PSG

PERCHLORATE STUDY GROUP

A coalition of aerospace, defense, chemical and allied industries

July 10, 2012

Thomas Carpenter
Designated Federal Officer
Science Advisory Board (MC-1400R)
1200 Pennsylvania Ave, NW
Washington, DC 20460

RE: Public Comment for U.S. EPA's Science Advisory Board Panel on Perchlorate MCLG

Dear: Mr. Carpenter

The Perchlorate Study Group (PSG) is pleased to provide the attached document*, Scientific Comments on US EPA's Life Stage Considerations And Interpretation Of Recent Epidemiological Evidence To Develop A Maximum Contaminant Level Goal For Perchlorate*, for the consideration of the agency's Science Advisory Board (SAB) panel review of the approaches being considered to derive a perchlorate maximum contaminant level goal (MCLG).

Among the many points presented, we would direct your attention to a fundamental issue that goes to the charge to the panel. It is essential that the SAB review the *entirety* of the perchlorate literature over the past six decades-- not rely solely on the very narrow scope of additional research done since 2005. Failing to take the full body of relevant science into account would prevent the panel from making judgments based on the best available science, as required by the Safe Drinking Water Act.

The breadth of pre-2005 research constitutes overwhelming evidence in support of U.S EPA's original determination *not* to regulate perchlorate. So, too, that robust record provides the basis for the National Research Council's 2005 report, *Health Implications of Perchlorate Ingestion.* Those findings from the National Academies of Science were further informed by a public review process.

None of the post-2005 studies have occasioned findings changing the foundational science. *None* of these studies changes the recognized no observed effect level (NOEL) — for a *non-adverse* effect — on which the extremely conservative NRC and US EPA perchlorate Reference Dose is based. *None* of these studies demonstrates that adverse effects occur at doses below the NOEL for *any* life stage. *None* of these studies provides evidence that any population is more sensitive than the fetus of the potentially hypothyroid mother.

It is absolutely necessary for the SAB panel to take into account the extraordinary, foundational scientific record in order to comprehensively evaluate EPA's approach to perchlorate regulation. That record can also enable the panel to credibly assess whether the agency has met its own guidelines for weight of evidence and data quality. So, too, it is necessary in order for the agency to meet its threshold statutory obligation to apply the best available science under the Safe Drinking Water Act.

Thank you for the opportunity to present the attached comments. Please do not hesitate to contact me or Dr. Richard C. Pleus if we can provide any additional information to you or the SAB panel.

Sincerely,

Jonathan Bode
Chair
Perchlorate Study Group

cc:
Hon. Lisa Jackson, Administrator, U.S. EPA
Hon. Bob Perciasepe, Deputy Administrator, U.S. EPA
Dr. Vanessa T. Vu, Director, U.S. EPA Science Advisory Board Staff Office
Nancy Stoner, U.S. EPA Acting Administrator for Water

Scientific Comments on:
U.S. EPA's LIFE STAGE CONSIDERATIONS AND INTERPRETATION OF RECENT EPIDEMIOLOGICAL EVIDENCE TO DEVELOP A MAXIMUM CONTAMINANT LEVEL GOAL FOR PERCHLORATE

Prepared for:

THE PERCHLORATE STUDY GROUP

July 10, 2012

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ABBREVIATIONS AND SELECTED DEFINITIONS

Agency	United States Environmental Protection Agency.
ATSDR	Agency for Toxic Substances and Disease Registry.
CDC	Centers for Disease Control.
DoD	United States Department of Defense.
fT3	Free T3.
fT4	Free T4.
fTI	fT4 index.
GLP	Good Laboratory Practices
in vitro	A study conducted outside a living organism in an artificial environment.
in vivo	A study conducted in a living organism.
IUI	Iodide uptake inhibition. Reduction of iodide uptake into the thyroid through the NIS. Measured by Radioactive Iodide Uptake or RAIU.
LOAEL	Lowest Observable Adverse Effect Level. The lowest exposure level at which there is biologically significant increase in frequency or severity of adverse effects between the exposed population and its appropriate control group.
MCL	Maximum Contaminant Level. A federally enforceable standard set by U.S. EPA.
µg/L	Microgram per liter. A unit of mass concentration defined as the concentration of one microgram of a substance per unit volume of the mixture equal to one liter; equivalent to a part per billion.
mg/d	Milligrams of chemical per day. Daily doses of a chemical are often described in these units; they are not normalized for weight.
mg/kg-d	Milligrams of chemical per kilogram of body weight per day. Daily doses of a chemical are often described in these units, which are normalized for weight. This is important as an identical dose in mg/d could be different in a 70 kg adult versus a 10 kg infant.
NPDWR	National Primary Drinking Water Regulation
NHANES	National Health and Nutrition Examination Survey I, II.
NIS	Sodium iodide symporter. An ion pump that actively transports an iodide ion along with two sodium ions across the membrane into certain cells, particularly thyroid epithelial cells; perchlorate can transiently block this uptake.
NOAEL	No Observable Adverse Effect Level. The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they

	are not considered adverse or precursors of adverse effects.
NOEL	No Observable Effect Level. An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control.
NRC	National Academy of Sciences National Research Council.
OEHHA	Office of Environmental Health Hazard Assessment (California Environmental Protection Agency).
PBPK	Physiologically-based pharmacokinetic.
PHG	Public Health Goal. Drinking water goal set by the State of California.
PSG	Perchlorate Study Group. A group of users and manufacturers of perchlorate. It includes Lockheed Martin Corp., Aerojet Corp., ATK, and American Pacific Corp.
ppb	Part per billion.
RfD	Reference Dose. 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.
SAB	United States Environmental Protection Agency Science Advisory Board.
SDWA	Safe Drinking Water Act.
T3	Triiodothyronine. A thyroid hormone, more potent than T4; can be bound to other molecules and measured as total T3, or as the fraction available for the body to use as free T3, which is more biologically relevant.
TT3	Total T3.
T4	Thyroxine. A thyroid hormone, that is also is a precursor of T3, a more potent thyroid hormone; can be bound to other molecules and measured as total T4, or as the fraction available for the body to use as free T4, which is more biologically relevant.
TT4	Total T4.
Tg	Thyroglobulin. An iodine-containing protein found in the thyroid gland that is involved in the production of the T4 and T3 hormones.
TGL	Total goitrogen load. The combined exposure to all substances that cause IUI, particularly nitrate, thiocyanate, and perchlorate.
TSH	Thyroid stimulating hormone. A pituitary hormone that stimulates the production of thyroid hormones.
UF	Uncertainty Factor.
U.S. EPA	United States Environmental Protection Agency.

U.S. FDA	United States Food and Drug Administration.
White Paper	U.S. EPA, 2012a. *White Paper: Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate*.
WHO	World Health Organization.

EXECUTIVE SUMMARY

The United States Environmental Protection Agency (U.S. EPA or the Agency) is presently evaluating the appropriate concentration at which to regulate perchlorate in drinking water. On May 18, 2012, the Agency publicly released a paper titled *White Paper: Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate* (the White Paper or U.S. EPA, 2012a). The White Paper is intended to provide scientific information to U.S. EPA's Science Advisory Board (SAB) to assist the SAB in its evaluation of the science in support of U.S. EPA's proposed regulatory action. The initial meeting of the SAB is scheduled for July 18 and 19, 2012, in Washington D.C.

We provide these comments to the SAB so panel members can: (1) quickly and efficiently understand the most important and relevant aspects of the scientific literature available to date that focus upon exposure to perchlorate; and, (2) fully appreciate the enormous breadth of that scientific database. Of particular import is to note that the scientific database has been established over several decades. This entire database is robust and supports the conclusion that no adverse effects are expected at environmental levels; this includes the narrow group of studies undertaken since 2005, which are the primary focus of the White Paper.

Summary of key comments and recommendations

- SAB should evaluate the entirety of the perchlorate literature derived over the past six decades, not rely unduly on the narrow scope of research done since 2005. In the period following the 2005 National Academies of Science National Research Council (NRC) report, *no* reliable scientific evidence has been developed that demonstrates that doses below the Greer (2002) No Observable Effect Level (NOEL) cause *any* effect on *any* subpopulation. That many studies report low levels of exposure does not change the foundational science of the pharmacology and toxicology of perchlorate. Examining the entirety of human studies using a weight-of-evidence approach, it is overwhelmingly clear that perchlorate at environmental levels is unlikely to cause adverse health effects.
- At exposure levels anticipated under the Agency's drinking water program, the toxicological literature for perchlorate has demonstrated no adverse effect on human health at environmental levels. U.S. EPA's White Paper provides no evidence that perchlorate exposure has resulted in *any* of the effects attributed to hypothyroidism, iodine deficiency or congenital hypothyroidism (CH).
- The SAB should consider whether U.S. EPA's White Paper meets the Agency's own guidelines related to the scientific processes of peer review, weight-of-evidence and quality of information, for the following reasons:
  - Calculations of lower thyroid hormone reserves in infants are based on assumptions rather than data and do not provide predictive reliability.
  - Values for some key parameters such as urinary clearance are not supported by the scientific evidence.
  - The White Paper infers an iodine uptake inhibition (IUI) greater than 0% is "adverse." It is not. As noted by NRC, IUI is a non-adverse event.
- The SAB should note that the point of departure for the current Reference Dose (RfD) is based on a NOEL—for a <u>nonadverse</u> effect—rather than a No Observable <u>Adverse</u> Effect Level (NOAEL).

