessments evaluate IUI and hypothyroidism in the fetus, we believe the better view is that both the NAS committee and the OIG draft report are correct and that the differences are contextual or a matter of semantics.

There are more recent studies such as Vermiglio *et al.* (2004) and Kooistra *et al.* (2006) that may provide more insight on the issues of hypothyroxinemia even though there are questions of how well iodine sufficiency was evaluated. (Moreover, these studies do not evaluate any IUI or any of the other NIS stressors that the OIG has defined). However, it is also appears that it would be useful to consult with clinical thyroid endocrinologists to further elucidate the subtleties of these clinical studies.

Regardless of whether hypothyroidism or hypothyroxinemia are defined as the first adverse effect, both the NRC and the OIG use IUI as a critical biochemical step and if a chemical or chemical does not cause an increase in IUI, then there can be no progression to an adverse effect.

4.4 Iodine Deficiency

Iodine sufficiency is an important human health topic. The manner in which iodine sufficiency is measured in people and the determination of what is sufficient are two key subjects that often are not discussed adequately. OIG correctly points out that none of the stressors (nitrate, thiocyanate, or perchlorate) directly cause iodine deficiency. The cause of iodine deficiency is a lack of iodine in the diet which is easily remedied by eating a balanced diet or taking supplemental vitamins with iodine. A cumulative risk assessment for chemicals that inhibit iodide uptake is a useful tool for scientific assessment to determine what concentrations are sufficient to inhibit iodide uptake and what duration is needed to deplete iodide stores that might lead to changes in thyroid hormones.

The manner in which iodine sufficiency is measured is by quantifying the amount of iodine excreted in urine. Iodine levels in urine are highly variable during the day and even day to day. Creatinine

adjustments can be made to account for some of the variability.[8] The standard method for measuring iodine in urine is the collection of urine over a 24-hour period. The advantage of total urine collected over the 24-hour period is that it accounts for iodine excretion and its variability during the day; however, it still cannot account for day-to-day variations which may be significant. The disadvantage and a common reason that it is not conducted is that it requires substantially more effort on the part of the study subjects and the investigators, which translates into more time and resources to conduct the study. Twenty-four-hour urine collections are generally limited to clinical studies, while epidemiological studies rely on the more common, but inferior method of "spot urine collection." As the term indicates, urine is collected once. Creatinine adjustment and controlling the time of day the sample is collected help to minimize some of the variability, but there remains no method to give an accurate depiction of the status of iodine sufficiency in a single subject.

How is iodine deficiency defined? The World Health Organization (WHO) criteria for assessing iodine status within any given population is that a population's median urine iodine value based on spot urine iodine measurements is optimal between 100 and 200 µg/liter, with no more than 20% of individual values less than 50 µg/liter (WHO/UNICEF/ICCIDD, 1994). According to WHO criteria, a population is mildly iodine deficient when its median urine iodine based on spot urine samples is between 50 and 99 µg/liter; moderate deficiency is a population median between 20 and 49 µg/liter; and less than 20 µg/liter is severe deficiency (WHO/UNICEF/ICCIDD, 1994).

Given the great variability of iodine content of food in the United States (Pearce *et al*., 2004; Pennington *et al*., 1991), it would be unlikely that a given individual would continue to ingest foods with low iodine over an extended period of time. This is confirmed in a one-year study of 15 euthyroid men in Denmark (an area of mild to moderate iodine deficiency) that shows long-term iodine status cannot be determined from a single 24-hour urine measurement (Andersen *et al*., 2001).

With that basis, we suggest OIG review their assessment. First, the OIG considers a single spot urine iodine test to be useful in assessing an individual's iodine status. As evidence, the OIG report states that an "approach to avoiding this potential for excess iodide exposure during pregnancy is to simply measure the [urine iodine concentration] UIC during prenatal care. If the UIC is below 150 µg/L, the use of an iodide-containing prenatal vitamin is warranted. If the UIC is above 150 µg/L, the use of an iodide supplementation may not be necessary, and eliminates the potential risk of inducing excess iodide intake." We agree that this would be an ideal solution if a clinical test was available that accurately assessed an individual's iodine status.

Second, the OIG should reevaluate the significance of individuals within a population with cross-sectional spot urine iodine levels below a certain cut-off, specifically levels less than 100 µg/L or less than 50 µg/L. The OIG report states that the "NHANES III survey documents that 8.1% of males and 15.1% of females are moderately iodide deficient (*i.e.*, urinary iodide concentrations < 50 µg/L) (Hollowell, 1998). The NHANES III survey identifies that 14.9 % ± 1.2 of the U.S. women of childbearing age are moderately iodide deficient and that 6.9 % ± 1.9 of pregnant U.S. women are moderately iodide deficient (Hollowell, 1998)." Thirdly, the OIG also states the NRC noted that "… distribution of iodide values measured in a spot urine sample is broader than values measured repeatedly in individual subjects (Anderson *et al*., 2001), this leads to overestimation of the number of subjects with both low and high values (emphasis added; NRC, 2005)." In short, the NRC opinion

[8] Creatinine is usually produced at a fairly constant rate by the body and actively secreted as fairly constant rate in urine whereas iodine is not.

demonstrates that the percentage of U.S. women of childbearing age and pregnant women who are moderately iodide deficient due to the limitations of a single urine spot test is overestimated. Several published papers by noted researchers have stated agreement with the NRC in recent years as discussed below. Other examples exist.[9]

The comparison of spot and 24-hour urine measurements from three studies (Greer *et al*., 2002; Tellez *et al*., 2005; Costeira *et al*., 2009), found there was no correlation in urine iodine measurement in individuals from one spot urine sample to the next. Overall, it appears (and one would expect) that 24-hour urine excretion measurements are more reproducible than spot urine measurements. Most significantly, urinary iodide levels in subjects from Greer *et al*. (2002) were not different based on perchlorate dose.

4.5 Protein binding by other anions

Proteins present in the serum are able to bind free anions to render the anions biologically unavailable. Even if an individual receives a certain dose or has a measured concentration in the body, if proteins (*e.g*., albumin) in the serum bind, for example, 50% of the free anion, the effective dose is 50% of the total dose. If it is known that a certain proportion of perchlorate, thiocyanate, and nitrate are all bound to serum proteins, this proportion must be carefully evaluated (*e.g.,* the concentration of the stressor to the thyroid adjusted) from the total dose in the cumulative risk assessment.

The OIG is correct in their assumption that free perchlorate, thiocyanate, and nitrate are the critical entities that compete for iodide transport at the NIS. This critical concept was not included in Tonacchera *et al.* (2004) or in any of the other supporting studies. The OIG report is likely correct in their application of an estimate that thiocyanate is approximately 50% protein bound, but it is less clear that the absence of protein binding factors for nitrate and perchlorate are justifiable. Although there are known significant differences between rodents and humans with respect to protein binding, enough is known regarding protein binding of various anions to estimate parameters for the Tonacchera model.

The 2005 NRC report states that "plasma-protein binding is often a source of substantial species differences in chemical disposition, as was evident for perchlorate. On the basis of the serum data of Greer *et al.* (2000), humans had a lower capacity for binding perchlorate than did rats (for example, the capacity for plasma-protein binding was 500 ng/hr-kg in humans *versus* 3,400 ng/hr-kg in rats),

[9] Laurberg *et al.* (2007) illustrated this misinterpretation by citing "a recent study of iodine deficiency in Spanish schoolchildren (Serra-Prat *et a*l., 2008). A crosssectional study of 987 four-year-old children gave a mean UIC of 214µg/L (median 189 µg/L), which is not low. Nevertheless, it was concluded that 7.8% of the children had iodine deficiency, because 7.8% of urinary samples had an iodine concentration of 100 µg/L." Similarly, Zimmerman (2008) concluded that "the median UI is often misinterpreted. Individual iodine intakes and, therefore, spot UI concentrations are highly variable from day-to-day, …and a common mistake is to assume that all subjects with a spot UI <100mg/l are iodine deficient." Hollowell and Haddow (2007) analyzed the NHANES III (1988-1994) data for women of reproductive age and women who were pregnant. They concluded that in "the NHANES III survey…we believe that an otherwise normal individual may excrete a concentration of iodine <50µg/L at the time of study, but this value does not necessarily reflect the long-term pattern for that individual." Most recently, Caldwell *et al*. (2008) analyzed spot UI levels for 2,526 participants, aged six years and older, participating in NHANES 2003–2004. They reported that the "median UI level for the general U.S. population in 2003–2004 was 160 µg/L (95% confidence interval [CI] 146–172), and 11.3 ± 1.8% of the population had a UI level below 50 µg/L . Children had a higher UI level than adolescents and adults. Among all (pregnant and nonpregnant) women of reproductive age, the median UI level was 139 µg/L (95% CI 117–156), 15.1 ± 3.2% women had a UI level <50 µg/L ... These findings affirm the stabilization of the UI level and the adequate iodine nutrition in the general U.S. population since 2000."

whereas the binding affinities and dissociation constants were similar" (NRC, 2005).

Harris *et al.* (1998) reported that chloride, bromide and perchlorate were all similarly bound to transferrin, a plasma protein that normally binds iron. Merrill *et al.* (2005) noted "reversible binding of perchlorate to nonspecific human plasma proteins has been qualitatively demonstrated in other studies (Carr, 1952). Additionally, the model [in the Merrill *et al.* study] indicated the existence of plasma binding, as without it the model underestimated serum perchlorate at 0.1 mg/kg-day, while simulations at 0.5 mg/kg-day produced adequate fits. Hence, at the lower level, plasma binding represents a larger proportion of the overall amount of serum perchlorate. Serum levels from the 0.02 mg/kg-day dose group were below the detection limit and thus could not be compared to model predictions. The plasma protein affinity for perchlorate was assumed to be similar to that used in the male rat model, given the same proteins (albumin and prealbumin) appear to be responsible (Carr, 1952; Merrill *et al*., 2003)." Okabe *et al.* (1993) found the effectiveness of thiocyanate, perchlorate, iodine, and bromide in reducing T4 binding was in the following order: thiocyanate > iodine > perchlorate > bromide.

Taken together, the literature suggests the binding of monovalent anions other than thiocyanate does occur. By including a binding constant for thiocyanate, but not for perchlorate or nitrate, the overall perceived risk from thiocyanate may be artificially low. Binding terms for perchlorate and nitrate should be estimated from the literature and applied to the overall OIG model.

4.6 Up- and Down-regulation of NIS Expression

Over the past decade, research regarding thyroid auto-regulation and the regulation of expression of the NIS has proliferated. Evidence suggests that the NIS is up-regulated in response to iodine deficiency in the thyroid. Up-regulation of the NIS would include the number of NIS increasing; thus, when an individual is iodine deficient, the body increases the number of NIS "pumps" such that the thyroid is more effective at capturing iodide. This is a common adaptive mechanism that many tissues, including the thyroid gland, use to maintain hormone homeostasis. The OIG report appears to consider up-regulation of NIS expression, but doesn't incorporate it into its risk assessment. The OIG report states that "iodide deficiency is frequently and incorrectly associated with hypothyroidism and increased TSH…" and that "TSH-independent auto-regulation is often overlooked or actually not known to younger Western trained physicians …"

Likewise, the NRC report also acknowledged the up-regulation of NIS expression, but did not incorporate it into their risk assessment. They state that "rats compensated for the inhibition within 5 days of perchlorate administration, most likely by increasing the expression of NIS in the thyroid. The data suggest that compensation occurs more quickly in rats because rats have a smaller reserve capacity of thyroid hormones than humans."

Dohán *et al.* (2003) provided perhaps the most comprehensive review of the NIS regulation at the time of the NRC report, stating that "up-regulation of thyroid NIS expression and iodide uptake activity by TSH [thyroid stimulating hormone] has been demonstrated not only in rats *in vivo* but also in the rat thyroid-derived FRTL-5 [Fisher rat thyroid cell line] cell line and in human thyroid primary cultures. TSH up-regulates iodide uptake activity by an increase in NIS transcription. "Both NIS mRNA and NIS protein levels decreased significantly after either 1 or 6 days of iodide administration. NIS mRNA levels were already significantly reduced at 6 hours following the injected single dose of iodide. In contrast, a significant decrease of NIS protein levels was detected

only at 24 hours." After TSH withdrawal, a reduction of iodide uptake activity is observed in FRTL-5 cells. This is a reversible process, as iodide uptake activity can be restored by TSH. The NIS half-life is approximately 5 days in the presence and approximately 3 days in the absence of TSH" (Dohán *et al.*, 2003). Other studies support up- or down-regulation of NIS during changing levels of iodine (*e.g.*, Eng *et al*. (1999)[10]; Wagner *et al*. (2002)[11]; Merrill *et al*. (2003)[12]; Pedraza *et al.* (2006)[13]; Nordén *et al.* (2007)[14]; and Bizhanova *et al*. (2009).[15]

The OIG report would benefit by the inclusion of this information and the incorporation of it into its assessment. For example, the serum binding could be incorporated into the Tonacchera model using free anions *versus* total anions as such:

$$TIU \propto ([I] \times [NIS\ expression]) / (1.22 + ([free\ perchlorate] + [free\ nitrate]/240 + [free\ thiocyanate]/15)$$

Where the concentration of each anion is expressed in µmol/L and [NIS expression] is a estimation of the total membrane bound NIS receptors in the thyroid at the point in time.

5.0 CONCLUSIONS

The OIG analysis confirms that the U.S. EPA's current RfD of 0.0007 mg/kg-d is conservative and no adverse effects are expected when exposures are below the RfD. The OIG was conservative in the interpretation of the iodine data, NIS up-regulation, and protein binding. The OIG analysis provides more information regarding the most sensitive populations, pregnant women and their fetuses. Although the OIG used a nontraditional method for a risk assessment, the conclusions they reached are consistent with a large body of evidence that has been previously published and reviewed. This evidence shows that no adverse effects occur to people when exposed to environmental concentrations of perchlorate. The OIG also demonstrates that reducing the concentration of perchlorate in drinking water from 24.5 ppb to 6 ppb would result in no meaningful reduction in risk to the public. Overall, the cumulative risk assessment was scientifically-based and an appropriate method for assessing the risk of perchlorate for regulatory decision making. However, there are several topics which we have discussed where the OIG could usefully provide further support for their model and thereby strengthen their arguments.

[10] Eng *et al.* (1999) found that although "serum TSH levels were unchanged …both NIS mRNA and protein were decreased at 1 and 6 days after chronic iodide ingestion. NIS mRNA was decreased at 6 and 24 h after acute iodide administration, whereas NIS protein was decreased only at 24 h."

[11] Wagner *et al.* (2002) found that "stimulation of thyrocytes with TSH (0.1-10 U/ml) …results in a dose- and time-dependent up-regulation of NIS expression reaching a maximum at 10 mU/ml TSH (2211 +/- 761 copies) … after 24h."

[12] Merrill *et al.* (2003) studied the inhibition of thyroid 125I– uptake after drinking water exposure to perchlorate. TSH-induced up regulation of NIS compensated for competitive inhibition of thyroid I– uptake by perchlorate across doses in the drinking water study" (Merrill *et al*., 2003).

[13] Pedraza *et al.* (2006) reported that their "present data; therefore, confirm an important role of thyroid autoregulatory responses in the efficient adaptation to a mild degree of ID. A possible role of the sodium/ iodide symporter in ID has hardly been addressed experimentally in rats."

[14] Nordén *et al.* (2007) reported that "TSH increased the NIS expression >100-fold after 48 h and 5- to 20-fold after prolonged stimulation. It was concluded that down-regulation of NIS is the likely explanation for (131)I-induced thyroid stunning.

[15] Bizhanova *et al.* (2009) demonstrated that "Thyroid-stimulating hormone (TSH) and iodide regulate iodide accumulation by modulating NIS activity via transcriptional and post-transcriptional mechanisms."

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7.0 REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 2008. Toxicological Profiles for Perchlorate.

Andersen S, Pedersen KM, Pedersen IB, Laurberg P., "Variations in urinary iodine excretion and thyroid function. A 1-year study in healthy men," *Eur J Endocrinol.* 2001 May; v. 144 no. 5: pp. 461-5.

Belzer RB, Bruce GM, Peterson MK, Pleus RC. 2004. Using comparative exposure analysis to validate low-dose human health risk assessment: The case of perchlorate. Comparative Risk Assessment and Evnironmental Decision Making. New York. Kluwer.

Bizhanova A, Kopp P., "The Sodium-Iodide Symporter NIS and Pendrin in Iodide Homeostasis of the Thyroid," *Endocrinology* 2009 Feb 5 [Epub ahead of print].

Caldwell KL, Miller GA, Wang RY, Jain RB, Jones RL., "Iodine status of the U.S. population, National Health and Nutrition Examination Survey 2003-2004," *Thyroid*. 2008 Nov; v. 18 no. 11: pp. 1207-14.

Carr JE, Conn JB, Wartman TG. 1952. The binding of metal ions by ACTH: a property correlated with biological activity. *Science* 116(3021):566-8.

Das Gupta PK, Kirk AB, Dyke JV, Ohira S. 2008. Intake of iodine and perchlorate and excretion in human milk. *Environ. Sci, Technol.*

Doerge DR, Chang HC., "Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo," *J Chromatogr B Analyt Technol Biomed Life Sci*. 2002 Sep 25; v. 777 no. 1-2: pp. 269-79.

Doerge DR, Sheehan DM., "Goitrogenic and estrogenic activity of soy isoflavones," *Environ Health Perspect.* 2002 Jun; v. 110 Suppl 3: pp. 349-53.

Dohán O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N., "The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance," *Endocr Rev.* 2003 Feb; v. 24 no. 1: pp. 48-77.

Eng PH, Cardona GR, Fang SL, Previti M, Alex S, Carrasco N, Chin WW, Braverman LE., "Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein," *Endocrinology* 1999 Aug; v. 140 no. 8: pp. 3404-10.

Glinoer D., "Iodine nutrition requirements during pregnancy," *Thyroid*, 2004; v. 16: pp.947–948.

Greer MA, Goodman G, Pleus RC, Greer S. E., "Health Effects Assessment for Environmental Perchlorate Contamination: The Dose Response for Inhibition of Thyroidal Radioiodine Uptake in Humans," *Environ. Health Perspect*. 2002; v. 110 no. 9: pp. 927-937.

Gibbs JP. 2009. Comment on "Intake of Iodine and Perchlorate and Excretion in Human Milk."

Environmental Science and Technology.

Harris WR, Cafferty AM, Abdollahi S, Trankler K., "Binding of monovalent anions to human serum transferring," *Biochim Biophys Acta.* 1998 Apr 2; v. 1383 no. 2: pp. 197-210.

Hollowell JG, Haddow JE., "The prevalence of iodine deficiency in women of reproductive age in the United States of America," *Public Health Nutr.* 2007 Dec; v. 10 no. 12A: pp. 1532-9.

Ikeda T, Nishikawa A, Imazawa T, Kimura S, Hirose M., "Dramatic synergism between excess soybean intake and iodine deficiency on the development of rat thyroid hyperplasia," *Carcinogenesis* 2000 Apr; v. 21 no. 4: pp. 707-13.

Ikeda T, Nishikawa A, Son HY, Nakamura H, Miyauchi M, Imazawa T, Kimura S, Hirose M., "Synergistic effects of high-dose soybean intake with iodine deficiency, but not sulfadimethoxine or phenobarbital, on rat thyroid proliferation," *Jpn J Cancer Res.* 2001 Apr; v. 92 no. 4: pp. 390-5.

Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ, Neonatal Effects of Maternal Hypothyroxinemia During Early Pregnancy. *Pediatrics,* 2006; v. 117 no. 1: pp. 161-167.

Laurberg P, Andersen S, Bjarnadóttir RI, Carlé A, Hreidarsson A, Knudsen N, Ovesen L, Pedersen I, Rasmussen L., "Evaluating iodine deficiency in pregnant women and young infants-complex physiology with a risk of misinterpretation," *Public Health Nutr.* 2007 Dec; v. 10 no. 12A: pp. 1547-52.

Merrill EA, Clewell RA, Gearhart JM, Robinson PJ, Sterner TR, Yu KO, Mattie DR, Fisher JW. 2005. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat," *Toxicol Sci.* 2003 Jun; v. 73 no.2: pp. 256-69. Epub 2003 Apr 15.

National Research Council of the National Academies (NRC). 2005. Health Implications of Perchlorate Ingestion. Committee to Assess the Health Implications of Perchlorate Ingestion, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies. Washington, D.C.: *The National Academies Press.*

Nordén, MM, Larsson F, Tedelind S, Carlsson T, Lundh C, Forssell-Aronsson E, Nilsson M., "Down-regulation of the sodium/iodide symporter explains 131I-induced thyroid stunning," *Cancer Res.* 2007 Aug 1; v. 67 no.15: pp. 7512-7517.

Pearce EN, Pino S, He X, Bazrafshan HR, Lee SL, Braverman LE., "Sources of dietary iodine: bread, cows' milk, and infant formula in the Boston area," *J Clin Endocrinol Metab*, 2004; v. 89: pp. 3421–3424.

Office of Inspector General (OIG). 2008. Scientific Analysis of Perchlorate. External Review Draft. Assignment No. 2008-0010.

Okabe N, Hokaze M. 1993. Effect of inorganic anions on the binding of thyroxine by bovine serum albumin. *Chem Pharm Bull (Tokyo)* 41(3):430-2.

Pedraza PE, Obregon MJ, Escobar-Morreale HF, del Rey FE, de Escobar GM., "Mechanisms of adaptation to iodine deficiency in rats: thyroid status is tissue specific. Its relevance for man," *Endocrinology.* 2006 May; v. 147 no. 5: pp. 2098-108. Epub 2006 Feb 2.

Pennington JA, Schoen SA., "Contributions of food groups to estimated intakes of nutritional elements: results from the FDA total diet studies, 1982– 1991," *Int J Vitam Nutr Res*, 1996, v. 66: pp. 342–349.

Son HY, Nishikawa A, Ikeda T, Imazawa T, Kimura S, Hirose M., "Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet," *Jpn J Cancer Res*. 2001 Feb; v. 92 no. 2: pp. 103-8.

Tellez RT, Chacon PM, Abarca CR, Blount BC, Van Landingham CB, Crump KS, Gibbs JP, "Long-term Environmental Exposure to Perchlorate through Drinking Water and Thyroid Function during Pregnancy and the Neonatal Period," *Thyroid*, 2005; v. 15 no.9: pp. 963-987.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, and Gibbs J. 2004. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid.* 14 (12):1012-9.

U.S. Environmental Protection Agency (EPA). 1989. Risk Assessment Guidance for Superfund (RAGS), Volume I. Human Health Evaluation Manual, Part A. Interim Final. Office of Solid Waste and Emergency Response, United States Environmental Protection Agency. Washington, D.C. EPA/540/1-89/002. December.

U.S. Environmental Protection Agency (EPA). 2007. Concepts, Methods, and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures, and Effects: A Resource Document. Environmental Protection Agency Office of Research and Development, National Center for Environmental Assessment, Aμgust 2007. (EPA/600/R-06/013F).

U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS). 2005. Perchlorate and Perchlorate Salts.

U.S. Environmental Protection Agency (EPA). 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (NCEA-1-05-3), External Review Draft. Washington, D.C.: Office of Research and Development. January 16.

Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, Castagna MG, Mattina F, Violi MA, Crisa A, Artemisia A, Trimarchi F., "Attention Deficit and Hyperactivity Disorders in the Offspring of Mothers Exposed to Mild-Moderate Iodine Deficiency: A Possible Novel Iodine Deficiency Disorder in Developed Countries," *J. Clin Endocrinol & Metabol* 2004; v. 89 no. 12: pp. 6054-6060.

Wagner S, Aust G, Schott M, Scherbaum WA, Feldkamp J, Seissler J., "Regulation of sodium-iodide-symporter gene expression in human thyrocytes measured by real-time polymerase chain reaction," *Exp Clin Endocrinol Diabetes* 2002 Nov., v. 110 no. 8: pp. 398-402.

WHO/UNICEF/ICCIDD, Indicators for assessing iodine deficiency disorders and their control

through salt iodization, WHO/Nut, 1994; v. 6: p. 36.

Zimmermann MB., "Methods to assess iron and iodine status," *Br J Nutr.* 2008 Jun; v. 99 Suppl 3: pp. S2-9.

To: the EPA Office of Inspection General.

From: Herwig Opdebeeck, MSc

RE**: Use and application of a cumulative risk assessment approach to characterizing the public health risk from a low TIU during pregnancy and lactation**

Dear Deputy Inspector General,

I am a Master in Science and a scientific consultant.

Attached you will find comments that indirectly are related to the use and application of a cumulative risk assessment approach to characterizing the public health risk from a low TIU during pregnancy and lactation.

Regards,

Herwig Opdebeeck
Rue de Latigny 3
CH-1955 Chamoson, Switzerland

Should a cumulative risk assessment, including nitrate and thiocyanate and lack of iodine be used for perchlorate risk assessment?

Acronyms

DW: Drinking Water
NAS: National Academy of Science
RfD: NAS Reference dose for perchlorate= 0.0007 mg/kg-day
RSC: Relative Source Contribution
G-RSC: goitrogen RSC expressed in PEC
PEC: Perchlorate Equivalent Concentration of TGL.
TGL: Total Goitrogen Load expressed in PEC: [Perchlorate] + [nitrate/150] + [thiocyanate/15]
UF: Uncertainty Factor

The purpose of these comments is to give a reply to the above question and at the same time raise 2 critical issues not addressed in this OIG analysis:

1. Which drinking water Relative Source Contribution(RSC) that should be used i.e. <1 or =1 or > 1, in order to derive a drinking water(DW) Maximum Contaminant Level (MCL) from the Reference Dose(RfD), with inclusion of the Total Goitrogen Load (TGL) into the RSC and its consequences. It should be kept in mind that the perchlorate RfD, contrary to most other contaminant RfDs, was based on a human dose response trial and therefore the RSC is at least =1.

2. Drawing the attention on the fact that the iodine insufficient female U.S. population has a TGL about half of that of the iodine sufficient female and general U.S population which implies that they are least susceptible to iodine uptake inhibition, if they would have been iodine sufficient.

The answer on the above question is therefore a 2 step answer:

1. Step 1 of the answer

If the question would mean: "*Should a cumulative risk assessment be used to derive an MCL from the NAS RfD by applying not only a perchlorate RSC but also a PEC (Perchlorate Equivalent Concentration) RSC*" then the answer is yes.

1.1. Introduction

The perchlorate NAS (National Academy of Sciences) RfD (Reference Dose) of 0.0007 mg/kg-day is based on the Greer 2002 dose response clinical study.
NAS 2005 did not suggest whether to apply a RSC (Relative Source Contribution) and also did not suggest whether to include nitrate, thiocyanate and the lack of iodine to arrive at a perchlorate MCL for DW. It did not consider this to be part of its mandate.
Also EPA's OIG did not go this last step.
That is unfortunate since the last step to derive a MCL from an RfD is made by applying the RSC.

1.2. Determination of the perchlorate RSC and goitrogen RSC

1.2.1. Should the RSC be smaller, equal or larger than 1?
Before defining how much in absolute value the RSC should be , the question should be first posed whether the RSC should be <1 or = 1 or even > 1.

First some background

-Mostly in toxicological risk assessments and consequently in determining RfDs and MCLs contaminant adverse effect levels are used as benchmark.
For this reason most trials are done on rats or other animals and the results extrapolated to humans.
The Greer 2002 trial had as benchmark a No Observable Effect Level (NOEL), which is a non adverse effect level.
So the trial could be done and has been done on humans.
This is rather exceptional.

- Most of the rat trials are such that intake of the contaminant other than the doses are avoided as much as possible (thru food and drinking water).
When MCLs for humans are derived from those animal trials the DW RSC is normally <1 since a food and DW contaminant background can be expected for the humans.
The Greer trial on humans does not imply such a lateral adjustment and the RSC is either =1 or >1.

RSC=1 or RSC>1?

RSC =1: when the RfD is derived from a dose response trial without food and without DW background
There are no other sources besides the doses administered. Therefore the dose administered is directly translated into MCL according to the formula: NOEL/UF = RfD and MCL= RfD x70kg/2l.

RSC> 1: when the RfD is derived from a dose response trial with food and/or with DW background.

1.2.2. RSC for perchlorate taking only perchlorate background into account

If in the Greer trial the participants would have had neither food nor DW perchlorate background the MCL would have been:
MCL = (RfD x BW/DWL) x RSC = (0.7ug/kg-day) x 70kg/2 lx1= 0.7 x35 x1 = 24.5 ug/l =24.5 ppb.
Yet the Greer participants had food and DW perchlorate background.

Therefore the RSC is >1.

Still this perchlorate background in food and DW was very low (see tables 1-3, col.8 on page 5)

Therefore a RSC =1 can be used in first instance.

Example: for nitrate and fluoride US-EPA and CAL-EPA did not apply a MCL <1 but a MCL=1

1.2.3. RSC taking into account the total goitrogen background (TGB)

The Greer participants also had a perchlorate equivalent background in food and DW from thiocyanate and nitrate.

Those food and DW goitrogen loads however are significant, about 100 times the perchlorate background, expressed in PEC (the perchlorate background represents 1% of the total goitrogen load, see following table and tables 1-3, col.5,7,9 and 10, page 5).

Total population (without outliers Iodine > 2000 µg)												
	T4	TSH	Nitrate µg	Nitrate (PEC)	SCN µg	SCN (PEC)	ClO4 µg	TGL (in (PEC))	ClO4/TG %	Iodine µg	I/ClO4	I/TGL
	5.4	4.9	59857	399	2507	167	5.1	571	0.9	240	47	0.42
	6.6	1.6	63321	422	2876	192	4.8	619	0.8	229	48	0.37
	7.2	1.6	58824	392	2526	168	5.4	566	1.0	247	46	0.44
	7.7	1.6	61427	410	2308	154	5.5	569	1.0	219	40	0.38
	8.3	1.6	58675	391	2349	157	6.0	554	1.1	245	41	0.44
	8.8	1.8	60640	404	2414	161	5.4	571	1.0	217	40	0.38
	9.6	1.6	62425	416	2098	140	5.2	561	0.9	233	45	0.42
	11.6	1.6	53173	354	2063	138	4.9	497	1.0	207	42	0.42
Mean	*8.2*	*2.1*	*59793*	*399*	*2393*	*160*	*5.3*	*563*	*0.9*	*230*	*43*	*0.41*
1	2	3	4	5	6	7	8	9	10	11	12	13

Col. 5 shows an average nitrate PEC of 400 ppb, col.7 an average SCN PEC of 160 ppb and col. 8 an average of 5 ppb perchlorate resulting in a 1% perchlorate contribution in the total goitrogen load (col. 10).

Now the average urinary perchlorate level in the US is 5.3.ug/l(see above table , col.8) This roughly corresponds with an oral perchlorate dose of 0.075ug/kg-day(Blount et al.). Therefore, since the average perchlorate intake represents less than 1 % of the total goitrogen intake, the total equivalent perchlorate uptake is about 7.5 ug /kg-day. Therefore the total oral goitrogen load of the Greer participants is 7 +7.5 ug /kg-day= 14.5ug/kg-day so the RSC is 14.5 /7= 2.1

This can be explained as following:

MCL = (RfD x BW/DWL) x RSC=(BW/DWL X NOEL/10) x RSC = 35/10x NOEL x RSC

The Greer NOEL of 0.7ug/kg-day is not an absolute NOEL but a lower dose-only NOEL.. The absolute NOEL is the perchlorate dose NOEL + the background perchlorate level + the background equivalent perchlorate level (see fig. 1 page 4).

Since the background perchlorate level is insignificant, the perchlorate dose NOEL + perchlorate background equivalent NOEL= 7 + 7.5= 14.5ug/kg-day = effective NOEL = Total NOEL = T-NOEL.
So T-NOEL = 2.1x NOEL and NOEL = T-NOEL/2.1

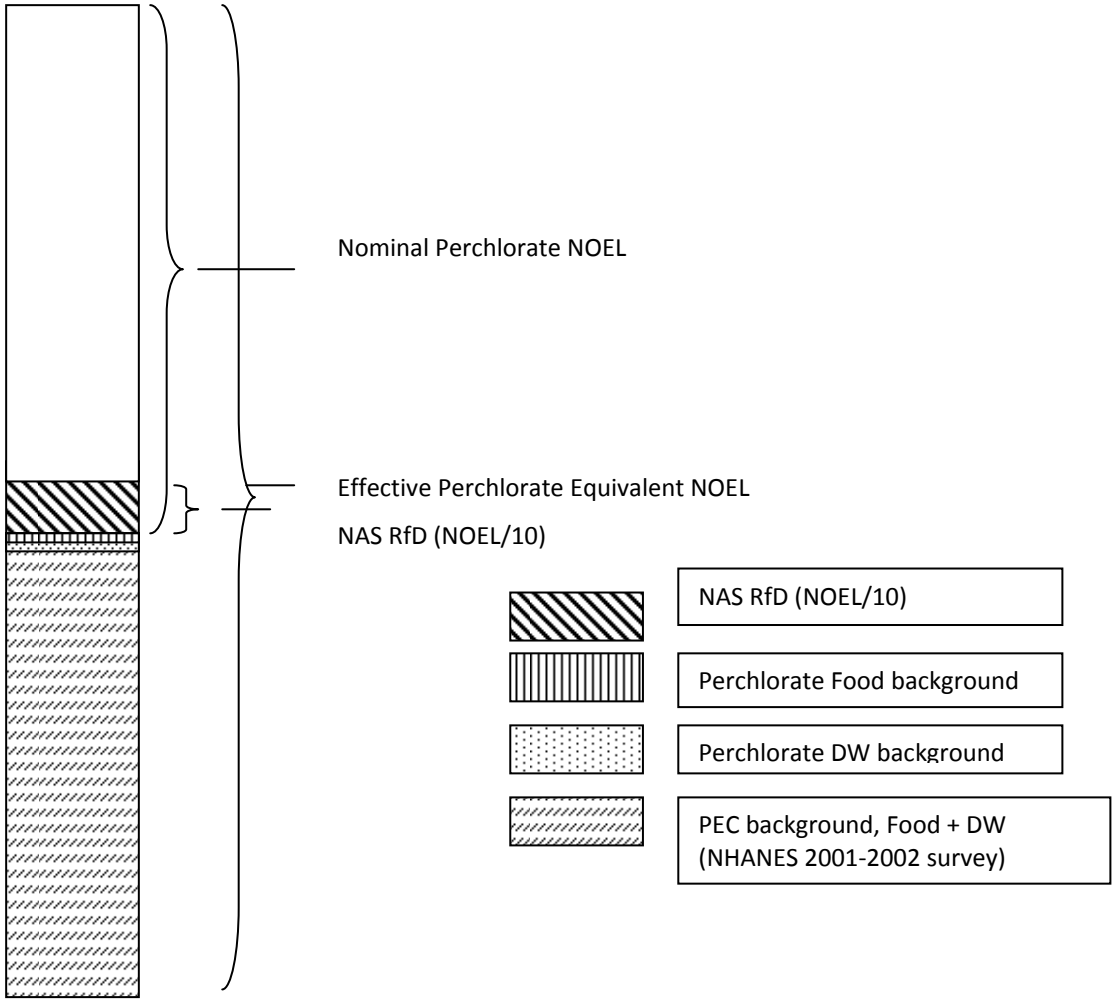
Thus the MCL for perchlorate is 35/10x T-NOEL x RSC
The RSC linked to T-NOEL =1 since there are no other sources neither form perchlorate nor from other goitrogens.

Thus the MCL for perchlorate = 35/10x 14.5 = 35/10 x NOEL x 2.1 which shows that the RSC=2.1.

Thus to correct for the contribution of the other goitrogens or in other words to allow for the higher effective T-NOEL, the RSC of the dose perchlorate should be 14.5/7=2.1
It follows that the MCL for perchlorate alone = 35/10x NOEL x 2.1 = 35/10 x 7 x 2.1 =51 ppb

All this is illustrated in the following fig.1:

Fig. 1: Greer 2002 NOEL, NAS RfD, Perchlorate and PEC background



Nevertheless, for example, the Office of Policy and Planning, New Jersey Department of Environmental Protection (NJDEP) applied a RSC of 0.20 and consequently arrived at a proposed MCL of 5 ppb (MCL= 0.0007 mg/kg/day x 70 kg x 0.2 = 0.0047 mg/L or 5 ug/L) instead of more than 24.5ppb, see:
http://www.nj.gov/dep/watersupply/perchlorate_mcl_10_7_05.pdf

Note: The perchlorate, nitrate and thiocyanate food and DW background of the Greer participants are assumed to be representative for those of the general U.S. population. If this

were not so then the perchlorate NOEL and RfD would not be so either and therefore could not be used as the basis for deriving a MCL either.

1.3. What would be the additional consequences if a RSC <1 would be applied instead?

If, by error, nevertheless a perchlorate RSC <1 would have been applied(as shown in the example above), a goitrogen adjusted RSC <1 should then logically be applied as well .That RSC however would then end up being << 1 because of its dominant share and therefore the DW MCL for perchlorate would become extremely stringent, well below 1 ppb and well below the natural background even though its contribution is insignificant. That would certainly not be “a meaningful opportunity for health risk reduction” since the lower the contribution of perchlorate in DW compared to food and to the other goitrogens the more stringent the DW MCL would have to be, so stringent that it would become meaningless.
This confirms via another way that applying a perchlorate RSC < 1 is erroneous.

Step 2 of the answer

If the question would additionally mean*: “Should a risk assessment including lack of iodine be used to decide how iodine uptake inhibition by goitrogens should be combated?”* then the answer is yes as well.

This is indeed amply and convincingly demonstrated in the OIG analysis based principally on the Tonacchera work and confirmed by other evidence.

What was not mentioned in the OIG analysis was that the U.S. iodine insufficient female population, besides an inadequate iodine containing diet, seems to have a generally unbalanced diet characterised by a low consumption of vegetables.
We refer again to the NHANES 2001-2002 data on perchlorate (CLO4), nitrate (NO3) and thiocyanate (SCN).

NHANES 2001-2002 : RELATIONSHIP IODINE WITH ClO4 AND TGP

TGPL = Total Goitrogen Load = [ClO4]/1 + [NO3]/150 + [SCN]/15

TABLE 1

1	2	3	4	5	6	7	8	9	10	11	12	13
						Women <100ug/l (without outliers Iodine > 2000 µg)						
	T4	**TSH**	**Nitrate µg**	**Nitrate (PEC)**	**SCN µg**	**SCN (PEC)**	**ClO4 µg**	**TGL (in (PEC))**	**ClO4/TG %**	**Iodine µg**	**I/ClO4**	**I/TGL**
	5.9	1.6	33655	224	1994	133	3.7	361	1.0	61	16	0.17
	6.9	1.7	35353	236	1654	110	4.9	351	1.4	62	13	0.18
	7.5	1.4	33047	220	1597	106	2.8	330	0.9	59	21	0.18
	7.9	1.4	31715	211	1447	96	2.7	311	0.9	59	22	0.19
	8.5	1.6	24077	161	882	59	2.3	222	1.0	53	23	0.24
	9.1	1.7	31355	209	1035	69	2.1	280	0.7	61	29	0.22
	9.8	1.3	43926	293	1607	107	2.5	402	0.6	56	22	0.14
	11.8	1.5	27509	183	1689	113	1.9	298	0.7	51	26	0.17
Mean	*8.4*	*1.5*	*32579*	*217*	*1488*	*99*	*2.9*	*319*	*0.9*	*58*	*22*	*0.19*

TABLE 2

						Women >100ug/l (without outliers Iodine > 2000 µg)					
	T4	**TSH**	**Nitrate µg**	**Nitrate (PEC)**	**SCN µg**	**SCN (PEC)**	**ClO4 µg**	**TGL (in (PEC))**	**ClO4/TG %**	**Iodine µg**	**I/ClO4**	**I/TGL**
	5.6	2.0	67915	453	2379	159	6.5	618	1.1	305	76	0.71
	6.8	1.4	66304	442	2511	167	5.6	615	0.9	341	99	0.96
	7.4	1.6	56527	377	2107	140	4.9	522	0.9	250	65	0.78
	8.1	1.6	76281	509	2636	176	5.3	690	0.8	250	64	0.60
	8.6	1.6	70570	470	2699	180	5.4	656	0.8	266	71	0.68
	9.2	1.5	68955	460	2311	154	6.3	620	1.0	284	77	0.74
	10.0	1.5	61800	412	2102	140	6.4	559	1.2	267	58	0.77
	11.8	1.6	63503	423	1939	129	6.1	559	1.1	268	78	0.96
Mean	*8.4*	*1.6*	*66482*	*443*	*2336*	*156*	*5.8*	*605*	*1.0*	*279*	*74*	*0.78*

TABLE 3

						Total population (without outliers Iodine > 2000 µg)						
	T4	**TSH**	**Nitrate µg**	**Nitrate (PEC)**	**SCN µg**	**SCN (PEC)**	**ClO4 µg**	**TGL ((in PEC))**	**ClO4/TG %**	**Iodine µg**	**I/ClO4**	**I/TGL**
	5.4	4.9	59857	399	2507	167	5.1	571	0.9	240	47	0.42
	6.6	1.6	63321	422	2876	192	4.8	619	0.8	229	48	0.37
	7.2	1.6	58824	392	2526	168	5.4	566	1.0	247	46	0.44
	7.7	1.6	61427	410	2308	154	5.5	569	1.0	219	40	0.38
	8.3	1.6	58675	391	2349	157	6.0	554	1.1	245	41	0.44
	8.8	1.8	60640	404	2414	161	5.4	571	1.0	217	40	0.38
	9.6	1.6	62425	416	2098	140	5.2	561	0.9	233	45	0.42
	11.6	1.6	53173	354	2063	138	4.9	497	1.0	207	42	0.42
Mean	*8.2*	*2.1*	*59793*	*399*	*2393*	*160*	*5.3*	*563*	*0.9*	*230*	*43*	*0.41*
1	2	3	4	5	6	7	8	9	10	11	12	13

The first table is for women with <100 ug/l urinary iodine (women<100), the second for women with >100 ug/l urinary iodine (women>100), the third for the total population. (The 3 tables do not include individuals with >2000 ug/l urinary iodine).

From these tables the following observations can be made:

-Women with iodine levels < 100 have a total goitrogen load(TGL) expressed in PEC that is about 50% lower than women > 100 and than the overall population (columns 9).

-Split into the constituents, their perchlorate, nitrate and thiocyanate levels, over the entire T4 spectrum (Col. 2), are about 50%, 50% and 40% lower respectively (columns 5, 7 and 8).

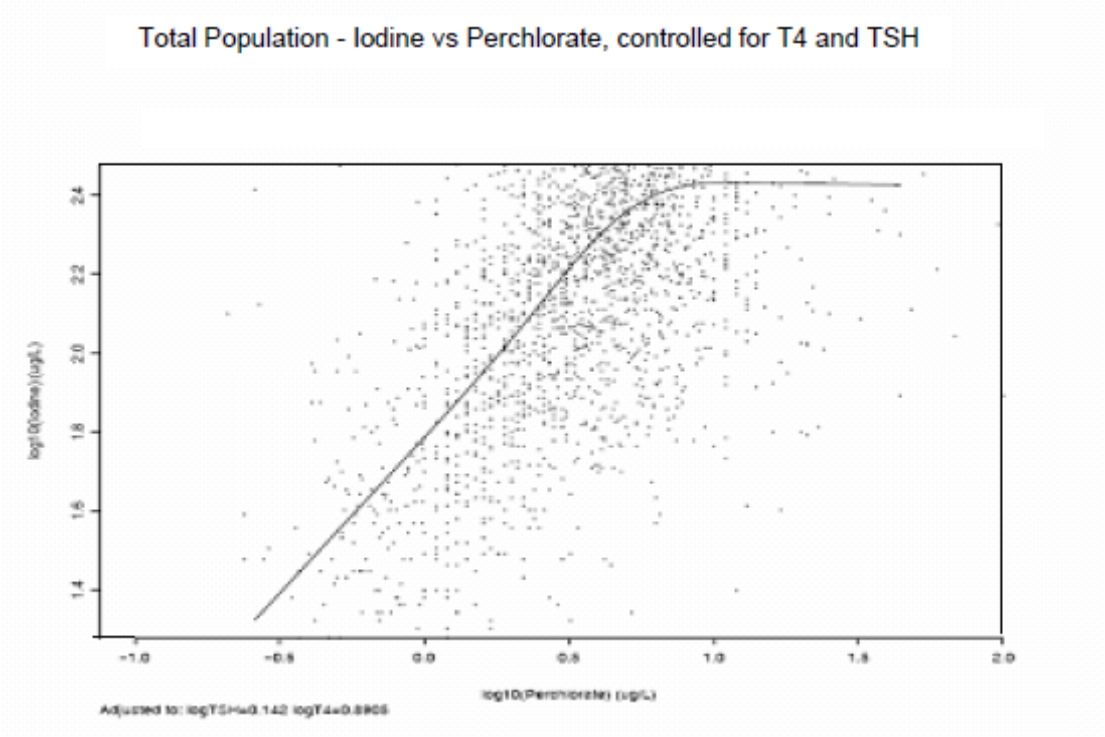
- **This means that women with insufficient iodine levels happen to be those that would be least susceptible to iodine uptake inhibition, if they would have been iodine sufficient.**

-As indicated also in the OIG study, perchlorate for all population groups represent less than 1% of the total goitrogen load expressed in PEC (columns 10).

- It confirms what OIG suggests: lowering the perchlorate intake by any rate (30, 60 or 100%) would indeed not diminish NIS iodine uptake inhibition, also not for iodine-insufficient (<100) women since the NIS iodine uptake inhibition potential caused by TGL which is more than 100 times larger, is already 50 % lower than for iodine sufficient women (>100). So besides the fact that, as OIG showed, eliminating all perchlorate even the background level would still leave 99% of PEC untouched, this remaining 99% is only half that of that of iodine sufficient women.

-Nevertheless women < 100 have a ratio of iodine to PEC equal to about 1/5 for every T4 percentile while women > 100 have about a ratio equal to ½ over the whole T4 spectrum (columns 13) which means that, even though the goitrogen load for iodine-insufficient women is only about half that for iodine sufficient women, their iodine inhibition potential is 2.5 times higher.

-For perchlorate this is explained by the fact that perchlorate, at low levels, is strongly related to iodine: see next figure:



Source: Gail Charnley, Perchlorate: Overview of Risks and Regulation, Food and Chemical Toxicology, 2008

This relationship suggests that low levels of perchlorate are naturally linked to iodine (both are formed in the atmosphere and both are found in naturally nitrate -rich soils in the US S-W deserts).
So women with low natural background levels of perchlorate have low natural background levels of iodine. For higher background levels of perchlorate the link with iodine ceases to exist.
So women with higher levels of iodine not only tend to have higher levels of natural perchlorate but additionally some from other (point) sources.

-For the other 2 goitrogens this suggests that those iodine-insufficient women also consume significantly less vegetables than the iodine-sufficient population. This could then also explain the higher non- natural levels of perchlorate from vegetables irrigated with synthetic perchlorate containing water such as the Colorado River. Nevertheless those synthetic perchlorate levels are themselves still insignificant compared to the total goitrogen load.

-All this strengthens the argument made by OIG that in the US population the potential iodine uptake inhibition does not lay with goitrogens and even less with perchlorate but clearly with a lack of iodine.

.

Conclusion

1. *Should a cumulative risk assessment be used to derive an MCL from the NAS RfD by applying not only a perchlorate RSC but also a PEC (Perchlorate Equivalent Concentration) RSC*?

The answer is yes.

1. Contrary to what is usual, the clinical trial on which the NOEL and the RfD for perchlorate is based is a trial on humans and not a trial on animals.

2. This implies that the RSC is either =1 or >1; this depends on the background contamination.

3 The background contamination of perchlorate is insignificant therefore in first instance the RSC would be =1.

4. However, in the case of perchlorate, to derive a MCL from the RFD a RSC taking into account the entire goitrogen load (TGL) i.e. the perchlorate equivalent concentration(PEC), should be applied.

5. This TGL was found to be significant and therefore the RSC is >1 and consequently this would cause the MCL to be larger than 24.5 ppb.

6. An RSC of 2.1 was calculated and therefore the MCL found was 51 ppb applying the same UF=10.

2. Should a risk assessment including lack of iodine be used to decide how iodine uptake inhibition by goitrogens should be combated?

The answer is yes

1. Since iodine insufficient women in the US have a total goitrogen load about half of that of iodine sufficient women confirms once more that the problem is iodine insufficiency.
2. In the US, low iodide nutritional status seems to be linked to a diet poor in vegetables. Or in other words people with a healthy diet sufficiently rich in vegetables tend to have a higher goitrogen load compensated by largely sufficient iodine. Health conscious people tend to ingest more goitrogens more than compensated by iodine intake (probably through food such as fish and other seafood or through iodine supplements).
3. Higher goitrogen load and a healthy diet seem, together with adequate iodine levels, to go hand in hand.
4. As a final conclusion, only a holistic approach decreases goitrogen related health risk: a healthy diet and a sufficient intake of iodine. Focusing on perchlorate is a useless distraction.

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Technical Review of

OIG Scientific Analysis of Perchlorate (Working Draft)

Final Report

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EXECUTIVE SUMMARY

ICF has reviewed the document *OIG Scientific Analysis of Perchlorate (Working Draft)* and provided a "marked-up" version of the text. This report provides ICF's responses to specific technical questions posed by EPA's Office of the Inspector General (OIG) related to the Scientific Analysis.

> *In ICF's opinion, which risk assessment approach is more appropriate to address this public health issue: A single chemical risk assessment or cumulative risk assessment? And why? Should the OIG refrain from recommending the use of a cumulative risk assessment approach and refocus the OIG audit to the issues associated with a single chemical risk assessment approach of perchlorate?*

Response: The general cumulative risk assessment paradigm can be used in any risk assessment where the adverse effect of concern results from the action of more than one stressor. In the case of perchlorate, there is sufficient evidence for the effect of multiple stressors to justify a risk assessment using "cumulative" risk principals. As discussed in Section 2, however, evaluating perchlorate health risks according to the most recently articulated cumulative risk framework (Teuschler 2007) using the most recent epidemiological findings would probably lead to the evaluation of risks based on data for the "whole mixture" rather than for individual components. Analyses of the newest NHANES III data (Blount et al. 2006b, Steinmaus et al. 2007) suggest that neither simple dose additivity nor effect additivity will be applicable to estimating risks to fetal development posed by perchlorate and other thyroid stressors. As discussed in Section 5, additional statistical analysis of the NHANES III data (and possibly additional data that is currently being gathered) would be necessary to fully characterize the nature of the interactions between chronic perchlorate exposures and other thyroid stressors before a usable exposure-response model can be developed.

With regard to OIG's recommendation, given the nature of the available data, it is inevitable that future EPA risk assessments for perchlorate will also take into account the simultaneous impacts of thiocyanate, iodide insufficiency, and other thyroid stressors. This can be done either explicitly using a "cumulative risk" paradigm or implicitly by estimating perchlorate risks over a set of "background" exposures to other stressors. Given the current uncertainty related to the nature of the interactions between perchlorate exposures and other stressors, it appears unwise for OIG to advocate a particular risk assessment methodology.

> *If the use of a cumulative risk assessment approach is appropriate, is the strategy recommended by the NAS Committee on Toxicity Testing and Assessment of Environmental Agents for the development of a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by the environmental agent appropriate?*

Response: Whether or not an explicit "cumulative" risk assessment model is adopted, some aspects of the Committee's recommendations will be applicable to perchlorate and others will not. The features of the "21st Century" methodology that are most applicable to perchlorate include the identification and characterization of a "toxicity pathway" or "key event" (Lambert and Lipscomb 2007) as the basis for the assessment, the explicit consideration of "background"

exposures to multiple stressors that contribute to the adverse effect, and quantification, to the extent possible, of the magnitude of variations of "host susceptibility." These principals also underlie current risk assessment practice.

Some of the Committee's recommendations are not applicable to perchlorate risk assessment, however, given the current state of knowledge. As discussed in Section 3, the Committee's recommendations are focused primarily on risk assessments of new chemicals for which there is little or no data from human studies and limited data from animal toxicity testing. Reliance on *in vitro* test results in such cases is sensible, especially if the "toxicity pathway" for the chemical is known so that the *in vitro* testing can be focused specifically in processes leading to adverse effects. In the case of perchlorate, however, the availability of data from numerous human and animal studies makes it unnecessary, and undesirable, to rely on *in vitro* data (such as sodium-iodide symporter, or NIS, inhibition) as the primary basis for quantitative risk assessment.

> *Is the subsequent pharmacokinetic modeling to identify a safe human exposure level that prevents the environmental chemical from reaching a toxic tissue concentration appropriate?*

Response: Because we have strong reservations about the use of *in vitro* tests as the primary basis for the perchlorate risk assessment, we also think physiologically based pharmacokinetic (PBPK) modeling will not play the dominant role in assessing safe human exposures envisioned in the Committee's paradigm. As discussed in Section 3, we believe that a possible role for the Clewell et al. (2007a) PBPK model would be to refine perchlorate dose estimates from the NHANES III (2001-2002) data set.

> *If the strategy of the NAS Committee on Toxicity Testing and Assessment of Environmental Agents is appropriate, is the Tonacchera Model an appropriate model to implement the strategy? If so, characterize and document the Tonacchera Model's uncertainties, limitations, and other issues that would need to be further addressed by EPA and/or the scientific community.*

Response: Our review of the Tonacchera et al. (2004) model found no technical problems with the experimental procedures and statistical analysis (see Section 4 and Appendix A). Its findings are generally consistent with previous and subsequent studies of human NIS kinetics and its inhibition by perchlorate and other ions. It is important to note, however, that the *in vitro* system used by Tonacchera et al. (2004) has a number of limitations that reduce its utility for quantitative risk assessment. In short, the *in vitro* model does not replicate important features of thyroid physiology that would affect maternal responses to thyroid stressors. It measures only instantaneous NIS inhibition, not thyroid iodide uptake and concentration over time (see Appendix B). In addition, it does not consider the complex and extensive network of controls in the hypothalamus-pituitary-thyroid (HPT) axis and liver that exists precisely to compensate for phenomena such as variations in short-term NIS inhibition and maintain maternal thyroid hormone levels. *In vitro* measurements of NIS inhibition appear to be poor predictors of the key event in perchlorate neurodevelopmental toxicity, which is reduced maternal thyroxine (T_4) levels during the first trimester of pregnancy.

If the Tonacchera Model is not appropriate, what model would ICF recommend (i.e., is there an appropriate model from which to construct a cumulative risk assessment from at this time)? Or how would ICF recommend how to proceed in conducting a cumulative risk assessment on this public health issue (e.g., as suggested, the uptake of iodide might not be the critical biological step to model)?

Response: In Section 5, we outline a possible approach to perchlorate health risk assessment that makes use of recent epidemiological data not available to previous risk assessment efforts. The key studies that enable this new approach are the analyses by Blount et al. (2006b) and Steinmaus et al. (2007) of the relationship between perchlorate exposure, urinary thiocyanate, smoking patterns, and iodide intake in the NHANES III (2001-2002) data. The basic approach would involve:

1. Identify the degree of serum free T_4 (fT_4) reduction in the first trimester of pregnancy that is likely to be of concern from the point of view of avoiding impairment of fetal neurodevelopment based on data epidemiological studies by Pop et al. (1999, 2003) and Kooistra et al. (2006), possibly using a benchmark dose methodology.

2. Fully characterize the statistical relationships and interactions between perchlorate exposure, urinary thiocyanate, cotinine/smoking history, iodine sufficiency, and other potentially confounding factors in the NHANES III (2001-2002) data set for reproductive-age women. Develop a comprehensive statistical model describing the relationships among the key exposure variables, confounders, and their effects on total T_4 (tT_4) levels.

3. Use the statistical model developed in the previous step to predict the daily dose of perchlorate (or the combination of perchlorate and thiocyanate) that would result in proportional serum tT_4 reductions equivalent to the level of concern for fT_4 derived in Step 1, when considered in conjunction with a reasonable set of other "background" exposures.

4. Recognizing that the calculations to this point in the assessment have been based on data from reproductive-age women and not from women in the first trimester of pregnancy, consider whether an additional degree of conservatism is needed in estimating a perchlorate (or perchlorate + thiocyanate) dose that is likely to be without harm to the developing fetal nervous system. If so, apply an appropriate uncertainty factor.

Major technical problems to be resolved in conducting this analysis would be the "unraveling" of the complex interactions between perchlorate, thiocyanate, smoking, and other stressors in the NHANES III data and evaluating the comparability of fT_4 changes measured in the Pop et al. (1999, 2003) studies to the tT_4 changes measured in the NHANES III data. Depending on when the assessment is conducted, additional data related to perchlorate-T_4 relationships in reproductive-age and pregnant women may be available from the 2007-2008 NHANES or other sources.

Comment on the NAS Committee's recommendation for the need to add iodide to all prenatal vitamins.

Response: It is hard to disagree with the desirability of maintaining adequate dietary iodine intake during pregnancy, and this concern is emphasized by the expanding database showing a strong relationship between iodine insufficiency during pregnancy and susceptibility to other thyroid stressors. On the other hand, there appears to be little support in the clinical and epidemiological literature for the desirability of universal iodine supplementation. There is a relatively narrow range between the recommended dietary intake for pregnant women (about 250 µg/day) and "excessive" iodine intake (500 µg/day) as defined by the World Health Organization (WHO). Excessive iodine intake, just like iodine insufficiency, can be associated with increased risk of goiter and other symptoms of thyroid dysfunction. Because there are wide regional variations in dietary iodide intake, it might be better to leave decisions about iodine supplementation to individual clinicians. We note that the Institute of Medicine (IOM) recommends increased iodine intake in pregnant women but stops short of recommending universal iodine supplementation, perhaps for this reason.

1. INTRODUCTION

1.1 Overview

ICF has been engaged by EPA's Office of the Inspector General (OIG) to undertake a technical review of the document *OIG Scientific Analysis of Perchlorate (Working Draft)* (U.S. EPA 2008). This report describes OIG's evaluation of the toxicological and epidemiological data related to human exposures to perchlorate and other thyroid stressors. In addition, it derives a Reference Dose (RfD) for human exposures to perchlorate and other thyroid stressors recognizing the important role played by simultaneous exposures to other "goitrogens" (chemicals that appear to act on thyroid iodide uptake by the same mechanism) and the important role of iodine insufficiency in contributing to the disturbance of thyroid function in pregnant women, fetuses, and infants. The RfD calculation is derived based on an analysis of the relative potency of perchlorate, thiocyanate, and other ions in the inhibition of iodide transport by the NIS in the thyroid gland. Estimates of potency are derived primarily based on data from an *in vitro* study by Tonacchera et al. (2004) supported by analyses of epidemiological studies of human populations exposed thiocyanate and perchlorate either occupationally, in diet, or in the course of volunteer clinical studies. Reviews of human exposure studies are used to estimate levels of Total Iodide Uptake (TIU) inhibition that appear to be associated with adverse effects on fetal and children's health, and a cumulative risk assessment is conducted for combined effect of the thyroid stressors perchlorate, thiocyanate, nitrate, and iodine insufficiency.

The overall conclusion of the analysis is that perchlorate exposures, at concentrations that have been found historically to occur in drinking water and diet, contribute only an insignificant portion of the total exposure to thyroid stressors (as measured in TIU) experienced by human populations when the presence of thiocyanate and other stressors are taken into account. An important implication of this finding is that reducing perchlorate exposures alone, even eliminating them entirely, will not significantly lower the overall thyroid stressor load or decrease the risk of adverse effects in sensitive populations. Given the important role played by iodine insufficiency in determining susceptibility to goitrogen exposures according to the TIU model, the OIG analysis suggests that a much more effective approach to limiting adverse effects from perchlorate (and other thyroid stressors) would be to mandate the use of iodine nutritional supplements during pregnancy.

1.2 Issues Addressed in this Review

In the Scope or Work for this review, OIG identified a number of important technical issues upon which it would like to receive comments. These included the applicability of a "cumulative risk" framework to evaluate the combined effects of multiple stressors on the thyroid, the use of the Tonacchera et al. (2004) *in vitro* analysis of NIS inhibition as the basis for the cumulative risk assessment, the validity of a specific characterization of "non-adverse" and "adverse" effects of perchlorate (and goitrogen) exposures, and the use of selected epidemiological studies to make inferences about the relative contribution of perchlorate and other stressors to increased risk of adverse effects. Subsequent discussions with OIG staff

resulted in the identification of a somewhat narrower range of key questions[1] that they particularly wished to have addressed, namely:

1. In ICF's opinion, which risk assessment approach is more appropriate to address this public health issue: A single chemical risk assessment or cumulative risk assessment? And why? Should the OIG refrain from recommending the use of a cumulative risk assessment approach and refocus the OIG audit to the issues associated with a single chemical risk assessment approach of perchlorate?

2. If the use of a cumulative risk assessment approach is appropriate, is the strategy recommended by the NAS Committee on Toxicity Testing and Assessment of Environmental Agents for the development of a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by the environmental agent appropriate? Is the subsequent pharmacokinetic modeling to identify a safe human exposure level that prevents the environmental chemical from reaching a toxic tissue concentration appropriate?

3. If the strategy of the NAS Committee on Toxicity Testing and Assessment of Environmental Agents is appropriate, is the Tonacchera Model an appropriate model to implement the strategy? If so, characterize and document the Tonacchera Model's uncertainties, limitations, and other issues that would need to be further addressed by EPA and/or the scientific community. If the Tonacchera Model is not appropriate, what model would ICF recommend (i.e., is there an appropriate model from which to construct a cumulative risk assessment from at this time)? Or how would ICF recommend how to proceed in conducting a cumulative risk assessment on this public health issue (e.g., as suggested, the uptake of iodide might not be the critical biological step to model)?

4. Comment on the NAS Committee's recommendation for the need to add iodide to all prenatal vitamins.

The remaining sections of this report address each of these issues more or less in order. There is some overlap among the various issues, and some of the either/or choices posed in the questions could not be addressed unconditionally. For example, the Tonacchera et al. (2004) results may be relevant to the risk assessment irrespective of whether the NAS/NRC Committee framework is employed, and some of the Committee recommendations could be relevant whether or not a cumulative risk methodology is selected.

Thus, Section 2 generally addresses the applicability of a cumulative risk assessment framework to perchlorate risk assessment with emphasis on recent developments in cumulative risk methods by EPA and other experts. Section 3 provides a brief review of the NAS/NRC Committee's recommendations for increased use of *in vitro* study results as the basis for quantitative risk assessment related to the utility of PBPK modeling to inform risk assessments. Section 4 reviews the Tonacchera et al. (2004) methods and results and evaluates their potential relevance in risk assessment. Finally, Section 5 integrates the information presented in the

[1] M. Wilson (EPA/OIG) e-mail to W. Mendez (ICF) April 3, 2008

previous sections and presents a methodology for a somewhat idealized "revised" perchlorate risk assessment that would make use of the most currently available data and methods.

Also enclosed with this document is a "marked-up" version of the OIG Scientific Analysis containing ICF's comments on the text.

2. APPLICABILITY OF MULTICHEMICAL (CUMULATIVE) RISK ASSESSMENT TO PERCHLORATE RISK ASSESSMENT

2.1 Background on Cumulative Risk Assessment

The risk assessment framework currently in use by EPA and other regulatory and advisory bodies consists of a range of methods and approaches growing out of a 1983 report by the National Research Council (NRC 1983). The report defined four key components of the risk assessment process: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification entails reviewing toxicological, clinical, and epidemiological data about the chemical(s) of concern, weighing the evidence that a chemical causes toxic effects, and evaluating the generalizability of toxic effects to humans. The dose-response assessment describes the magnitude of the toxic effect(s) as a function of exposure concentration or an appropriate dose metric, for a specified time frame of exposure. Exposure assessment is the quantitative and/or qualitative evaluation of chemical contact with the receptor. Risk characterization uses all of the information generated in the previous steps of the risk assessment to develop risk estimates for the exposure scenarios of interest. While risks may be assessment qualitatively, it is preferable to relate specific patterns of exposure to quantitative risks.

The risk assessment paradigm has been expanded and elaborated significantly since 1983 as the scientific community has recognized the complexities of chemical exposure scenarios and toxic mechanisms. One problem that frequently arises is that humans are exposed to multiple chemicals through multiple routes of exposure, and risk assessment methodologies have been developed to address these multi-pathway and multi-chemical exposures.[2] Some of the earliest multi-chemical (cumulative) risk assessments were conducted at contaminated waste sites with multiple pollutants, many of which had very little toxicity data. Out of necessity, methods were developed so that some form of quantitative risk assessment can be conducted when data are not available on the whole mixture to which receptors are exposed (which is almost never the case) or when high-quality toxicity data are available for only a few of chemicals present (which is frequently the case). The general strategy employed to support such assessments originally involved: (1) grouping chemicals in the mixture according to the type of adverse effects they cause (e.g., cancer) or with regard to the organ system that they affect (e.g., liver) and (2) looking for defensible ways to sum the different adverse effects or effects in different organ systems, with relatively little consideration of toxicological mode of action. (See for example U.S. EPA 1989.)

There has been a great deal of research on the general and specific problems associated with cumulative risk assessment since the general approach was first developed in the 1980s (U.S. EPA 2000, 2002b, 2003). The most important advances have been better definition of the

[2] Legislative mandates have also affected the science of risk assessment. For example, as early as 1980, Congress stated in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) that whole mixtures must be evaluated for their potential to adversely affect human health. In 1996, Congress passed the Food Quality and Protection Act (FQPA), requiring EPA to consider both aggregate (i.e., multiple routes of exposure) and cumulative (i.e., multiple chemical) effects when conducting risk assessments for pesticides and other food contaminants.

criteria that allow components of chemical mixtures to be grouped (by similarity of mechanism or similarity of endpoint), improvements in methods for combining risks within groups (dose additivity, effect additivity, and variations within both of these), and better incorporation of toxic mode of action into cumulative risk calculations (Lambert and Lipscomb 2007). The importance of "simple similar" or "simple dissimilar action" (i.e., either dose or response addition is applicable) is becoming much better understood (U.S. EPA 2002b, Teuschler 2007). These concepts are discussed in more detail with respect to perchlorate/goitrogen toxicity in the remainder of this section.

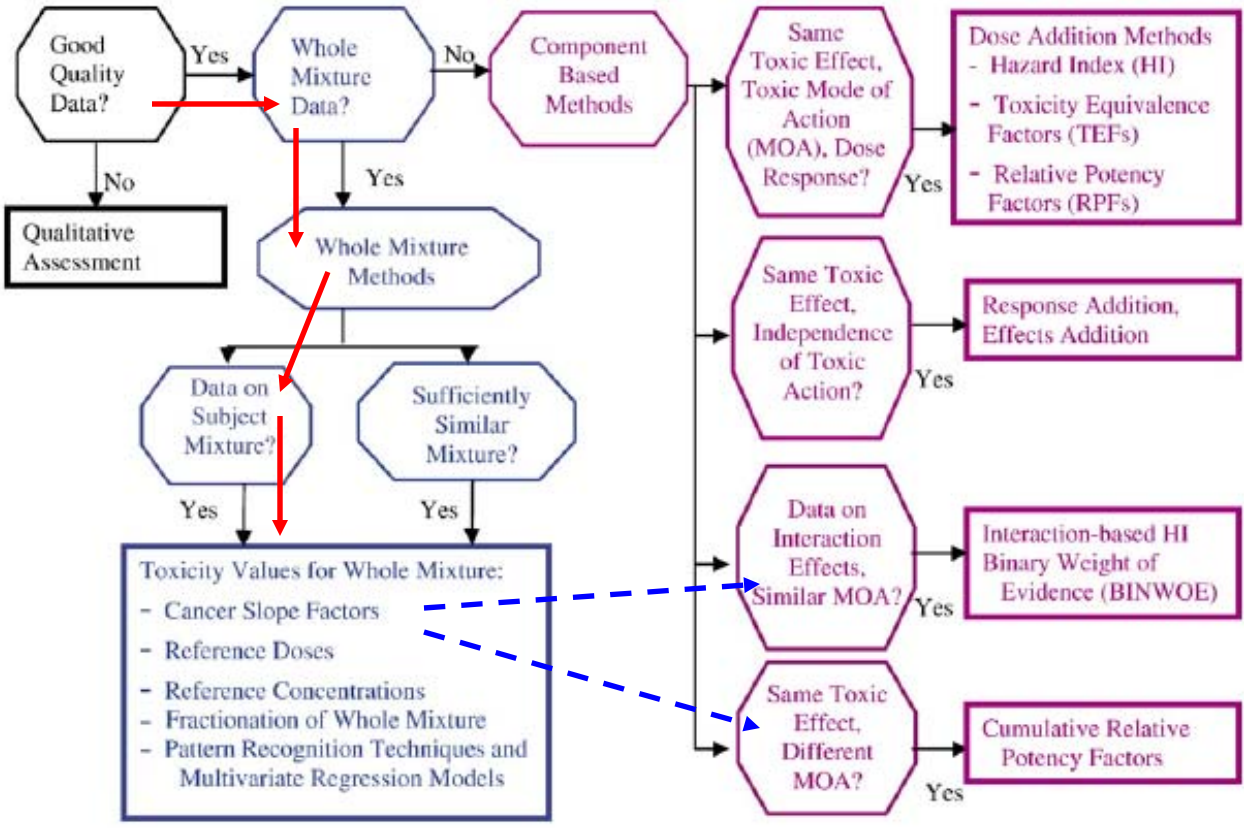
One important feature of "cumulative risk assessment" is that the general method can be applicable to a very wide range of exposure situations, in fact to any risk assessment where multiple stressors (chemical exposure or other factors) contribute to risk. In some sense, most modern risk assessments are "cumulative" in that they implicitly or explicitly take into account the effect of one or more "background" exposures in addition to the effect of the "index chemical" or principal toxicant. Application of "cumulative risk" methodology to a mixture of chemicals or stressors, however, does not always allow the application of dose- or effect-additivity that is generally considered to be the hallmark of "mixtures" risk assessment. Where data are available showing the effects of exposure to the mixture in question in a human population that is similar to the population of concern, it is preferable to use that data in the risk assessment rather than to try and estimate risks for the individual components. In addition, when there is evidence that the individual mixture components do not interact additively to cause the key event leading to adverse effects, it is likewise not appropriate to base a risk assessment on dose- or effect-additivity.

We believe that, strictly speaking, a "cumulative risk" method is entirely appropriate (in fact, necessary) for perchlorate. We also believe, however, that proper application of the latest cumulative risk paradigm (Teuschler 2007) results not in dose-additivity, but in the decision to use data on general population combined exposures to perchlorate, thiocyanate, and other thyroid stressors as the basis for the risk assessment.