- There is sufficient scientific evidence that the fetus of the potentially hypothyroid pregnant woman is the most sensitive population. U.S. EPA has not provided any science-based evidence that another life stage is more sensitive.
- U.S. EPA has provided no evidence demonstrating the effects or severity of perchlorate exposure will differ in children compared to adults. In contrast, studies in humans and in animals do not demonstrate that the neonate is more susceptible to perchlorate exposure.
- Close evaluation of the post-2005 studies cited in the White Paper does not support an association between exposure to environmental levels of perchlorate and adverse health effect. No adverse health concern at any life stage would be expected based on exposure to perchlorate at the point of departure dose or lower.
- The tone used by U.S. EPA to describe the perchlorate science appears biased in that it assumes that adverse effects must exist at very low levels of exposure, even though available data do not support this assertion. For example, the White Paper states "there are currently no data available to directly link perchlorate to neurobehavioral effects in infants and children." However, the toxicological and medical database for perchlorate have several studies that have in fact reported no effect of perchlorate on neurodevelopment.
- The level of IUI that would be associated with exposure to perchlorate at the RfD is minimal when compared to the IUI associated with other environmental goitrogens naturally present in many foods, particularly nitrate and thiocyanate.
- NRC has stated, "The committee notes that effects of downstream IUI by the thyroid have not been clearly demonstrated in any human population exposed to perchlorate, even at doses as high as 0.5 mg.kg per day."

This document is divided into seven sections: Section 1, Introduction; Section 2, The Agency must adhere to its own information quality guidelines; Section 3, A brief review of science database prior to and including 2005; Section 4, Life stage assessment; Section 5, Use of physiologically-based pharmacokinetic (PBPK) modeling; Section 6, Human studies; and Section 7, Conclusions and integration of the data. We have briefly summarized the primary comments and scientific discussion in each section in this Executive Summary.

NRC has stated

The committee notes that effects downstream of inhibition of iodide uptake by the thyroid have not been clearly demonstrated in any human population exposed to perchlorate, even at doses as high as 0.5 mg/kg per day.

Section 1. Introduction

In 2008, U.S. EPA determined that perchlorate did not meet the Safe Drinking Water Act (SDWA) requirements for regulation. The Agency announced that it therefore did not intend to regulate the chemical (U.S. EPA, 2008a). Subsequently, on August 19, 2009, U.S. EPA publicly initiated a reconsideration of its decision not to regulate perchlorate and asked for public comments on a document titled *Alternative Approaches to Analyzing Scientific Data Related to Perchlorate in Drinking Water*. In that document the Agency asked for comments on PBPK modeling, alternative Health Risk Levels (HRLs) based on life stages, occurrence analysis, and consideration of studies published since U.S. EPA adopted the NRC-recommended RfD—a set of questions which the Agency appears to have asked the SAB (via the White Paper) to consider as well. In February 2011, U.S. EPA announced its intent to regulate perchlorate under the SDWA.

Section 2. Safe Drinking Water Act Criteria and Information Quality Requirements

The White House Office of Management and Budget (OMB) has set forth guidelines to ensure and maximize the quality, objectivity, utility, and integration of information disseminated by Federal Agencies (OMB, 2002). U.S. EPA developed its own set of guidelines which correspond to the OMB guidelines. Both sets of guidelines apply to "influential scientific information" that U.S. EPA disseminates to the public. The White Paper clearly qualifies as "influential scientific information" under the definition of that phrase included in the guidelines. Accordingly, U.S. EPA must, in compliance with its own guidelines, adhere to the scientific processes of peer review, weight-of-evidence, and quality of information. The SAB should therefore consider whether this White Paper has complied with these and other requirements set forth in the U.S. EPA guidelines. Regrettably, we find that they have not.

Section 3. Scientific database for perchlorate prior to 2005

The scientific database for estimating acceptable exposure limits for perchlorate, which encompasses more than 60 years of scientific data, is robust with a plethora of reliable studies, including human studies. However, the White Paper focuses only upon work reported after the 2005 release of the NRC report on *Health Implications of Perchlorate Ingestion*—a small set of studies in comparison to the substantial weight-of-evidence presented in the several decades of perchlorate research available in the scientific literature. A review of this robust database suggests one of the most important considerations to note is that the perchlorate RfD is based on a NOEL for a non-adverse effect rather than a higher, less conservative NOAEL. In the words of the NRC, this provides a "…a reasonable and transparent approach to the perchlorate risk assessment."

The point of departure represents a dose at which there is no change over background for the parameter evaluated—IUI in humans. Greer et al. (2002), the seminal perchlorate study from which the value is obtained, is not the only human clinical study conducted for this endpoint. However, Greer sets forth the most conservative (low) NOEL compared to other studies. The Drinking Water Equivalent Level (DWEL), using standard U.S. EPA default values, for the NOEL in Greer et al. (2002) would be 245 ppb. This means that at that level and below, there is no measureable effect in the human body.

Section 4. Comments to the SAB regarding life stages

The White Paper states:

> ***There are currently no data available to directly link perchlorate to neurobehavioral effects in infants and children.***

The White Paper and the perchlorate database provide sufficient scientific evidence that the fetus of the potentially hypothyroid pregnant woman is the most sensitive population. Thus, the current, scientifically justified sensitive population is the pregnant woman and her fetus. The Agency has not provided any science-based evidence that another life stage is more sensitive. There are a number of studies regarding gestational exposures to perchlorate where adverse effects, including neurobehavioral effects in infants and children, were evaluated. In these studies, for the periods examined, perchlorate exposure was not associated with neurobehavioral effects.

The Agency asks:

How should U.S. EPA consider the following life stage factors in deriving Maximum Contaminant Level Goals (MCLG)? [Our response to each query is set forth below.]

- **Life stage specific differences in body weight and food and drinking water intake**

  Response: There are a number of data quality issues with studies the Agency used for the values chosen. These include: the relative source contribution (RSC), data for the food consumption values, body weight, drinking water intake rates, the study populations, and the use of a recall study.

- **Differences in greater severity and permanence of potential adverse effects in neonates, infants and young children compared to adults**

  Response: There is no scientific evidence provided by the Agency demonstrating the effects or severity of perchlorate exposure will differ in children compared to adults.

- **Shorter half-life and lower reserves for thyroid hormone in infants compared to adults**

  Response: The methodology used by the Agency to calculate lower thyroid hormone reserves (turnover rates) in infants is based on assumptions which are not data driven and thus do not provide predictive reliability. The White Paper reports thyroxine (T4) half-life values from neonates with severe congenital disorders, patients that have had acute pediatric T4 overdoses, or premature neonates treated with T4 and who had died. The SAB should carefully consider whether the White Paper provides appropriate scientific justification for the use of these values, taking into account that the scientific literature does not support the use of these values. The use of T4 half-lives from neonates with CH as a surrogate value for normal neonates also is not valid.

- **Urinary clearance rates in neonates are slower than in other life stages**

  Response: Evaluation of renal clearance rates of other non-metabolized pharmaceuticals similar to perchlorate, suggests that children and neonates have at minimum equal to adult clearance rates, although data report slightly higher rates. It is not apparent why the White Paper did not consider this type of information in its evaluation, as this evidence suggests that children and neonates are not more sensitive than the fetus.

  There are no reliable data that demonstrate that environmentally relevant levels of perchlorate are sufficient to cause any thyroidal hormone or pituitary hormone (TSH) changes.

- **Intrauterine exposure to perchlorate and impact on thyroid status in fetuses**

  Response: There are no reliable data that demonstrate that environmental levels of perchlorate cause any thyroidal hormone or pituitary hormone (*i.e.* thyroid stimulating hormone (TSH)) changes. The White Paper reviews a number of studies regarding gestational exposures where there exist populations with substantial changes in thyroid hormones which are due to exposure to contaminants other than perchlorate.

Section 5. Comments to the SAB regarding PBPK modeling

The Agency asks:

> ***Pertaining to PBPK evidence, how should [U.S. EPA] consider PBPK modeling to derive an MCLG for perchlorate?***

Response: We have some concerns with the parameters that were modified by the Agency; nonetheless, the predicted levels of IUI are low and are indistinguishable from fluctuations resulting from differences in diet and feeding styles. No adverse health concern at any life stage would be expected based on exposure to perchlorate at the point of departure dose (7 µg/kg-d) or lower. The PBPK model can be used to reinforce that the current RfD drinking water equivalent of 24.5 ppb and the interim health advisory of 15 ppb are conservative and health protective of all subpopulations.

The predicted levels of IUI are low and are indistinguishable from fluctuations resulting from differences in diet and feeding styles.

> ***What are the strengths and limitations of the two PBPK model results described in this effort?***

Response: The output of this model is limited to a single agent, perchlorate, that represents at most only 2% of the daily goitrogen load and does not account for exposures to other goitrogens or other thyroid active agents. Furthermore, input parameters are not selected from consistent points within their distributions and uses of values for some key parameters (*e.g.*, urinary clearance) are not supported by the scientific evidence. Finally, it is well-understood that the human hypothalamic–pituitary–thyroid (HPT)-axis can compensate for changing environmental stimuli. The model does not account for these mechanisms and likely overestimates IUI for a particular dose.

Section 6. Comments to the SAB regarding human studies

The Agency asks:

> ***How should [U.S. EPA] consider the post-NRC epidemiology data in deriving an MCLG?***

Response: Since the 2005 NRC Report, no scientific evidence has been developed that reliably reports doses lower than the Greer et al. (2002) NOEL that cause any effect on any subpopulation. If one considers all of the scientific data for this chemical (*e.g.*, weight-of-evidence), there are only five studies which might be used to suggest environmental doses are implicated for changes in thyroid or pituitary hormones. However, these studies are analyses of biomonitoring data or ecological epidemiological studies and, therefore, cannot determine causation. These few studies, when weighed against the vast literature on perchlorate, fail to provide a sufficient weight-of-evidence to change the current understanding of the science of perchlorate.