2.2 A "Cumulative Risk Methodology" Applied to Perchlorate

Figure 2-1 provides a schematic diagram of the major steps in a cumulative risk assessment as described by Teuschler (2007). The diagram illustrates the major decision points in the assessment and the recommended methods that become available when specific decisions are taken. As shown in the figure, there are three major decision points: (1) deciding whether there is "good quality data," (2) deciding whether good data are available for the mixture as a whole, and (3) if not, deciding which of the component methods are most applicable to the individual components of the mixture. The approach summarized in the diagram, however, is highly idealized; decisions are not always clear-cut, and there may be competing data sets that suggest different courses of action. Above all, the method summarized in the diagram does not provide operational directions for the two most important steps of any risk assessment: (1) identifying the critical adverse effect and key events on the causal pathway leading to the critical effect and (2) identifying the most appropriate data set for use in estimating risks that the critical adverse effect will occur.

Figure 2-1. Conceptual Framework for Evaluating Chemical Mixtures Applied to Perchlorate (framework adapted from Teuschler 2007)



(The arrows indicate possible paths for perchlorate when the methodology is applied.)

2.2.1 Identification of Critical Adverse Effect and Key Event(s)

Risk assessment efforts for perchlorate (and for other agents) are often complicated by a lack of clarity about the identity of a "critical adverse effect." For purposes of risk assessment, the critical adverse effect is defined as the adverse effect occurring at the lowest exposure (or dose) in the most sensitive population group. Uncertainty about which effect is "critical" should be resolved by reviewing the available data; if uncertainty remains about which effect is critical, then separate assessments may be performed for each effect.

In the case of perchlorate and similar thyroid stressors, there are a number of candidates for the critical adverse effect. These include adverse neurodevelopmental effects on the first-trimester fetus, adverse neurological effects resulting from maternal hypothyroxinemia (low fT_4) later in pregnancy, and neonatal toxicity associated with perchlorate exposures in human milk. There are several reasons why the first of these qualifies as the critical effect. First, there are good mechanistic reasons having to do with the unique sensitivity of the fetus during this period. For approximately the first twelve weeks of development, the fetus has no functioning thyroid tissue and develops the capacity to synthesize and regulate the levels of its own thyroid hormones only slowly over the next two months. Second, important steps in the development of

the central nervous system (neurogenesis, differentiation, and migration) occur starting in the first trimester. These processes are dependent on adequate levels of triiodothyronine (T_3), the deiodinated form of the major thyroid hormone, T_4 (Morreale de Escobar et al. 2007, Obregon et al. 2005). Later during pregnancy, the fetus has a functional thyroid and is better able to maintain adequate T_3 levels.

Epidemiological data also support the selection of first trimester neurodevelopmental impairment as the key adverse effect. Epidemiological studies by Pop et al. (1999, 2003) on a cohort of Dutch women characterized the relationship between reduced maternal fT_4 levels and impaired neurobehavioral development to infants. An initial study conducted in 1999 reported that infants of women who had fT_4 levels below the 5th and 10th percentiles seen in the population at 12 weeks' gestation showed signs of neurological impairment compared to children of mothers with normal fT_4 levels during this period (Pop et al. 1999). A follow-up study observed mental and motor function defects in a subset of the original children at one and two years, demonstrating that adverse effects associated with low maternal fT_4 in early pregnancy persist into early childhood (Pop et al. 2003). A more recent study by Kooistra et al. (2006) also conducted in the Netherlands reported comparable result to those described by Pop et al. (1999, 2003). Kooistra et al. (2006) observed that newborns of mothers with first-trimester hypothyroxinemia demonstrated signs of neurodevelopmental impairment at three weeks of age, whereas infants of mothers who had f T_4 values that were between the 50th and 90th percentiles did not show signs of neurological damage.

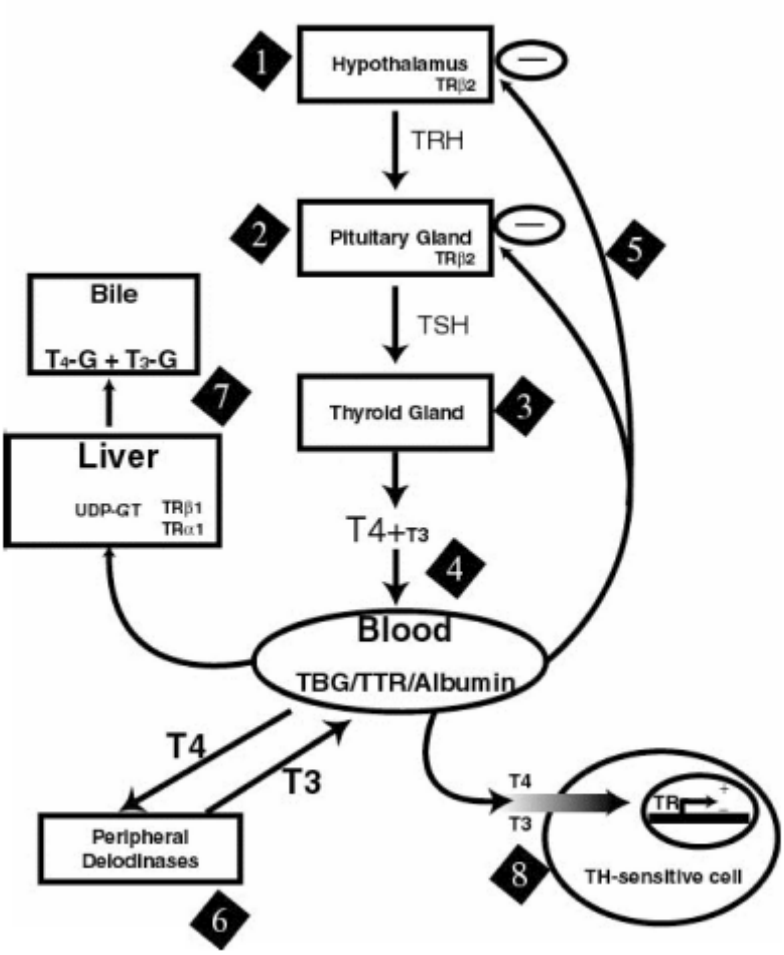
Importantly, Pop et al. (2003) also reported that children of women with low fT_4 levels in the first trimester of pregnancy, followed by a return to normal fT_4 levels in the second and third trimesters, achieved test scores similar to those reported for children whose mothers were euthyroid throughout pregnancy. Children of mothers who experienced hypothyroxinemia only during the second or third trimester also had normal test scores. Taken together, these studies strongly support the idea that first-trimester T_4 deprivation is the necessary precondition for long-lasting (possibly permanent) neurodevelopmental impairment in children.

In addition to identifying the critical effects, these studies provide evidence indicating that the key event that affects neurodevelopmental impairment in the newborn is low maternal fT_4 in the first trimester of pregnancy that is not compensated for in the second and third trimesters of pregnancy. In the general population, the likelihood of this key event is influenced by a wide range of factors, including iodide deficiency, goitrogen exposures, exposures to other agents (e.g., PCBs and pesticides) that act on the thyroid, and maternal HPT axis controls and other physiological factors (e.g., human chorionic gonadotropin [HCG], estrogen levels, liver deiodinases), and by other less understood genetic factors.

The identification of maternal hypothyroxinemia during the first trimester as the most likely key event in the toxicity pathway is based on two considerations. First, maternal T_4 levels represent an important "nexus" in the myriad control pathways that govern the overall thyroid "economy" (see Figure 2-2). Serum T_4 levels represent the most important signal that is monitored by the HPT axis and controlled by changes in levels of thyroid stimulating hormone (TSH) secretion and by other physiological mechanisms. Second, as discussed above, maternal

hypothyroxinemia has been clearly identified by human epidemiological studies as being directly associated with the critical adverse effect.

Figure 2-2. Simplified Schematic of Major Thyroid Hormone Control Pathways (Source: Zoeller et al. 2007)



The same cannot be said for other physiological indicators of thyroid function. For example, there are no human data linking short-term NIS inhibition to adverse effects. In fact, the available data (human occupational and volunteer studies) suggest that relatively high levels of NIS inhibition can be well-tolerated for short periods without adverse effects on the thyroid economy. In addition, as shown in Figure 2-2, there are many other physiological processes and controls that interact to determine overall T_4 levels. Some of these processes have no doubt evolved precisely to maintain T_4 levels in the presence of short-term NIS inhibition.

2.2.2 Selection of Data for Use in the Perchlorate Risk Assessment

Appendix C provides a brief summary of previous risk assessment efforts for perchlorate and shows how the analyses have evolved as new data have become available. Assessments have progressed from the use of animal studies (U.S. EPA 2002c) to the use of human volunteer

studies (Dollarhide et al. 2002, ICF 2004, Cal/EPA 2004, NRC 2005) and epidemiological studies (Strawson et al. 2003, Gibbs 2006, Crump and Gibbs 2005). In each assessment, the analysts provided reasons as to why the data set that they chose was the best and most appropriate. They did not always agree as to which study was best, but there has always been general agreement that: (1) high-quality human data should be used when they are available in preference to animal studies and *in vitro* data and (2) human studies that measure key events in populations similar to the most sensitive group are preferable to studies that record precursor events in populations that differ from the population of concern.

Most of the risk assessments conducted since 2002 selected data from the Greer et al. (2002) volunteer study as the most appropriate for risk assessment, primarily because it is unique among human studies in that it directly measures, rather than infers, human perchlorate doses, and because it documents changes in both iodide uptake and changes in thyroid hormone levels. Nevertheless, it is not an ideal study due to the short-term nature of exposures and because (as noted by OIG) the reported results do not account for potential impacts of variations in iodide intake and exposure to other thyroid acting agents. Similarly, the epidemiological studies of perchlorate exposure available until quite recently also have limitations arguing against their use in risk assessment for neurobehavioral development. Reviewing these studies is beyond the scope of this analysis, but in general, epidemiological studies of perchlorate risks prior to 2006 have one or more of the following weaknesses:

- Perchlorate (and other goitrogen exposures) were estimated (in "ecological" studies) rather than measured.
- Some measured endpoints (e.g., neonatal thyroid function) were not relevant to the key adverse effect.
- Some populations (e.g., in the Chilean epidemiological studies) had histories of extremely high iodine exposures and high incidence of thyroid dysfunction (goiter).
- Other studies did not measure or estimate iodide intake
- Occupational studies were conducted primarily in males already acclimated to high perchlorate exposures.[3]

For these reasons, we found little to recommend selection of epidemiological studies prior to 2006 in support of a quantitative risk assessment for perchlorate. Rather, two newer studies (Blount et al. 2006a, 2006b, Steinmaus et al. 2007) appear to exhibit most of the desirable qualities for risk assessment. Using NHANES III data, Blount et al. (2006a) measured low-level chronic perchlorate exposures in 100 percent of 2,880 urine samples collected. Exposure to perchlorate was correlated with reduced fT_4 and increased TSH in women of child-bearing age with low levels of urinary iodine (<100 μg/L) but not in iodine-sufficient women. Blount et al. (2006b) also examined the effects of co-exposures to nitrate and thiocyanate (as estimated from urinary excretion) and did not find that either correlated consistently with fT_4 or TSH levels. Steinmaus et al. (2007) expanded the analysis of NHANES III data and observed that urinary thiocyanate and cotinine (i.e., smoking) interact to increase the slope of the effect of perchlorate on levels of fT_4. Their analysis found, however, that thiocyanate and smoking individually were not statistically significant predictors of fT_4 levels. These two studies present data from a large

[3] Some of these limitations apply with equal force to available epidemiological studies of exposures to thiocyanate.

sample of a population (reproductive-age women in the United States) that is very similar to the population of concern (pregnant women in the United States). Additionally, while they do not directly measure the adverse critical effects, they do report on the key event (reduced T_4 levels) that is highly predictive of the adverse effects. Finally, they provide estimates of individual exposures not only to perchlorate, but also to thiocyanate as well as information on an exhaustive catalogue of potential confounding variables including race/ethnicity, age, smoking history, previously diagnosed thyroid disease, and use of medications that could effect thyroid hormone levels.

Therefore, as discussed in more detail in Section 5, we would select the NHANES III (2001-2002) data as the most suitable for risk assessment. Like the previously conducted studies, the NHANES III data have their limitations (also discussed in Section 5), but overall, these data provide important new information concerning the relationships between perchlorate exposures, exposures to other thyroid stressors, and increased risk of hypothyroxinemia.

2.3 Application of the Cumulative Risk Methodology to Perchlorate

Having identified the critical adverse effect and key events in the "toxicity pathway" (see Section 3) and having identified the most appropriate data set for use in risk assessment, it is now appropriate to apply the cumulative risk methodology described by Teuschler (2007) to perchlorate. (As noted in Section 2.1, we have already determined that applying this methodology is appropriate given the known impacts of simultaneous exposures to perchlorate, thiocyanate, and other thyroid stressors.)

Referring back to Figure 2-1, we can answer the first question as to whether we have "Good Quality Data." As indicated by the red arrow in the upper left-hand corner of the diagram, the answer is "yes." The next determination needed is whether we have data on "the Whole Mixture." The answer to this question is again "yes" because our selected data set (NHANES III) measures exposures to perchlorate, goitrogens, and other thyroid stressors typical of those that would be experienced by the population of concern (pregnant women). We therefore move down to the "Whole Mixture Method" box and decide whether we have "Data on the Subject Mixture." The answer is again "yes" for the reasons outlined above. The methodology then sends us on to the "Toxicity Values for Whole Mixture" box.

At this point, the application of the Teuschler (2007) methodology becomes less clear-cut. The "Toxicity Values for Whole Mixture" box includes only a limited set of alternative approaches for estimating safe exposure levels, such as establishing an RfD for the mixture taken as a whole. As discussed in more detail in Section 5, it is not really clear how to proceed at this point because we believe that additional statistical analysis of the NHANES III data are needed to more adequately elucidate the interactions between iodine insufficiency, perchlorate exposure, thiocyanate exposure, and other confounding factors. Depending on the results of that analysis, it might be possible to develop some kind of comprehensive statistical model for predicting risk as a basis of the combined impact of the various stressors. That might lead back (in effect) to one of the more complicated approaches for addressing mixture components shown in the lower right-hand corner of the diagram (the dotted blue arrows).

2.4 Summary

The cumulative risk paradigm is clearly appropriate for use in implementing a perchlorate risk assessment. After carefully identifying the critical adverse effect and key events preceding the adverse effect and selecting the most appropriate data set, the methodology leads to a hybrid of the "whole mixture" approach and some variant of an approach for characterizing the adverse effects of interacting mixture components. It is most unlikely that application of the cumulative risk methodology to perchlorate would result in the selection of dose- or effect-additivity based on short-term NIS inhibition.

3. APPLICABILITY OF NRC COMMITTEE'S "TOXICITY TESTING IN THE 21ST CENTURY" PRINCIPALS TO PERCHLORATE RISK ASSESSMENT

In June 2007, the National Research Council (NRC) Committee on Toxicity Testing and Assessment of Environmental Agents published a report "Toxicity Testing in the 21st Century: A Vision and a Strategy" (NRC 2007), which proposed new approaches to characterizing the toxicity of chemicals and for assessing human health risks associated with chemical exposures. The report proposes a number of important changes in the way toxicity testing is performed and in the methods used to assess risks. In this section, we discuss the degree to which the principals put forward by the NRC Committee may be applicable to conducting a human health risk assessment for perchlorate exposure and discuss the role of PBPK modeling, a key element of the NRC recommendations, in the assessment of safe human doses. As noted previously, the applicability of the NRC Committee proposals to perchlorate can be evaluated irrespective of the specific risk assessment paradigm (single chemical or cumulative risk) that is chosen to evaluate perchlorate risks.

3.1 Overview of NRC Approach

The publication of the NRC report (NRC 2007) culminates a long process during which experts from regulatory agencies, academia, and stakeholders with concerns related to environmental health, animal welfare, and regulatory compliance costs considered possible changes to the way in which risks from chemicals are identified, characterized, and quantified. In the 2007 report, the NRC Committee presents a "long-range vision and strategic plan to advance toxicity testing and considers its vision within the current regulatory framework." (p. 2)

The overall objectives ("design criteria") behind the report were:

> "…(1) to provide broad coverage of chemicals, chemical mixtures, outcomes, and life stages, (2) to reduce the cost and time of testing, (3) to use fewer animals and cause minimal suffering in the animals used, and (4) to develop a more robust scientific basis for assessing health effects of environmental agents." (p. 3)

Thus, the report does not just propose a new framework for risk assessment, but also a new, more efficient and cost-effective approach to gather data to support risk assessments, based on a better understanding of the fundamental mechanisms through which chemicals cause adverse effects in humans:

> "Fresh thinking and the use of emerging methods for understanding how environmental agents affect human health will promote beneficial changes in testing of these agents and in the use of data for decision-making. The envisioned change is expected to generate more robust data on the potential risks to humans posed by exposure to environmental agents and to expand capabilities to test chemicals more efficiently." (p. 2)

The core of the NRC proposal involves a transition from the current approach of assessing human toxicity using studies of "apical events" (health effects in animal studies) to assessing risks based on more "basal" events (alterations in cellular function that can lead to

adverse effects in humans). The approach is based on the identification of "toxicity pathways," which are "…cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects…" (p. 4). *In vitro* tests are used to study the effect of potentially toxic chemicals on the critical cellular pathways, and the results are used to predict the concentrations of chemicals (or key metabolites) that would disturb these pathways in humans. In the NRC report, a number of potential "toxicity pathways" are identified for specific agents. Essentially all of the examples cited refer to changes in gene expression profiles induced by toxic agents. There is, however, no reason that other types of cellular mechanisms (such as thyroid iodide uptake inhibition) might also be identified as toxicity pathways. This issue will be discussed in more detail later in this section.

Once the toxicity pathway is identified by *in vitro* tests, risks are then assessed by estimating the exposures levels in environmental media that would result in the potentially toxic chemical (metabolite) levels in the target organs. PBPK models are intended to play a role in this process:

> "…physiologically based pharmacokinetic modeling would then be used to predict human exposures that lead to tissue concentrations that could be compared with the concentrations that caused perturbations in vitro…" (p. 9)

That is, pharmacokinetic data from more conventional animal tests (as well as *in vitro* studies) would be used to inform the risk assessment decision. As an example, the perchlorate PBPK model developed by Clewell et al. (2007a) uses human and animal data to estimate perchlorate concentrations in maternal and fetal tissues in response to specified perchlorate intakes in diet and drinking water. In theory, such a model could be used to identify perchlorate intake levels resulting in a "critical" concentration of perchlorate in maternal blood. In practice, however, the Clewell et al. (2007a) model does not incorporate information related to HPT axis controls on iodide uptake that are necessary to draw any conclusions about perchlorate effects on maternal thyroid hormone levels. This aspect somewhat limits its utility in fulfilling the function foreseen by the NRC committee.

The NRC committee paradigm also proposes a role for human exposure data, where they are available, namely in the assessment of "background" exposures and receptor variability:

> "…human data would provide information on background chemical exposures and disease processes that would affect the same toxicity pathway and provide a basis for addressing host susceptibility quantitatively…" (p. 9)

That is, human data could be used to characterize exposure patterns for mixtures of agents thought to affect the same endpoint, identify highly exposed populations, or identify "susceptible" populations where environmental exposures result in greater responses (biomarkers or adverse health outcomes). These data would also be used to quantitatively estimate the differences in response within and across exposed populations. The epidemiological studies by Blount et al. (2006b) and Steinmaus et al. (2007) discussed in Section 2 provide good examples of analyses characterizing the differences in sensitivity to thyroid-affecting agents across groups (men, iodine-sufficient and iodine-insufficient pregnant women) and evaluating the nature of

interactions in exposures to different thyroid-acting agents (perchlorate, thiocyanate/smoking, nitrate).

3.2 Limitations of NRC Approach Applied to Perchlorate

To place this issue in context, however, the proposed NRC reforms to toxicity testing and risk assessment are as yet untested. More specifically, ICF is not aware of any quantitative human health risk assessment for any agent that has been exclusively grounded with *in vitro* test results, supported by PBPK modeling to assess critical target organ doses, and by human exposure data to assess receptor variability in response. Identifying "toxicity pathways" in the sense intended by the NRC Committee, quantifying the effects of chemical agents on these pathways, and characterizing the relationship of the toxicity pathways to human health risks is a very complex undertaking. (Perchlorate is a case in point.) EPA and academic researchers are just now beginning to study the impacts of chemical exposures on gene expression patterns and cellular control pathways suspected of being linked to adverse effects for a few well-studied agents, and much more research is needed before a workable risk assessment paradigm based on these effects is developed.

Thus, applying the entire NRC paradigm to the perchlorate risk assessment is unlikely to be successful. While there is a large amount of data related to the potential "toxicity pathways" (including NIS inhibition), the relative importance of the myriad acute and longer-term physiological responses and control pathways in determining maternal thyroid hormone levels during pregnancy is not known. Similarly, the relative impacts of perchlorate and other exposures (goitrogens and other agents) on these many interlocking pathways are also largely unknown. In summary, there are too many control pathways and compensatory mechanisms between the thyroidal NIS and maternal T_4 levels to allow clear identification of a single toxicity pathway or characterize the effects of all of the environmental stressors on those pathways. The quantitative connection between the Tonacchera et al. (2004) results in acute *in vitro* tests and the critical toxic event (decreased maternal T_4 in the first trimester) is insufficiently characterized to allow reliance on those results for risk assessment.

Another aspect of the NRC proposal is that it is intended for use primarily on a large, as-yet unknown, universe of chemicals for which significant animal and human data are not available. It is intended not only to improve the mechanistic basis for risk assessment, but also to conserve expensive animal test resources. In the case of perchlorate, however, ICF does not believe that the NRC committee would recommend reliance on *in vitro* test results to support a risk assessment for which so much high-quality human and animal data are available. As discussed in more detail in Section 5, ICF believes that there are sufficient high-quality human epidemiological data to support a risk assessment, irrespective of the results of the *in vitro* test results of Tonacchera et al. (2004) and others.

3.3 NRC Principals that Are Applicable to Perchlorate

Applying the NRC Committee recommendations is not an "all-or-nothing" decision, and their applicability does not depend on whether perchlorate risks are viewed in a single- or

multiple-chemical framework. Indeed, some of the NRC principals are eminently applicable to perchlorate.

The NRC idea of defining a "toxicity pathway" is clearly important. The recognition that chemical impacts on cellular control pathways are the basic phenomena underlying adverse effects cannot be emphasized enough. In fact, risk assessment models based on key biochemical and physiological processes (biologically based dose-response [BBDR] models) have been a "Holy Grail" of risk assessment for some time. As discussed in Section 2, EPA researchers are working to develop a systematic framework for incorporating mode of action ("key events") into risk assessment for chemical mixtures (Lambert and Lipscomb 2007), and this general principal is clearly applicable to perchlorate. Another notable attempt in this regard is the joint effort sponsored by EPA, CIIT, EPRI, and their contractors to develop a biologically-based dose response model for inorganic arsenic (Clewell et al 2007b).

Another concept included in the NRC paradigm that is clearly applicable to perchlorate is the consideration of "background" exposures and exposures to multiple stressors that could contribute to the critical adverse effect. It goes without saying that whatever specific model is used to characterize the risk associated with perchlorate exposure, it will be necessary to consider exposures to other goitrogens, other thyroid-active agents, and stressors such as iodine insufficiency. This is true whether perchlorate adverse effects are estimated in an explicit multi-chemical context or estimated individually in the context of a "background" of combined thyroid stressors.

Finally, the NRC recommendation for quantitatively addressing variations in "host susceptibility" deserves strong endorsement. Variations in susceptibility can contribute significantly to the overall uncertainty in quantitative risk assessment as is clearly the case for perchlorate. The available data show that women react more strongly to iodide insufficiency, perchlorate, and other goitrogen exposures than men and that identifiable physiological changes during pregnancy strongly influence thyroid hormone levels. As will be discussed in more detail in Section 5, there are high-quality epidemiological studies that characterize the extent of variation in maternal T_4 levels through pregnancy (Casey et al. 2007) and show the quantitative relationships between variations in first-trimester T_4 levels and adverse neurological outcomes in infants and children (Pop et al. 1999, 2003, Kooistra et al. 2006). Similarly, Blount et al. (2006a) have studied the distributions of perchlorate exposures in reproductive-age women, and Blount et al. (2006b) and Steinmaus et al. (2007) have characterized the statistical relationships between women's exposures to perchlorate and other goitrogens, iodide insufficiency, and changes in T_4 levels. As will be discussed in Section 5, an "ideal" risk assessment for perchlorate should involve explicit (quantitative) analyses of the relationships between variations in exposures and variations in response (maternal T_4 levels) and analyses of interactions among thyroid stressors in causing changes in T_4 levels.

3.4 The Role of PBPK Modeling in Perchlorate Risk Assessment

A key feature of the NRC paradigm discussed above is that *in vitro* test results would be coupled with PBPK modeling in performance of the risk assessment. The idea is that the *in vitro* test data will identify target organ doses (chemical or metabolite concentrations at key receptors)

that could be associated with disturbances of normal cellular functions, and that PBPK modeling could then be used to estimate human doses that would result in the critical target organ doses.

As discussed in the previous sections, we do not believe that *in vitro* test results are likely to be the primary source of data for a perchlorate risk assessment. Thus, it does not appear that PBPK modeling would play the central role envisioned by the NRC Committee in a perchlorate risk assessment. As discussed in Section 2, ICF believes that the best data set to support a revised risk assessment would the NHANES III data on the relationship between perchlorate, other thyroid stressor exposures, and changes in T_4 levels. If this data set were used, the role of PBPK modeling would probably be limited to refining the human dose estimates developed by Blount et al. (2006b) based on measurements of urinary perchlorate. The PBPK model developed by Clewell et al. (2007a) would probably be well-suited for this task. It is not clear, however, the extent to which the perchlorate dose estimates for the NHANES III data would be improved beyond those already derived by Blount et al. (2006b) on the basis of simple creatinine normalization. Application of the Clewell et al. (2007a) model would, after all, require similar assumptions to be made regarding creatinine excretion and other physiological parameters in the exposed population, and even then there would be little basis for judging the relative quality or accuracy of the PBPK dose estimates versus those derived using simpler methods.

4. UTILITY OF TONACCHERA MODEL IN PERCHLORATE RISK ASSESSMENT

In the previous two sections, we discussed the applicability of different risk assessment paradigms (single-chemical versus multi-chemical cumulative, the NRC "21st Century" recommendation for the use of *in vitro* data) to the assessment of the human health risks associated with perchlorate exposures. In this section, we briefly review the potential applicability of the data and analyses presented by Tonacchera et al. (2004) in providing information to support such an assessment.

4.1 Overview of Tonacchera et al. (2004) Study and Model

Tonacchera et al. (2004) present the results and analysis of a set of experiments in which the gene for the human sodium-iodine symporter (NIS) is introduced into, and expressed by, Chinese Hamster Ovary (CHO) cells. The investigators present evidence showing that the NIS protein is expressed on the surface membrane of the cells and that it can actively transport iodide ions into the cells. They also studied the degree to which iodide uptake is inhibited by different concentrations of perchlorate as well as by other ions (thiocyanate, nitrate) known to inhibit iodine uptake than the NIS. Iodide uptake is assayed by measuring the uptake of radioactive iodide in to the cells over a time course of 45 minutes in the presence of different concentrations of total iodide and inhibitor ions in the external medium.

Tonacchera et al. were not the first to "clone" the NIS gene or to study the inhibition of the NIS by perchlorate and other anions, and their general findings (iodide uptake rates or V_{max} and the relative potency of NIS inhibition by different individual ions) are similar to those seen by previous investigators (Dai et al. 1996, Eskandari et al. 1997, Yoshida et al. 1997, 1998). The major innovation in the Tonacchera et al. (2004) study was the measurement of NIS inhibition by simultaneous exposures to mixtures of inhibitors rather than by individual ions. In addition, they fitted a statistical model to the data on mixtures of inhibitors that strongly supported the idea that such mixtures act additively through a common competitive mechanism to inhibit iodide transport into cells. Using their model, they assigned relative NIS inhibition potencies to perchlorate (1.0), thiocyanate (1/15), and nitrate (1/240), which they showed could be used to reliably predict the extent of NIS inhibition by mixtures of these ions across the entire range of concentrations that had been studied. They then discussed the implications of their findings with regard to establishing safe levels of exposure to perchlorate and other ions.

4.2 Evaluation of Tonacchera et al. Model

We have briefly reviewed the experimental procedures and statistical methods used to interpret the results of the Tonacchera et al. experiments. To summarize, the basic experimental procedures appear to be appropriate and seem to accurately characterize the relative degree of instantaneous NIS inhibition under conditions (iodide and goitrogen levels) not too different from "normal" environmental exposures. As noted above, the basic kinetic parameters for iodide, perchlorate, thiocyanate, and nitrate derived by Tonacchera et al. are also consistent with those seen by other investigators (Dai et al. 1996, Eskandari et al. 1997, Yoshida et al. 1997, 1998), providing further confidence in the overall reasonableness of the estimates. As discussed

later in this section, however, the *in vitro* gene expression system they used does not match the *in vivo* physiological conditions of NIS operation in all regards. In addition, Tonacchera et al. measured only short-term (instantaneous) NIS inhibition, not long-term thyroid iodide uptake.

As part of our review of the Tonacchera et al. model, we obtained spreadsheets containing the radioactive iodide uptake (RAIU) data used to derive the multi-ion inhibition model from Dr. John Gibbs. Our analysis was confined to a review of data selection (treatment of outlying observations, etc.) and evaluation of the statistical methods that were used to fit the model for NIS inhibition. The results of our analysis are described Appendix A. To summarize briefly, we found relatively few discrepant (apparent outlier) observations, and the inclusion or exclusion of these observations made little difference in the overall results of the statistical modeling. The general form of the statistical model reported by Tonacchera et al. was appropriate for analysis of the data, and consistent with the independent competitive inhibition of the NIS by the various anions. Our application of the same "Hill" model gave results (relative inhibitory potencies for perchlorate, thiocyanate, and nitrate) very similar to those found by Tonacchera et al. We obtained different estimates of parameter variances, but this may have been due to slight differences in details of statistical model implementation (likelihood functions) or in different software packages.

Thus, on the whole, we found no major technical problems with the Tonacchera et al. (2004) study to the extent that this could be confirmed without a formal data audit. The consistency of their results with those from other laboratories and the good statistical fit to a model that represents independent competitive inhibition by the various ions reinforces the overall level of confidence in the quality of the Tonacchera et al. results.

4.3 Applicability and Limitations of the Tonacchera et al. Model in Perchlorate Risk Assessment

In Sections 2 we discussed our reservations about the role of "classical" (additive) cumulative risk models and the use of *in vitro* results (in general) for perchlorate risk assessment. Our overall conclusion, which will be further elaborated in Section 5, is that any new health risk assessment for perchlorate is likely to be based on analyses of data from recently published epidemiological studies with considerations of biochemical mechanism playing a minor role. This conclusion is established first on the principal that quantitative risk assessment should be based on data from suitable human studies, if they are available, in preference to data from animal or *in vitro* studies. In the particular case of perchlorate, there is also the concern that short-term NIS inhibition, or even short-term inhibition of thyroid iodide uptake, is rather far-removed from the "key event" (reduced maternal T_4 levels) leading to adverse effects on the fetus (see Section 2.2). In our 2004 analysis of perchlorate toxicity (ICF 2004), we cited the following reasons for <u>not</u> choosing short-term iodide uptake inhibition as the key event for assessing perchlorate toxicity:

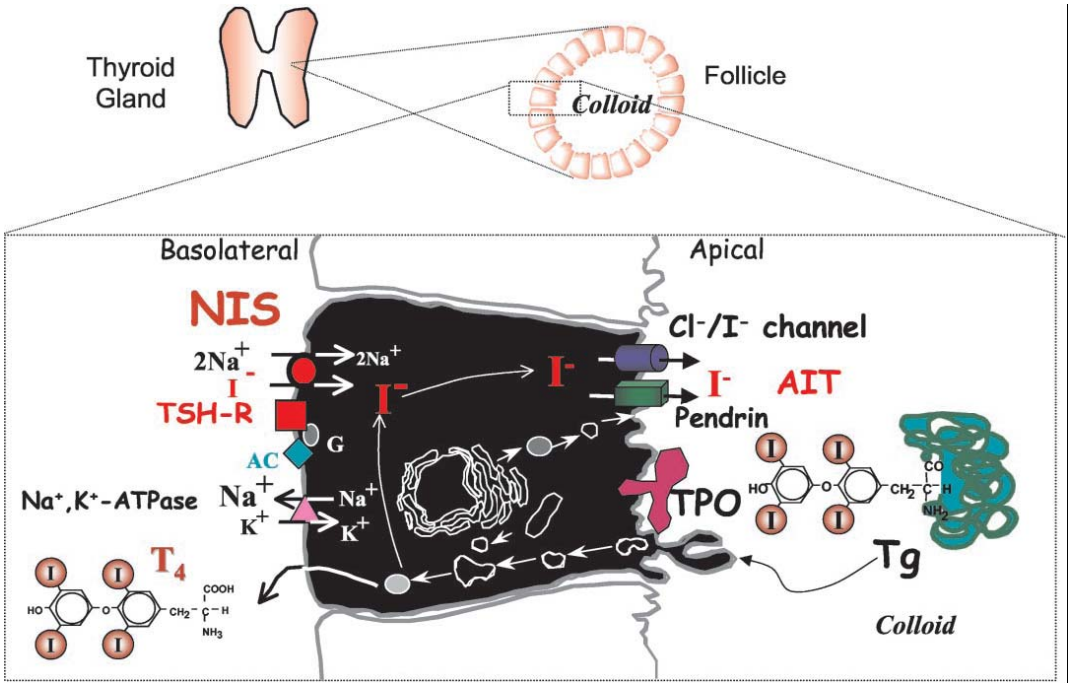
> "We disagree with this choice based on the judgment that short-term iodine uptake inhibition, like TSH, is a poor predictor of potential adverse effects of prolonged perchlorate exposures in sensitive populations. Many studies of the interactions between iodine intake, pregnancy, and maternal hypothyroxinemia (Burrow et al. 1994; Chan and

Kilby 2000; Zoeller 2003) indicate that the interaction between iodine intake, HPT axis homeostatic responses, and changes in circulating hormone levels are complex, time-dependent, and highly variable."

Since that time, much new data on perchlorate toxicity has become available, but it has not changed our basic opinion; in fact it has strengthened it, as discussed below.

Short-term NIS inhibition (as measured by Tonacchera et al. 2004) is too far removed from the "key event" to be a useful predictor of risk. In the first place, the *in vitro* test system employed by Tonacchera et al. does not adequately mimic the physiology of even short-term thyroid iodide accumulation. Figure 4-1 illustrates the position of the NIS and other transporters and physiological processes that affect iodide uptake and thyroid hormone synthesis and release in a thyroid cell.

Figure 4-1. Overview of Follicular Cell Control Iodine Pathways (Source: Dohan et al. 2003)

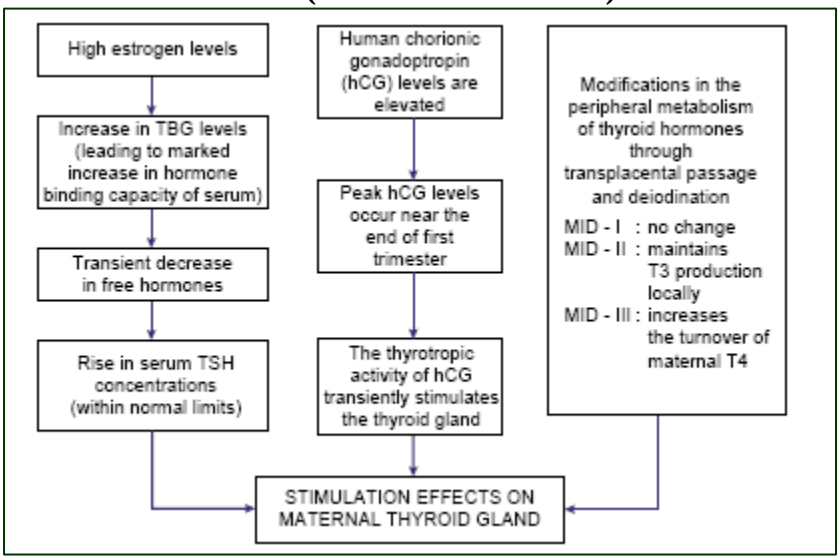


These features include cell polarity with the NIS being positioned exclusively on the basolateral (or basal membrane), a pre-existing iodine concentration gradient in the cell, and a pre-existing (and energy-linked) sodium gradient maintained by the sodium-potassium ATPase that drives iodide concentration in the cell above the levels in circulation. In Appendix B, we use a simple PBPK model to explore the differences between the patterns of short-term NIS inhibition and thyroid iodide uptake that would be expected solely on the basis of the physical geometry of the thyroid.

In addition to cell configuration, there are thyroid-specific transporters that drain iodide and other ions through the apical membrane; this process is tightly coupled with iodine organification, which is mediated by thyroperoxidase (TPO). Finally, there is the mobilization of thyroid hormones from the colloid back into the follicular cell and the TSH-driven control of NIS and other enzyme expression. None of these specific processes are present in the CHO cells used by Tonacchera et al.

In particular, the HPT axis signaling mechanisms (see Figure 2-2) provide oversight of the "thyroid economy" during pregnancy, exercising controls on the NIS and a range of other genes to assure adequate iodide uptake and the maintenance of adequate thyroid hormone levels. In addition, pregnancy itself imposes an additional set of physiological changes on the HPT axis (see Figure 4-2). The response of these systems to perchlorate and other goitrogen exposures is not well understood (in fact, it is pretty much unknown) compared to the impact of these ions on short-term NIS inhibition.

Figure 4-2. Regulation of Thyroid Function during Pregnancy (Source: Glinoer 2004)



None of these control processes are active (or at least active in the same way) in the *in vitro* system used by Tonacchera et al. to measure NIS inhibition. Thus, it is very hard to infer the effects perchlorate, thiocyanate, and nitrate on thyroid iodine uptake or maintenance of maternal thyroid hormone levels based solely short-term NIS inhibition. As will be discussed in Section 5, recent epidemiological data suggest that the relative effect of chronic perchlorate and thiocyanate on T_4 levels in reproductive-age women, whatever the mechanism, is not additive. Thus, there is strong evidence that "something else is going on" (i.e., some mechanism other than NIS inhibition is dominant in controlling maternal T_4 levels).

5. PROPOSED METHODOLOGY FOR A REVISED PERCHLORATE RISK ASSESSMENT

In this section, we propose what we believe to be a defensible approach to conducting a revised human health risk assessment for perchlorate exposures. This approach builds on the experience of previous perchlorate risk assessments (summarized in Appendix C) and takes into account the availability on new human data related to the impact of perchlorate and other environmental exposures on thyroid hormone levels.

5.1 Selection of Data Set for Risk Assessment

As discussed in Section 2, the most important question in selecting studies to be used for risk assessment is which studies provide the best information for predicting the occurrence of adverse effects in the most sensitive human population. Thus, there is a clear preference for acceptable-quality human data over even high-quality animal toxicity studies and over *in vitro* studies. Similarly, there is a preference for human studies that match, as closely as possible, the health status, life stage, gender, and general exposure conditions facing the most sensitive receptors. Where causal mechanisms are complex, there may be studies that report biomarkers of exposure and non-adverse precursor events as well as, or instead of, the adverse health outcomes of concern. In this case, studies that measure effects on the most "down-stream" effect, or the precursor events most strongly correlated with the health outcome of interest, are preferred to those reporting events more distant from the health effect of interest. Similarly, studies that have the highest potential (power) to detect adverse effects or precursor events are preferred to those with lower power. This translates into a strong preference for studies with larger numbers of subjects over smaller data sets.

Once the highest-quality studies are identified, this implies that these studies will be the primary sources of information used to estimate dose- or exposure-response relationships for the populations of concern. This does not mean that data from other sources will be ignored. If there is a "close call" such that two or more studies appear to provide useful quantitative evidence for risk assessment, all the relevant studies may be used independently or together to provide alternative risk estimates (in the extreme, a systematic statistical analysis of a large number of studies is called "meta-analysis"). In addition to providing alternative dose-response estimates, more limited data sets (e.g. from animal studies and *in vitro* tests) can also be used to clarify mode of action, to suggest approaches to analyzing the "critical" data sets, and to test the credibility of the derived risk results. As discussed in Section 3, for example, PBPK modeling can be a useful tool for estimating human "target organ" dose in response to specific exposure patterns.

Consistent with the above considerations, a revised risk assessment would ideally be based on a study of perchlorate and goitrogen exposure impacts on T_4 levels and neurobehavioral development patterns in a large population of "typical" North American pregnancies. This population would naturally include some portion of iodine-insufficient women, which would allow the characterization of the variation in impacts associated with variations in iodine nutritional status. In the absence of this ideal data set, one would instead look for the largest, most well-documented data set in a population that is most similar to pregnant North American

women and whose exposures to perchlorate, goitrogens, and other thyroid stressors we believe is most similar to theirs.

Given these considerations, it is clear that the NHANES III (2001-2002) survey data analyzed by Blount et al. (2006b) and Steinmaus et al. (2007) comes the closest to the ideal and is indeed greatly superior to any data set previously available. The data analyzed by Blount et al. (2006b) included spot urine samples from a systematically weighted sample of 2,880 individuals across the United States for perchlorate, thiocyanate, and iodine as well as data on serum tT_4, TSH, and cotinine (a nicotine metabolite indicative or recent smoking). The data set also includes demographic and socioeconomic data, self-reported smoking history, information on pre-existing thyroid disease, and use of medications that could affect thyroid function. The study population included 1,854 females, approximately 1,100 of whom were reproductive age.

These data do have some limitations. The NHANES survey, while it does allow analyses of the relationship between measures of perchlorate and thiocyanate exposure, smoking, and the previously identified "key event" (reduced serum T_4 levels), does not provide data on the critical health endpoint (neurodevelopmental impairment in children). Also, there were too few pregnant women in the sample to draw any firm conclusions about the relationships between urinary perchlorate and thiocyanate distributions, smoking, and other confounding variables in the population of most interest. Thus, inferences related to changes in thyroid hormone levels must be based on analyses of the entire data set (all reproductive-age women).

Another limitation to the study is that all of the urine analyses are from single "spot samples" and all serum hormone measurements were conducted only once per subject. This limitation introduces a degree of uncertainty into any attempts to characterize the relationships between long-term perchlorate levels and time-averaged thyroid hormone levels, both of with are expected to exhibit substantial temporal variability. As pointed out by the investigators (Blount et al. 2006, Steinmaus et al. 2007), these limitations are likely to reduce the sensitivity of their analyses of the relationships between perchlorate exposure and T_4 levels, but they are not likely generate spurious correlations. Finally, the NHANES III data include measurements of tT_4 rather than fT_4. The latter measure is considered to be a better indicator of overall thyroid hormone status because it is the free hormone that is believed to interact with thyroid hormone receptors (Zoeller et al. 2007). Methods for addressing limitations of the data (which are less severe than those of other data sets) are discussed below.

5.2 Methodology for a Revised Perchlorate Risk Assessment

While there are a number of substantial technical problems to be resolved, the basic risk assessment strategy might involve the following general steps:

1. Identify the degree of serum T_4 reduction in the first-trimester of pregnancy that is likely to be of concern from the point of view of avoiding impairment of fetal neurodevelopment.

2. Fully characterize the statistical relationships and interactions between perchlorate exposure, urinary thiocyanate, cotinine/smoking history, iodine sufficiency, and other

3. Use the statistical model developed in the previous step to predict the daily dose of perchlorate (or the combination of perchlorate and thiocyanate) that would result in serum T_4 reductions equivalent to the level of concern derived in Step 1, when considered in conjunction with a reasonable set of other "background" exposures.

4. Recognizing that the calculations to this point have been based on data from reproductive-age women and not from women in the first trimester of pregnancy, consider whether an additional degree of conservatism is needed in estimating a perchlorate (or perchlorate + thiocyanate) dose that is likely to be without harm to the developing fetal nervous system. If so, apply an appropriate uncertainty factor.

All of these steps would require a substantial degree of professional judgment, as well as carefully designed quantitative analyses, as discussed below.

5.2.1 Identification of the Degree of fT_4 Reduction that is of Concern

Data for Step 1 could come from the epidemiological studies of Pop et al. (1999, 2003) and Kooistra et al. (2006). All three studies detected statistically significant adverse effects on neurobehavioral development in infants and toddlers born to mothers who had fT_4 levels below the tenth percentile found in the entire population of pregnancies that were studied. The average week 12 maternal fT_4 level in the Pop et al. (1999, 2003) studies was 13.1 pM/L, while the 10^{th} percentile level in the study population was 10.4 pM/L, which corresponds to a reduction of approximately 21 percent.

These data provide a starting point for estimating the fT_4 level of concern associated with adverse neurodevelopmental effects. They suggest that an approximately 20 reduction in fT_4 should be of concern from the standpoint of impaired fetal neurodevelopment. The premise behind this judgment is that reduced fT_4 level is indeed the "key event" and that a reduction in fT_4 due to perchlorate exposure (or any other cause) has the same effect on development as reduced fT_4 levels that were low, as assumed in this study, due to insufficient iodine intake.

These studies provide a reasonable estimate of the extent of fT_4 reduction that <u>is</u> associated with adverse effects. Determining the degree of fT_4 reduction that is <u>not</u> likely to be associated with adverse effects will be more complicated. These studies, which are based on an initial population of only 220 pregnancies, clearly have limited statistical power to identify a "threshold" above which reductions in fT_4 levels are not of concern. A decision on the degree of fT_4 reduction that is "safe" would inevitably a science policy decision, but it can be informed by quantitative analysis. If, for example, the original test data from either or both of the Pop et al. (1999, 2003) studies were available, a benchmark dose-type analysis could be used to derive statistical confidence limits on the fT_4 level likely to be associated with some specific level of

effect on developmental test scores. These results could then be incorporated into subsequent calculations.

5.2.2 Statistical Analysis of NHANES III Data

Steinmaus et al. (2007) conducted a thorough analysis of relationships and interactions among variables in relation to changes in tT_4 levels in the NHANES III data. Briefly, they found that perchlorate dose (as estimated using urinary perchlorate corrected for creatinine secretion) was the strongest, most consistent predictor of tT_4 levels in women with urinary iodide below 100 μg/L. While smoking and urinary thiocyanate alone were not significant predictors of tT_4 levels, they found a strong interaction between smoking status, urinary thiocyanate, and cotinine concentrations. That is, being a smoker, having high serum cotinine, or having high urinary thiocyanate increased the magnitude of the observed effect of perchlorate dose on tT_4 levels.

If these data are to be used to estimate a useful and robust model for predicting T_4 changes as a function of perchlorate, iodine, and smoking, additional work needs to be done to further clarify the relationships and interactions among these variables. The regression results reported by Steinmaus et al. (2007) suggest that perchlorate is the key determinant of serum fT_4 among the variables that were analyzed with thiocyanate having no independent effect but, rather acting as a "potentiator" that increases the impact of perchlorate by a constant proportion across the entire range of perchlorate dose. This finding is not consistent with what would be predicted based on the additive inhibition of the NIS by perchlorate and thiocyanate that has been observed with *in vitro* experiments. Given this unexpected result, it would be advisable to do an exhaustive investigation of all the potential interactions among the key exposure variables in the model. (The Blount et al. and Steinmaus et al. studies explored only a limited subset of possible interactions and correlations.)

Detailed analyses of all the correlations in the data set are called for, perhaps along with a polynomial regression that included terms for all of the first-order interactions among the variables. Once the relationships among the potential explanatory variables are clarified, then the effects of the individual variables (perchlorate, thiocyanate, and smoking) can be captured in a single statistical model that can be used to predict exposure combinations that would result in changes in specified changes in tT_4 levels based on the NHANES III data set. As discussed in Section 2, depending on the results of the statistical analyses, it might then be possible to draw on previously developed methods for establish health criteria for mixtures of interacting stressors.

5.2.3 Estimation of Perchlorate (Goitrogen) Doses Associated with Potentially Adverse Changes in fT_4 Levels

There would be two sub-steps in this part of the risk assessment. The first is to come to an understanding of the relationship between the changes in fT_4 levels (as measured in the Pop et al. studies) and the changes in tT_4 levels measured in the NHANES III data. The second sub-step is then to use the statistical model derived described in Section 5.2.2 to estimate the perchlorate/goitrogen dose that would cause the specified proportional reduction in tT_4 levels (as converted from changes in fT_4 levels) identified as being associated with adverse effects.

Substantial data exist related to the relationship between fT_4 levels and tT_4 levels in single individuals and populations. It is well understood that the great majority of T_4 in human circulation is bound to thyroxine binding globulin (TGB) or other proteins and that only a very small proportion is "free" to interact with hormone receptors. Estimates of the proportion of T_4 that is typically free in euthyroid adults range from about 0.03 percent (Refetoff 2007) to 0.1 percent (Zoeller et al. 2007), with the majority of the estimates concentrated around the lower end of this range. The important question for the risk assessment, however, is not the exact value of the fT_4/tT_4 ratio in normal adults, but whether the ratio varies systematically between first-trimester pregnant women (the Pop et al. study population) and in mostly non-pregnant reproductive age females (the NHANES III subjects). If the ratio is not systematically different, then it would be reasonable to assume that a 21 percent change in fT_4 (the change associated with adverse effects) would be equivalent to a 21 percent change in tT_4 as measured in NHANES III, in terms of increased risk of adverse effect. That assumption would allow a simple, direct translation between the study showing adverse effects associated with fT_4 changes to the study that predicts changes in tT_4 as a function of perchlorate/goitrogen exposure.

The "translation" of fT_4 changes seen by Pop et al. (1999, 2003) to the tT_4 changes seen in the NHANES III data may not be quite as straightforward as just discussed. It is well-known that pregnancy (specifically the surge of HCG production) causes an increase in the concentration of TGB in serum, which theoretically could reduce the level of fT_4 and the fT_4/tT_4 ratio (Neale et al. 2007). Pregnancy is also associated with increased iodine secretion, T_4 deiodination, and other physiological changes that might make it more difficult to directly equate changes in fT_4 and tT_4 levels. There is some evidence, however, that an offsetting increase in T_4 production occurs early in the first trimester and that the increase in TGB levels tends to occur later in pregnancy so, that fT_4/tT_4 ratios in the first trimester may not be significantly disturbed from pre-conception levels (Morreale de Escobar et al. 2007). Addressing this issue in sufficient detail to support a risk assessment would require a systematic review of the clinical and epidemiological literature bearing on the question. Based on the very limited research done for this review, however, it appears that support will be found for adopting a simple ratio with a value near 1.0 for translating proportional changes in fT_4 in the Pop et al. (1999, 2003) studies to changes in tT_4 in the NHANES III data. If necessary, sensitivity analyses can be used to test the impact of a range of assumptions related to fT_4/tT_4 ratios.

After identifying the change in fT_4 that may be associated with adverse effects, deriving a model describing the relationship between perchlorate (goitrogens) and tT_4, and developing a method for translating changes in fT_4 to changes in tT_4, the next step would be to estimate the perchlorate (goitrogens) exposure predicted to result in the proportional fT_4 change of concern. For purposes of the following illustration, we assume that proportional changes in fT_4 translate into equal proportional changes in tT_4.

In section 5.2.2, we described how we would develop a statistical model predicting tT_4 changes in the NHANES III data based on perchlorate and other exposures. This model would be of the following general form:

$$tT_4 = f(\text{perchlorate, thiocyanate, smoking, covariates}) \quad (1)$$

Where f() could represent a multiple linear regression or a similar multivariate model, the precise formulation of which would be determined in the actual risk assessment. Let P be the proportional change in fT_4 of concern from the standpoint of adverse neurodevelopmental effects. Recall that the value for P might take the form of a benchmark dose, the lower 95 percent confidence limit on the estimated change on fT_4 associated with, for example, a one standard deviation change in the test scores used to measure development.

To estimate the perchlorate (goitrogen) dose of concern, we would then solve Equation (1) for the combined exposure(s) resulting in a proportional change of P in tT_4 relative to some baseline of exposures. The nature of the solution to Equation 1 depends on the results of the statistical analyses conducted in Section 5.2.2. If it were found that perchlorate was, in fact, the principal driver of tT_4 changes, then the equation could be used to determine a perchlorate dose resulting in a change of P in tT_4 given some assumed (typical or upper percentile) background levels of urinary thiocyanate and other covariates in the NHANES III data set. If instead it were found that perchlorate and thiocyanate exerted independent or interacting effects on tT_4, then the solution to Equation 1 might take the form of a "frontier" of combined perchlorate dose and urinary thiocyanate resulting in a change P in tT_4 levels for women with iodine intake less than 100 µg/day. In this case, the decision as to which point(s) on the frontier were most relevant from the standpoint of risk management would be a science policy decision. Again, decision makers could use the results of the model to define a "conservative" background thiocyanate level (based on the distribution or urinary thiocyanate in the NHANES III data) and estimate the perchlorate dose that would result in a predicted change in tT_4 levels of P or less. [Note again that we have assumed equivalence between proportional changes in fT_4 and tT_4. If the data indicate that such a conversion was not justified, the allowable dose(s) of perchlorate (and thiocyanate) would need to be calculated using an "R" value different from 1.0.]

5.2.4 Considerations Affecting the Degree of Health Protectiveness

Each step in the proposed approach described in the preceding sections has its own associated uncertainties. The NHANES III data are subject to a number of limitations that affect our ability to accurately characterize the relationships we are concerned with (between perchlorate/goitrogen exposure and changes in T_4 levels). The statistical models that would be developed to analyze that data will come with built-in statistical uncertainty (as measured in variances and confidence levels), and there will be uncertainty as to whether the models themselves are correctly specified. There is also uncertainty associated with the assessment of the reduction in fT_4 levels associated with neurodevelopmental impairment, which can only be partially addressed by estimating lower confidence limits using benchmark dose methods. Finally, as discussed in Section 5.2.3, the question of whether to treat proportional changes in fT_4 measured in one study the same as changes in tT_4 in another study of a different population is also subject to a substantial degree of quantitative uncertainty.

One way to look at this step in the analysis is to consider the analogy to EPA's "standard" approaches to estimating health criteria (U.S. EPA 2002a). The estimate of the proportional change in fT_4 associated with adverse effect can be thought of as a "point of departure" (POD) for establishing an RfD or similar criterion. Once the POD is estimated, then

specified types of uncertainty factors (UFs) can be used to account for the various types of uncertainty in the models used to estimate it. Most of the conventionally used UFs, however, would not be relevant in this assessment. (This is a consequence of using a high-quality human data set.)

The critical data comes from humans; thus, there would be no need for an interspecies UF. The presumed exposure duration in the NHANES III data is chronic or continuous, or in any event very similar to the exposure duration of concern with regard to the population of concern. Thus, there is no need for a UF to account for differences in duration of exposure. The point of departure would most likely be a benchmark dose low (BMDL) and not a lowest observed adverse effect level (LOAEL). Thus, current EPA practice (and statistical common sense) indicates that there is good reason not to employ a UF to compensate for using a LOAEL.

An argument could be made for including an "FQPA" UF to account for adverse effects in children. We do not think that an FQPA UF is justified, however, because it is meant to account for the greater sensitivity of children when the critical study is conducted in healthy adults. The critical event in this risk assessment is reduced T_4 levels in pregnant women, and the critical data set (NHANES III) addresses T_4 changes in a very similar population (reproductive-age women). Also, the sensitivity of the fetus would be addressed directly in this assessment through the use of the Pop et al. (1999, 2003) data on the relationship between reduced T_4 levels and impaired neural development. Finally, given the extraordinary depth of the available data related to perchlorate toxicity, it does not appear reasonable to include any additional margin of safety (database UF) to account for limitations in the available data.

Consideration should be given, however, to the inclusion of an intra-species UF (UF_I), which is used to capture potential differences in response to exposures between the study population from which the POD was derived and the most susceptible population. In this risk assessment, the study population (reproductive-age women) does differ in important respects from the most susceptible group, women in the first trimester of pregnancy. These differences arise due to the changes in thyroid physiology that are known to occur in early pregnancy. As noted earlier in this section, the UF_I might also be considered to account for differences in exposure patterns between the study and susceptible populations.

When considering what value to assign to the UF_I, it is customary to break it down into two subfactors, one uncertainty factor for pharamacokinetic differences and another one for pharmacodynamic differences (IPCS 2001). Because the maximum value assigned to the UF is 10, each sub-factor can take a value between 1.0 and 3.0 (or more properly between one and the square root of ten). The pharmacokinetic factor is meant to account for differences in the absorption, metabolism, and elimination of the agent in question between the study subject and most susceptible population, while the pharmacodynamic factor is meant to account for differences in the sensitivity of the two populations to the same target organ dose of the agent or its active metabolite. In the case of perchlorate, it is not clear that this breakdown is very useful. Because the combined thyroid economy of mother and fetus is so complicated, it is difficult to cleanly separate pharmacokinetics from pharmacodynamics. If this assessment were undertaken in earnest, a considerable degree of thought would need to be given to the potential magnitude of differences in sensitivity to perchlorate (goitrogen) impacts on thyroid hormone levels between

non-pregnant and pregnant women. It is not likely that the final value for the UF_I could be decided on the basis of a simple pharmacokinetics-pharmacodynamics dichotomy. Careful analyses of the available data, however, would most likely yield important insights as to the physiological bases for differences in sensitivity and suggest a reasonable range of values for UF_I.

5.2.5 Summary of the Proposed Risk Assessment Process

The major innovation in the proposed assessment would be the use of data from recently published epidemiological studies that was not available when previous assessments were performed. The NHANES III (2001-2002) data set of perchlorate exposures and thyroid hormone levels in reproductive-age women represents a unique new resource that directly measures a key event in a population very similar to the most susceptible group. The steps involved in actually deriving the quantitative health criterion (something like an RfD) are relatively straightforward; perhaps the most technically difficult step in the assessment would be assessing the comparability of changes in fT_4 during pregnancy and changes in tT_4 in non-pregnant reproductive-age females, as discussed in the Section 5.2.4. The actual statistical analysis of the NHANES III data should be relatively straightforward (although the result might not be as easily interpretable as one would like). The final step in the analysis, deciding how much of a "margin of safety" to build in to account for differences in sensitivity between the study population and the most sensitive groups, would be a science policy decision. That decision, however, can be informed by a great deal of quantitative information from the available epidemiological and clinical studies.

Good human data on a well-defined key event in a population similar to the most sensitive group should be given precedence over data from less predictive human studies, animal studies, and *in vitro* test results. Such secondary information could well enter into the decision about the degree of conservatism to incorporate into the health criterion to account for uncertainty. For example, if the epidemiological data indicate that perchlorate, rather than thiocyanate, is the key factor affecting women's T_4 levels during pregnancy, knowledge about the relative potency of perchlorate, thiocyanate, and other goitrogens from *in vitro* studies might suggest a higher degree of conservatism, or suggest that an allowable perchlorate exposure be estimated assuming a higher (or lower) background of exposures to other thyroid-active chemicals.

As discussed in Appendix C, the selection of the data set that is used for risk assessment largely determines the result of the assessment, within a relatively narrow range. The outcome of a risk assessment based on the NHANES III data will likewise lead to risk estimates that fall within a relatively narrow range (according to the standards of risk assessment), irrespective of what methods are used to analyze the data or what assumptions are made about the appropriate degree of conservatism to incorporate into the health criteria that are derived. The NHANES III data, whose study subjects and exposure conditions so closely match those of the most susceptible population, will constrain the result within relatively narrow limits, limits that are narrower than if a less suitable data set had been chosen. Having such a high-quality human study reduces the degree of uncertainty, and, as explained above, obviates the need for most of the UFs that would customarily be considered in assessing risks from lower-quality data.

To get an approximate idea of the magnitude of human health risk criteria might be derived from the NHANES III (2001-2002) data, we note that Blount et al. (2006b) has estimated that a change from the minimum perchlorate dose seen in the study subjects (0.19 μg/L) to the 95th percentile (13 μg/L) would be associated with reduction in average tT_4 of 1.64 μg/dL, which is equal to approximately 20 percent of the average tT_4 level (8.27 μg/dL). Even though this calculation does not take into account the effect of thiocyanate exposures, it provides an order-of-magnitude estimate of what the POD for maternal T_4 reduction might be. The average equivalent drinking water concentration corresponding to these calculations is about 6 μg/L, which is at the lower end of the range of RfD values derived in previous perchlorate risk assessments (see Appendix C). Using the same data, Steinmaus et al. (2007) estimated that the average tT_4 level in a current smoker with urinary iodine less than 100 μg/L and perchlorate exposure greater than the median seen in the NHANES III data set would be 1.25 μg/dL lower than the average value in an iodine-sufficient never-smoker with perchlorate exposures below the median. However, we believe that additional statistical analysis of the NHANES III data are needed to clarify the interrelationships among perchlorate exposures, urinary thiocyanate, smoking history, and other covariates in this database before deriving a defensible "safe level" of exposure

There is yet another alternative available with regard to conducting a risk assessment for perchlorate, however. As just discussed, selection of the NHANES III (2001-2002) data to assess perchlorate risks would probably result in health criteria (estimated "safe" exposures) that are somewhat lower (slightly more stringent) than those derived in most previous assessments based on human data. In addition, as noted above, the existing analyses of the NHANES data seem to indicate that perchlorate, not thiocyanate, is the dominant driver of reduced T_4 levels in pregnant women. Thus, the NHANES III data challenge two preconceptions (alternatively, scientific hypotheses) about the nature of perchlorate- (and goitrogen-) associated risks that were found persuasive by many risk assessors prior to the publication of the Blount et al. (2006) and Steinmaus et al. (2007) studies.

It is possible (another science policy judgment) that the NHANES III data could be found to be too inconsistent with previous studies to be used as the primary basis for a risk assessment. If that were the case, EPA could refrain from conducting a revised risk assessment until additional data related to the relationships between perchlorate exposure and maternal T_4 levels are generated. For example, the Centers for Disease Control (CDC) is currently conducting another round of urinary perchlorate sampling as part of the 2007-2008 NHANES. In addition, Dr. Elizabeth Pearce of Boston University has been gathering data relating to indices of perchlorate exposure, iodine intake, and thyroid hormone levels from several cohorts of pregnant women, but these data have not yet been published in full. When these data become available, they may further clarify the relationships between environmental perchlorate exposures and T_4 levels, and may (or may not) provide confirmation of the findings from the NHANES III (2001-2002) data. As a policy judgment, EPA (or any other risk assessor) might consider not conducting a risk assessment until these additional data are available. We did not find any obvious technical shortcomings in the published analyses of the NHANES III (2001-2002) data, however, that seriously undermine their credibility or that would disqualify these data from being used as the basis for a risk assessment.

6. RECOMMENDATION FOR IODINE SUPPLEMENTATION

The OIG also requested that ICF comment on the merits of the recommendation made by the NRC Committee to Assess the Health Implication of Perchlorate Ingestion (NRC 2005) that "iodine be included all prenatal vitamins." This recommendation, made in recognition of the importance of preventing maternal hypothyroidism in assuring healthy fetal development, acquires greater significance in light of the recent evidence that perchlorate and other goitrogen exposures most strongly (or perhaps exclusively) affect maternal thyroid function in women with inadequate iodine intake.

To help establish some context, we note that the NRC Committee recommendation is one of many made by WHO (2005), IOM (2001), and other clinicians and researchers (Glinoer 2004, Delange 2007, Morreale de Escobar et al. 2007, Zimmerman 2008) regarding the importance of maintaining adequate dietary iodine intake during pregnancy. There is clearly a consensus among experts in maternal nutrition and endocrinology that pregnancy is associated with increased demands on the thyroid that necessitate increased iodine intake to maintain normal thyroid function, with the recommended daily intake increasing from 150 μg/day for non-pregnant adults to 220-250 μg/day for pregnant women.

In the face of this unanimity, and considering the well-established relationship between impaired maternal thyroid function and adverse effects on the fetus, it is hard to disagree with a recommendation for maintaining adequate maternal iodine intake. One possible concern associated with the adoption of universal iodine supplementation is the rather narrow range of acceptable iodine intake that is apparently required to maintain health thyroid function. For example, WHO identifies an "excessive iodine intake" level of 500 μg/day (WHO 2005). The basis for setting this level is the observation that too much iodine can also inhibit thyroid function, and that populations with high iodine intake often have an increased incidence of goiter and other signs of thyroid dysfunction. Therefore, there might be some concern about further increasing the iodine intake of pregnant women in areas where normal dietary iodine intake is already approaching levels considered excessive.

Furthermore, factors in addition to iodine status (and goitrogen exposure) can affect thyroid dysfunction during pregnancy. For example, selenium is a critical cofactor in the iodine organification reaction involving the thyroperxoidase enzyme, as well as a cofactor involved in the deiodinase-catalyzed T_4-T_3 conversion in the liver (Brauer et al. 2006). Thus, adequate selenium intake is also required to assure proper thyroid function. Yet other factors may affect maternal thyroid function through mechanisms that are not fully understood. For example, Casey et al. (2007) found that among a study population of 17,298 pregnancies, the women with "isolated hypothyroidism" (low T_4 levels) during early pregnancy were "older, heavier, and more often multiparous" than women whose thyroid function had been normal during pregnancy. Thus, while iodine supplementation might indeed reduce the incidence of thyroid disruption in pregnant women, there is no assurance that it would eliminate it, or that it would eliminate the effects of other thyroid stressors.

The NRC Committee, while recognizing that iodine supplementation during pregnancy was in principal desirable, none the less noted:

> "…further research is needed to measure more precisely the extent of, and risk factors for, iodide deficiency, particularly in pregnant women and their offspring…"

and their recommendation for iodine in prenatal vitamins was less than categorical:

> "…while studies are being conducted, the committee emphasizes the importance of ensuring that all pregnant women have adequate iodide intake and, as a first step, recommends that consideration be given to adding iodide to all prenatal vitamins."

From the standpoint of OIG's question, we cannot disagree with the NRC recommendation that "consideration be given" to adding iodine to maternal vitamins. We suggest that the appropriate bodies who should "give consideration" to this question are institutions such as WHO and IOM rather than EPA. We note that while IOM does recommend increased iodine intake for pregnant women, it does not go as far as recommending universal iodine supplementation, perhaps recognizing that such decisions should best be made by clinicians on a case-by-case basis.

7. REFERENCES

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL. (2006a) Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ Health Perspect 114:1865–1871.

Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL. (2006b) Perchlorate exposure of the US population, 2001–2002. J Expo Sci Environ Epidemiol 17(4):400-7. [Online 18 October 2006].

Brauer VF, Schweizer U, Köhrle J, Paschke R. (2006) Selenium and goiter prevalence in borderline iodine sufficiency. Eur J Endocrinol. 155(6):807-12.

Braverman LE, He XM, Pino S, Cross M, Magnani B, Lamm SH, Kruse MB, Engel A, Crump KS, Gibbs JP. (2005) The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. J Clin Endocrinol Metab 90(2):700–706.

Burrow GN, Fisher DA, Reed Larsen P. (1994) Maternal and Fetal Thyroid Function. New England Journal of Medicine 331(16):1072-1078.

Cal/EPA. (2004) Final Public Health Goal for Perchlorate in Drinking Water. Available from: Pesticide & Environmental Toxicology Section, Office of Environmental Health Hazard Assessment.

Casey BM, Dashe JS, Spong CY, McIntire DD, Leveno KJ, Cunningham GF. (2007) Perinatal significance of isolated maternal hypothyroxinemia identified in the first half of pregnancy. Obstet Gynecol. 109(5):1129–35.

Chan S, Kilby MD. (2000) Thyroid hormone and central nervous system development. Journal of Endocrinology 165:1-8.

Clewell RA, Merrill EA, Gearhart D, Robinson PJ, Sterner TR, Mattie DR, Clewell HJ. (2007a) Perchlorate and Radioiodide Kinetics Across Life Stages in the Human: Using PBPK Models to Predict Dosimetry and Thyroid Inhibition and Sensitive Subpopulations Based on Developmental Stage. Journal of Toxicology and Environmental Health, Part A 70: 408–428.

Clewell HJ, Thomas R, Gentry PR, Crump KS, Kenyon EM, El-Masri HA, Yager JA. (2007b) Research toward the development of a biologically based dose response assessment for inorganic arsenic carcinogenicity: A progress report. Toxicology and Applied Pharmacology 222:388–398.

Crump KS, Gibbs JP. (2005) Benchmark calculations for perchlorate from three human cohorts. Environ Health Perspect. 113(8):1001-8.

Dai G, Levy O, Carrasco N. (1996) Cloning and characterization of the thyroid iodide transporter. Nature 379:458–460.

Delange, F. (2007) Iodine Requirements during Pregnancy, Lactation, and the Neonatal Period and Indicators of Optimal Iodine Nutrition. Public Health Nutrition 10(12A):1571–1580.

Dohan O, Portulano C, Basquin C, Reyna-Neyra A, Amzel LM, Carrasco N. (2007) The Na+/I-Symporter (NIS) Mediates Electroneutral Active Transport of the Enviromental Pollutant Perchlorate. Proceedings of the National Academy of Sciences 104(51):20250-20255.

Dohan O, de la Vieja A, Paroder V, Reidel C, Artani M., Reed M, Ginter CS, Carrasco N. (2003) The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical Significance. Endocrine Reviews 24(1):48–77.

Dollarhide J, Zhao Q, Dourson M. (2002) Reference dose for perchlorate based on human studies. Available from: Toxicology Excellence for Risk Assessment, 1757 Chase Ave. Cincinnati, OH 45223.

Dunn JT. (1998) Editorial: What's Happening to Our Iodine? Journal of Clinical Endocrinology and Metabolism 83(10):3398-3400. (as cited in U.S. EPA 2008)

Eskandari S, Loo DD, Dai G, Levy O, Wright EM, Carrasco N. (1997) Thyroid Na^+/I^- symporter. Mechanism, stoichiometry, and specificity. J Biol Chem 272:27230–27238.

Gamper N, Stockand JD, Shapiro MS. (2005) The Use of Chinese Hamster Ovary (CHO) Cells in the Study of Ion Channels. Journal of Pharmacological and Toxicological Methods 51:177-185.

Gibbs, JP. (2006) A Comparative Toxicological Assessment of Perchlorate and Thiocyanate Based on Competitive Inhibition of Iodide Uptake as the Common Mode of Action. Human and Ecological Risk Assessment 12(1):157 – 173. http://dx.doi.org/10.1080/10807030500430567

Glinoer D. (2004) The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. Best Practice & Research Clinical Endocrinology & Metabolism 18(2):133–152.

Greer M, Goodman G, Pleus R, Greer S. (2002) Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. Environ Health Perspect 110(9):927-37.

ICF. (2004) Recommendation for an Oral Intake Reference Dose (RfD) for Perchlorate. Prepared for: National Aeronautics and Space Administration (NASA), Environmental Management Division. ICF Consulting, Fairfax, VA

Institute of Medicine (IOM). (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press, Washington DC.