Section 7. Comments to the SAB regarding integration of information

The Agency asks:

> ***How can [U.S. EPA] best use the total body of information to derive a health protective MCLG, while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values?***

Response: Many of the studies published since the NRC evaluation report only low levels of *exposure* to perchlorate. These studies demonstrate more clearly that exposure to perchlorate comes from sources that are both natural and anthropogenic. These newer epidemiological and

biomonitoring data do not change the foundational science of the pharmacology and toxicology of perchlorate, nor do they demonstrate that adverse effects occur at doses below the NOEL for IUI of perchlorate. Some of these studies also suffer from important methodological weaknesses that affect the interpretation of their data. For scientific work related to an endocrine system that has normal but dynamic fluctuations on a daily basis, experimental design is critical. Thus, timing of sampling, appropriate clinical analysis, (*e.g.*, full clinical panel of thyroid hormone measurements), and attention to a sound and robust database should be considered. It would not follow the Agency's own data quality guidelines, for example, to place undue emphasis on a few studies that are not consistent with the literature, have methodological issues, and are not designed to make causative determinations.

> ***How can [U.S. EPA] use the available data to estimate reductions in adverse health effects (i.e., dose response) that are likely to result from reducing perchlorate levels in drinking water?***

Response: The available data strongly supports the conclusion that there cannot be any reductions in adverse health effects from reducing perchlorate level, because adverse effects have not been demonstrated at the RfD or at any level below the established NOEL. The RfD is 10-fold below the level at which there is no change in background levels of a non-adverse effect. The Agency has presented no scientific data purporting to establish that any other population other than the pregnant woman is the most sensitive.

1.0 INTRODUCTION

1.1 Purpose of these comments

The Agency has released a document titled *White Paper: Life Stage Considerations and Interpretation Of Recent Epidemiological Evidence To Develop A Maximum Contaminant Level Goal For Perchlorate* (White Paper), dated May 18, 2012. The Perchlorate Study Group (PSG), a group of users and manufacturers of perchlorate, requested Intertox provide an assessment of the science presented in the White Paper. We conduct our work to the standards of our profession. The White Paper and comments are expected to be provided to the Scientific Advisory Board (SAB) panel to assist in their review of the depth of the scientific database for perchlorate.

1.2 How this paper is organized

We provide a history of the Agency's regulatory review of perchlorate. We then summarize critical scientific information developed prior to the NRC evaluation in 2005 and provide comments on the Agency's specific charge questions to the SAB, along with discussions of some of the newer assertions presented by the U.S. EPA in the White Paper. Perchlorate is one of the most well studied environmental contaminants and has an extensive scientific database.

2.0 SAFE DRINKING WATER ACT CRITERIA AND INFORMATION QUALITY REQUIREMENTS

In 2008, U.S. EPA determined that perchlorate did not meet the SDWA requirements (U.S. EPA, 2008a) for federal regulation. At the time, U.S. EPA decided not to propose a National Primary Drinking Water Regulation (NPDWR) for perchlorate, as it would not present "a meaningful opportunity for health risk reduction for persons served by public water systems." This decision was based on a Health Reference Level (HRL) for perchlorate in drinking water of 15 ppb, calculated based on an assumed relative source contribution (RSC) for drinking water of 62% (U.S. EPA, 2008). In February, 2011, U.S. EPA, reversing its preliminary determination, announced its intent to regulate perchlorate under the SDWA.

Under the SDWA, U.S. EPA is required to publish Maximum Contaminant Level Goals (MCLGs) and NPDWR <u>if all</u> the following statutory requirements are met:

> (a) the contaminant may have an adverse effect on the health of persons;
>
> (b) the contaminant is known to occur in public water systems with a frequency and at levels of public health concern; <u>and</u>
>
> (c) in the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems (U.S. EPA, 1996).

All three of U.S. EPA's statutory criteria for setting a standard are unmet in this case. At exposure levels anticipated under the drinking water program, the toxicological literature for perchlorate has demonstrated no adverse effect on human health at environmental levels. Second, data from U.S. EPA's first cycle of the Unregulated Contaminant Monitoring Rule (UCMR1) demonstrates perchlorate does not consistently occur in public water systems at levels that may cause adverse effects in humans. Lastly, even though this criteria is to be based on the sole discretion of the Administrator, because the first two requirements are not met there is no meaningful opportunity for health risk reduction.

In December 2011, U.S. EPA announced the creation of a SAB panel for perchlorate and requested nominations. On May 30, 2012, U.S. EPA announced the panel and released the White Paper. The

White Paper includes four sets of questions based on Life Stage Considerations, Physiologically-Based Pharmacokinetic Analysis, Human Studies Conducted Since the 2005 NRC Report, and Integration of Information.

U.S. EPA states "Interested members of the public may submit relevant written or oral information on the topic of this advisory activity, and/or the group conducting the activity, for the SAB to consider during the advisory process" (U.S. EPA, 2012b). This document responds to that invitation.

2.1 Requirements for data quality in conducting risk assessments

The Agency is required to use scientific information to make informed decisions. Under the guidelines issued by the Office of Management and Budget (OMB), U.S. EPA is responsible for ensuring and maximizing the quality, objectivity, utility, and integration of information disseminated by the Agency (OMB, 2002). These guidelines apply to information U.S. EPA disseminates to the public, including the White Paper.

Regarding risk assessments, the Agency writes:

> [U.S. EPA] conducts and disseminates a variety of risk assessments. When evaluating environmental problems or establishing standards, [U.S. EPA] must comply with statutory requirements and mandates set by Congress based on media (air, water, solid, and hazardous waste) or other environmental interests (pesticides and chemicals). Consistent with [U.S. EPA's] current practices, application of these principles involves a "weight-of-evidence" approach that considers all relevant information and its quality, consistent with the level of effort and complexity of detail appropriate to a particular risk assessment. In our dissemination of influential scientific information regarding human health, safety or environmental risk assessments, [U.S. EPA] will ensure, to the extent practicable and consistent with Agency statutes and existing legislative regulations, the objectivity of such information disseminated by the Agency by applying the following adaptation of the quality principles found in the [SDWA] Amendments of 1996:
>
> (A) The substance of the information is accurate, reliable and unbiased. This involves the use of:
>
> > (i) the best available science and supporting studies conducted in accordance with sound and objective scientific practices, including, when available, peer reviewed science and supporting studies; and
> >
> > (ii) data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justifies the use of the data).
>
> (B) The presentation of information on human health, safety, or environmental risks, consistent with the purpose of the information, is comprehensive, informative, and understandable (U.S. EPA 2002).

2.2 Does the Agency adhere to the data quality requirements it sets?

The Agency has published guidelines for information quality for use in risk assessments. The SAB should evaluate whether the Agency is fulfilling its own mandate.

First, the SAB should inquire whether the White Paper has been peer reviewed in a manner consistent with a document that will have special importance and whether that peer review was external. One of the components of the Information Quality Guidelines is peer review (U.S. EPA, 2002).

Second, a "weight-of-evidence" approach is required. The Agency states that:

> Consistent with U.S. EPA's current practices, application of these principles involves a "'weight-of-evidence' approach that considers all relevant information and its quality, consistent with the level of effort and complexity of detail appropriate to a particular risk assessment (U.S. EPA, 2002).

In contrast, the White Paper focuses on work reported after the release of the NRC report *Health Implications of Perchlorate Ingestion* released in 2005. The SAB will benefit from valuable scientific work that was conducted prior to (and including) the NRC assessment. There was substantially more research conducted prior to the 2005 NRC review than after, and the SAB should not limit their examination to post-2005.

Regarding information data quality, U.S. EPA's guidelines state:

> **Consistent with U.S. EPA's current practices, application of these principles involves a *"'weight-of-evidence' approach that considers all relevant information and its quality, consistent with the level of effort and complexity of detail appropriate to a particular risk assessment."***

Third, quality of information is another key concept in the Agency's guidelines. There is no evidence of adverse health effects caused by exposure to environmental levels of perchlorate. The White Paper states (on page 13), that:

> There are currently no data available to directly link perchlorate to neurobehavioral effects in infants and children.

In this statement, the Agency implies there are limitations in the scientific data. However, the toxicological and medical database for perchlorate is rich, especially when compared to many other environmental contaminants. There is no scientific indication that perchlorate exposure to levels at or near the current RfD will cause the implied neurobehavioral effects.

These comments are intended to ensure the SAB is provided with a fuller picture of toxicological and medical literature for perchlorate—both pre-2005 as well as post-2005. Given the short timeline and vast literature on perchlorate, the SAB faces a substantial task.

3.0 SCIENTIFIC DATABASE FOR PERCHLORATE PRIOR TO 2005

Although the White Paper focuses on work reported after the 2005 release of the NRC report *Health Implications of Perchlorate Ingestion*, substantially more research was conducted prior to the 2005 NRC review than after. As such, the SAB should consider both the pre- and post-2005 data in an assessment of the perchlorate science. Specifically:

- The point of departure for the U.S. EPA RfD[1] for perchlorate is based on a NOEL for a non-adverse effect rather than a NOAEL. In the words of the NRC, this provides "…a reasonable and transparent approach to the perchlorate risk assessment."
- The point of departure represents a dose at which there was no change over background for the parameter evaluated─IUI in humans. These data were obtained from a study by Greer et al. (2002), although this is not the only human clinical study conducted for this endpoint. Greer et al. (2002) provides the most conservative (low) NOEL compared to other studies. If one were to develop a Drinking Water Equivalent Level (DWEL) corresponding to the

[1] 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.

NOEL using standard U.S. EPA default exposure assumptions (*i.e.*, consumption of 2 L/d of water by a 70 kg person), that concentration would be 245 ppb. Exposure to this water concentration and below would result in no measureable effect on IUI, which is itself a non-adverse effect.

- Derivation of the current RfD assumes that the most sensitive population is the pregnant woman. Other life stages have been considered and none have been scientifically demonstrated as more sensitive.

The point of departure for the U.S. EPA RfD for perchlorate is based on a NOEL for a non-adverse effect rather than a NOAEL. In the words of the NRC, this provides "…a reasonable and transparent approach to the perchlorate risk assessment."

If one considers the totality of the scientific data (*e.g.*, the weight-of-evidence) for perchlorate, only five publications could be interpreted to suggest that environmental levels are associated with changes in thyroid or pituitary hormones.[2] However, these studies rely on analysis of biomonitoring data or are ecological epidemiological studies, they suffer a number of methodological weaknesses, cannot assess causation, and specifically are not designed to directly test the scientific hypothesis. Further, the body of literature strongly indicates that the perchlorate dose would need to consistently attain levels that cause approximately 70% or higher IUI. Lastly, we analyzed the same data set used in three of these studies (National Health and Nutrition Examination Survey (NHANES) 2001-2002) using a more complete panel of thyroid chemistry analytical data obtained from reanalysis of stored serum samples that was recently released by the Centers for Disease Control (CDC). This reanalysis also utilized a more robust measure of IUI exposure (perchlorate equivalent concentration, based on urinary levels of perchlorate, nitrate, and thiocyanate, adjusted for their relative potency to cause IUI). In our reanalysis we were not able to demonstrate consistent and biologically plausible associations between urinary IUI concentrations and thyroid effects (CDC, 2004; 2011a; 2011b).[3]

3.1 Mechanism of action of perchlorate

Perchlorate interacts with the sodium iodide symporter (NIS), a membrane-bound protein on the basal side of the thyroid cell (known as the thyrocyte). With sufficient dose, perchlorate can reduce the uptake of iodide from the blood to the thyroid. In the thyroid, iodide is used in the organification of thyroid hormone. Therefore, given sufficient dose and exposure, reduction in thyroid iodide stores over time would result in reduced production of thyroid hormone. However, the thyroid has stores of iodide such that short-term fluctuations in iodide uptake will cause no effect. The reduction in iodide uptake must be sustained for months or more to deplete existing stores. Further, in situations when IUI is increased—as is common with biological receptors—there is regulation of NIS numbers (*e.g.*, up-regulation).

[2] The five studies are Blount et al. (2006), Steinmaus et al. (2007), and Schrienemachers (2010), all three of which are based on a single study population; Cao et al. (2010); and Steinmaus et al. (2010).
[3] Manuscript by Bruce et al. (2012) submitted for publication.

Because perchlorate has a short half-life, some have questioned whether exposure to perchlorate might cause an acute effect. Toxicologically speaking, "acute" describes effects seen after one high dose or an exposure of less than 24 hours. No published study has demonstrated an effect after one exposure to perchlorate at environmental levels. A therapeutic dose is required to cause acute effects. The medical literature reports therapeutic doses from 200 mg to over 1 gram given on a daily basis (the equivalent of 100,000 to 500,000 ppb in drinking water using standard U.S. EPA default assumptions[4]). At exposures lasting several weeks to months (more commonly termed "subchronic"), the scientific literature does not demonstrate any evidence of adverse effect. Thyroxine (T4) has a half-life of approximately 8 days and must be sufficiently depleted for developmental effects to occur. A single perchlorate dose in excess of 1 gram per day would most likely cause some side effects (see U.S. FDA Adverse Reaction Database), but based on the science to date, will not decrease serum thyroid hormone levels without daily exposure for weeks.

At exposures lasting several weeks to months the scientific literature does not demonstrate any evidence of adverse effect.

3.2 Perchlorate is not the only agent that can inhibit iodide uptake

The level of IUI associated with exposure to environmental perchlorate is minimal compared to the IUI associated with other environmental goitrogens naturally present in many foods. Nitrate and thiocyanate are less potent (Tonacchera et al., 2004) but more plentiful inhibitors of NIS activity than perchlorate (Belzer et al., 2004). The potency of nitrate and thiocyanate relative to perchlorate has been demonstrated *in vivo* (Wyngaarden et al., 1952, 1953; Greer et al., 1966) and *in vitro* (Tonacchera et al., 2004). The effects of perchlorate are much smaller than the effects of either nitrate or thiocyanate (Belzer et al., 2004; U.S. EPA, 2010; Tarone et al. 2010). As noted by the Agency for Toxic Substances and Disease Registry (ATSDR), "Nitrate and thiocyanate are widely distributed in nature and, because both anions also inhibit RAIU [radioactive iodide uptake], as demonstrated by Tonacchera et al. (2004), should also be included in the discussion of the effects of inhibition of the NIS by anions (ATSDR, 2008)."

The potential for perchlorate to inhibit iodide uptake cannot be distinguished from the effects of other NIS inhibitors (De Groef et al., 2006; Tarone et al., 2010). Because exposure to nitrate and thiocyanate would continue, and given perchlorate's very low contribution to IUI (less than 2 %) attempts to limit exposure to perchlorate are unlikely to provide an actual public health benefit.

The potential for perchlorate to inhibit iodide uptake cannot be distinguished from the effects of other NIS inhibitors (nitrate and thiocyanate). Thus, the premise underlying U.S. EPA's attempt to isolate the effect of perchlorate is unlikely to provide a public health benefit.

3.3 Perchlorate's use as a medication

The scientific literature on perchlorate comprises more than six decades of scientific inquiry, largely because of perchlorate's use as a drug during the 1950s and 1960s to treat hyperthyroidism (*e.g.*, as caused by Graves' disease, a form of autoimmune hyperthyroidism resulting from autoactivation of the TSH receptor). Perchlorate is still used for treating some thyroid-related diseases and in diagnostics, although it is no longer the drug of choice for hyperthyroidism. Perchlorate's medicinal use provided scientists with valuable information about its toxicity. Where data gaps remained,

[4] 1g = 1,000 mg; Drinking Water Equivalent for 1,000 mg perchlorate / 2 L of water per day = 500 mg/L or 500 ppm or 500,000 ppb; Drinking Water Equivalent for 200 mg would be 100 ppm or 100,000 ppb.

animal and human studies were performed in the 1990s and early 2000s.

There were few side effects from therapeutic exposures up to 4.1 mg/kg-d (equivalent to approximately 144,000 ppb in drinking water) and these side effects were nonthyroidal. Nonthyroidal side effects varied but included nausea and vomiting, skin rashes, fever, enlarged lymph nodes, and nonspecified kidney dysfunction, with an incidence of 3-4 % in patients taking 400 to 600 mg/d (4.1 to 6.2 mg/kg-d; Wolff, 1998). In patients taking 1,000 to 2,000 mg/d (10.3 to 20.5 mg/kg-d), the incidence was 16-18% (Wolff, 1998).

Thyroidal and nonthyroidal side effects have been reported at doses that exceed 4.1 mg/kg-d. The most serious side effects were reports of hemotoxic effects (agranulocytosis and aplastic anemia) in 13 individuals, seven of whom died, receiving 6 to 14 mg/kg-d (Wolff, 1998; Soldin et al., 2001[5]). This effect is inconsistent with perchlorate's thyroidal mechanism of action and may have been due to contaminated medication (Clark, 2000). No hemotoxic effects have ever been reported at doses below 6 mg/kg-d, nor has there been a repeat of these side effects since. Morgans and Trotter (1960) remark on the similarity in rate of side effects between their own study (180 patients receiving 4.1 to 10.3 mg/kg-d with a 3% side effect incidence) and that of Crooks and Wayne (1960) (200 patients receiving 4.1 to 10.3 mg/kg-d with a 2% side effect incidence).

Hypothyroidism has been reported only at doses of 5.7 mg/kg-d (equivalent to 199,500 ppb in drinking water) and up. Typically, when patients are being treated for a disease, doses are given based on animal and human studies, experience, and manufacturers' recommendations. However, refining a dosage up or down for a particular person is not uncommon. Three studies that gave patients doses of 5.7 to 10.3 mg/kg-d report some degree of hypothyroidism as a result of perchlorate treatment in a total of eight individual patients (Smellie, 1957; Newnham et al.1988; Martino et al., 1966). These three studies also report successful treatment of hyperthyroidism in these eight patients and others in the study populations. The first study in juvenile females (Smellie, 1957) reported "slight" hypothyroidism (the definition of "slight hypothyroidism" was not reported) following therapeutic doses of 5.6 and 6.8 mg/kg-d (assuming an average body weight of 31.8 kg) in an 8 and 6 year old with Graves' disease. In both cases, hypothyroidism appeared in less than 3 months and the patient's dose was immediately reduced. The hypothyroid symptoms disappeared when the dose was reduced to 4.5 mg/kg-d in both cases and the patients became euthyroid. The four other case studies reported in Smellie (1957) had no side effects and well controlled euthyroidism with doses of 4.5 to 6.8 mg/kg-d for up to 3 years.

Two other studies reported hypothyroidism as an effect following perchlorate treatment with doses greater than 8.2 mg/kg-d. These were three patients receiving 8.2 mg/kg-d (assuming a 70 kg adult; Newnham et al. (1988)) and three patients receiving approximately 10.3 mg/kg-d (Martino et al., 1966). There are no reports of hypothyroidism with doses of 4.1 mg/kg-d or lower (Figure 1).

[5] Soldin et al. (2001) cites Hobson (1961), Johnson and Moore (1961), Fawcett and Clark (1961), Krevans et al. (1962), Gjemdal (963), Barzilai and Sheinfeld (1966).