IPCS (International Programme on Chemical Safety). (2001) Guidance Document for Use of Data in Development of Chemical-Specific Adjustment Factors (CSAFs) for Interpsecies Differences and Human Variability in Dose/Concentration-Response Assessment. World Health Organization. WHO/PCS/01.4

Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ. (2006) Neonatal Effects of Maternal Hypothyroxinemia during Early Pregnancy. Pediatrics 117:161-167.

Lambert JC and Lipscomb JC. (2007) Mode of action as a determining factor in additivity models for a chemical mixture risk assessment. Reg Tox and Pharm 49(3):183-194.

Lamm SH, Braverman LE, Li FX, Richman K, Pino S, Howearth G. (1999) Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. J Occup Environ Med 41(4):248–260.

Merrill E, Clewell R, Robinson P, Jarabek A, Gearhart J, Sterner T, Fisher J. (2005) PBPK Model for Radioactive Iodide and Perchlorate Kinetics and Perchlorate-Induced Inhibition of Iodide Uptake in Humans. Toxicological Sciences 83:25-43.

Morreale de Escobar G, Obregon MJ, Escobar del Rey F. (2007) Iodine Deficiency and Brain Development in the First Half of Pregnancy. Public Health Nutrition 10(12A):1554–1570.

National Research Council (NRC). (2007) Toxicity Testing in the 21st Century: A Vision and a Strategy. Committee on Toxicity Testing and Assessment of Environmental Agents. National Academies Press, Washington DC. http://www.nap.edu/catalog/11970.html

National Research Council (NRC). (2005) Health Implications of Perchlorate Ingestion. Committee to Assess the Health Implications of Perchlorate Ingestion. National Academies Press, Washington DC. http://www.nap.edu/catalog/11202.html

National Research Council (NRC). (1983) Risk Assessment in the Federal Government: Managing the Process. Committee on the Institutional Means for Assessments of Risks to Public Health. National Academies Press, Washington DC.

Neale DM, Chung Cootauco A, Burrow G. (2007) Thyroid Disease in Pregnancy. Clin Perinatol 34:543–557.

Obregon MJ, Escobar del Ray F, Morreale de Escobar G. (2005) The effects of iodine deficiency on thyroid hormone deiodination. Thyroid 15(8):917-929.

Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL. (1999) Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. ClinEndocrinol 50:149–155.

Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. (2003) Maternal hypothyroxinemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin Endocrinol 59:282–288.

Refetoff S. (2007) Section 3a. Thyroid Hormone Serum Transport Proteins: Structure, Properties, Genes and Transcriptional Regulation. In: Throid Disease Manager (online reference resource). http://www.thyroidmanager.org/Section3/3a-frame.htm

Steinmaus C, Miller MD, Howd R. (2007) Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001–2002 National Health and Nutrition Examination Survey. Environmental Health Perspectives 115(9):1333-1338.

Strawson J, Zhao Q, Dourson M. (2003) Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. Available from: Joan Strawson, Toxicology Excellence for Risk Assessment, 1757 Chase Ave., Cincinnati, OH 45223.

Teuschler LK. (2007) Deciding which chemical mixtures risk assessment methods work best for what mixtures. Tox and App Pharm 223: 139-147.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J. (2004) Relative Potencies and Additivity of Perchlorate, Thiocyanate, Nitrate, and Iodide on the Inhibition of Radioactive Iodide Uptake by the Human Sodium Iodide Symporter. Thyroid 14:1012-1019.

U.S. Environmental Protection Agency (EPA). (2008) OIG Scientific Analysis of Perchlorate (Working Draft). Office of the Inspector General (OIG). March 26, 2008.

U.S. Environmental Protection Agency (EPA). (2003) Framework for Cumulative Risk Assessment. Risk Assessment Forum. EPA/630/P-02/001F

U.S. Environmental Protection Agency (EPA). (2002a) A review of the reference dose and reference concentration processes. Risk Assessment Forum. Washington, DC. Report No. EPA/630/P-02/002A.

U.S. Environmental Protection Agency (EPA). (2002b) Guidance on Cumulative Risk Assessment of Pesticide Chemicals that have a Common Mechanism of Toxicity. Office of Pesticide Programs. Washington, DC.

U.S. Environmental Protection Agency (EPA). (2002c) Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization, External Review Draft (NCEA-1-0503).

U.S. Environmental Protection Agency (EPA). (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Risk Assessment Forum. EPA/630/R-00/002

U.S. Environmental Protection Agency (EPA). (1998) Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information, Review Draft (NCEA-1-0503).

U.S. Environmental Protection Agency (EPA). (1989) Risk Assessment Guidance for Superfund. Volume 1. Human Health Risk Assessment. Part A. Office of Emergency and Remedial Response. EPA/540/1-89/002

World Health Organization (WHO). (2005) Proceedings of the WHO Technical Consultation on control of iodine deficiency in pregnant women and young children. Geneva, Switzerland.

Yoshida A, Sasaki N, Mori A, Taniguchi S, Mitani Y, Ueta Y, Hattori K, Sato R, Hisatome I, Mori T, Shigemasa C, Kosugi S. (1997) Different electrophysiological character of I^-, $ClO4^-$, and SCN^- in the transport by Na^+/I^- symporter. Biochem Biophys Res Commun 231:731–734.

Yoshida A, Sasaki N, Mori A, Taniguchi S, Ueta Y, Hattori K, Tanaka Y, Igawa O, Tsuboi M, Sugawa H, Sato R, Hisatome I, Shigemasa C, Grollman EF, Kosugi S. (1998) Differences in the electrophysiological response to I^- and the inhibitory anions SCN^- and $ClO4^-$, studied in FRTL-5 cells. Biochim Biophys Acta 1414:231–237.

Zimmerman M. (2008) The Adverse Effects of Mild-to-Moderate Iodine Deficiency during Pregnancy and Childhood: A Review. Thyroid 17(9):829-835.

Zoeller RT, Tan SW, Tyl RW. (2007) General Background on the Hypothalamic-Pituitary-Thyroid (HPT) Axis. Critical Reviews in Toxicology 37:11–53.

Zoeller, RT. (2003) Challenges Confronting Risk Analysis for Potential Thyroid Toxicants. Risk Analysis 25(1):143-162.

APPENDIX A: EVALUATION OF TONACCHERA ET AL. (2004) STATISTICAL MODEL

A.1 Methods

The paper by Tonacchera et al. (2004) describes a series of 26 experiments investigating the radioactive iodide uptake (RAIU) in Chinese hamster ovary (CHO) cells transfected with human sodium iodide symporter. In each experiment, various mixtures of four anions were added to different wells and the radioactivity counts per minute of radioactive sodium iodide were measured. The four anions evaluated were nitrate (NO_3^-, "n"), thiocyanate (SCN^-, "t"), perchlorate (ClO_4^-, "p"), and nonradioactive sodium iodide (I^-, "i"). A statistical model based on the Hill model was fitted to the data. The model is of the form:

$$\text{Counts per minute} = C_0/\{1 + [(t/T50) + (i/I50) + (n/N50) + (p/P50)]^r\} + \text{Error}.$$

This same model can also be rewritten in the perchlorate equivalent concentration (PEC) form:

$$\text{Counts per minute}/C_0 = P50^r/\{P50^r + [PEC]^r\} + \text{Error},$$

$$PEC = 1 + \beta_T \times t + \beta_I \times i + \beta_N \times n.$$

In these equations we have:

C_0 = background counts per minute, varies by experiment (parameter)
t = SCN^- thiocyanate concentration in medium (μmol/L)
T50 = thiocyanate concentration resulting in 50 percent RAIU inhibition when acting alone (parameter)
i = I^- nonradioactive iodide concentration (μmol/L)
I50 = nonradioactive iodide concentration resulting in 50 percent RAIU inhibition when acting alone (parameter)
n = NO_3^- nitrate concentration (μmol/L)
N50 = nitrate concentration resulting in 50 percent RAIU inhibition when acting alone (parameter)
p = ClO_4^- perchlorate concentration (μmol/L)
P50 = perchlorate concentration resulting in 50 percent RAIU inhibition when acting alone (parameter)
r = shape constant (parameter)
Error = (assumed to be normally distributed with mean = 0 and constant variance $sigma^2$)
β_T = P50/T50 (NIS inhibition potency of thiocyanate relative to perchlorate)
β_N = P50/N50 (NIS inhibition potency of nitrate relative to perchlorate)
β_I = P50/I50 (NIS inhibition of iodide relative to perchlorate)

These models were fitted either with an estimated value of the "Hill term" (r) or with r set equal to 1.0. This approach reproduces to the extent possible the methods reported in Tonacchera et al. (2004). The final selected model had r equals 1, which differs only slightly from the estimated value.

A.2 Results

Using the raw data provided by Dr. John Gibbs, we refitted the same model to the data using maximum likelihood and the SAS® NLIN procedure. The results are shown in Table A-1, which gives the Tonacchera et al. parameter estimates (from their Table 2) and the results of our calculations. The parameter estimates are quite close. The biggest differences are for the iodide parameter, I50, for which the paper gave estimates of 33.9 and 36.6 for the r estimated and r = 1 models, but ICF calculations gave 31.7 and 38.3, respectively. Since the maximum likelihood procedure applied to a non-linear model is an iterative procedure and may converge to a different solution using different starting points, these differences are not a major concern. More significant are the differences between the estimated 90% confidence intervals, which were computed using a profile likelihood approach. ICF estimated the confidence intervals by computing the likelihood at the maximum likelihood values plus or minus 5% and then applying a quadratic approximation. The Tonacchera et al. intervals agree well with ICF's except for the I50 parameter, for which the ICF intervals were two or three times as wide. These differences seem to be too large to be due to software differences although it is possible that Tonacchera et al. used a more accurate algorithm to compute the confidence intervals.

Table A-1. Comparison of Tonacchera et al. (2004) and ICF Parameter Estimates

	Parameter Estimates (90 percent LCL, UCL)	
	Tonacchera et al.	**ICF Analysis**
Model with fitted "r" parameter		
P50	1.27 (1.23, 1.32)	1.27 (1.21, 1.34)
T50	19.3 (18.4, 20.0)	19.2 (18.3, 20.2)
I50	33.9 (29.8, 35.8)	31.7 (21.7, 41.7)
N50	297 (283, 312)	296 (278, 315)
r	1.04 (1.02, 1.08)	1.04 (1.01, 1.08)
sigma	1716	1716
Model fitted with "r" set = 1.0		
P50	1.22 (1.19, 1.26)	1.23 (1.17, 1.29)
T50	18.7 (17.9, 19.3)	18.7 (17.9, 19.7)
I50	36.6 (29.3, 38.6)	38.3 (26.5, 50.2)
N50	293 (279, 309)	294 (276, 314)
r	1 .0 (constrained)	1 .0 (constrained)
sigma	1722	1722

Reviews of plots of the studentized model residuals versus anion concentrations and plots of the observed versus predicted counts indicated no obvious problems (heteroskedasticity, correlated residuals) with the fitted models. We also evaluated an alternative model where the errors were assumed to have a variance equal to the mean. This model corresponds to assuming that the counts per minute are Poisson distributed, which is often a reasonable assumption for count data. However, to simplify the likelihood calculations we approximated the counts as being normally distributed with a variance equal to the estimated mean (this approximation is

valid when the counts are large). We found this model to have a much lower log-likelihood than the Tonacchera et al. model, so the original model is preferred.

We also evaluated the effects of potential outliers on the parameter estimates. First, we noted that raw data included 21 observed counts that were flagged as Outliers, although the flags referred to additional Excel worksheets that were not included in the data set. We fitted the Tonacchera et al. models (r estimated or r =1) with and without these 21 outliers. We also fitted the models after removing three statistical outliers. These statistical outliers had a studentized residual of +/- 3.993 or greater.[4] As shown in Table A-2, the parameter estimates are not very sensitive to excluding either set of "outliers."

Table A-2. Effect of Outlier Removal on Parameter Estimates

Model	Outlier Treatment[1]	Observ-ations	Estimated Parameter Values					
			P50	T50	I50	N50	r	sigma
r estimated	None	776	1.27	19.2	31.7	296	1.04	1716
	Remove "flagged" points	765	1.31	19.6	31.8	303	1.06	1657
	Remove points with t>4	773	1.27	19.5	32.7	293	1.04	1658
r = 1.0	None	776	1.23	18.7	38.3	294	1	1722
	Remove "flagged" points	765	1.25	18.9	41.0	302	1	1668
	Remove points with t>4	773	1.23	19.0	39.4	292	1	1663

Notes: 1. "flagged points" are those identified in the original spreadsheets as being outliers. "Points with t>4" are points with studentized residuals greater than 4.0 or less than –4.0.

Tonacchera et al. (2004) used a likelihood ratio test to compare their model with a simpler model that assumed that the background counts per minute is the same for all 26 experiments. They reported a Chi-square test statistic of 1,639 with 25 degrees of freedom (26 experiments minus 1). ICF repeated this analysis using the models with r estimated and r = 1 and obtained almost the same results (Table A-3). A crucial issue for the analysis is how the "experiments" are defined, which is not fully specified in the paper or in the raw data. We assumed from the fact that they reported 25 degrees of freedom for this test that in their Table 1, experiments with the same number but a different letter (e.g. 13A and 13B) were all tested at the same time and were thus part of the same numbered physical experiment with the same background count. This assumption is supported by the fact that our parameter estimates based on this interpretation agreed very well with those in the paper (Table A-3).

[1]There were 776 observed counts. The studentized residuals are equal to the differences between the observed and predicted counts per minute divided by the estimated standard deviation of the difference. The studentized residuals are approximately standard normally distributed and independent. Thus if there are no outliers, the probability that all 776 studentized residuals are +/- 3.993 or greater is 0.05.

Table A-3. Likelihood Test Comparison versus a Model Assuming No Variation in Counts across Experiments

Model	C_0 Specification	P50	T50	I50	N50	r	sigma	Chi Sq	P-value
r estimated	Constant	1.56	24.0	108.0	467	1.14	4924	1636	$< 10^{-6}$
	Varies	1.27	19.2	31.7	296	1.04	1716		
r = 1.0	Constant	1.44	22.6	130.5	458	1	4944	1637	$< 10^{-6}$
	Varies	1.23	18.7	38.3	294	1	1722		

Finally, we attempted to replicate Tonacchera et al.'s evaluation of potential interactions between the NIS inhibitory activity of the various ions. Tonacchera et al. evaluated possible synergistic or antagonistic effects among the NIS inhibitors by adding interaction terms to their model. Using the PEC (perchlorate equivalent concentration) formulation, the interaction between SCN^- and ClO_4^- can be included in the model by adding in a term $\beta \times t \times p$ to the equation for PEC. We refer to this as the "tp" interaction. Other interactions and second-order (concentration-squared) terms are defined similarly. Tonacchera et al. reported that they tested the tp, np, and tn interactions and found them not significant with p-values of 0.18 or greater. Table A-4 shows the results from our analyses of interactions using likelihood ratio tests for adding in either a single interaction term (e.g., "tp") or a second-order term (e.g., "tt"). The results in Table A-4 confirm that the tp, np, and tn interactions are not significant and so can be excluded from the model. However, the results show that the ti interaction is extremely significant (p-value < 0.000001) and the ip, tt, and nn interactions or square terms are significant at p <= 0.05. From an experimental point of view, since the nonradioactive iodine was held constant in each experiment, it is understandable why the interactions ti, in, and ip were not considered. These interactions can be statistically estimated from the entire set of experiments, however, because the nonradioactive iodine levels tested in different experiments were 0, 1, and 10 μmol/L. The "take-home lesson" from these calculations is that the observed results are consistent with the PEC formulation, and that no statistically significant interactions among perchlorate, thiocyanate, and nitrate inhibition of the NIS were detected.

The Tonacchera et al. (2004) model assumes that all the measured counts, and therefore the error terms, are statistically independent. It is plausible that there could be some dependence between measured values in the same experiment. This dependence could be modeled, for example, using a mixed model formulation, treating the C_0 values as being randomly drawn from a suitable distribution (e.g., normal). It is not clear how this would affect the overall result of the modeling. On the whole, the qualitative fit to the data is quite good, and the form of the model is consistent with a competitive inhibition model with a single binding site.

Table A-4. Tests for Interactions among NIS Inhibiting Ions

Model	Interaction/ Second Order Term[1]	P50	T50	I50	N50	r	sigma	Interaction Coefficient Value	P-value
r estimated	None	1.27	19.2	31.7	296	1.04	1716	--	--
	ti	1.22	20.6	17.9	283	1.05	1688	0.0026	0.000
	tn	1.28	19.0	31.3	292	1.05	1712	-0.00005	0.074
	tp	1.26	19.1	30.0	295	1.05	1714	-0.00752	0.186
	in	1.28	19.4	36.4	285	1.04	1712	-0.00007	0.065
	ip	1.22	19.8	54.5	305	1.04	1704	-0.02154	0.001
	np	1.26	19.3	31.3	293	1.05	1714	-0.00063	0.184
	tt	1.24	20.5	33.4	294	1.02	1709	0.00015	0.012
	nn	1.26	19.1	33.5	334	1.03	1707	0.000002	0.005
	ii	1.27	19.2	28.7	296	1.04	1716	-0.00041	0.959
	pp	1.25	19.4	30.1	296	1.06	1711	-0.008219	0.033
r = 1.0	None	1.23	18.7	38.3	294	1	1722	--	--
	ti	1.17	20.1	20.9	281	1	1694	0.0026	0.000
	tn	1.23	18.5	38.7	291	1	1719	-0.00004	0.174
	tp	1.22	18.6	37.6	294	1	1721	-0.00467	0.418
	in	1.24	18.9	44.7	283	1	1718	-0.00007	0.062
	ip	1.18	19.3	69.5	304	1	1709	-0.0222	0.001
	np	1.22	18.7	38.4	292	1	1720	-0.00047	0.321
	tt	1.22	20.6	35.3	293	1	1709	0.00018	0.001
	nn	1.23	18.8	38.2	340	1	1710	0.000002	0.001
	ii	1.23	18.8	72.9	295	1	1721	0.0015	0.812
	pp	1.17	18.8	38.7	294	1	1723	-0.01378	0.252

Notes: 1. p = ClO_4^-, t = SCN^-, i = I^-, n = $NO3^-$. For example, "tp" denotes the interaction term between SCN^- and ClO_4^- while "tt" denotes the SCN^- second order (squared) term.

APPENDIX B: EVALUATION OF THE TONACCHERA MODEL

B.1 Objectives of PBPK Analysis

The *OIG Scientific Analysis of Perchlorate* (U.S. EPA 2008) makes use of the Tonacchera et al. (2004) study as the basis for a cumulative risk assessment for perchlorate and other NIS inhibitor (goitrogen) ions. Tonacchera et al. provide a model (the "Tonacchera Model" hereafter) that estimates the extent of sodium-iodide symporter (NIS) inhibition as a function of the concentrations of the inhibitor ions perchlorate, thiocyanate, and nitrate.

The Tonacchera et al. experimental set-up consisted of placing Chinese hamster ovary (CHO) cells transfected with human NIS in a medium of given ion concentrations and allowing the cells to take up radioiodide over the course of a 45 minute experiment. Then, the cell iodide contents were compared in the presence of the various iodide and inhibitor concentrations. It was found that perchlorate equivalent concentration (PEC), as defined in the study, could capture the overall inhibition of iodide uptake by the combinations of the three inhibitor ions (see also Appendix A).

As discussed in Section 4, several aspects of the Tonacchera study may limit its applicability to human risk analyses. First, the CHO cells differ in structure compared to human thyroid cells. They are epithelial-like cells which are not polar and "constitutively" (that is, continuously) express the NIS symporter over the entire cell surface. Colloid cells in the thyroid in contrast, are "polar" cells, and express the NIS only on the "basal" or "basolateral" surface that is in contact with the blood stream. Also, the amount of NIS that is synthesized and "trafficked" to the surface of the colloid cells is variable and under the control of TSH-driven signaling. Also, the thyroid follicular cells exist in a fixed geometric relationship within the follicle, with a net iodine flow entering the cell through the basal membrane and a transporter-mediated outward flow through the apical membrane into colloid. All of these processes are again influenced by TSH and other signaling mechanisms. Thus, while they are widely used in the study of ion-channels due to the ease with which they can be grown and transfected, the results obtained using CHO cells may not be applicable to the native cell (Gamper et al. 2005).

In addition, the CHO cells were not "pre-loaded" with iodine, perchlorate, or any of the test ions at the beginning of the Tonacchera assay. In contrast, in human cells chronically exposed to iodide, perchlorate and other ions *in vivo,* the concentrations of these ions would build up over time to some steady-state or pseudo steady-state levels. In humans, the rate of iodide uptake is driven by the competition between the iodide concentration gradient, (with generally high iodine concentrations inside the cell), and the sodium electrochemical potential gradient (lower sodium concentration in the cell), which is coupled to NIS activity. The NIS uptake tends to transport a net charge (two sodium ions, one iodide) across the cell membrane, but for the charge balance for perchlorate is not clear (Dohan et al. 2007). Thus, the presence of the iodide and inhibitor ions in the thyroid cells at the time of the dose (an effect not seen in the Tonacchera et al. system) would affect the electrochemical potential and could change the absolute and relative amounts of the ion that are transported by the NIS.

Finally, the medium into which the CHO cells were placed during the Tonacchera experiment likely provided a nearly constant concentration of iodide and inhibitor ions over the duration of the experiment. Although it is not possible to determine from the paper itself, the ionic composition of the medium likely did not change over the course of the run, suggesting the cells had an "infinite" supply of iodine and inhibitor mass at the specified concentration over the course of the relatively short experimental time. Thus, the Tonacchera experiment likely captures the maximum possible iodide uptakes at a specified PEC. In the human body, nearly 90% of an administered dose of iodide leaves the body as urine (page 118 of *OIG Scientific Analysis of Perchlorate*, citing Dunn 1998). In addition, dietary doses of iodide likely occur on half-daily to daily timescales and are not replenished over a 45 minute time span. Thus, the human thyroid sees temporally varying arterial concentrations of iodide and the inhibitors, and it is the long term average (that is, longer than 45 minutes) of the iodide uptake which determines the level of intracellular iodide that is drawn on for thyroid hormone synthesis.

For these reasons, we tested the Tonacchera Model to determine whether the *in vitro* experiment suitably captures the dynamic behavior of iodide inhibition in humans over long time scales. To this end, we compared the Tonacchera predicted levels of NIS inhibition to the predictions from two versions of the Merrill-Clewell physiologically-based pharmacokinetic (PBPK) model (e.g. Merrill et al. 2005, Clewell et al. 2007a). This model has been peer reviewed, the parameters have been optimized for the use in pregnant humans, and the Clewell version of the model is used to convert serum concentrations to equivalent doses in *OIG Scientific Analysis of Perchlorate*.

B.2 Development of PBPK Models

To evaluate the Tonacchera Model and its applicability to the human thyroid, two different Excel-based PBPK models were built, both of which are based on the human Merrill-Clewell model. The models model iodide and perchlorate pharmacokinetic behavior using parameters listed in Merrill et al. (2005) and Clewell et al. (2007a)[5], and the model is expanded to include thiocyanate and nitrate by making assumptions of how the chemical-specific parameters for these ions compare to those for perchlorate.

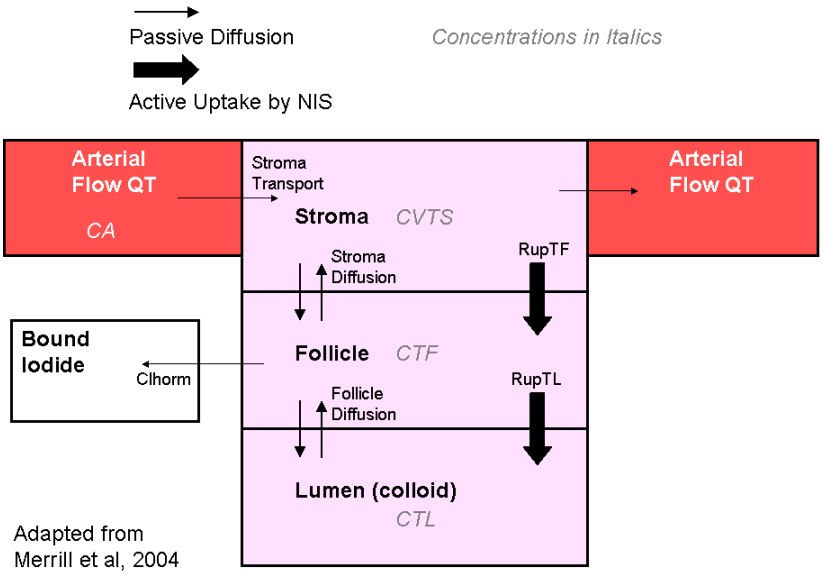
The first model is a thyroid-only model, which was constructed to compare a functioning human thyroid with the CHO cells in the Tonacchera Model. Figure B-1 below shows the general layout of the model, taken from the full human body model of Merrill et al. (2005). The thyroid-only model built here assumes a fixed concentration of the ions in the arterial flow, so that the arterial flow is, in effect, an infinite source of iodide and inhibitor mass to the thyroid. The thyroid then takes up iodide into the stroma (connective tissue), follicle, and lumen (colloid), and the follicle loses mass of free iodide due to the formation of bound iodide. Specifying a fixed arterial concentration makes the assumption that in the full human body, the first-order balance that maintains an observed serum concentration is between the source in the gastrointestinal (GI) tract and the removal by the kidneys. The depletion of arterial ion mass due

[5] The Clewell study is the most recent, so the parameter values listed in that study are used in the modeling. However, the Clewell study is a follow-up of the Merrill study, and only a few of the parameters were updated from the Merrill values. Thus, the Clewell values were used for all updated parameters where values were available for both ions, and the Merrill parameters were used for the parameters that were not updated.

to the uptake and conversion in the thyroid is assumed to be a higher order term in the balance. This assumption is consistent with the observation that the urinary iodide concentration tends to be 90 percent of the dose in humans (page 118 of *OIG Scientific Analysis of Perchlorate*, citing Dunn 1998).

In addition to the thyroid-only model, a simplified full-body model was built to investigate how iodide removal by the kidney and thyroid uptake affect the arterial mass after a single dose of the ions. Figure B-2 below shows the general layout of the model, again taken from the full human body model of Merrill et al. (2005). To avoid the full complexity of the Merrill/Clewell model, the red blood cell compartment was excluded and all tissues other than the thyroid and the kidney were lumped into an "Other Tissue" compartment. The dose is administered by assuming an initial arterial concentration, rather than introducing it through the GI tract. This assumption allows direct comparison of iodide uptake with the thyroid-only model.

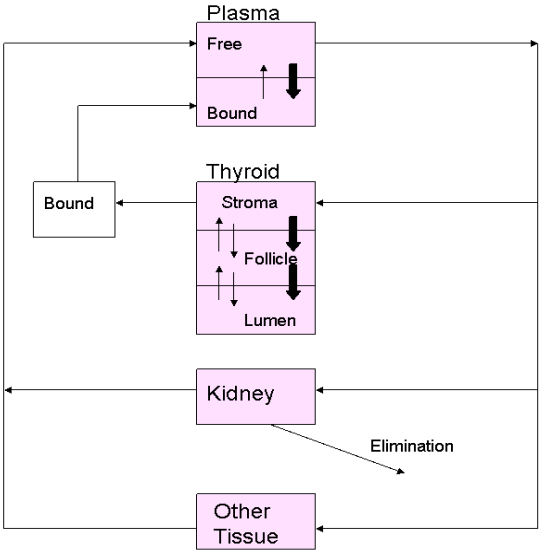
Figure B-1. Diagram of Thyroid-Only Model



Both of these models were built in Excel VBA using standard difference equations for mass balance in each compartment. Masses were converted to concentrations by dividing by the compartment volumes, which were assumed to be constant. Iodide uptake inhibition by perchlorate, thiocyanate, and nitrate was modeled using a competitive inhibition form of the Michaelis-Menten kinetic equation, as in the Merrill/Clewell model. At run time, the user can specify whether all three inhibitors are present or whether only perchlorate is present. The model is written in terms of mass rather than in terms of moles, so conversions are necessary to directly compare the predictions with the Tonacchera Model. In a single run, the model first assumes that iodide and any of other user-specified inhibitor ions are present, and iodide uptake is inhibited. In the second pass, the model assumes that only iodide is present. The model then compares the total uptake of mass by the colloid and lumen when the inhibitors are included to the uptake of mass when the inhibitors are absent. This ratio is referred to as the "relative

uptake," to match the terminology in the Tonacchera et al. paper, and this model metric is compared with the NIS relative uptake (extent of inhibition) reported in the Tonacchera et al. paper.

Figure B-2. Diagram of Simplified Full-Body Model



In the human body, NIS uptake is partially coupled to a sodium gradient, and inclusion of this effect in the uptake terms was considered. However, after contacting both R. Clewell and E. McLanahan, we learned that data for such a parameterization are not readily available and that sodium is usually assumed to be present in quantities that have little effect on the NIS uptake.

Initial concentrations in the serum and in the thyroid compartments can be set by the user, along with the volumes of the tissues, the partition coefficients, the Michaelis-Menten maximum capacities (V_{max}), the Michaelis-Menten constants (K_m), the permeability cross products, and the clearance/production rates. Where possible, these values were set to the same values used in Clewell et al. (2007a) because these represent optimized and tested parameter values. The exceptions include the "other tissue" partition coefficients (which were set equal to the slowly perfused tissue values in Clewell et al. (2007a) because these values are intermediate between the fat tissue and the richly perfused tissue values) and the "other tissue" volume, which was set by assuming that 80 percent of the body is perfused and by subtracting the thyroid, kidney, and blood volumes from that total.

The Merrill/Clewell model parameters have been optimized to agree with existing kinetic data for iodide and perchlorate. However, thiocyanate and nitrate kinetic parameter values were not available there or elsewhere in the literature. Thus, in nearly all cases, these parameters were set equal to the perchlorate parameters. The exceptions include the arterial concentrations, which are specified by the user, and the K_m values. For the K_m values, we converted the potencies for thiocyanate and nitrate given in Tonacchera et al. (2004) to relative mass potencies (rather than molar potencies), and we multiplied the perchlorate K_m by the potencies to estimate

the thiocyanate and nitrate K_m. This assumption is equivalent to dividing the thiocyanate and nitrate concentrations by the potency factors in the inhibition uptake equation and then assuming they have the same K_m as perchlorate. To see this, the effective K_m iodide in the presence of inhibitor ions is given by:

$$K_{m,eff} = K_{m,I}\left(1+\frac{[ClO_4^-]}{K_{m,P}}+\frac{[SCN^-]}{K_{m,T}}+\frac{[NO_3^-]}{K_{m,N}}\right)$$

where $K_{m,eff}$ is the effective iodide Michaelis-Menten (M-M) parameter, and $K_{m,I}$, $K_{m,P}$, $K_{m,T}$, and $K_{m,N}$ are the iodide, perchlorate, thiocyanate, and nitrate M-M parameters, respectively. If instead the inhibition uptake is expressed using the equivalent perchlorate concentration, then:

$$K_{m,eff} = K_{m,I}\left[1+\frac{1}{K_{m,P}}\left([ClO_4^-]+[SCN^-]\beta_T+[NO_3^-]\beta_N\right)\right]$$

where the β values are the inverse of the relative perchlorate potencies, as in Tonacchera et al. (2004). Comparison of the two equations indicates that setting:

$$K_{m,T} = \frac{K_{m,P}}{\beta_T} \quad \text{and} \quad K_{m,N} = \frac{K_{m,P}}{\beta_N}$$

in the first equation ensures that the overall iodide uptake will be consistent with the assumption of an equivalent perchlorate concentration.

This normalization was done for the terms that capture the NIS uptake from the stroma to the follicle, but it was also done for the uptake from the follicle to the lumen and from the free plasma to the bound plasma. There is uncertainty surrounding these assumptions, since these uptakes are not mediated by the NIS and may have different relative potencies; however, in the absence of available data, it was assumed that the same relative potencies prevailed.

Table B-1 shows the chemical-specific model parameters used, where allometric scaling assuming a body weight of 70 kg has already been performed on the variables with asterisks. The italicized variables indicate parameters that are needed for the full model but not the thyroid-only model. Table B-2 shows the model parameters which are not chemical-specific. The volumes all assume the compartment density is 1 kg/L, since Merrill et al. (2005) gives volumes in terms of percent of body weight.

Table B-1. Chemical-Specific Parameters Used in the Models

Variable Description	Units	Iodide Value	Perchlorate Value	Thiocyanate Value	Nitrate Value	Source for Iodide and Perchlorate Values	Source for Thiocyanate and Nitrate Values
Initial stroma concentration	ng/L	User Defined	User Defined	User Defined	User Defined	N/A	N/A
Initial follicle concentration	ng/L	User Defined	User Defined	User Defined	User Defined	N/A	N/A
Initial lumen concentration	ng/L	User Defined	User Defined	User Defined	User Defined	N/A	N/A
Initial arterial blood concentration (free)	ng/L	User Defined	User Defined	User Defined	User Defined	N/A	N/A
Initial bound concentration in blood	*ng/L*	*User Defined*	*User Defined*	*User Defined*	*User Defined*	*N/A*	*N/A*
Initial bound concentration in thyroid	*ng/L*	*User Defined*	*User Defined*	*User Defined*	*User Defined*	*N/A*	*N/A*
Initial kidney concentration	*ng/L*	*User Defined*	*User Defined*	*User Defined*	*User Defined*	*N/A*	*N/A*
Initial Tissue concentration	*ng/L*	*User Defined*	*User Defined*	*User Defined*	*User Defined*	*N/A*	*N/A*
Perm. Cross Prod. From stroma to follicle*	L/hr	7.00E-03	7.00E-03	7.00E-03	7.00E-03	Clewell et al. 2007a	Same as Perchlorate
Perm. Cross Prod. From follicle to lumen*	L/hr	1.05E-03	7.00E-01	7.00E-01	7.00E-01	Clewell et al. 2007a	Same as Perchlorate
Partition coefficient for follicle	Unitless	0.15	0.13	0.13	0.13	Merrill et al. 2005	Same as Perchlorate
Partition coefficient for lumen	Unitless	7	7	7	7	Merrill et al. 2005	Same as Perchlorate
Partition coefficient for kidney	*Unitless*	*1.09*	*0.99*	*0.99*	*0.99*	*Merrill et al. 2005*	*Same as Perchlorate*
Partition coefficient for Tissue	*Unitless*	*0.2*	*0.3*	*0.3*	*0.3*	*Merrill et al. 2005 (slowly perfused)*	*Same as Perchlorate*

* These variables require allometric scaling, and the values in the papers were multiplied by a 70 kg body weight.

Table B-1 (Continued). Chemical-Specific Parameters Used in the Models

Variable Description	Units	Iodide Value	Perchlorate Value	Thiocyanate Value	Nitrate Value	Source for Iodide and Perchlorate Values	Source for Thiocyanate and Nitrate Values
Clearance by organification*	L/hr	0.7	N/A	N/A	N/A	Merrill et al. 2005	N/A
*Hormone Secretion**	*L/hr*	*8.40E-05*	*N/A*	*N/A*	*N/A*	*Merrill et al. 2005*	*N/A*
*Plasma unbinding**	*L/hr*	*0.063*	*1.75*	*1.75*	*1.75*	*Merrill et al. 2005*	*Same as Perchlorate*
*Urinary Excretion**	*L/hr*	*3.5*	*3.5*	*3.5*	*3.5*	*Clewell et al. 2007a*	*Same as Perchlorate*
Maximum velocity for follicular uptake*	ng/hr	8.54E+06	4.20E+05	4.20E+05	4.20E+05	Clewell et al. 2007a	Same as Perchlorate
Maximum velocity for lumen uptake*	ng/hr	7.00E+09	1.19E+06	1.19E+06	1.19E+06	Clewell et al. 2007a	Same as Perchlorate
*Maximum velocity for plasma binding**	*ng/hr*	*1.40E+04*	*3.50E+04*	*3.50E+04*	*3.50E+04*	*Merrill et al. 2005*	*Same as Perchlorate*
MM constant for follicular uptake	ng/L	4.00E+06	1.60E+05	1.44E+06	2.40E+07	Merrill et al. 2005	Perchlorate Normalized by Potencies from Tonacchera
MM constant for lumen uptake	ng/L	1.00E+09	1.00E+08	9.00E+08	1.50E+10	Merrill et al. 2005	Perchlorate Normalized by Potencies from Tonacchera
MM constant for plasma binding	*ng/L*	*7.80E+05*	*1.80E+04*	*1.62E+05*	*2.70E+06*	*Merrill et al. 2005*	*Perchlorate Normalized by Potencies from Tonacchera*

* These variables require allometric scaling, and the values in the papers were multiplied by a 70 kg body weight.