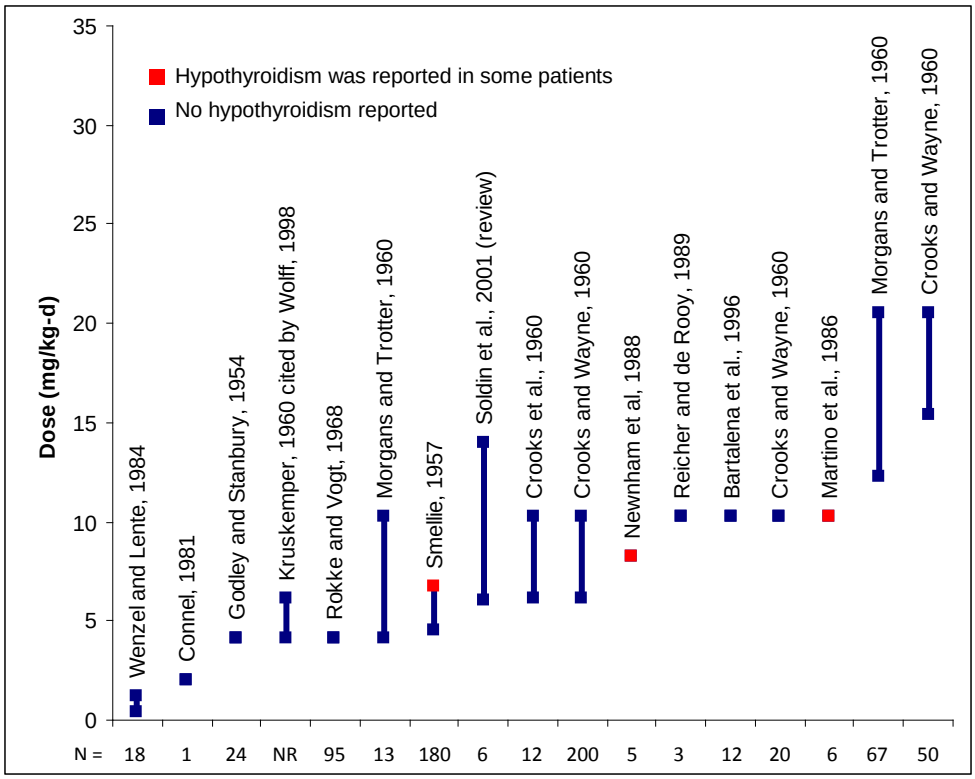

Figure 1. Summary of clinical studies where perchlorate was administered to patients and incidence of hypothyroidism

Doses and dose ranges reported in several therapeutic studies and the number of study participants in the dose group (N on X-axis). Some studies are listed multiple times as multiple dose groups were presented. There were three studies in which hypothyroidism was reported as an effect (red boxes) of perchlorate treatment: two juvenile females who received a higher weight-normalized dose than the adults (Smellie, 1957); three patients that were the subject of the Newnham et al. (1988) case studies report; and three adult patients (Martino et al. (1986). The authors report that when the initial dose was decreased, the patients became euthyroid. For reference, the NOEL dose determined in Greer et al. (2002) is 0.007 mg/kg-d, several orders of magnitude below the lowest dose depicted here, and is not visible on this chart due to scale.

3.4 Animal studies conducted to address U.S. EPA data requirements

Since U.S. EPA began evaluating perchlorate in the early 1990s, scientists from government, industry, and academia have made important contributions to reducing uncertainties about the risks of perchlorate to public health. Between late 1990s and early 2000s, at least 12 toxicological studies in animals were conducted according to U.S. EPA protocols. These included pharmacokinetic, subchronic, developmental, and immunotoxicology studies and a multigenerational reproductive study, using a range of doses. These studies were funded by PSG and the Department of Defense (DoD), conducted using Good Laboratory Practices (GLP), U.S. EPA guidance, with data directly transmitted to U.S. EPA.

3.5 National Academies of Science: NRC review of perchlorate in 2005

3.5.1 Summary of the NRC findings

In 2005, the NRC was asked to review the data on the health effects of perchlorate and U.S. EPA's 2002 risk assessment. The NRC conducted an examination of the perchlorate literature and evaluated the risks associated with ingestion of perchlorate at environmental levels. The report, published in

2005, concluded:

- At doses above environmental levels but below therapeutic doses, IUI is the only consistently documented effect of perchlorate exposure in humans. There is no scientific evidence to suggest that low doses of perchlorate will cause any other effect.
- "…effects downstream of inhibition of iodide uptake by the thyroid have not been clearly demonstrated in any human population exposed to perchlorate, even at doses as high as 0.5 mg/kg per day."
- As shown in Greer et al. (2002), IUI is only observed at doses greater than 0.007 mg/kg-d (equivalent to 245 ppb in drinking water). The NOEL for IUI reported in Greer et al. (2002) is consistent with other clinical studies.
- Extensive human and animal data demonstrate that no adverse effect will occur if no IUI occurs.
- IUI is a reversible biochemical phenomenon and is not an adverse effect. Thyroid hormone (T4 or triiodothyronine (T3)) changes are not necessarily adverse because these levels adapt in response to various environmental stimuli (*e.g.*, temperature, illness, or diet) on a daily and seasonal basis.
- Basing the risk assessment on a dose at which no effect, specifically a non-adverse effect, occurs is conservative and health protective compared to using a dose at which adverse effects occur. Other human clinical studies have been conducted and Greer et al. (2002), has the lowest NOEL of the studies.
- Perchlorate is water soluble, has a half-life in humans of approximately eight hours, and does not bioaccumulate.
- Perchlorate's reversible inhibition of the NIS is documented in many experiments, both *in vitro* and *in vivo*. Perchlorate competitively causes IUI; it neither mimics a hormone nor directly stimulates a response. The inhibition of the NIS exhibits the sigmoidal dose-response curve commonly observed in toxicology and pharmacology—as dose increases, so does the response. IUI precedes the potential adverse effect of hypothyroidism by several biochemical steps.
- The mechanism of action of perchlorate on the thyroid gland, including the delineation of what is and what is not considered an adverse effect, is summarized in Figure 2.

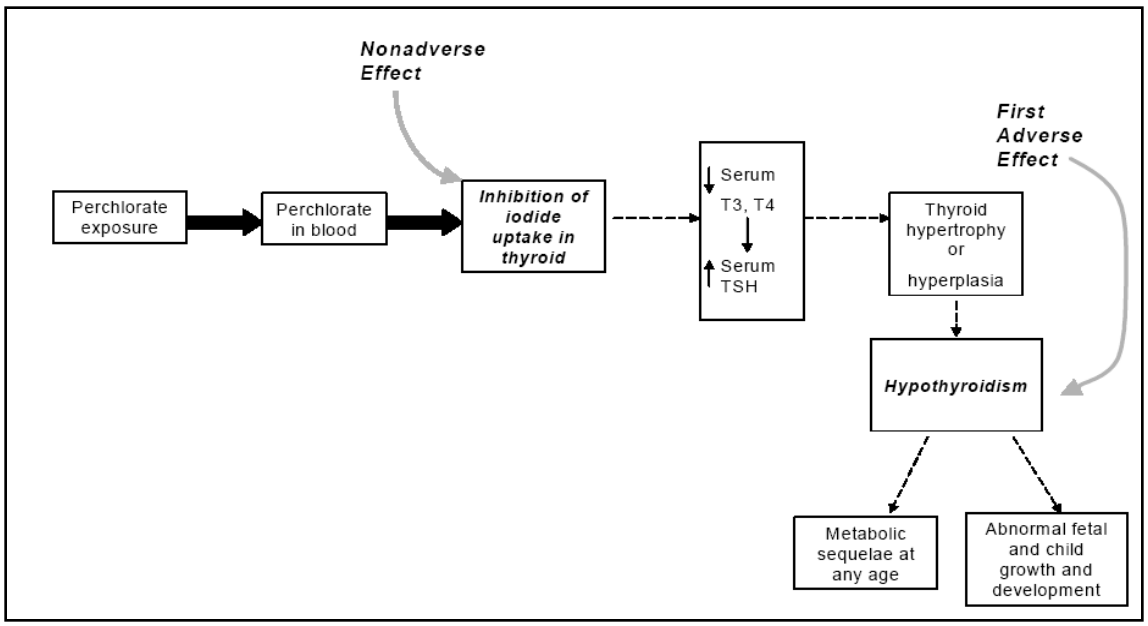

Figure 2. Mechanism of action for perchlorate as summarized in the NRC (2005) report
The NRC's suggested mode-of-action model of perchlorate toxicity in humans. Solid arrows represent outcomes that have been observed in humans with perchlorate doses greater than 0.007 mg/kg-d. Dashed arrows represent outcomes that have not been clearly demonstrated in humans exposed to perchlorate but that are biologically possible in the absence of adequate compensation. The thyroid response to increased serum TSH and an independent increase in thyroid iodide uptake would raise T3 and T4 production to normal and therefore usually prevent the later steps from occurring (NRC, 2005).

3.5.2 There is no reliable scientific study that shows environmental levels of perchlorate cause any adverse effect

The NRC report stated:

> The available epidemiologic evidence is not consistent with a causal association between exposure during gestation to perchlorate in the drinking water at up to 120 ppb and changes in thyroid hormone and TSH production in normal-birthweight, full-term newborns;

and that:

> The evidence from chronic, occupational-exposure studies and ecologic investigations in adults is not consistent with a causal association between perchlorate exposure at the doses investigated and hypothyroidism or other thyroid disorders in adults.

However, NRC also stated

> …that no studies have investigated the relationship between perchlorate exposure and adverse outcomes among especially vulnerable groups, such as low-birthweight or preterm infants. The available studies did not assess the possibility of adverse outcomes in the offspring of mothers who were exposed to perchlorate and had a low dietary iodide intake. Finally, there have been no adequate studies of maternal perchlorate exposure and neurodevelopmental outcomes in infants.