Table B-2. Model Parameters Which Are Not Chemical-Specific

Other Inputs	Units	Value	Source
Total Cardiac Output	L/h	1155	Merrill et al. 2005
Kidney blood flow	*L/h*	*202.125*	*Merrill et al. 2005*
Thyroid blood flow	L/h	18.48	Merrill et al. 2005
Stroma volume**	L	0.005796	Merrill et al. 2005
Follicle volume**	L	0.012033	Merrill et al. 2005
Lumen volume**	L	0.00315	Merrill et al. 2005
*Plasma volume***	*L*	*3.08*	*Merrill et al. 2005*
*Kidney volume***	*L*	*0.308*	*Merrill et al. 2005*
*Tissue volume***	*L*	*52.591*	*Assumes a total of 80% perfused*
Length of run	min	User Defined	N/A
Time step	min	User Defined	N/A

** Volumes assume a density of 1 kg/L

The time step for the model was fixed and was set to one second. In general, running with a shorter time step did not alter the results, indicating that one second was sufficient to resolve all mass transfers appropriately. The model was then typically run for 45 model minutes, in order to equal the duration of the Tonacchera experiment. The model was also run for longer times to discover the sensitivity of the relative uptake to the duration of the run (as discussed in the next section). Both the full and thyroid-only models ran for 45 model minutes in just a few seconds, allowing many model explorations to be performed.

The arterial concentrations of iodide and inhibitor ions were then set to a variety of values to span human-relevant parameter space in order to compare the relative uptakes in the model to the uptakes in the Tonacchera Model. To decide which concentration ranges were appropriate, the authors looked at the typical ranges for the inhibitors as reported in *OIG Scientific Analysis of Perchlorate* (page 75, and repeated in Table B-3, below). These upper and lower bound concentrations were converted to ng/L and the modeled parameter space went from half the lower bound up to double the upper bound. For iodide, only urinary concentrations were available, and the free iodide serum concentrations are likely much lower. Thus, a wide range of iodide concentrations were examined to evaluate the sensitivity of the relative uptake curves to the choice of iodide concentration.

Table B-3. Typical Serum Concentrations in Humans and Concentration Ranges Evaluated in the Models

	Iodide	Perchlorate	Thiocyanate	Nitrate
Typical Ranges (μmol/L)		0.0047	10-120	10-140
Typical Values (μmol/L)	7***	0.0013	40	40
Model Starting Value (μmol/L)	0.37	0.0001	5	5
Model Ending Value (μmol/L)	19	0.14	240	281
Model Starting Value (ng/L)	1.00E+04	10	2.9E+05	3.10E+05
Model Ending Value in (ng/L)	5.01E+05	1.39E+04	1.39E+07	1.74E+07

***This concentration is a urinary concentration, serving as a surrogate for the serum concentration.

B.3 Results: Comparison of PBPK Model Predictions with Tonacchera Model

In this section, the results from the PBPK models are compared with the Tonacchera Model. First, the models are run with only iodide and perchlorate, since these parameters have been optimized by Merrill/Clewell. Second, thiocyanate and nitrate are added using the approximated parameters. In the first case, relative uptake is plotted as a function of perchlorate concentration, while in the second, relative uptake is plotted as a function of PEC.

B.3.1 Results From the Models Including only Iodide and Perchlorate

Figure B-3 shows the relative iodide uptake for the thyroid-only and full models, as well as the relative NIS inhibition values for the Tonacchera Model. Here, the model concentrations have been converted to μmol/L for comparison with the Tonacchera Model, and concentrations are plotted on a logarithmic scale. The range of perchlorate concentrations extends beyond the human-relevant range, in order to demonstrate the full shape of the relative uptake curves. Overall, the predicted thyroid iodide uptake from the thyroid-only model agrees well with the inhibition data from the Tonacchera Model. However, the full model predicts higher relative thyroid uptakes for a given perchlorate concentration than would be predicted based on the NIS inhibition seen the Tonacchera Model.

These differences can probably be ascribed to differences in the structure of the full-body model compared to the thyroid-only setup and Tonacchera assay system. In the Tonacchera Model experimental set-up, CHO cells were exposed to a prescribed environmental concentration, and it is likely that the mass in that environment was not significantly depleted over the short (45 minute) assay. Similarly, the thyroid-only model, which assumes the thyroid is exposed to a constant arterial concentration, allows build up of ion mass in the thyroid without depletion of the surrounding media (in this case, the serum). However, in the full model, the arterial concentration decreases over the course of the 45 minute run since kidney elimination is included and it is assumed that the person does not get another dose of any ion during the 45 minutes. Figure B-4 shows the time series of the iodide arterial concentration over the course of a 45 minute run, with an initial concentration of 1.5E+05 ng/L. The iodide concentration quickly decreases by a factor of four within a minute and then slowly decreases over the remainder of the run. After 45 minutes, the arterial concentration is 5.4 times smaller than the initial concentration, indicating the constant-arterial-concentration assumption in the thyroid-only model (and, by extension, the Tonacchera Model) is likely not justified and overestimates the inhibition of the iodide uptake.

Figure B-3. Relative Inhibition Iodide Uptake in the Full Model, the Thyroid Only Model, and the Tonacchera Model for a Wide Range of Perchlorate Concentrations

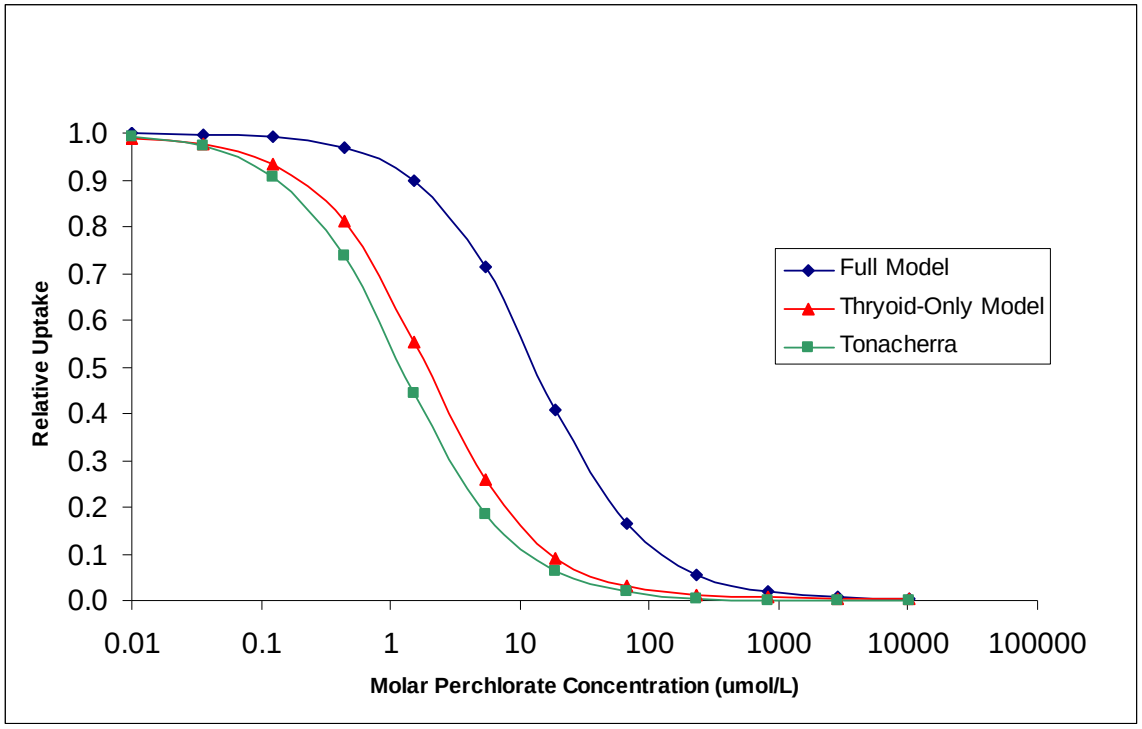
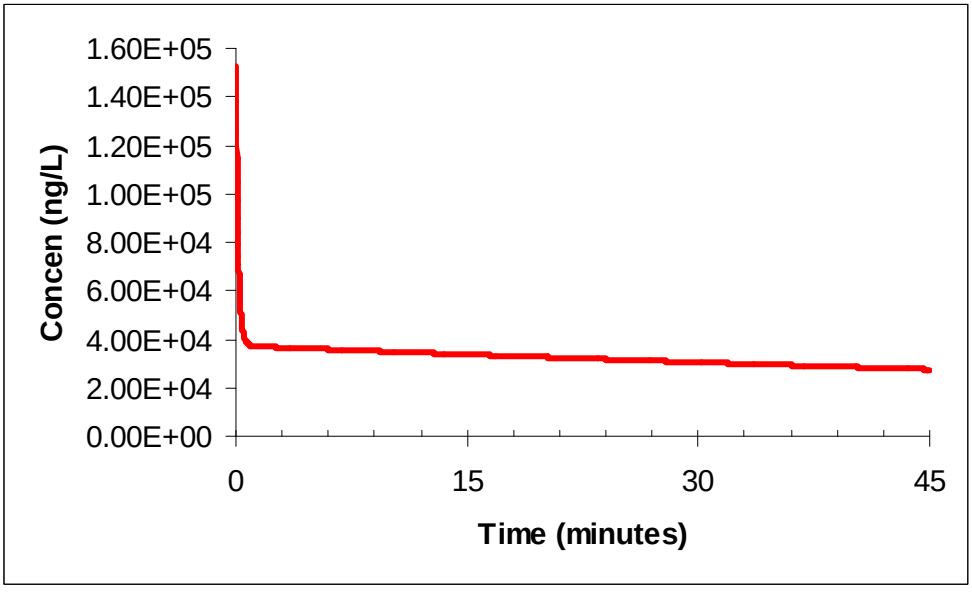


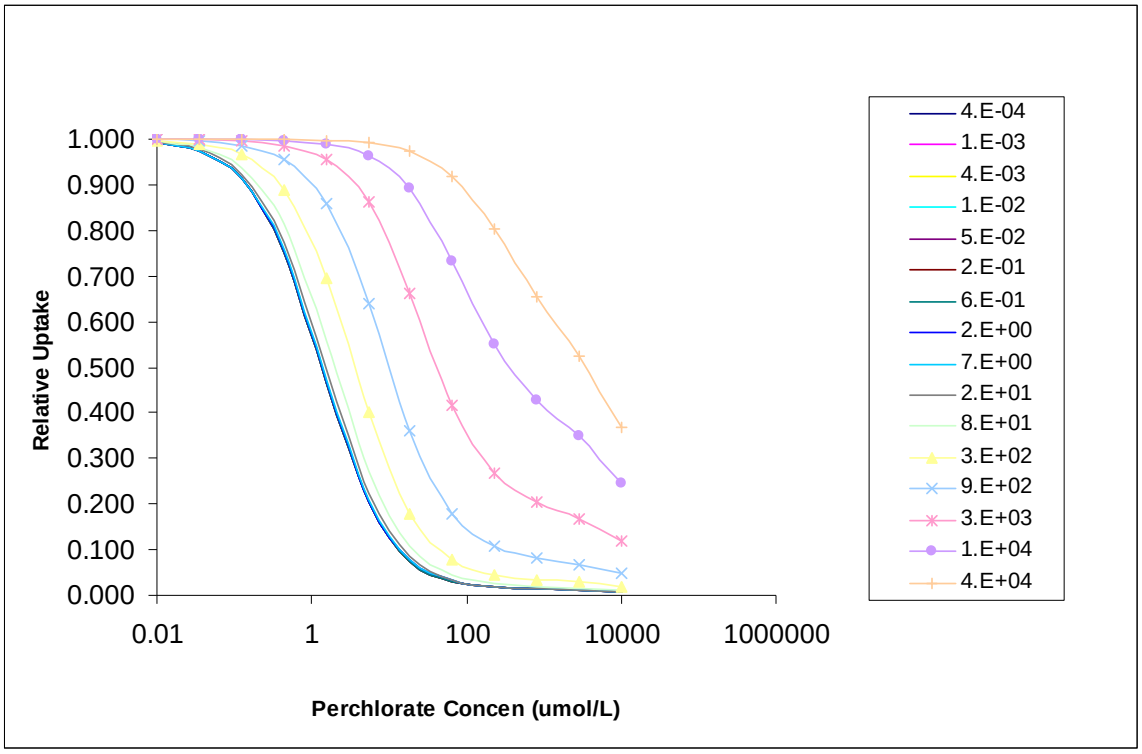
Figure B-4. Iodide Time Series in a 45 Minute Full Model Run



We also compared the predicted iodide uptake in the thyroid-only model with predicted degree of NIS inhibition from the Tonacchera et al. system. To do this, the model was run over a range of both perchlorate and iodide arterial concentrations. Figure B-5 shows the relative uptake as a function of the perchlorate concentration for a variety of iodide arterial concentrations, where the arterial concentrations are given in µmol/L. The curves are nearly

identical for iodide concentrations up to approximately 80 µmol/L, and then the relative uptake is larger for the same perchlorate concentration at larger iodide concentrations. The typical urinary iodide concentration was given as 170 µg/L or about 7 µmol/L in *OIG Scientific Analysis of Perchlorate*. This at best is an upper bound for the long-term free iodide concentration in the serum under typical conditions. Thus, at human-relevant long-term iodide concentrations, the thyroid-only model predicts iodide concentrations that are consistent with the NIS inhibition predicted by the Tonacchera Model.

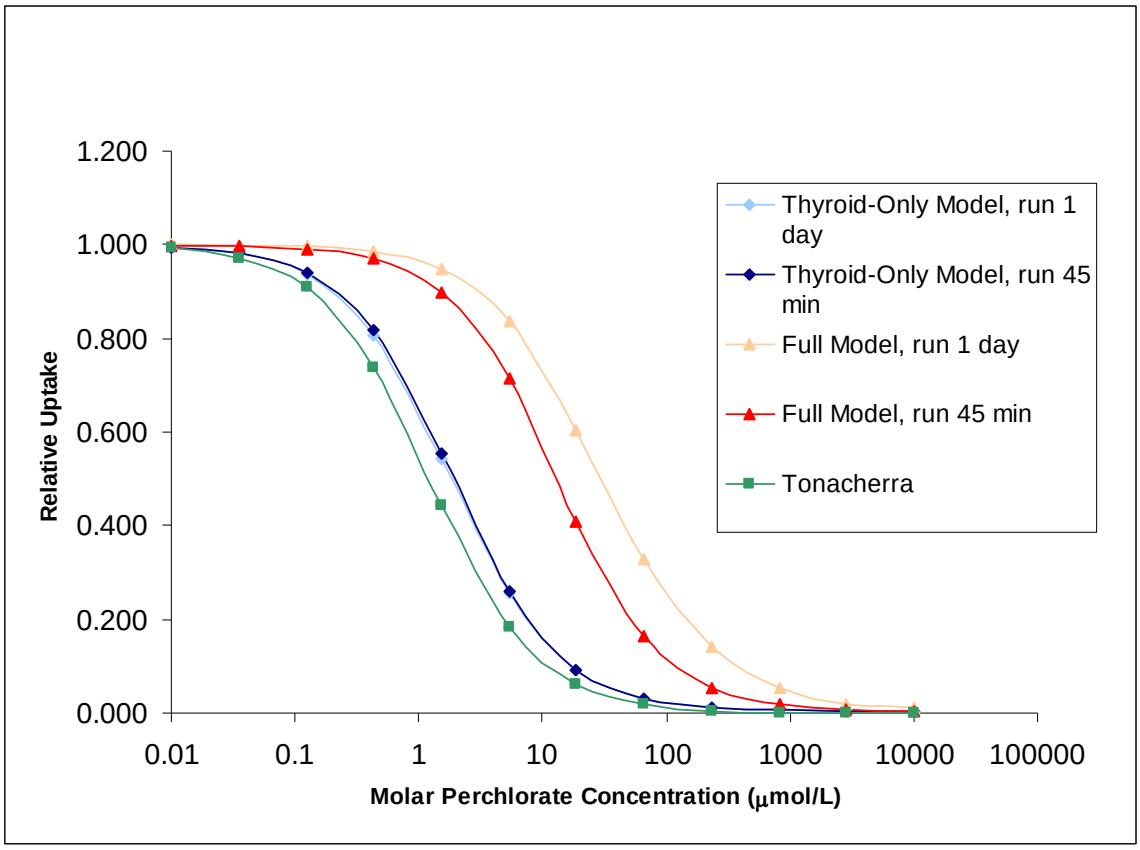
Figure B-5. Relative Inhibition Iodide Uptake as a Function of Perchlorate Concentration for a Variety of Iodide Concentrations in the Thyroid-Only Model



To obtain the results presented thus far, the PBPK models were run for 45 minutes, in order to be directly comparable to the Tonacchera experiments. However, as seen in Figure B-4, the full model predicts a steadily decreasing arterial concentration during that time. To determine the effect of running the model for longer time periods, both models were run for a full day (again assuming no additional dose in the full model). Figure B-6 compares the uptake curves in runs of 1 day to those in runs of 45 minutes. For the thyroid-only model, the curves are nearly identical when the model run is extended, since the serum provides a constant supply of ion mass; however, the full model predicts higher relative uptakes when the model is run longer for the same perchlorate concentration. At low perchlorate concentrations, this difference is minimal, since perchlorate is not effectively inhibiting the iodide uptake. At intermediate perchlorate concentrations, the arterial perchlorate concentrations are predicted to decrease during the course of the full model run, so the overall iodide uptake is less strongly inhibited than when the perchlorate arterial concentrations are artificially held constant. At high perchlorate concentrations, the inhibition is effective even though the perchlorate concentrations are gradually falling, and the relative uptake is low for all models. It seems reasonable to expect that

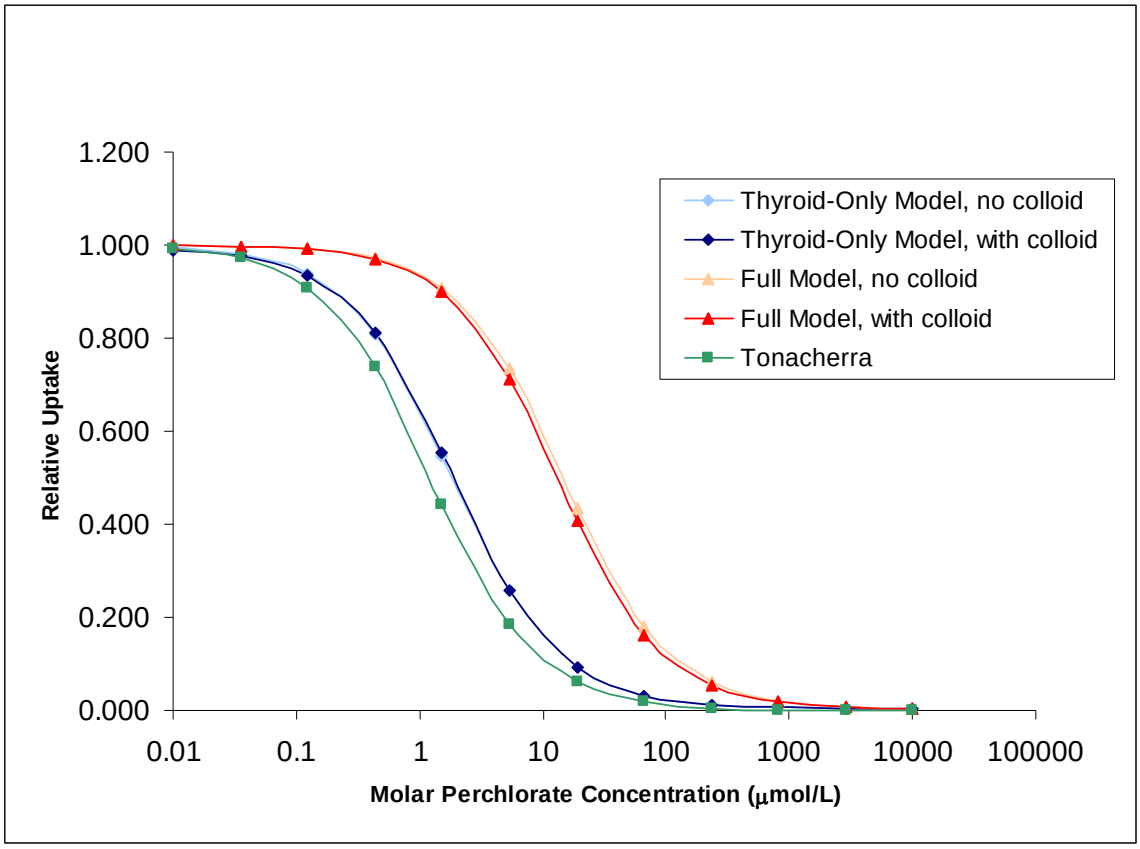
the full model, with the assumption that no additional iodine or perchlorate dose is administered during the run, is appropriate for a 1 day simulation; however, at longer time periods, one would expect a dietary source of iodide (and the other ions) would introduce a new source of ion mass to the serum.

Figure B-6. Comparison of the Relative Thyroid Iodide Uptake as a Function of Perchlorate Concentration when the Thyroid-Only and Full Models are Run for 1 day and for 45 Minutes



The Tonacchera experiment uses CHO cells which have follicle cells but which do not contain a colloid compartment as in the human thyroid. To determine the effects of excluding the colloid from the PBPK models, the models were run for 45 minutes and the lumen permeability cross product and V_{max} values were set to zero. This effectively eliminates the lumen from the models. Figure B-7 compares the thyroid-only model and full model relative uptakes as a function of perchlorate concentration when the colloid is either included or excluded. The Tonacchera Model relative uptake is shown as well for comparison. The model curves with and without the colloid are nearly identical, indicating that the presence of the lumen does not greatly change the iodide uptake inhibition in the models. This result would suggest that the structure of the CHO cells does not limit their applicability to human thyroid cells. However, the uptake by the lumen is parameterized using the simple Michaelis-Menten uptake equation and may not adequately capture the kinetics of the human thyroid colloid.

Figure B-7. Comparison of the Relative Uptake as a Function of Perchlorate Concentration when the Thyroid-Only and Full Models Are Run With and Without a Colloid Compartment

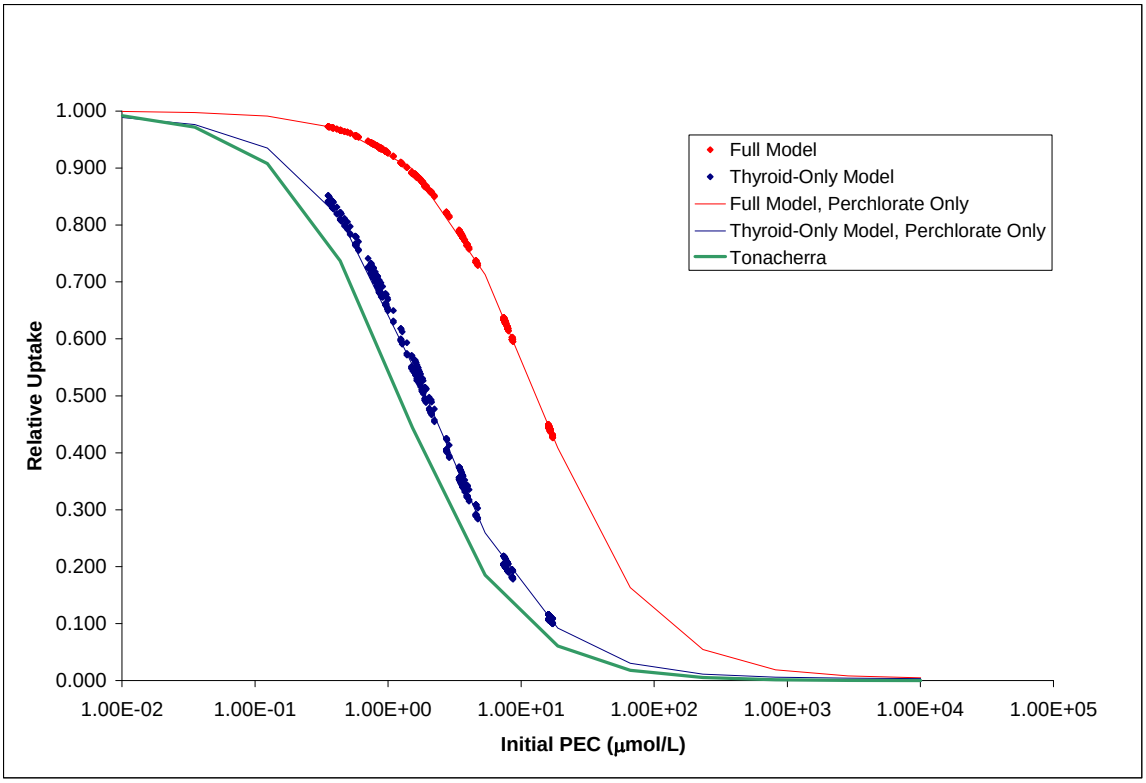


B.3.2 Results From the Models Including Iodide, Perchlorate, Thiocyanate, and Nitrate

The results reported above all assume that perchlorate is the only inhibitor present. The Merrill/Clewell model has kinetic parameters for both iodide and perchlorate that have been optimized using human data. However, no such optimization has been performed for thiocyanate and nitrate. In this subsection, we present model results when all inhibitors (perchlorate, thiocyanate, and nitrate) are included. The thiocyanate and nitrate kinetic parameters are assumed to be the same as those for perchlorate, aside from the K_m values. As discussed previously, these have been normalized using the potency factors in the Tonacchera Model. Figure B-8 shows the relative thyroid iodide uptake inhibition uptake as a function of PEC using the full model and thyroid-only model and using a range of ion concentrations in the humanly-relevant portion of parameter space (see Figure B-3). Also shown for reference are the predictions from the full model and the thyroid-only model when only perchlorate is included, and the predicted NIS inhibition from the Tonacchera Model. Because the nitrate and thiocyanate inhibition were modeled in a way that is consistent with the idea of the PEC from the Tonacchera et al. model, the model results including all ions lie along the same curve as the model results including only perchlorate and iodide. However, the full model continues to

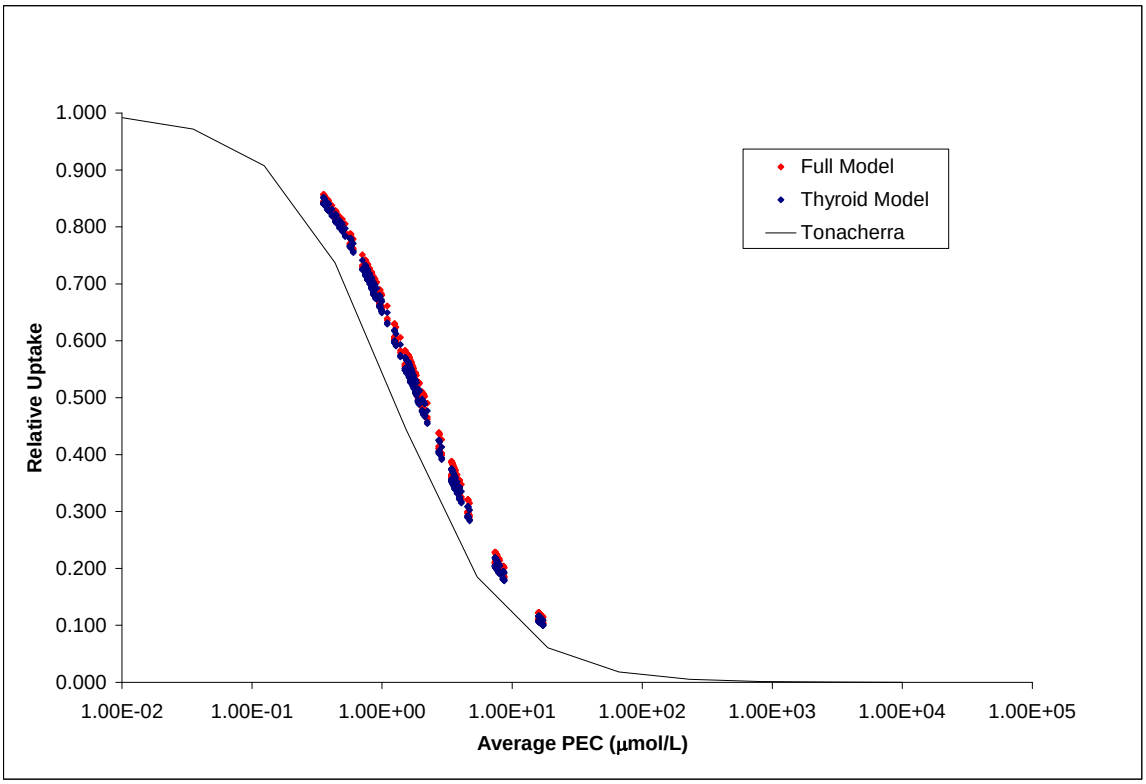
exhibit higher relative uptakes for the same PEC, compared to the thyroid-only model and the Tonacchera Model.

Figure B-8. Relative Iodide Inhibition Uptake as a Function of the Initial Equivalent Perchlorate Concentration for Ion Concentrations in Human-Relevant Ranges



In Figure B-8, the uptakes are plotted as a function of the initial PEC values. However, as discussed above, the arterial concentrations tend to decrease during the course of the 45 minute run in the full model. They remain constant in the thyroid-only model by design. Thus, the *average* arterial PEC may better capture the actual arterial concentration from which the thyroid extracts ion mass. Figure B-9 shows the relative iodide inhibition uptake as a function of the average PEC using the full model and thyroid-only model and using a range of ion concentrations in the humanly-relevant portion of parameter space. To make this calculation, it was observed that the average concentrations were generally about 6.7 times lower than the initial concentrations for perchlorate, thiocyanate, and nitrate. Thus, the initial arterial concentrations were multiplied by 6.7, so that the average concentrations were nearly identical to the initial concentrations in Figure B-8. This technique assumes that the ranges used to find the human-relevant parameter space were derived from measurements of long term average serum concentration, not an instantaneous spike after a dose. In general, the full model predicts relative uptakes that are similar to the thyroid-only model when the average arterial concentration is used as the PEC. Both the full and thyroid-only model predict thyroid uptake inhibition that differs slightly from the results seen in the Tonacchera et al. in experiments.

Figure B-9. Relative Iodide Inhibition Uptake as a Function of the *Average* Equivalent Perchlorate Concentration for Ion Concentrations in Human-Relevant Ranges



B.4 Conclusions

The Tonacchera Model was evaluated using adapted forms of a peer reviewed iodide and perchlorate PBPK model (the Merrill-Clewell model, using parameters for the pregnant human from Clewell et al. 2007a). Evaluation was performed using a thyroid-only model, which is exposed to a constant-concentration serum and thus has an infinite supply of ion mass at the specified arterial concentration, and a full body model, in which the thyroid is exposed to diminishing arterial concentrations due to elimination by the kidneys. The models were run first with perchlorate as the only inhibitor, and then thiocyanate and nitrate were included.

Overall, the thyroid-only model is predicts short-term thyroid uptake inhibition that is generally consistent with the NIS inhibition results from the Tonacchera Model, both with perchlorate alone, and when all three inhibitors are included. The modeled relative iodide uptake inhibition is fairly insensitive to the assumed serum iodide concentrations within the range of physiologically plausible iodide concentrations. In addition, the modeled relative uptakes are not sensitive to the presence of a colloid compartment, which tends to have slow back diffusion into the follicle.

However, the results from the full model were less consistent with the short-term NIS inhibition results from the Tonacchera Model. The efficient kidney elimination of iodide causes dramatic changes in the arterial concentration in the first few minutes of the model run, and the

relative thyroid uptakes predicted by the full-body model are larger for a given perchlorate concentration than predicted by the Tonacchera Model NIS inhibition. Both the iodide and the perchlorate serum concentrations change in parallel over the course of the run. However, because the iodide uptake is relatively insensitive to the iodide concentrations at low perchlorate concentrations but is sensitive to the perchlorate concentrations, the overall inhibition is less effective and the relative uptakes are higher.

When the other ions were added, the relative uptakes predicted by the full and thyroid only model plotted as a function of the PEC fell along the same curves as the perchlorate-only relative uptakes. This result is not unexpected, since the thiocyanate and nitrate K_m values were estimated in a fashion consistent with the PEC definition used by Tonacchera et al. The same K_m normalizations were performed for the follicle, lumen, and free iodide uptakes, which may not be a realistic assumption, because these processes are not mediated by the NIS. When thiocyanate and nitrate K_m values become available in the literature, this model comparison should be undertaken again, and quite possibly it will be observed that the true thyroid inhibition does not behave similarly for identical PEC with different perchlorate, thiocyanate, and nitrate concentrations.

The Tonacchera CHO cells were likely exposed to a medium that had nearly constant ion concentrations over the course of the 45 minute experiment, as in the thyroid-only PBPK model. Thus, the initial PEC concentration is the relevant concentration to use when plotting the relative uptakes. However, in the full model, the ion concentrations fall dramatically over the first few minutes of the run, and then fall more gradually for the remainder of the run. If the relative uptakes are plotted as a function of the average ion concentrations instead of the initial concentrations, the uptake curves are in line with the thyroid-only model and roughly agree with the Tonacchera Model.

In a risk assessment, it is necessary to determine the long-term effects of chronic doses of inhibitor ions. If measurements of population serum concentrations are available (and particularly if multiple samples from the same individual are available), then they could be used to estimate average arterial concentrations. However, *OIG Scientific Analysis of Perchlorate* often applies the Tonacchera Model normalized to dose rather than long-term average serum concentrations. The model analysis performed here indicates that even only 45 minutes after an applied dose, the inhibition calculated using the dose is higher than the inhibition calculated using the average serum concentrations. Care must also be taken when using the Clewell model to derive serum concentrations from doses or vice versa, since the timescale this relationship changes dramatically with different durations of the simulation.

The model results suggest that, if anything, the short-term NIS inhibition estimated by Tonacchera is conservative, and is higher than the extent of thyroid uptake inhibition seen for the same PEC in the full model. However, as discussed above, it is inherently difficult to compare short-term NIS inhibition as measured by Tonacchera et al. with time-averaged thyroid iodide uptake, and even more difficult to compare NIS inhibition results with overall thyroid function in response to chronic perchlorate, thiocyanate, and nitrate exposures. Neither Tonacchera et al. nor the available PBPK models include HPT axis mechanisms that dynamically control NIS

expression and activity, as well as the rates of many other processes “downstream” of the NIS that ultimately determine maternal responses to thyroid stressors.

APPENDIX C: SUMMARY OF PREVIOUS PERCHLORATE RISK ASSESSMENTS

As discussed in Section 5, in evaluating how best to conduct a risk assessment for perchlorate, it is useful to review the methods and data used in previous efforts. This Appendix presents a brief review of previous perchlorate risk assessments.

C.1 EPA's Risk Assessment (U.S. EPA 2002c)

Owing to its complex and mode of action and potential interactions with other thyroid stressors, the assessment of health risks associated with perchlorate poses a number of complex technical challenges. These problems were so complex that EPA took several years during the 1990s to develop a toxicokinetics/toxicodynamics testing strategy identifying specific studies that needed to be performed to support a risk assessment (U.S. EPA 1998). Studies recommended as part of the strategy included a 90-day subchronic bioassay, a genotoxicity assay, developmental (rabbit) and 2-generation reproductive (rat) studies (both of which would also examine thyroid histopathological endpoints), and an immunotoxicity assay. These studies were conducted in the late 1990s, and the results gradually became available starting in 2000. Thus, perchlorate is almost unique with regard to the breadth and depth of the toxicity test data available when EPA developed its risk assessment *Perchlorate Environmental Contamination Toxicological Review and Risk Characterization* (U.S. EPA 2002c). Basing its risk assessment on a controversial finding of changes in brain morphometry in 21-day-old rat pups exposed to low doses of perchlorate, EPA derived a very low RfD for protection against neurodevelopmental effects of 0.00003 mg/kg-day, corresponding to an average drinking water concentration of 1 μg/L, assuming humans received 100 percent of their perchlorate intake from water. The methodology and results of that assessment are summarized in Table C-1 along with a number of subsequent risk assessments undertaken by other parties after EPA's analysis was published.

While EPA's 2002 assessment was well-supported by animal toxicity studies, the Agency was somewhat handicapped in its choice of data in that a temporary moratorium was in effect that precluded EPA's use of data from human volunteer studies. Aside from the Greer et al. volunteer study (2002) that was just being completed, there were no high-quality human clinical or epidemiological studies of perchlorate exposure available at the time when EPA began its work. A number of stakeholders questioned EPA's decision to base the RfD on a poorly defined endpoint of unknown clinical significance that was highly discrepant with endpoints from other toxicity studies.

C.2 Risk Assessments Prior to NRC (2005)

In 2002, Greer et al. reported the results of a clinical study of the impacts of perchlorate exposures on 31 adult volunteers. Subjects were given doses of 0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day perchlorate as soluble salts in drinking water for 14 days. Changes in 8- and 24-hour radioactive iodide uptake were measured, along with serum levels of total T_4 (tT_4), free T_4 (fT_4), total T_3 (tT_3), and TSH and urinary iodide excretion. The results of this high-quality clinical study were then used by several groups to derive dose-response, RfD values, and equivalent average drinking water concentrations, as shown in Table C-1. The justification for using the

Greer et al. (2002) study was that, despite its shortcomings (small number of subjects, short duration), the availability of a defensible human study should be given precedence over the results of the more questionable animal studies.

The various groups that used the Greer et al. (2002) study for risk assessment selected different endpoints, dose-assessment models, and uncertainty factors (UFs) to derive RfD values for perchlorate exposure and equivalent average drinking water concentrations (EADWCs) ranging from 65 μg/L (Dollarhide et al. 2002) to 6 μg/L (Cal/EPA 2004). Another group (Strawson et al. 2003) used the results of an epidemiological study of Chilean school children exposed to up to 100 μg/L perchlorate in drinking water that found no adverse effects on serum T_4 levels. This group also arrived at an RfD of 65 μg/L, which equivalent to that estimated by Dollarhide et al. (2002). The approaches used in the various assessments are described in detail in ICF (2004) and will not be discussed further here.

C.3 NRC (2005) and Subsequently Published Studies

The results of more recent perchlorate risk assessments are summarized in Table C-2. As discussed previously, the NRC Committee to Assess the Health Implications of Perchlorate Ingestion (NRC 2005) also relied on the Greer et al. (2002) results to estimate an oral RfD for perchlorate and an equivalent average drinking water concentration. The NRC Committee identified the lowest dose administered in this study (0.007 mg/kg-day) as a NOAEL because it was the lowest dose at which no effect on iodine uptake was observed in this study. Even though they identified this dose as a NOAEL, the NRC Committee noted that inhibition of iodide uptake was not actually an "adverse" effect but instead was simply an indicator of the potential for adverse thyroid effects, which they identified as clinical hypothyroidism. Because the critical effect was non-adverse, the NRC elected to employ a LOAEL-NOAEL uncertainty factor of 1.0. They also elected to assign values of 1.0 to uncertainty factors for short duration of exposure and for database limitations. They did, however, assign a value of 10 for the intraspecies uncertainty factor, reflecting possible differences in sensitivity between the study subjects (healthy, non-pregnant volunteers) and the most likely sensitive population (pregnant women), yielding an RfD estimate of 0.0007 mg/kg-day. This dose is equivalent to a drinking water concentration of 25 μg/L (assuming 2.0 L/day water consumption by a 70-kg individual).

Table C-1. Summary of Perchlorate Risk Assessments through 2004 (Source: ICF 2004)

	ICF (2004)	U.S. EPA (2002c)	Cal/EPA (2004)	TERA (Dollarhide et al. 2002)	TERA (Strawson et al. 2003)
Critical Study	Greer et al. (2002)	Argus (1998, 2001)	Greer et al. (2002)	Greer et al. (2002)	Crump et al. (2000a)
Species	Human adult volunteers	Sprague-Dawley rats	Human adult volunteers	Human adult volunteers	Human children (6-8 years of age) volunteers in Chile
Type of Study	Controlled, clinical, 14 days	Neurohistological/ neurodevelopmental toxicity	Controlled, clinical, 14 days	Controlled, clinical, 14 days	Population-based cross-sectional
Doses	0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day	0, 0.01, 0.1, 1.0, 3.0, and 10.0 mg/kg-day	0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day	0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day	0, 4-7, and 100 ppb in drinking water
Critical Study Endpoint(s)	Change in tT_4, fT_4, or T_3 levels after 14 days of exposure	Changes in brain morphometry in pups on postnatal day (PND) 21 sacrifice and decreased T_4/increased TSH in dams of effected pups at various pre-/ postnatal time intervals	5% decrease in 24-hour iodide uptake after 14 days exposure	20% decrease in 24-hour iodide uptake after 14 days exposure	Change in T_4 levels following lifetime exposure, including during gestation
Point of Departure (mg/kg-day)	$BMDL_{10}$ = 0.18	LOAEL = 0.01	$BMDL_{05}$ = 0.0037	$BMDL_{20}$ = 0.02	NOAEL = 0.006 (free-standing)
Uncertainty Factors (UFs)[1]	UF_H = 10 UF_{DUR} = 3 UF_{DB} = 3	UF_H = 3 UF_{DUR} = 3 UF_L = 10 UF_{DB} = 3	UF_H = 10 UF_{DUR} = 1	UF_H = 10 UF_{DUR} = 1	UF_H = 3 UF_{DUR} = 1
RfD (mg/kg-day)	0.0013	0.00003	0.00037	0.002	0.002
EADWC (ppb)[2]	40	1	6 (PHG)	65	65

1. UFs: UF_H = intra-individually in humans; UF_{DUR} = exposure duration; UF_{DB} = database deficiencies; UF_L =LOAEL to NOAEL extrapolation
2. EADWC = Equivalent Average Drinking Water Concentration

Table C-2. Summary of Perchlorate Risk Assessments Since 2006

	NRC (2005)	Crump and Gibbs (2005)	Gibbs (2006)
Critical Study	Greer et al. (2002)	Greer et al. (2002), Braverman et al. (2005), Lamm et al. (1999)	Tonacchera et al. (2004), other epidemiological studies
Species	Human adult volunteers	Human adult volunteers (Greer et al. 2002); male workers (Braverman et al. 2005, Lamm et al. 1999)	Humans
Type of Study	Controlled, clinical, 14 days	Controlled, clinical, 14 days (Greer et al. 2002); cross-sectional measurements thyroid parameters before, after three-day occupational exposures (Braverman et al. 2005, Lamm et al. 1999)	Multiple human case and epidemiological studies, *in vitro* NIS inhibition studies of perchlorate, thiocyanate
Doses	0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day	0, 0.007, 0.02, 0.1, and 0.5 mg/kg-day (Greer et al. 2002); 0.4 – 392 mg/shift (Braverman et al. 2005); <1 – 35 mg/day (Lamm et al. 1999)	Various perchlorate and thiocyanate dose metrics (smoking used as thiocyanate surrogate in some cases)
Critical Study Endpoint(s)	(Lack of) iodine uptake inhibition	One standard deviation change in fT_4 and TSH (Greer et al. 2002); change in fT_4 index (FTI) and TSH (Braverman et al. 2005, Lamm et al. 1999)	"Non-adverse:" goiter, thyroglobulin levels, and RAIU "Adverse:" myxedema, clinical hypothyroidism, protein-bound I, fT_4, tT_4, and TSH
Point of Departure (mg/kg-day)	NOAEL = 0.007	$BMDL_{1SD}$ [1] Greer et al. (2002) 0.64 – 0.79 Braverman et al. 2005, Lamm et al. 1999 (combined data) 0.18 – 0.56 (FTI) 0.36 – 0.92 (TSH)	Equivalent perchlorate dose No adverse effects: 0.1-0.26 Non-adverse effects: 0.35 – 0.44 Adverse effects: > 0.56
Uncertainty Factors (UFs)[2]	$UF_H = 10$ $UF_{LOAEL} = 1$ $UF_{DUR} = 1$ $UF_{DB} = 1$	Not calculated	Not Used
RfD (mg/kg-day)	0.0007	Not calculated	Not calculated
EADWC (ppb)[3]	25	Not calculated	No adverse effects: 3,500 - 9,100[4] Non-adverse effects: 12,300 – 15,400 Adverse effects: > 19,6000

1. $BMDL_{1SD}$ = Benchmark Dose Low (lower confidence limit) for a one-standard deviation change in the measured endpoint
2. UFs: UF_H = intra-individually in humans; UF_{DUR} = exposure duration; UF_{DB} = database deficiencies; UF_L =LOAEL to NOAEL extrapolation
3. EADWC = Equivalent Average Drinking Water Concentration
4. Calculated by ICF from the Gibbs (2006) effects levels, assuming 2.0 L/day water consumption by a 70-kg person

Two other assessments have also been published more recently. Crump and Gibbs (2005) employed a benchmark dose methodology to calculate lower confidence limits on the daily perchlorate doses that resulted in specified changes in thyroid function parameters (5 percent, 10 percent, one standard deviation) in the Greer et al. (2002) study and in two occupational studies of subjects who worked at the same plant (Braverman et al. 2005, Lamm et al. 1999). Based on the Greer et al. (2002) study fT_4 data, they calculated BMDL values between 0.64 and 0.73 mg/kg-day; the BMDL values for changes in TSH concentrations were 0.70-0.79 mg/kg-day. When the combined Braverman et al. (2005) and Lamm et al. (1999) occupational data were used, the range of calculated BMDL values for fT_4 index (FTI) was 0.18 to 0.56 mg/kg-day. The BMDL values for changes in TSH calculated for this cohort were 0.36-0.92 mg/kg-day. Gibbs and Crump (2005) did not calculate RfD values or equivalent average drinking water concentrations corresponding to these benchmark dose estimates. These results, however, can be compared to those generated by ICF (2004); in that analysis, we calculated a range of $BMDL_{10}$ values for different measures of thyroid hormone levels ranging from 0.18 to 0.58 mg/kg-day. These values correspond to equivalent average drinking water concentrations ranging from 40 to 136 μg/L when a total UF value of 100 is used. Both the Gibbs and Crump (2005) and the ICF (2004) analyses share the limitations; the data on which they were based came from short-term studies of healthy non-pregnant adult volunteers and male workers rather than from pregnant women (the identified susceptible population).

Finally, Gibbs (2006) recently published a risk assessment for perchlorate that is explicitly based on the idea that perchlorate "safe" doses can be estimated by reference to the relative potency of NIS inhibition by perchlorate and thiocyanate. Summarized briefly, Dr. Gibbs analyzed the results of a number of clinical case studies, occupational and volunteer exposure studies, and epidemiological studies of smokers to characterize the dose of thiocyanate associated with "non-adverse" and "adverse" effects (see Table C-2). Then, he used *in vitro* data (primarily the Tonacchera et al. 2004 study of short-term NIS inhibition) to calculate equivalent perchlorate doses that would be expected to cause the same types of effects. As shown in Table C-2, he concluded that perchlorate equivalent doses in the range of 0.12 to 0.26 mg/kg-day were not likely to be associated with any adverse or non-adverse effects, while doses in the range of 0.35 to 0.44 mg/kg-day could be associated with the defined non-adverse effects. The defined adverse effects were only predicted to occur at perchlorate equivalent doses of greater than 0.56 mg/kg-day. Dr. Gibbs did not calculate RfD values, nor did he suggest that any uncertainty factors would be needed to establish doses for sensitive populations. As shown in the bottom of Table C-2, the daily doses that he predicts would be required to induce non-adverse and adverse effects are very much greater than those derived in the other risk assessments (>12,000 μg/L).

C.4 Lesson Learned from Previous Assessments

With the exception of U.S. EPA (2002c) all of the studies summarized in Tables C-1 and C-2 were conducted after a well-documented human volunteer study (Greer et al. 2002) was completed. It is interesting to note that the majority of the risk assessments conducted since 2002 selected this study as the most appropriate data set for risk assessment, despite the availability of a number of occupational and epidemiological studies. All of the assessors recognized the limitations of the study: the small number of subjects, the short duration, and the

use of healthy non-pregnant volunteers. However, it was clearly a better choice than any of the available animal data and clearly better than the available occupational and epidemiological studies. A key principal to note is that, despite the different approaches to analyzing and interpreting the Greer et al. (2002) data, selection of the best available human data set determined the basic form of all the subsequent risk assessments. The lone exception is Gibbs (2006), but we believe that his choice of critical human studies and classification of "non-adverse" and "adverse" thyroid effects is of questionable value for assessing *in utero* neurodevelopment risks.

The other major commonality among all the risk assessments is that, despite the universal recognition that perchlorate and thiocyanate share at least one common biochemical mechanism contributing to the critical adverse effect, they are all "single chemical" assessments. In none of these assessments is there any explicit consideration of the joint effect of perchlorate and other goitrogens, other thyroid-active agents, iodine insufficiency, or hormonal impacts of early pregnancy. This is certainly a limitation of these assessments. It is not necessarily a fatal limitation, however, because these assessments all acknowledge the problem and in different ways attempt to implicitly account for all these other affects. This is done in two ways.

First, it is assumed that the exposed subjects, to a large extent, are affected by other stressors (goitrogens, iodine deficiency, and other thyroid toxins) that are somehow "typical" of the identified sensitive population. Thus, the effects on thyroid function found by Greer et al. (2002) represent the marginal (additional) effects of perchlorate over a set of background exposures to thiocyanate and other goitrogens received by the study subjects. Therefore, the effects seen in these subjects are taken to be useful predictors of the marginal effects of perchlorate exposure that would be seen in pregnant women experiencing (roughly) the same background exposures. This problem of multifactoral causation frequently arises in chemical risk assessment and is not unique to perchlorate. One technically defensible way that this problem is addressed is to use the best data set available and to establish, to the extent possible, that the study population and most sensitive groups receive more-or-less equivalent "background" exposures.

The second way in which the "single chemical" assessment accounts for multiple chemical exposures (and for variations in receptor sensitivity) is through the use of uncertainty factors. To the extent that the "background" exposures in the study population are suspected to be different from those experienced by the most sensitive group, this can be incorporated into the "intraspecies" uncertainty factor. For example, the belief that pregnant women might be exposed to more thiocyanate than the Greer et al. (2002) subjects would argue for a larger intraspecies uncertainty factor. If data were available, potential differences in exposure to thiocyanate (and other thyroid stressors) could be evaluated, and this evaluation would support the selected UF value. All too often, however, there are few or no quantitative data to evaluate the differences in background exposures between subjects in the experimental human study and the most susceptible population (and such is the case for perchlorate).[6] That is why risk assessments tend

[6] OIG's suggestion that the iodine status of the Greer et al. (2002) subjects be investigated to shed more light on the role of iodine deficiency on the iodine uptake and thyroid changes seen in the study subjects is a good one. Dr. Gay Goodman is currently conducting such an analysis, and preliminary results will be available soon. Owing to the

to rely on the intraspecies uncertainty factor to address background exposure differences, as well as intraspecies variations in susceptibility.

The final, and most important aspect of the perchlorate risk assessments (which again, is common to all risk assessments), is that the quality and limitations of the "best" data set determine not only the general nature of the assessment but also, within broad ranges, determine the results of the assessment. Greer et al. (2002) found, as expected, dose-dependent inhibition in short-term RAIU but little or no effect on other indices in thyroid function (TSH, T_3, T_4 levels) over the dose range that they tested. All of the risk assessments relying on Greer et al. (2002) are forced to conform to these findings, and it is no surprise that they all come to similar conclusions with regard to the magnitude of risk. Despite the differences in the endpoints that were selected, the statistical models used to analyze the data, and even the different decisions that were made regarding UF values, all of the assessments that are based on Greer et al. (2002) established RfD values and equivalent average drinking water concentrations that differ by only about a factor of ten (from 6 to 65 μg/L). Once the Greer et al. (2002) study is selected, it is very hard to overcome its limitations by reference to the specifics of a specific biological mode of action. It is to be expected that this principal will also apply to a risk assessment conducted based on more recent epidemiological data.

small number of study subjects, however, that analysis may not provide much insight into this issue, and it does not address the other limitations of Greer et al. (2002) as a "critical" study for risk assessment.

Title: ICF's Oral Presentation to the OIG on ICF's Technical Review (Dated May 9, 2008) of the OIG Scientific Analysis of Perchlorate (Working Draft – Dated March 26, 2008)

Purpose: To document the content of ICF's presentation to the OIG and the discussion between ICF and the OIG concerning ICF's technical review.

May 21, 2008 -- 12:00 noon to 4:00 pm/EST

Attendees:

EPA-OIG:

Bill Roderick (BR)
Eileen McMahon (EM)
Eric Lewis (EL)
Michael Wilson (MW)

ICF:

William M. Mendez, Jr., Ph.D.
Jennifer Welham

Conclusion:

In 1997, former EPA Administrator Carol Browner directed EPA to embrace the cumulative risk approach on all future major risk assessments. ICF's oral presentation on their Technical Review indicates that "cumulative risk" principals are clearly applicable to perchlorate. However, ICF's recommends using a "whole mixture" method to perform a cumulative risk assessment while the OIG used a "component based" approach (i.e., dose addition method) to perform a cumulative risk assessment.

ICF's cumulative risk assessment approach of using a "whole mixture" method is accomplished by fitting a statistical model to the NHANES 2001-2002 epidemiological data using a decrease in thyroxine (T_4) during the first trimester of pregnancy as the critical effect. This selection of a critical effect contradicts the National Academy of Sciences (NAS) Committee's established science policy that the total iodide uptake (TIU) by the thyroid is the critical effect and does not take into account the increase sensitivity of the fetal thyroid during the 2^{nd} & 3^{rd} trimesters or the risk during nursing when the brain is still developing. Both Blount and Steinmaus analyses of the NHANES 2001-2002 epidemiological data are examples of this approach, but ICF indicates that these statistical models are incomplete and need further development. Furthermore, the increased toxicity of perchlorate reported in the Blount analysis is inconsistent with the known mechanism of toxicity for perchlorate and has not been corroborated in adverse effects observed in humans (Pearce 2008, Pearce 2007) or any other source of data. An independent evaluation of the Blount analysis suggests that the reported relationship between perchlorate dose and the predicted changes in total thyroxine (tT_4) may be an artifact (Lamm 2007). ICF's oral presentation allowed us to discuss the limitations with the "whole mixture" approach to cumulative risk assessment and ICF's interpretation of the

NHANES data. ICF's approach uses a single data source (i.e., selective) which has not corroborated by any other data source. The statistical model disagrees with the perchlorate toxicity reported by all other studies and this difference is not explained or accounted for by the "whole mixture" approach. Consequently, ICF did not provide sufficient information to warrant a recommendation to EPA to conduct cumulative risk assessment based on a "whole mixture" approach.

By contrast, the "component based" method is the "hallmark approach" for conducting a cumulative risk assessment. Furthermore, EPA policy is to assume components of a chemical mixture produce toxic effects through addivity. The mechanistic interaction of the four sodium iodide symporter (NIS) stressors meets all of EPA's risk assessment guidance requirements for using the dose addition method. The NAS Committee established science policy when setting the total iodide uptake (TIU) by the thyroid as the critical effect. The OIG used the Tonacchera Model which defines the dose addition relationship between the four NIS stressors and allows the TIU (i.e., the critical effect) to be calculated. The OIG identified the TIU level at which subtle mental deficits occur in children and corroborated the Tonacchera Model results against the observations of seven human studies. The OIG approach addresses the exposure to all four NIS stressors across all the critical life stages (i.e., fetal and neonate) when the brain is still developing. The OIG found that the TIU is both a good predictor and a good explanation for the observed toxicity of perchlorate. Consequently, using the Tonacchera Model in a dose addition cumulative risk assessment is consistent with EPA policy, NAS findings, EPA risk assessment guidance, and observed effects in humans. Therefore, OIG's potential recommendation that EPA continues the development and refinement of the cumulative risk assessment initiated by the OIG is supported. Furthermore, this dose addition method of cumulative risk assessment concludes that correcting iodide deficiency during pregnancy and nursing is the most effective and efficient approach to address this public health.

Summary:

Note: This workpaper documents the topics and issues discussed during the ICF's oral presentation. ICF's PowerPoint presentation (PPP) (Dated May 22, 2008; presentation rescheduled and given on May 21, 2008) provides the basic sequence and summary of information presented during the presentation. However, ICF's full opinion is presented in ICF's Technical Review (Dated May 9, 2008).

Dr. Mendez began ICF's oral presentation providing an overview of the draft OIG Scientific Analysis of Perchlorate (See page 2 of ICF's PPP). Dr. Mendez stated that ICF was recommending a revised approach for conducting a human risk assessment of perchlorate. However, MW clarified that the OIG's analysis was not to initiate a new perchlorate risk assessment, but to check and validate the appropriateness and health protectiveness of the NAS recommended reference dose (RfD). MW identified that the NAS committee used an unconventional approach of deriving an RfD using a no observed effect level (NOEL) and asserted their approach was a conservative. Since no adverse effects are observed for perchlorate exposure in any human populations, there is no human perchlorate data set from which to derive an RfD using the conventional risk assessment approach. MW stated the OIG used a cumulative

risk assessment approach using adverse effect from excess sodium iodide symporter (NIS) inhibition exposure from thiocyanate in male, female and from nitrate in school-aged children to check the appropriateness of the NAS recommended RfD. Furthermore, MW stated that the OIG evaluated the NAS' application of a 10 uncertainty factor (UF) to the point-of-departure (POD). MW indicated that the OIG audits by identifying procedures or criteria for an activity and determine if they were followed. However, MW stated that NAS identified that there are no clear criteria for when a UF is applied and the size of the UF to be used. The application of UFs in risk assessments is a matter of scientific judgment (NAS 2005, page 29). As a result, MW stated that the OIG used a cumulative risk assessment approach to assess the health protectiveness of the application of UFs to the perchlorate POD. MW also indicated that "ground breaking" data would be needed to invalidate and throw out EPA's approved perchlorate RfD before a new perchlorate risk assessment is needed.

Single Versus Cumulative Risk Assessment

Dr. Mendez states that cumulative risk approach is clearly applicable, entirely appropriate (in fact, necessary) for perchlorate (see page 6 of ICF's PPP; page 5 of ICF's Technical Review). Using the cumulative risk process characterized by Teuschler, Dr. Mendez identifies the following two approaches to applying cumulative risk to perchlorate: "Whole Mixture" versus "Component based" (see page 5 of ICF's PPP; adapted from Teuschler 2007). With the availability of the Blount analysis (Blount 2006) of the NHANES 2001-2002 epidemiological data (i.e., the results occurred after the NAS Committee's report), Dr. Mendez states that ICF prefers the "whole mixture" risk assessment method over an individual "component based" method using the principal of dose-addition (e.g., use of the Tonacchera Model). Dr. Mendez states that a "multivariate regression model" (i.e., a statistical model) using the NHANES 2001-2002 epidemiological data is probably the best approach to account for the interaction between NIS inhibitors. In addition, Dr. Mendez describes the "component based" method (a.k.a. dose addition method) as the "hallmark approach" for conducting a cumulative risk assessment.

MW identified that in 1997, a former EPA Administrator Carol Browner issued guidance directing EPA to embrace cumulative risk assessments on all future major risk assessments. MW commented that the current regulatory approach on perchlorate does not identify, address, or incorporate the following into the environmental decision making: the contribution of other NIS stressors to the public health issue (thiocyanate, nitrate, and the lack of iodide). Therefore, ICF and the OIG conceptually agree that a cumulative risk assessment approach is needed for perchlorate, but differ on the methodology on how to accomplish a cumulative risk assessment.

Identification of the Key Event Leading to an Adverse Effect

Dr. Mendez states the first step in a "whole mixture" risk assessment is to identify the key event leading to the adverse effect from perchlorate exposure. ICF proposed using hypothyroxinemia (i.e., reduced T_4, normal thyroid stimulating hormone (TSH) during first trimester of pregnancy as the critical effect). Dr. Mendez used the Pop study (Pop 1999, Pop 2003) to identify that about a 20% decrease in T_4 levels during the first trimester is associated

with mental deficits in the offspring. However, Dr. Mendez stated that the exact degree of reduction in T_4 levels during the first trimester that results in an adverse effect needs to be better defined and characterized. Dr. Mendez states that the reduction in T_4 during the first trimester of pregnancy is the furthest upstream biological event that is known to be associated with the adverse affect (i.e., irreversible fetal neurological impairment). Dr. Mendez states the first "precursor event" (i.e., reduction of T_4) is strongly associated with and strong predictor of an adverse outcome.

MW stated that using a reduction in T_4 during the first trimester of pregnancy is not consistent with science policy established by the NAS Committee. The NAS Committee in essence established science policy on perchlorate by stating that hypothyroidism (i.e., decrease in T4; increase in TSH) was not to be used for the risk assessment of perchlorate (page 166). The NAS Committee identified the inhibition of iodide uptake as the critical non-adverse effect and used this upstream event of the adverse effect to derive a conservative, health protective RfD (page 15). MW indicated that the NAS Committee's logic recognizes that the lack of adequate iodide uptake precedes all thyroid mediated adverse effects from perchlorate. MW also indicated that ICF (while under contract to NASA) already presented this proposal to the NAS Committee to select the decrease in T_4 as the key event and the NAS Committee rejected it. MW inquired that if the NAS Committee has established science policy to use the inhibition of iodide uptake as the critical effect for a perchlorate risk assessment and not the reduction of T_4 during pregnancy, why is ICF recommending the later? Dr. Mendez indicated that the NAS Committee's position is just the opinion of a group of clinicians in which he disagrees with their assessment. However, the NAS is recognized by Congress, OMB, and EPA as the highest leading scientific organization in the U.S. Dr. Mendez is entitled to his opinion, but the OIG can not disregard a NAS Committee scientific finding without evidence that the NAS Committee finding is wrong. At this time, the OIG does not have any such evidence. To the contrary, the OIG has found and documented subtle adverse effects in children whose mother's had low TIU during pregnancy, but the low TIU was not low enough to induce a decrease in T_4. Although a decreased T_4 during the 1^{st} trimester of pregnancy is known to cause mental deficits in the child, the critical effect should be a low TIU during pregnancy and nursing because TIU is a more sensitive measure of thyroid stress and correctly identifies the first occurrence of the slightest adverse effect in the child at a lower observed thyroid stress level.

Furthermore, MW also indicted that the NRC Committee's vision and strategy for toxicity testing is to identify and to use the perturbed biological step as the basis for characterizing the risk. The NRC's logic is by avoiding the initial biological perturbation; all downstream adverse effects are avoided (see NRC's Figure S-2). MW pointed out that multiple biological steps occur in the production and distribution of thyroid hormones. The potential for a reduction in T_4 levels during pregnancy is the combined result of any or all the biological steps being perturbed by concurrent chemical exposures. MW indicated that using the reduction in T_4 as the critical effect consumes the entire hypothalamus-pituitary-thyroid (HPT) axis's compensatory capacity to maintain thyroid hormone production to address only the perturbation of a low iodide uptake. MW indicated that the process to derive a perchlorate RfD needs to allow a sufficient amount of untapped HPT axis compensatory capacity to be available to address non-iodide uptake perturbations of the thyroid. MW indicated that ICF's approach does not address the concurrent perturbations of the other thyroid hormone biological steps unrelated to

iodide uptake. In other words, all of the thyroid's compensation capacity is tied up correcting for an abnormally low uptake of iodide from perchlorate and is, therefore, not able to address additional known exposures to thyroid stressors.

MW stated that ICF's proposed critical effect is inconsistent with the biology of brain development and with the concerns expressed by Office of Children's Health and others. MW stated that brain development is still occurring in the second and third trimester and during nursing (up to about 30 months). Any disruption in an adequate supply of T_4 during the 2nd and 3rd trimester and during early childhood when the brain is still developing can still potentially cause adverse mental deficits. MW specifically identified that beginning in the 2nd and continuing in the 3rd trimester, the fetal thyroid is supplying the majority of T_4 for brain development. MW stated that from umbilical cord measurements, the maternal T_4 is known to provide up to 40% of the T_4 while the fetal thyroid needs to generate the remaining 60% of the T_4 supply. MW stated that EPA is required to protect all life stages (i.e., not just the first trimester) from excessive exposure.

MW indicated the problem with using the reduction of T_4 during the 1st trimester is that the fetal thyroid is less able to adapt to iodide deficiency (i.e., a measure of the total iodide uptake (TIU) of the thyroid) than the maternal thyroid. MW described that a low TIU causes the fetal thyroid to have a high turnover rate. As a result of this increased sensitivity of the fetal thyroid to an inadequate uptake of iodide, adverse effects can be observed in offspring without maternal T_4 being reduced. Therefore, offspring can be born with adverse effects from pregnancies in which the mother never experienced a lowered T_4 level, but had a low TIU due to mild iodide deficiency. The non-cognitive adverse effects have been documented in the offspring of mothers with a low TIU without a reduced T_4 level which include the following: a decreased reaction time to stimuli, an increased frequency of neonates born with elevated TSH resulting in an increased frequency of mild thyroid dysfunction later in childhood (Calaciura 2002). MW pointed out that the impact on neurocognitive and psychomotor function has not been studied in this subpopulation (i.e., neonates born with elevated TSH). MW stated that during mild iodide deficiency during pregnancy, tracer studies in mild hypothyroid neonatal rats (i.e., elevated TSH without below normal free thyroxine (fT_4)) have observed an increased D2 deiodinase activity of the central nervous system (CNS) in order to compensate in order to for a decrease supply of triiodothyronine (T_3) in the CNS. This indicates that during mild iodide deficiency (i.e., although the maternal thyroid hormone levels are normal), the fetal brain tissue is compensating for an insufficient supply of thyroxine (T_4). MW indicated that the maternal total iodide uptake (TIU) level should never reach such a low level as to stress the fetal thyroid and require the fetus to activate the D2 deiodinase compensation mechanism to maintain T_3 levels in the brain

MW indicated that TIU levels during pregnancy qualitatively follow a dose-response curve. The lowest TIU levels during pregnancy have the highest frequency and severity of adverse effects. The lowest TIU levels occur in severe iodide deficient pregnancies (i.e., urinary iodide concentration (UIC) < 20 ug/L) resulting in a high frequency of the children being cretins (i.e., severe physical and cognitive problems). At slightly higher TIU levels during pregnancy, the frequency and severity of adverse effects are much less obvious and pronounced. This TIU level can be observed in moderate iodide deficit pregnancies (i.e., 20 ug/L < UIC < 50 ug/L)

where the offspring frequently have verbal skill problems (e.g., low reading comprehension), lower IQ scores, and have higher frequencies of attention deficit disorder. At still slightly higher TIU levels during pregnancy, the frequency and severity of adverse effects is barely detectable, but is still present. This TIU level can be observed in moderate iodide deficit pregnancies (i.e., 50 ug/L < UIC < 100 ug/L) where the offspring are observed to have decreased reaction times to stimuli, increased frequency of elevated TSH levels at birth which result in an increased frequency of mild thyroid dysfunction in childhood (Delange 1998).

In response to the adverse effects in offspring that result from a low TIU, but not so low of a TIU as to induce a reduced maternal T_4, Dr. Mendez stated his opinion and the commonly accepted scientific opinion that the adverse effect from perchlorate (i.e., a lower TIU) is the potential for impaired brain development. Dr. Mendez indicated he was not directly aware that a low TIU during gestation could induce a decrease in response to stimuli or the increased frequency of mild thyroid dysfunction in childhood of neonates born with elevated TSH levels. Furthermore, Dr. Mendez indicated that an elevated neonate TSH levels is not an adverse effect. MW stated that Delange considers neonatal TSH as the single indicator that focuses on potential brain damage (Delange 1998). MW agreed that cognitive function has neither been tested nor shown to occur in this subpopulation, but the increase frequency of mild thyroid dysfunction in later childhood in this subpopulation would qualify as an adverse effect. Dr Mendez responded that if a different adverse effect is identified for perchlorate exposure, additional study is needed to confirm it.

Interpretation and Application of the NRC Committee's Strategy and Vision for Toxicity Testing

Dr. Mendez states that the NRC Committee recommendation are intended to apply to chemicals with limited human epidemiological data or animal studies. Dr. Mendez identifies that in traditional risk assessment hierarchy of data human data is preferred over animal data which is subsequently preferred to *in vitro* data. Dr. Mendez states that *in vitro* test results should not be used as the basis of a perchlorate risk assessment.

MW stated that since ICF's Technical Review clearly stated an obvious difference of opinion of the NRC Committee's recommendations and there potential application to the perchlorate risk assessment, the OIG undertook the effect to interview Dr. Charnley, a member of the NRC Committee, to clarify the issue. MW characterized Dr. Charnley opinion that the NRC Committee's strategy and vision for toxicity testing is not a sufficiently developed approach to support setting a reference dose (RfD). However, Dr. Charnley added that the NRC Committee's approach is appropriate for "ground truthing"[1] the perchlorate RfD. In her opinion, if the *in vitro* dose-response model (i.e., the Tonacchera Model) is adequately predictive of adverse health outcomes, then it can be used both to validate the RfD and to evaluate potential health protection actions in the environmental decision making process. MW stated that the OIG Scientific Analysis of Perchlorate does not set a perchlorate RfD, but verifies the appropriateness and health protectiveness of the perchlorate RfD and also provides insight into the contribution of the other NIS stressors to this public health issue and potential actions that could be taken to address this public health issue.

[1] Corroborating.

MW indicated that the strength of using the *in vitro* data is that the approach allows the individual contribution of each of the NIS stressors to the total iodide uptake (TIU) to be identified and quantified. This can not be done in animal testing or in human studies. The Tonacchera Model mathematically characterizes the relationship between the four NIS stressors and the TIU and expresses in quantitative terms how the NIS biological step is perturbed. However, this mathematical characterization is only relevant if it predicts adverse effect in humans. If the mathematical model agrees with the observed effects in humans, this result would indicate that the lack of an adequate uptake of iodide is significant factor inducing an adverse effect. By contrast, if the mathematical model does not agree with the observed effects in humans, this result would suggest that the lack of an adequate uptake of iodide is not a significant factor inducing an adverse effect (i.e., the known mechanism of toxicity for perchlorate). The OIG has found the Tonacchera Model does agree with observed effects in humans.