Since the NRC report, studies have been conducted in some of these populations, but no study has

reported adverse effects from exposure to perchlorate at environmental levels (see Section 6, Comments to the SAB Regarding Human Studies).

3.5.3 Implications of the NRC findings for risk assessment

In a typical risk assessment, safety factors are applied to the NOAEL or the Lowest Observable Adverse Effect Level (LOAEL) to determine the RfD. However, as the NRC (2005) stated with emphasis, "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." In recommending a perchlorate RfD based on a nonadverse effect, the NRC (2005) emphasized it was taking a non-standard and unusually cautious approach:

> The committee emphasizes that its recommendation differs from the traditional approach to deriving the RfD. The committee is recommending using a nonadverse effect rather than an adverse effect as the point of departure for the perchlorate risk assessment. Using a nonadverse effect that is upstream of the adverse effects is a conservative, health-protective approach to the perchlorate risk assessment.

To derive an RfD, the NRC (2005) divided the NOEL by an Uncertainty Factor (UF) of 10 to account for potential variation in response by the most susceptible individuals in a population—hypothyroid or iodine-deficient pregnant women and their developing fetuses. Regarding database uncertainty, NRC states "the database is sufficient, given the point of departure selected—one based on inhibition of iodide uptake by the thyroid," and no further UF was applied.

U.S. EPA writes:

> The overall confidence in this RfD assessment is high because it is based on a no-effect level for a well-characterized biochemical precursor effect (iodide uptake inhibition), accompanied by a 10 fold uncertainty factor for susceptible populations. The RfD should protect the health of even the most sensitive populations, because a dose that does not inhibit thyroid iodide uptake will not affect thyroid function, even in subjects with an abnormal thyroid gland or a very low iodide intake (Integrated Risk Information System , 2005).

Since the NRC report, other agencies have conducted reviews of the scientific literature regarding the potential for adverse health effects from perchlorate exposure and have determined that doses equivalent to the current RfD of 0.0007 mg/kg-d (equal to 24.5 ppb in drinking water) are conservative and health protective (ATSDR, 2008; U.S. EPA, 2010).

4.0 COMMENTS TO THE SAB REGARDING LIFE STAGES

The White Paper poses the following charge questions related to Life Stages to the SAB:

> **There are currently no data available to directly link perchlorate to neurobehavioral effects in infants and children. How should [U.S. EPA] consider the following life stage factors in deriving an MCLG?**
>
> **• Life stage specific differences in body weight and food and drinking water intake.**
>
> **• Differences in greater severity and permanence of potential adverse effects in neonates, infants and young children compared to adults;**
>
> **• Shorter half-life and lower reserves for thyroid hormone in infants compared to adults; and**

- **Intrauterine exposure to perchlorate and impact on thyroid status in fetuses.**

Each of these charge questions will be addressed individually.

4.1 Regarding life stage differences in body weight and food and drinking water intake

U.S. EPA requested guidance on how to consider life stage factors in its MCLG calculation. The purpose of an MCLG is to define a drinking water concentration based on the RfD that takes into account exposures through other media (e.g., food). However, using drinking water intake values with no empirical basis is scientifically unsound. Specifically, the life stage factors

- use data that are not derived from scientific experiments but from a summary obtained in a survey that relies on subject recall rather than direct measurement;
- rely on methods that are incapable of measuring, estimating, or predicting the effects of a chemical on the body; and
- produce results that are qualitative at best.

Using water intake and body weight as the sole variables in a life-stages analysis makes general assumptions about numerous body functions in lieu of actual data. The White Paper is essentially using a simple mathematical formula and from that deriving a potential MCLG. There is no science embedded in the formula. An infant's weight alone does not account for the physiological or pharmacological parameters that would be critical in understanding developmental differences. What is missing is a scientific accounting of absorption, distribution, metabolism, excretion, the physical-chemical properties that may or may not play a role in how the chemical may react differently in the body, as well as other parameters, regardless of developmental state.

Using water intake and body weight as the sole variables for calculations about effect makes general assumptions about numerous body functions in lieu of actual data. In effect, this calculation does not account for absorption, distribution, or excretion, regardless of developmental state. This calculation implicitly assumes that the body's disposition of perchlorate is directly proportional to body weight. There are no scientific data to support these assumptions.

The question posed is not a foundational science issue (*e.g.*, toxicology, pharmacology, biochemistry, *etc.*), but one of risk assessment, and in particular, an artifact of establishing a DWEL. The MCLG calculation presented is an arithmetic calculation based on only three variables: the RfD, an RSC, and life stage specific water intake rates per body weight. Of these, the only scientifically defensible value is that upon which the RfD is based— the point of departure derived from Greer et al. (2002).

One important purpose of converting the RfD (in units of mg/kg-day) to a DWEL (in units of µg/L or ppb) is to make it easier to compare water concentrations of contaminants to government guidelines. Generally accepted risk-assessment practice involves conservatively selecting a higher than average water intake and a lower than average body weight. These default values are not symmetrically representative of the population. The water consumption (2 liters) is approximately equal to the 88th percentile water intake for adults aged 20 through 64 (U.S. EPA, 1997). The body weight value of 70 kg is approximately equal to the 27th percentile of the distribution for adult males aged 18-74 and the 70th percentile of the distribution for adult females (U.S. EPA, 1997). Because the conversion uses the ratio of body weight to water intake, the use of lower percentiles in the numerator than are used in the denominator leads to a conservative MCLG.

There are a number of data quality issues with the values proposed by the Agency, including:

- The water intake by body weight is arbitrarily categorized by age (*i.e.*, what is physiologically unique about 6 months of age versus 5 months of age?).
- The consumption values proposed for the infant are based on a study in which the study participants recalled their own (or their infant's) consumption over two non-consecutive days; no direct measurements were taken. Recall studies are subject to error (termed recall bias) from lapses in memory about the amounts consumed (*i.e.*, what, when, and how much did I eat or drink?). If, for example, one or two individuals in the study overestimated intake, the data would be skewed (as observed by inspection) and the statistical estimation of the 90th and 95th percentile water consumption values would be overestimated. Moreover, the population size in this study does not meet U.S. EPA's data quality standards.
- The body weight and water consumption rate factors proposed are the upper 95th percentile water consumption rate to body weight ratios for infants aged 0-6 months (U.S. EPA, 2004). These ratios are based on water intake from all sources, including direct water intake (used for drinking) and indirect water intake (used in the final preparation of foods at home or restaurants).
- The drinking water intake rates are based on data collected in the USDA Continuing Study of Food Intakes by Individuals from 1994-1996 and 1998. This was a large study of dietary recall and was reported by U.S. EPA (2004) and Kahn and Strahlka (2008). Dr. Kahn and Ms. Strahlka were the primary authors of U.S. EPA (2004). Kahn and Strahlka (2008) use the same data set as reported in U.S. EPA (2004). However, for children, data are presented for finer age groups. For example, in U.S. EPA (2004), infants under one year old were grouped by birth to six months and six to 12 months. In Kahn and Strahlka (2008), infants under one year are grouped by birth to one month, one to three months, three to six months, and six to 12 months.
- The body weights presented by Kahn and Strahlka (2008) are lower than those reported in 1996-2000 NHANES, as presented by U.S. EPA in the Child Specific Exposure Factors Handbook (U.S. EPA, 2008b) and may not be representative of the general population. For one month olds, the Kahn and Strahlka (2008) mean, 90th and 95th percentile body weights are 20, 45, and 24% lower than the NHANES 1996-2000 data. The use of a lower body weight, as the White Paper suggests, serves to decrease the MCLG.
- Data are available from studies measuring nutrition from formula intake. These studies (including Fomon et al., 1969, 1971, 1975, 1977, 1983, 1995, 1999; Ryu et al., 1983; Shepherd et al., 1988; Ekstrand et al., 1994; Specker et al., 1997) used tightly controlled measurement of actual formula intake with a consistent ratio of formula to water. These studies present individual body weight and intake rates in infants based on actual measurements. An example of how these data differ from those reported by U.S. EPA (2004) and Kahn and Strahlka (2008) is the difference in the intake rates of infants less than one year old. Kahn and Strahlka (2008), which present finer age groups, report the mean intake rate to be 137 mL/kg-d, with a 90% confidence interval of 109 to 166 mL/kg-d. The formula measurement studies report a lower mean intake of 131 mL/kg-d with a 90% confidence interval of 128 to 133 mL/kg-d.

One way to gauge whether the 90th and 95th percentile values suggested in U.S. EPA (2004) are statistically unreliable is a simple comparison to blood volume. The average adult has a blood volume of approximately 5 L and drinks approximately 1.4 L/d of drinking water, or 27% of their

blood volume (U.S. EPA, 1997). It is well understood that infants and children consume more than adults when normalized for body weight, but using the intake rates suggested by U.S. EPA, the 95th percentile one month old infant would consume 2.6 L/d or 280% of their blood volume.[6] This is the equivalent of the average adult consuming 14 L/d or just less than four gallons, every day.

4.2 Regarding differences in greater severity and permanence of potential adverse effects in neonates, infants and young children compared to adults

U.S. EPA requested guidance on how to apply effects that are understood to occur with other thyroid stressors (*e.g.*, gestational hypothyroidism) to perchlorate.

There is no evidence that the effects or severity of perchlorate exposure will differ in children compared to adults. While it is well documented that untreated gestational or neonatal hypothyroidism can have effects in later life if not treated, environmental levels of perchlorate have not been reported to cause any adverse effects. Second, the papers cited in the White Paper report that when there are clinical thyroid values that indicate hypothyroidism that goes untreated, then adverse effects are noted. This fact has been known for decades; however, the data presented in the White Paper are unrelated to perchlorate.

While it is well documented that untreated gestational or neonatal hypothyroidism can have an effect in later life if not treated; environmental levels of perchlorate have not been reported to cause any adverse effects.

The studies cited in the White Paper do not support the White Paper's supposition. The study by Haddow et al. (1999) measured TSH in serum samples from 25,216 pregnant women in Maine, 62 of whom were identified as having been hypothyroid during pregnancy, 47 of whom had very high TSH levels (at or above the 99.7th percentile of all pregnant women), and 15 of whom had TSH levels that were moderately high and serum T4 levels that were moderately low (between the 98th and 99.6th percentile for all pregnant women). Fourteen of the 62 hypothyroid women were treated during their pregnancies for hypothyroidism; the rest were untreated. The study found that the children of the untreated hypothyroid women scored lower on IQ tests in comparison to the children of matched control women who had normal TSH levels during pregnancy, but were similar in other respects. Hypothyroidism in the U.S. is most often caused by chronic autoimmune thyroiditis, not iodine deficiency (Haddow et al., 1999). In the Haddow study, 77% of the hypothyroid women had chronic autoimmune thyroiditis. This study does demonstrate neurodevelopmental effects in children born to mothers with TSH levels greater than the 98th percentile. Although this study provides meaningful information regarding hypothyroidism and neurodevelopment, it is important to note that the subjects were likely hypothyroid. Environmentally relevant exposures to perchlorate have not been shown to inhibit iodide uptake. The authors do not suggest that a chemical exposure could be responsible for this severity of hypothyroidism. Iodine status was also not mentioned in the article.

The studies by Pop et al. (1999; 2003) followed a cohort of children born to mothers in southeastern Netherlands with free T4 concentrations at the low end of the range considered normal in that region, as well as with levels well-below the normal range. Maternal TSH levels were reported to be somewhere in the range considered normal in that region (this region is reported to have low iodine intake). Thus, the mothers can be considered to have been hypothyroxinemic but not hypothyroid. Based on their initial analysis, when the children were 10 months of age, the authors concluded that

[6] The blood volume of a 1 month old infant is 85 mL/kg (Dallman et al., 2003). The 95th percentile water intake for a 1 month old infant is estimated to be 238 ml/kg (U.S. EPA, 2009). The body weight of a 1 month old is given as 11 kg (Kahn and Strahlka, 2008).

"…low maternal plasma free T4 concentrations during early pregnancy may be an important risk factor for impaired infant development" (Pop et al., 1999). However, based on their subsequent analysis when the children were up to 2 years of age, the authors concluded:

> Maternal hypothyroxinemia during early gestation is an independent determinant of a delay in infant neurodevelopment. However, when free T4 concentrations increase during pregnancy in women who are hypothyroxinemic during early gestation, infant development appears not to be adversely affected.

Thus, according to the investigators' interpretation, there was no lasting effect on development in the absence of sustained hypothyroxinemia. Although the investigators indicated that the area in which the study was conducted is iodine-sufficient, it is important to note that no measures of iodine nutrition were obtained and it seems likely that the iodine intake of this Dutch cohort was marginal, according to the criteria described for a Belgian cohort by Glinoer et al. (1992). The authors do not relate the hypothyroxinemia noted in this study to exposure to perchlorate (or any other goitrogen).

NRC also reviewed studies by Haddow et al. and Pop et al. in their 2005 assessment. NRC found that the studies had significant limitations (*e.g.*, "…test scores were small, and the scores could be confounded by socioeconomic, educational, and other differences between the study groups…"). However, NRC states: "Nonetheless, if confirmed, they [the studies] emphasize the potential vulnerability of fetuses to decreases in maternal thyroid function." Thus, if there were thyroidal changes demonstrated by perchlorate at environmental levels, then it would be appropriate to consider that information in deriving MCLG. However, the NOEL for a non-adverse effect is equivalent to a DWEL of 245 ppb (using default assumptions), higher than concentrations of perchlorate found in the environment.

NRC chose to address any additional uncertainty by assigning a value for the intraspecies uncertainty factor. NRC states that:

> …the committee recommends use of a full factor of 10 to protect the most sensitive population—the fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The committee views its recommendation as conservative and health-protective, especially given that the point of departure is based on a nonadverse effect that precedes the adverse effect in the continuum of possible effects of perchlorate exposure" (NRC, 2005).

4.3 Regarding shorter half-life and lower reserves for thyroid hormone in infants compared to adults

The White Paper suggests that neonates are more susceptible due to higher turnover rate of intrathyroidal iodine reserves. The White Paper supposition is based upon this statement:

> …higher turnover rate of intrathyroidal iodine reserves compared to adults (17% versus 1% under iodide-replete conditions). The turnover rate for iodine reserves in neonates is increased to 62% and 125% under moderate and severe iodide deficiency conditions (Delange, 1998).

If there were scientific data that demonstrated that environmental levels of perchlorate <u>caused</u> clinically significant changes in thyroid or pituitary hormone levels, these data would be relevant. But there are no such data, nor does the White Paper provide a reliable assessment of the information available in the literature.

The Agency's assessment of iodine turnover rate is not scientifically supported. The iodine turnover rate is defined as the rate by which the iodine stored in the thyroid is metabolized into thyroid hormones. The turnover rate calculation is based solely on three small groups of data. They are the

urine iodine concentrations of neonates that were either iodine replete (n=13), mildly iodine deficient (n=4), or severely iodine deficient (n=10) (Delange, 1998). The turnover rates were presented as "estimated iodine turnover rate (%/day)." A scientific question remains as to how urine iodine is related to intrathyroidal turnover rate. The White Paper is not transparent on the process used by Delange (1998), and it is not apparent that this method would have any predictive reliability. Furthermore, not only was the sample size for each neonate group small, but there was no mention that the ages or genders of each group were matched, and no quantitative assessment of dietary iodine intakes were presented, so it is unclear how this critical factor was controlled for in the turnover rate calculation.

In direct contrast to the work cited by U.S. EPA, another study found that the iodine concentration in the urine of neonates with CH (n=23, 21.3 (SD=11.2) μg/dL), mothers of hypothyroid neonates (n=25, 16.3 (SD=7.5) and normal neonates (n=25, 14.4 (SD=4.6) μg/dL) and presumably intrathyroidal turnover rates, of these populations, were all fairly similar even in a background of CH (Nasir et al., 2010).

The Agency's assessment of thyroid hormone half-life rates is not scientifically supported. The White Paper reports that the half-life of T4 (3-3.6 days) and T3 (6 days) are shorter for neonates (3-6 days) than for adults (7-10 days) therefore making the claim that neonates are more susceptible to iodine deficiencies. The half-life values referenced are either from neonates with severe congenital disorders (Vulsma et al., 1989), patients that have had acute pediatric T4 overdoses (Lewander et al., 1989), or premature neonates treated with levothyroxine (L-T4) that had died (van den Hove et al., 1999). T4 elimination rates are influenced by thyroid status with T4 half-lives of euthyroid, hyperthyroid and hypothyroid patients at 6-7, 3-4 and 9-10 days, respectively (Levoxyl, 2010). One would expect differences in elimination rates between patients with CH and the normal healthy population (euthyroid). To suggest that the utilization of T4 half-lives from neonates with CH can be used for a surrogate value for normal neonates is not scientifically justifiable. The White Paper is not transparent with respect to the relevance, limitation and source of the information used to assess the half-life of T4 and T3 of normal neonates.

The White Paper is not transparent to the relevance, limitation and source of the information used to assess the half lives of T4 and T3 of normal neonates

The T4 half-life calculated by Vulsma et al. (1989) was based on cord serum values of neonates with total organification defect, a form of severe CH where neonates have the inability to iodinate thyroid proteins. That means that T4 measured in the cord originated from the mothers, not the neonates (Vulsma et al., 1989). Thus, the T4 half-life value of 3.6 days was not calculated from normal physiological conditions but from patients with an extremely rare autosomal recessive thyroid metabolism disorder (thyroid agenesis) and the T4 measured in the cord blood was transferred transplacentally from their mothers. The White Paper is not transparent with respect to the relevance, limitation and source of the information used to assess the half-life of T4 of normal neonates.

Lewander et al. (1989) measured T4 and T3 concentrations in 15 pediatric patients who had overdosed with thyroxin (1.5 to 8.8 mg) and were less than five years of age. They determined that the elimination half-life in seven patients was 2.8 ± 0.4 days for T4 and 6 ± 1.7 days for T3. The authors concluded that for these overdosed patients, the half-life of T4 is shorter and the half-life of T3 it is longer than when therapeutic doses are given. It is not appropriate to derive the half-lives of T4 and T3 in normal children from data taken from patients that have overdosed on thyroxine.

The concentrations of T4 and T3 in very premature, premature full-term, and full-term neonates that were treated with L-T4 were reported by van den Hoove et al. (1999). For pre-term neonates, L-T4

treatment increased thyroglobulin iodination and T4-T3 content, but did not increase thyroglobulin concentration in the thyroid (van den Hove et al., 1999). These findings are not derived from healthy neonates, but from post-mortem samples from neonates treated with L-T4, and included data from very pre-term (24-32 postmenstrual weeks) and pre-term (34-41 postmenstrual weeks) neonates. Again, with this reference, the White Paper is not transparent to the relevance, limitation, and source of information, The data used are not from a normal and healthy population of neonates, and it is also not an appropriate to make half-life comparisons of T3 and T4 between of neonates and children.