The "Whole Mixture" Approach to the Cumulative Risk Assessment of Perchlorate

Based on the newly available (i.e., post-NAS) NHANES 2001-2002 epidemiological data (i.e., the best available data set in Dr. Mendez's opinion), Dr. Mendez recommends a revised quantitative health risk assessment of perchlorate by using a "whole mixture" approach to the cumulative risk assessment of perchlorate by fitting a multivariate regression model (i.e., statistical model) to the NHANES 2001-2002 epidemiological data. The Blount analysis is an example of a statistical model fitted to the NHANES 2001-2002 epidemiological data. The Blount analysis predicted a decrease of 1.64 ug/dL in total T4 serum from a perchlorate exposure to 13 ug/day in women $\geq$ 12 yrs of age with a urinary iodide $\geq$ 100 ug/L to the NIS inhibition of perchlorate. The Steinmaus analysis is also an example of a statistical model fitted to the NHANES 2001-2002 epidemiological data. The subsequent Steinmaus analysis on the same NHANES 2001-2002 epidemiological data found that thiocyanate exposure potentates the effect of decreasing the total T_4 with increasing perchlorate exposure. Dr. Mendez stated that the Steinmaus statistical model is incomplete. Dr. Mendez stated that ICF's recommends further developing and improving the fit of the statistical model to the NHANES 2001-2002 epidemiological data by adding all significant interaction terms to the model and to generate confidence limits for each term for use in a risk assessment. Dr. Mendez stated that the statistical model should probably take the form of a exponential equation (e.g., total T4 = $e^{(\beta perchlorate + \beta(perchlorate \times thiocyanate)}$).

MW stated that a "whole mixture" approach to risk assessment is best suited when the ratio of chemicals in the mixture are fixed and defined, such as a pesticide mixture. MW stated that this is not the case with the four NIS stressors. An individual's exposure to each of the four NIS stressors can vary independently depending on the dietary preferences of the individual. So the exposure ratio of the four NIS stressors is not fixed. Furthermore, MW stated that a "whole mixture" approach to risk assessment is best suited when the chemicals in the mixture don't share a common mechanism of toxicity, but act on different biological pathways in different organ systems of the body. MW stated the mechanistic biochemistry is not sophisticated enough to figure out the interaction between several chemicals simultaneously affecting multiple organ

systems and/or multiple biological pathways in order to develop a "component based" dose addition model. So, scientist would be forced to rely on the "whole mixture" approach this type of risk assessment. However, for this public health issue, the four NIS stressors act only through a single common mechanism of toxicity (i.e., the uptake of iodide by the NIS) which can and has been modeled. Therefore, MW stated that the "whole mixture" approach is not well suited for this particular public health issue. Dr. Mendez did not disagree with this characterization, but just continued on with his oral presentation.

MW inquired about a basic contradiction with the Blount analysis. MW stated that the findings of the Blount analysis that 13 ug/day of perchlorate exposure induces about 20% reduction in total T_4 is not consistent with findings from all of the other perchlorate studies. The prediction that adverse effects from 13 ug/day of perchlorate indicates that perchlorate is much more toxic than previously documented. Dr. Mendez agreed with MW's basic characterization that either the rest of the perchlorate data is wrong and the Blount analysis is correct or the rest of the perchlorate data is correct and the Blount analysis is wrong. However, Dr. Mendez reaffirmed his position that the NHANES epidemiological data is the best data set in the population of concern with the background exposure typically found in the U.S.

On a related discussion point, Dr. Mendez agreed with MW's assessment that the increased toxicity of perchlorate reported by the Blount analysis is not explained by perchlorate's inhibition of NIS (i.e., perchlorate mechanism of toxicity) and that some unidentified mechanism of perchlorate toxicity would needed to be identified to explain this lower level of perchlorate toxicity. Since all four NIS stressors act through the same mechanism of toxicity, (i.e., the uptake of iodide), for only one to induce an adverse effect while the other three NIS stressors do not induce an adverse effect is not consistent with the known biological mechanism. If the Blount analysis is an accurate characterization of the toxicity of perchlorate, MW indicated that due to the relative low perchlorate exposure needed to induce adverse effects, adverse effects in the offspring of iodide deficient pregnant women should be relatively easy to identify. MW asked if Dr. Mendez new of any human studies in which adverse effects had been identified in the offspring of iodide deficient pregnant women exposed to 13 ug /day of perchlorate. Dr. Mendez indicated that he did not know of any such study results. MW stated that the Blount analysis results have not been seen or corroborated in any human perchlorate studies. Dr. Mendez agreed that the Blount analysis has not been corroborated by adverse effects seen in the offspring of iodide deficient pregnant women exposed to 13 ug/day of perchlorate.

In ICF's Technical Review, the Blount analysis was used to estimate a Drinking Water Equivalent Level (DWEL) of 6 ug/L. MW inquired how this value was determined. Dr. Mendez indicated that the Blount analysis indicates the ingestion of 13 ug/day of perchlorate induces the 20% reduction in total T4 which is sufficient to induce an adverse effect. Assuming the consumption of 2 liters of drinking water per day with no application of uncertainty factors give the estimated 6 ug/L. MW indicated that without any uncertainty factors, the 6 ug/L level is the equivalent of a lowest observed adverse effect level (LOAEL) and has no safety margin (i.e., adverse effects are expected to occur at 13 ug/day ingestion). MW indicated that by definition, an RfD is an exposure value "...that is likely to be without an appreciable risk of adverse health effects over a lifetime." Therefore, MW stated that a safety margin needed to be applied to the 6 ug/L (i.e., in order to be protective) so that the population's exposure does not exceed this

LOAEL resulting in adverse effects. MW indicated that applying 10x factor would be reasonable resulting in a DWEL more 0.6 ug/L. Dr. Mendez indicate that a safety margin is not necessary because the effect (i.e., decrease in T4) was measured in the population of concern with the typical U.S. background of exposure. Dr. Mendez further stated that this DWEL of 6 ug/L is on the low end of the previous calculated DWELs for perchlorate that ranged from 6 to 65 ppb (i.e., refer to Table C-1 in ICF's Technical Review). MW disagreed with this comparison and stated that the comparison is not appropriate (i.e., comparing apples and oranges). MW stated that all previous perchlorate RfDs incorporated the application of one or more uncertainty factors that made the RfD value substantially lower than the LOAEL at which adverse effects are expected to occur. The EPA perchlorate RfD was derived from a NOEL of 0.007 ug/kg-day. Assuming a 70 kg person and the LOAEL is no more than 10x greater than the NOEL, the estimated LOAEL equivalent from the Greer study would be at least roughly 4900 ug/day. MW implied that the correct comparison is the 13 ug/day LOAEL from the Blount analysis needs to be compared to the estimated 4900 ug/day LOAEL equivalent from the Greer study (Note: even the EPA's 2002 proposed RfD corresponding to a DWEL of 1ppb has a LOAEL of 700 ug/day). The Blount analysis suggests that the perchlorate toxicity is at least two orders of magnitude more toxic than what all of the previous studies would indicate. MW indicated that the Blount analysis is the outlying data point in the perchlorate that needs to be explained mechanistically and corroborated with observations of adverse effects in humans. MW indicated that the Blount biomonitoring data indicates that a portion of the U.S. population is already over 13 ug/day without the potential added exposure from water. MW indicated that if the Blount analysis is correct, cognitive adverse effects should be relatively easy to document in the offspring of mildly iodide deficiency women (i.e., UIC < 100 ug/L) because perchlorate exposure greater than 13 ug/day is not rare.

MW stated that the Blount analysis is attributing all of the predicted change in total T4 serum in women ≥ 12 yrs of age with a urinary iodide ≥ 100 ug/L to the NIS inhibition of perchlorate. MW indicated that this was inappropriate because multiple stressors impact the uptake of iodide not to mention other chemical exposures impacting other biological steps that could impact the supply of T_4 that could result in a decrease. MW stated that Dr. Charnley's paper states that the R^2 value of 0.240 reported in the Blount analysis shows that perchlorate accounts for only 3% of the variation seen in T_4 (Charnely 2008). R^2 is a statistic called the "proportion of variation explained" and represents the total response variation explained by the explanatory variable. MW indicated that if only 3% of the T_4 change predicted by the Blount analysis was from perchlorate, this would make the toxicity of perchlorate reported in the Blount analysis consistent with the bulk of the other perchlorate studies. MW asked Dr. Mendez to comment on this. Dr. Mendez indicated that this is not how the statistics work at which time Dr. Mendez went to the marker board to provide an explanation of the Blount and Steinmaus statistical models. The β-coefficient is the slope of the linear fit to the data. The R^2 represents a measure of how well the statistical model explains the data. A R^2 of less than 0.2 indicates that the β-coefficient does not explain the data particularly well [Note: R^2 value reported in the Blount analysis is only 0.240]. Dr. Mendez stated that the β-coefficient (i.e., the slope of the perchlorate term) is significant even if R^2 indicates that perchlorate exposure does not explain a lot of the scatter in the data. Dr. Mendez stated that both the Blount and Steinmaus statistical models are incomplete and could be improved.

Dr. Mendez stated that Blount Analysis indicates that perchlorate dose was the strongest, most consistent predictor of total T4 ($tT_{4)}$ levels in women with urinary iodide below 100 μg/L. MW disagrees because the decrease in tT_4 is only seen in iodide deficient women and not in iodide sufficient women. Therefore, the level of iodide nutrition in the women is the largest factor indicating the likelihood of a decreased tT_4. MW stated that the Blount Analysis supports the concept that iodide intake has the largest impact on the amount of iodide uptake by the thyroid and on predicting an adverse effect. In other words, if iodide intake is not deficient, there is no observed toxicity from perchlorate. MW stated that this is consistent with the clinical observations in Africa where iodide intake is increased to eliminate the adverse effects of excessive thiocyanate exposure from cassava consumption [Note: the amount of thiocyanate exposure remains the same, but increasing the iodide shifts the point at which thiocyanate is toxic]. MW states that the toxicity of perchlorate (i.e., a NIS inhibitor analogous to thiocyanate) is not fixed (i.e., the perchlorate RfD is not a single value), but a function of iodide nutrition. In other words, the higher the iodide intake; the higher the calculated perchlorate RfD. By converse, the lower the iodide intake; the lower the calculated perchlorate RfD. MW stated that the conventional use of a single RfD by risk assessors to describe and set a toxicity limit does not work for perchlorate. MW states that iodide exposure modulates the toxic effect of perchlorate and the other NIS inhibitors.

Dr. Mendez replied that defining a background of exposures typical of the United States (e.g., for thiocyanate exposure, iodide nutrition, nitrate exposure, etc.) in an epidemiological study allows a perchlorate RfD to be defined for the typical exposure conditions found in the U.S. population (i.e., use of the central tendency (average background) of the U.S. population). Dr. Mendez indicated this is why risk assessors can not use the Chilean data to set a perchlorate RfD because the Chilean population has a higher iodide nutrition than the U.S. population. MW stated that controlling for the background exposure in an epidemiological study marginalizes those factors and their contribution to an adverse outcome. This is the difference between using the outdated single chemical risk assessment approach which is designed to measure the impact from only a single chemical versus a cumulative risk assessment where the integrated impact and interaction of multiple stressors are evaluated concurrently. For this public health issue, MW indicated that adverse effects occur at the lower end of the iodide intake. By utilizing a typical background, the critical role and impact of iodide deficiency in the U.S. population on this public health issue is lost. This effect can be seen in Steinmaus analysis of the NHANES 2001-2002 epidemiological data which concludes that thiocyanate does not predict or cause the decrease in T_4 by itself but increases the adverse effect of perchlorate (i.e., potentiates). However, this conclusion from the Steinmaus statistical model (i.e., the "whole mixture" approach to cumulative risk assessment) does not agree with the known biological mechanism of thiocyanate in that it can inhibit the NIS by itself. Furthermore, the Steinmaus conclusion does not agree with the observation that thiocyanate can by itself induce decreases in T_4 and induce adverse effects in humans as seen in human studies in Africa and India. ICF's approach derives a perchlorate RfD unique for America background exposure, but does explain why thiocyanate is toxic oversees in Africa and India, but not in America. There is no known difference in the biology of African and/or Indian thyroids that would not account for the difference in the observed toxicity of thiocyanate. Therefore, the observed toxicity of a NIS inhibitor (e.g., thiocyanate or perchlorate) changes as a function the background exposure level to the other NIS stressors. This conclusion can also be seen in the Chilean epidemiological study. Perchlorate at

over 118 ug/day is not toxic in Chile when the mean UIC is 269 ug/L, but perchlorate is reported to be toxic in the Blount and Steinmaus analyses at 13 ug/L in the U.S. in women with a UIC < 100 ug/L. Using ICF's "whole mixture" own conclusion, obviously the toxicity of perchlorate is not fixed (i.e., perchlorate toxicity is not static, but dynamic) and is directly related to the background exposures to NIS stressors (i.e., in the Chilean study – the difference in iodide nutrition drives the resulting toxicity level of perchlorate). Therefore, controlling for the U.S. typical background to set a single perchlorate RfD value represents the major critical deficiency in a single chemical risk assessment in that it does not explain all the factors that contribute to an adverse outcome. By contrast, a cumulative risk assessment explains all the factors that contribute to an adverse outcome so that appropriate migration steps can be taken to address each of the contributing factors (e.g., numerically, the most important factor is the need to address iodide deficiency during pregnancy and nursing).

MW states that a statistical model of the NHANES 2001-2002 epidemiological data should not show a classic dose-response, but should have a bimodal response. MW explained that the compensatory mechanism of the HPT axis should cause the generation of a bimodal response. At lower levels of NIS stress, the HPT axis is able to compensate for an increasing amount of NIS stress (e.g., an increase in perchlorate exposure) and maintain a stable T_4 serum concentration (i.e., the initial increase in perchlorate dose should result in no change in thyroid hormones [a non-dose-response relationship). However, once the compensation capacity of the HPT axis is exhausted, any additional NIS stress (e.g., an excess exposure to perchlorate) generates a corresponding decrease in thyroid hormones (i.e., a normal dose response relationship). MW indicated that the Blount and Steinmaus models are linear response models and don't address bimodal response nature of the HPT axis. Dr. Mendez agrees and stated the Blount and Steinmaus statistical models should be improved by probably taking the form of a exponential equation (e.g., total T4 = $e^{(\beta \text{ perchlorate} + \beta \text{ (perchlorate x thiocyanate)})}$) to allow for the anticipated bimodal response of the thyroid to changes in stress levels. [Note: In MW opinion, the terms in the statistical model should reflect the known biochemical mechanism of the NIS stressors (e.g., the exposure term in the statistical model should be the total iodide uptake (TIU) parameter and not the individual NIS stressor(s)). The incorporation of a TIU term into Dr. Mendez's statistical model would take the form of total T4 = $e^{(TIU)}$; Dr. Mendez's statistical model does not have the TIU term included.]

MW stated that Blount analysis identifies a relationship between ug of perchlorate/L versus reduction in T4 for women ≥ 12 yrs of age with a urinary iodide ≥ 100 ug/L (Blount 2006; Table 6). However, the results of a single un-timed spot urinalysis can vary due to dilution or concentration of the urine (i.e., due to an individual's hydration status). A common technique to eliminate this variation in the spot urinalysis is to normalize the iodide urine concentration against creatinine resulting in iodide excretion being expressed as ug of iodide/g of creatinine (i.e., ug/g creatinine). MW identified that Lamm is reporting (Lamm 2007) being able to successfully repeat Blount's statistical analysis of the NHANES 2001-2002 epidemiological data only when using urinary iodide being measured as ug/L and the cut off being < 102 ug/L. Lamm reports a β-coefficient of -0.91 which agrees with Blount's report β-coefficient of -0.89 (i.e., negative slope). When Lamm repeats the statistical analysis again using only women of childbearing age (ages 15-44 years) and uses urinary iodide being measured as ug/g creatinine and the cut off being < 95 ug/g creatinine to define low iodide intake, Lamm reports a β-

coefficient of +0.25 (i.e., positive slope) which is found not to be statistically significant with a p = 0.75. MW asked Dr. Mendez to comment on Lamm analysis where the relationship between ug of perchlorate/L versus reduction in T4 is completely lost by defining low urinary iodide in terms of ug/g of creatinine versus ug/L. Dr. Mendez stated that Lamm has "never found a dose-response in any data set". Furthermore, Dr. Mendez stated that he could not comment on Lamm's findings since only the conclusions are provided in the abstract. Dr. Mendez stated that he would comment on Lamm's analysis only after it has been accepted and published in a peer review journal and had a chance to review the specifics of Lamm's statistical analysis in detail. Dr. Mendez attended the March 20 & 21, 2008 perchlorate symposium held during the Annual Meeting of the Society of Toxicology in Seattle, Washington and the symposium was titled: Perchlorate Exposures, Iodine Modulation of Effect, And Epidemiologic Associations: Implications for Risk Assessment (An Ancillary Program of the Annual Meeting of the Society of Toxicology); Sponsored by: the Kleinfelder Group. Dr. Mendez indicated that Lamm presented this analysis at the perchlorate symposium and that, in his opinion, Lamm's presentation was neither well received nor persuasive by the fellow scientist and statisticians attending.

Section 3.1.3.2 of EPA's Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models guidance on the use of environmental models requires all models to be corroborated before the model can be used as the basis of rule-making (i.e., regulation) (EPA 2003a). The Blount Analysis represents a "statistical model" of the toxicity of perchlorate and its findings need to be corroborated with others epidemiological data and other human exposure studies of perchlorate for it can be used as the basis to set a perchlorate RfD. MW stated that the findings of the Blount Analysis have not been corroborated. MW identified Pearce's study in a recent cohort of pregnant women exposed to perchlorate at these levels. Dr. Pearce studied 789 pregnant women (i.e., 396 from Cardiff, Wales; 311 from Turin, Italy; and 82 from Dublin, Ireland). Her finding show that low-level perchlorate exposure is not associated with altered T_4 or TSH levels among either iodide sufficient or iodide deficient pregnant women in their first trimester. MW provided Dr. Mendez a copy of Pearce's abstract and asked Dr. Mendez to comment on her study. Dr. Mendez stated that he was aware of her study and heard her present her findings at the March 20 & 21, 2008 perchlorate symposium held during the Annual Meeting of the Society of Toxicology in Seattle, Washington and the symposium was titled: Perchlorate Exposures, Iodine Modulation of Effect, And Epidemiologic Associations: Implications for Risk Assessment. Dr. Mendez indicated that he doubts that Dr. Pearce will be able to get her study published in a peer reviewed journal because, in his opinion, her epidemiological study utilizes poor statistics. Dr. Mendez indicated that he did not find Dr. Pearce's study persuasive.

[Subsequent to ICF's May 22 oral presentation, the OIG has learned of another pending epidemiological study by Dr. Pearce in 128 pregnant women in Los Angeles, California and 102 pregnant women in Cordoba, Argentina (Pearce 2008). The study also shows that low-level perchlorate exposure is not associated with altered T_4 or TSH levels among either iodide sufficient or iodide deficient pregnant women in their first trimester].

ICF's Evaluation of the Tonacchera Model

As part of the ICF Technical Review, ICF obtained the raw experimental data used by Tonacchera to develop the Tonacchera Model and performed an independent evaluation of the Tonacchera Model (See Appendix A of ICF's Technical Review for details). Since this evaluation and results from this evaluation were not part of ICF's oral presentation, MW asked Dr. Mendez to characterize ICF's evaluation of the Tonacchera Model and their results of this evaluation. Dr. Mendez stated that relative potency factors for the NIS inhibition have been determined before, but the Tonacchera experiment is unique in the all four NIS inhibitors were evaluated together in a mixture which resulted in a model of their inhibition interaction. Dr. Mendez did not elaborate on ICF's evaluation, but stated that Tonacchera Model accurately characterizes the NIS performance under the specific experimental conditions used. Dr. Mendez indicated that the qualitative fit of the Tonacchera Model to the experimental data is quite good, and the form of the Tonacchera Model is consistent with a competitive inhibition model with a single binding site. Dr. Mendez also indicated that ICF's evaluation of the Tonacchera Model suggests the short-term NIS inhibition estimated by Tonacchera Model is conservative (i.e., predicts a lower TIU than the expected long-term TIU estimated in the full model). However, Dr. Mendez issue is not the quality of the fit of the Tonacchera Model to the experimental *in vitro* data, but how accurately this short-term NIS inhibition model predicts an adverse affect for use as the basis of a risk assessment?

The "Component Mixture" Approach to the Cumulative Risk Assessment of Perchlorate

Dr. Mendez stated that the Tonacchera Model measures and models the instantaneous inhibition of the NIS (i.e., short term). The Tonacchera Model does not take into account the physiology of the thyroid and the compensatory mechanism of the HPT axis. Dr. Mendez expressed that the uptake of iodide is only one of the many thyroid processes affecting the key event (i.e., the decrease supply of T_4). MW agrees that a comprehensive model of all the thyroid processes is beyond the capability of science. However, MW indicated that classic descriptive toxicity testing identifies an external dose level that induces an adverse effect without knowing the internal biological mechanism(s) at work. Furthermore, MW indicated that the Blount analysis is modeling the NHANES data to identify an external dose that induces an adverse effect without knowing the internal biological mechanism(s) at work. MW indicated that the dose addition method is using the Tonacchera Model to identify an internal dose level (i.e., a TIU level) that induces an adverse effect (i.e., analogous to the above techniques). However, the dose addition method utilizes an increased understanding of the biological mechanism (e.g., the relative effect of the NIS stressors on the TIU) to evaluate how critical the total iodide uptake (TIU) is on inducing or predicting an adverse effect. If the TIU is predictive of an adverse effect, than the uptake of iodide is a critical biological step. However, if the TIU is not predictive of an adverse effect, than the inhibition of the NIS is not a critical biological step.

Based on the NHANES 2001-2002 epidemiological data, Dr. Mendez implied that the *in vitro* measurements of NIS inhibition appear to be poor predictors of neurodevelopment toxicity. However, by contrast, MW indicated that the OIG has corroborated the Tonacchera Model against the following studies and has shown that the TIU is both a good predictor and a good explanation for the observed toxicity of perchlorate:

- Perchlorate human exposure study (Greer)
- Explains the Chilean studies (Crump, Tellez)
- Perchlorate occupational study (Braverman)
- Excess thiocyanate studies in men and women (Banerjee)
- Excess nitrate exposure in school children (Tajtakova)
- NAS statement on the amount of iodide deficiency to generate hypothyroidism
- Explains the clinic observation that the toxicity of inhibitors is function of iodide nutritional status

MW asked Dr. Mendez to confirm that EPA's policy that in the absence of data to the contrary, EPA policy is to assume components of a chemical mixture produce toxic effects through additivity. Dr. Mendez agreed that this is EPA's policy. Furthermore, MW asked Dr. Mendez to confirm if the mechanistic interaction of the four NIS stressors meet the following EPA's risk assessment guidance requirement for dose addition:

- Same mechanism of toxicity (all four NIS stressors act to limit the amount of iodide uptake by the thyroid)
- Similar dose-response curves (i.e., same slopes, different intercepts)
- Act independently of one other, act as dilutions of one another
- Known relative potency factors (RPF) for each NIS stressor
- Behave similarly in terms of the primary physiologic processes (i.e., uptake, metabolism, distribution, and elimination)
- Human exposures of each chemical are known (including at various life stages)

Dr. Mendez agreed that the above EPA requirements for conducting a cumulative risk assessment using the dose addition method are meet by all four NIS stressors.

ICF's Comment on the OIG's Recommendation for Iodide Supplementation

Dr. Mendez stated that the OIG specifically requested ICF to comment on the OIG's recommendation addressing iodide supplement. Dr. Mendez approaches the issue of iodide supplementation as though it is a separate medical issue from the risk associated perchlorate exposure (i.e., this shows that Dr. Mendez is not approaching the public health issue from a cumulative risk assessment point of view which is based on the realization that the an adverse effect is the integrated effect of multiple stressors). Dr. Mendez stated that the consensus in the literature among scientist and clinicians is that maintaining a sufficient iodide intake before and during pregnancy is essential for the health of the developing child. However, Dr. Mendez stated that WHO and Institute of Medicine have not recommended universal iodide supplementation. Dr. Mendez stated that an excess of iodide intake during pregnancy can also lead to adverse

effects. As such, Dr. Mendez commented that recommendations on iodide supplementation should be left to professional organizations in the medical community. Dr. Mendez implied that such a recommendation exceeds EPA's authority and expertise.

MW replied that using a dose addition approach to cumulative risk assessment clearly indicates that iodide is the dominate stressor while perchlorate is only a minor NIS stressor. Therefore, this public health issue of an inadequate uptake of iodide during pregnancy can only effectively be addressed if iodide deficiency is corrected during pregnancy. Reducing perchlorate exposure below the RfD does not induce a large enough effect on the total iodide uptake to correct iodide deficiency. Hence, this is the basis for proposing this recommendation.

MW also stated that the NHANES data does not show a statistical increase in pregnant women with excessive iodide intake when taking iodide supplementation then those pregnant women not taking iodide supplementation. MW indicated that excessive iodide intake could be easily avoided by given iodide supplementation only to pregnant women (or women who want to become pregnant) whose urinary iodide concentration is tested and shown to be below average (e.g. UIC of 150 ug/L). Iodide supplementation in this subset of women (i.e., low urinary iodide) is unable to cause excessive iodide exposure.

MW also stated that the Framework of Cumulative Risk Assessment (EPA 2003) provides guidance that corrective action from a cumulative risk assessment might be outside of EPA's legislative mandates and that EPA would need to coordinate with other Agencies to effective address the public health issue. MW implied that federal agencies are not intended to work independently, but to work in cooperation and in conjunction with one another to address a public health issue. MW indicated that the OIG recommendation is intended to direct EPA to take the finding of the cumulative risk assessment to both the FDA and NIH in order to coordinate an effective response to correct inadequate iodide uptake during pregnancy.

Concluding Remarks

Dr. Mendez indicated that the development of a statistical model of the decrease in T_4 in iodide deficient women observed in the NHANES 2001-2002 epidemiological data was ICF's recommended approach of conducting a revised risk assessment of perchlorate. Dr. Mendez stated that he has not heard any persuasive arguments against using the NHANES epidemiological data to conduct a revised risk assessment. Dr. Mendez indicated that there was no compelling reason to base a perchlorate risk assessment on *in vitro* tests when quality epidemiological data is available.

Dr. Mendez commented that EPA's National Center for Environmental Assessment (NCEA) continues to develop the science of cumulative risk and that the OIG might want to contact Kevin Crofton, Jason Lambert, and/or Linda Teuschler for more information.

Dr. Mendez offered and indicated that if the OIG is interested, ICF is available under an additional contract to further develop the statistical model for the NHANES 2001-2002 epidemiological data.

Questions:

MW stated that adverse affects are documented to be observed in the offspring of mildly iodide deficient pregnant women (i.e., decreased reaction time, increased frequency of neonatal TSH, increased in thyroid dysfunction in childhood of leveled TSH neonates) who did not experience ICF's proposed critical effect of a 20% reduction in T_4 during the first trimester. MW asked how ICF's "whole mixture" approach to cumulative risk assessment would address the issue of the fetal thyroid's apparent increased sensitivity to the lack of an adequate iodide uptake during gestation. Dr. Mendez replied that if a different critical effect is identified, then an appropriate data set for that critical effect needs to be established and then it would have to be quantitatively evaluated to derive an RfD. Dr. Mendez's reply did not answer the question of how ICF's "whole mixture" approach addresses the increased sensitivity of the fetal thyroid. Therefore, MW concludes from his answer that the ICF's "whole mixture" approach does not address this concern and that if a low TIU in fetal thyroid was chosen as the critical effect, a different data set than the NHANES 2001-2002 epidemiological data would need to be identified and used. By contrast, the OIG identified a maternal TIU exposure not associated with either fetal thyroid stress or subtle adverse effects in childhood and used this maternal TIU exposure as the critical effect that the OIG Scientific Analysis of Perchlorate used to define the No Adverse Effect Level (NOAEL)) in order to protect and address the increase sensitivity of the fetal thyroid .

MW asked Dr. Mendez to follow-up with the issue to whether or not the Blount analysis and/or the Steinmaus statistical model used ug of perchlorate/L of urine or ug of perchlorate/g of creatinine. The Blount analysis of the NHANES 2001-2002 data reports a decrease in total T_4 levels in iodide deficient women with increasing perchlorate exposure when perchlorate exposure is measured in ug of perchlorate/L of urine. By contrast, Dr. Lamm reports that the decrease in total T_4 levels in iodide deficient women with increasing perchlorate exposure is lost when perchlorate exposure is measured in ug of perchlorate/g of creatinine. The issue is the relationship observed in the Blount analysis between reduced T_4 in iodide deficient women and perchlorate exposure should be observed when using either spot urinalysis data (i.e., ug/L) or spot urinalysis date controlled for dilution (i.e., urinary perchlorate adjusted for grams of creatinine).

Dr. Mendez stated and stressed multiple times during the presentation that the NHANES 2001-2002 data set represents the population of concern (i.e., the U.S. population) in the "background" of concern (i.e., what the U.S. population is exposed to). Dr. Mendez stated the Chilean study and those in Africa don't have the same "background" exposure to other chemicals as the U.S. population which does not make them appropriate studies from which to conduct a risk assessment on. EL asked Dr. Mendez why ICF relied on the Pop study (Pop 1999) when the study was conducted in Netherlands and does not represent a typical U.S. "background" exposure. Dr. Mendez indicated ICF relied on the Pop study because it was a well controlled study. Dr. Mendez did not answer the question. Dr. Mendez has used as a justification for the exclusive use and reliance on the NHANES 2001-2002 epidemiological data to calculate a perchlorate RfD is that the "background" exposure in this study is the background exposure

uniquely experienced by the U.S. population. Therefore, the use of uncertainty factors are not needed to adjust the RfD for differences between the U.S. background exposure and the background exposure of studies in other countries (i.e., Chilean epidemiological studies, endemic cretinism in Africa caused by excess exposure to cassava (i.e., thiocyanate) in iodide deficient areas, and abnormal thyroid hormones levels in India from excessive thiocyanate exposure). EL question points out an inconsistency is his explanation because the background in the Netherlands is different than the background in the U.S., but this critical issue of background does not prevent Dr. Mendez from using Pop's Netherlands study to defined the critical effect level.

EL asked Dr. Mendez if increasing the iodide nutrition in the iodide deficient women in the Blount analysis (Blount 2006) to above 100 ug/L UIC, would this action prevent the decrease in T_4? Dr. Mendez indicated that he could not answer this question. Dr. Mendez indicated that an additional study giving these iodide deficient women additional iodide would be needed to address this issue. The answer to this question was obvious. Women in the same study with sufficient iodide did not have a problem. Given the level of iodide was the only difference between the two groups, how do you ignore this conclusion except to state that somehow the study was flawed and is not considering some other undefined variable? Dr. Mendez's answer also indicates that he is only considering the findings from a "whole mixture" approach (e.g., the Blount analysis) because the clinical observations in endemic cretinism in Africa and the mechanism of toxicity of NIS inhibitors clearly indicate that additional iodide prevents the decrease in T_4.

EL asked Dr. Mendez what amount of perchlorate exposure would be theoretically needed to induce an adverse effect (i.e., the 20% decrease in T_4) in the iodide sufficient population of the Blount analysis (Blount 2006)? Dr. Mendez indicated that he could not answer this question. Dr. Mendez can not identify the perchlorate exposure level that induces an adverse effect (i.e., decrease in tT_4) in an iodide <u>sufficient</u> women. On page ES-2 of ICF's Technical Review, ICF states that the total iodide uptake (TIU) is weakly associated with and is a "poor predictor" of an adverse outcome and does not recommend the use of total iodide uptake (TIU) as the basis for a perchlorate risk assessment. However, the observations of the Blount analysis directly contradict this opinion. A decrease in tT4 is only observed in iodide deficient women (i.e., UIC < 100 ug/L) exposed to perchlorate, but no change in tT_4 levels is observed in iodide sufficient women (i.e., UIC > 100 ug/L) regardless of the amount of perchlorate exposure. Since TIU is directly linked to iodide nutrition (i.e., the higher the UIC, the higher the TIU; the lower the UIC, the lower the TIU), the Blount and Steinmaus analyses indicate that the best "predictor" for an adverse outcome is UIC level (i.e., iodide nutrition level). The UIC level is the best and principal predictor of the risk of an adverse effect. If the UIC is high, no adverse effects are observed. If the UIC is low, adverse effects are observed. Contrary to Dr. Mendez option, iodide intake is the key predictor of the risk for an adverse effect because the lack of iodide is the NIS stressor with the largest impact on the chances for an adverse outcome in this public health issue. [Note: All adverse effects in this public health issue occur in the bottom fifth of the population with low iodide intake (i.e., < 100 ug/L). Both the "component based" and "whole mixture" methods to cumulative risk assessment point to this same conclusion.]

References:

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL, 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. Environmental Health Perspectives 114(12):1865-1871.

Calaciura F, Motta RM, Miscio G, Fichera G, Leonardi D, Carta A, Trischitta V, Tassi V, Sava L, Vigneri R, 2002. Subclinical Hypothyroidism in Early Childhood: A Frequent Outcome of Transient Neonatal Hyperthyrotropinemia. Journal of Clinical Endocrinology & Metabolism 87(7): 3209-3214.

Charnley G, 2008. Perchlorate: Overview of Risks and Regulation. Food and Chemical Toxicology. (Epub ahead of print - March 10, 2008). doi:10.1016/j.fct.2008.03.006

EPA 2003. *Framework for Cumulative Risk Assessment*. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington Office, Washington, DC, EPA/600/P-02/001F.

EPA 2003a. Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models. Prepared by: The Council for Regulatory Environmental Modeling (CREM). Environmental Protection Agency, Office of Science Policy, Office of Research and Development, Washington, D.C. 20460. November 2003.

Lamm, S.H., Hollowell, J.G., Engel, A., Chen, R., 2007. Perchlorate, thyroxine, and low urine iodine association not seen with low creatinine-adjusted urine iodine among women of childbearing age. Thyroid 17 (s1), S-51.

National Academy of Sciences/National Research Council (NAS/NRC) (2007). Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press. Washington, DC

National Research Council/National Academy of Sciences (NRC/NAS), 2005. Health Implications of Perchlorate Ingestion. The National Academies Press, Washington, DC, 2005, (ISBN 0-309-09568-9).

Pearce, EN, Spencer CA, Mestman J, Lee R., Bergoglio LM, Mereshian P, He X, Leung AM, Braverman LE, 2008. Thyroid function is not affected by environmental perchlorate exposure in first trimester pregnant women from California and Argentina. Pending presentation at the annual meeting of the Endocrine Society, San Francisco, California, June 15-18, 2008.

Pearce, E.N., Lazarus, J.H., Smyth, P.P.A., He, X., Dall'Amico, D., Parkes, A.B., Burns, R., Smith, D.F., Maina, A., Leung, A.M., Braverman, L.E., 2007. Thyroid function is not affected by environmental perchlorate exposure in first trimester pregnant women. Thyroid 17 (s1), S-133 [abstract only].

Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL, 1999. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol 50:149-155.

Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ, 2003. Maternal hypothyroxinemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin Endocrinol 59: 282-288.

Téllez Téllez R, Chacón PM, Abarca CR, Blount BC, Van Landingham CB, Crump KS, Gibbs JP (2005). Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. Thyroid 15:963-975

Teuschler LK, 2007. Deciding which chemical risk mixtures risk assessment methods work best for what mixtures. Tox and App Pharm 223: 139-147.