The White Paper asserts that urinary clearance rates in neonates is slower than in other life stages; however, in an evaluation of clearance rates of renally excreted, non-metabolized pharmaceuticals, like perchlorate, children and neonates had a higher clearance rate than adults (Appendix A, Tables A-1 and A-2). In 2005, the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA) reported:

> As indicated in the [Public Health Goal (PHG)] document, perchlorate is not retained by the body to any significant extent. The increased relative fluid intake rate of infants, balanced by the increased urinary excretion rate, does not appear to lead to a higher blood concentration of perchlorate in infants, compared to adults. In their January 22, 2003 memorandum, United States Environmental Protection Agency (U.S. EPA) stated "The uptake and elimination kinetics of perchlorate for children are such that traditional adjustment of exposure based on body weight scaling results in exposure estimates equivalent to those for adults." No information was provided in the comments that indicate this conclusion was incorrect (Denton, 2005).

4.4 Regarding intrauterine exposure to perchlorate and impact on thyroid status in fetuses

The White Paper claims there are "no data to directly link perchlorate to neurobehavioral effects in infants and children." The concern with this statement is the implication that the level of scientific uncertainty is unacceptable—this implication is not correct. The White Paper reviews a number of studies regarding gestational exposures where adverse effects were evaluated and not found. It would be more correct to state that there are several studies that have reported no effect of perchlorate on neurodevelopment. There are also ecological epidemiological studies of perchlorate exposure that do not report adverse effects. There are studies where perchlorate is provided in breast milk to infants and no adverse effects are noted. ATSDR (2008) stated, "Thus far, there is no conclusive evidence that exposure to perchlorate produces developmental effects in humans."

The effects of perchlorate ingestion from contaminated drinking water on neonates was investigated in Las Vegas, Nevada where perchlorate levels reached 9 to 15 ppb for seven months versus Reno, Nevada where perchlorate was not detected (detection limit, 4 ppb) (Li et al., 2000). Analysis of T4 levels from more than 23,000 newborns in this study did not correlate to environmental perchlorate exposure. There was no evidence of thyroid function suppression in 9,784 newborns or 162 school-age children in Northern Chile where perchlorate concentrations of up to 120 ppb have been recorded in drinking water (Crump et al., 2000).

In humans, Tellez Tellez et al. (2005) reported no significant differences in thyroid health between offspring of pregnant women exposed to up to 114 µg/L (n=66) and offspring of pregnant women exposed to 0.5 µg/L (n=65). Amitai et al. (2007) reported no effect on the thyroid hormone levels of newborns exposed *in utero* to perchlorate in drinking water at levels of 340 ppb compared to newborns exposed *in utero* to <3 ppb. Follow up on these same children revealed no difference in neurodevelopmental indices between the groups of children (Amitai et al., 2008). See Section 6.1.4 for more detail on these studies.

Two studies have evaluated maternal urinary perchlorate and effects in newborns. Cao et al. (2010) reported no association between urinary perchlorate, nitrate, or thiocyanate and TSH and free T4 in blood. Using a non-standard approach of measuring TSH and free T4 (fT4) values from diaper urine, perchlorate was positively associated with both TSH and fT4. This, however, is opposite of what would be expected based on the known mechanism of action of perchlorate. When perchlorate was evaluated with thiocyanate and nitrate, it was not associated with TSH or fT4. Blount et al. (2010) reported no association between maternal (urine, serum) and fetal (cord blood) levels of perchlorate, thiocyanate, nitrate, and iodide compared to infant body weight, body length, and head circumference.

Two rat neurobehavioral studies were constructed to assess the potential for perchlorate to cause neurobehavioral effects in the offspring of mothers exposed to perchlorate in drinking water during pregnancy and postnatally. The results of these analyses revealed no statistically significant perchlorate-related effects. These studies were:

- A neurodevelopmental study conducted by York et al. (2004) that included 15 different neurobehavioral measures in four behavioral tests (passive avoidance testing, water maze testing, auditory startle habituation, and motor activity), and
- A subsequent motor activity study conducted by Bekkedal et al. (2000) that included nine different measures.

In both studies, female Sprague-Dawley rats were administered perchlorate in drinking water. In York et al. (2004) perchlorate was administered at doses of 0, 0.1, 1.0, 3.0, or 10.0 mg/kg-day, beginning at gestational day (GD) 0 and continuing through postnatal day (PND) 101. In Bekkedal et al. (2000), perchlorate was also administered at 0, 0.1, 1.0, 3.0, or 10.0 mg/kg-day, but beginning two weeks prior to conception and continuing through PND10. In both studies, neurobehavioral effects were measured in the offspring. A summary of relevant literature, which demonstrates the effect of antithyroid agents upon offspring, including several critical neurobehavioral measures is included in these comments (Figure 3). Appendix A, Table A-3 summarizes many studies, including those cited here.

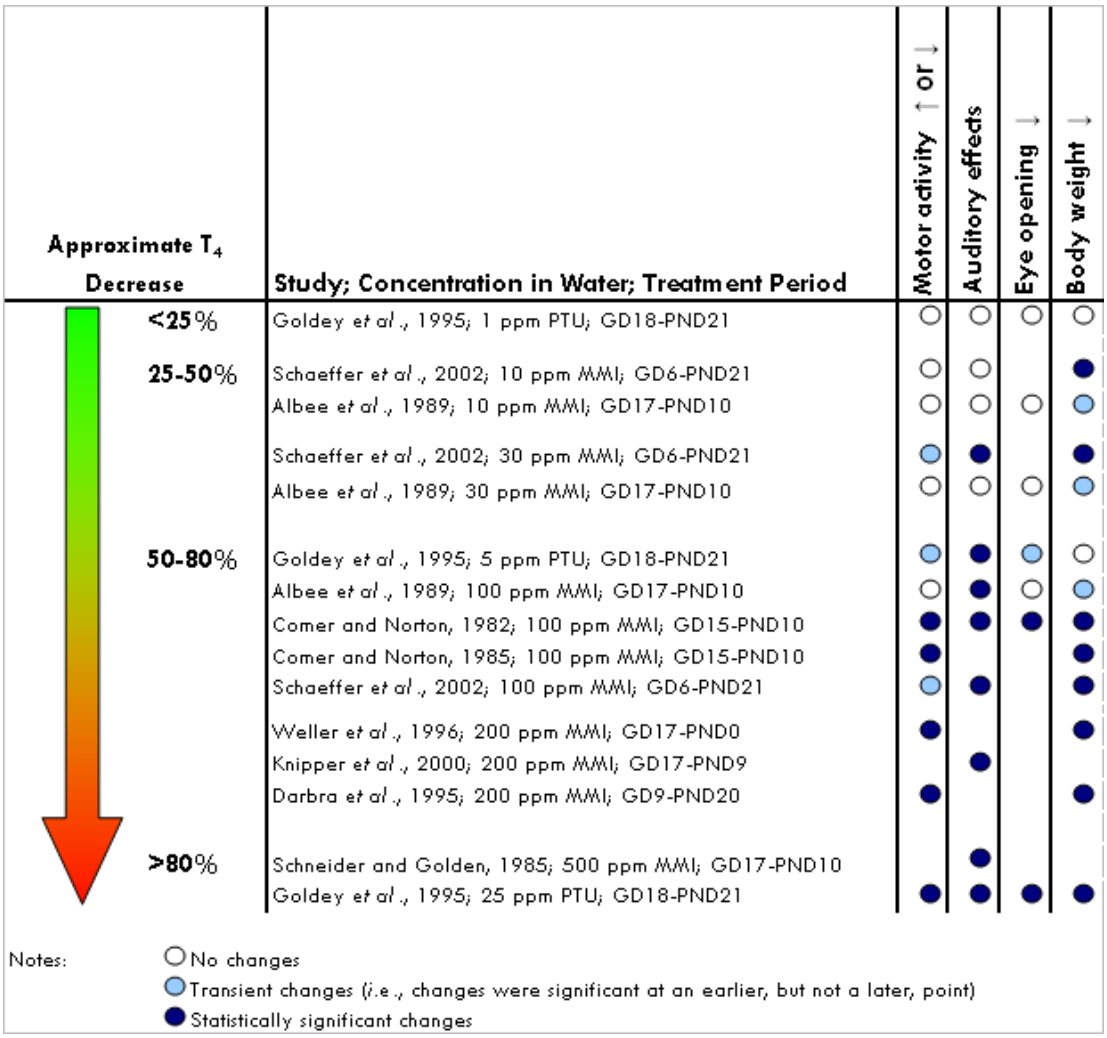

Figure 3. Developmental effects in offspring of dams treated with various doses of antithyroid agents

Summary of animal studies in which either PTU or MMI were administered to dams and the effects of those agents on T4 and developmental effects in offspring.

4.5 Regarding other information that may be useful in considering U.S. EPA's questions

4.5.1 Concur that the pregnant woman and her fetus are the most sensitive population that must be the basis for protecting public health

We concur with U.S. EPA, the National Academy of Sciences Nation Research Council (NRC), and others, that the pregnant woman and her fetus constitute the most sensitive population which an RfD must protect (Integrated Risk Information System, 2012; NRC, 2005; ATSDR, 2008).

In 2010, U.S. EPA (2010) concluded that "the most sensitive population for cumulative risk assessment purposes is the iodide-sensitive fetus and neonate during gestation and lactation whose mothers are iodide deficient." In 2005, OEHHA concluded:

> While an infant is one of the populations of concern, infants are not considered as sensitive as the pregnant woman and her fetus, based on both biological and effective

> dose-rate calculations. Although there is no recognized iodine-deficient fraction of the general population, pregnant women are considered to be more likely to be iodide deficient because they have a greater need for iodide and at the same time have a higher urinary iodide excretion rate (Denton, 2005).

The White Paper provides ample, compelling evidence that the fetus of the potentially hypothyroid pregnant woman is the most susceptible population. Similar to previous reports, other subpopulations are also mentioned to be sensitive; however, the White Paper provides no evidence that these subpopulations are more sensitive than the fetus.

In addition to studies of human populations, studies in animals do not demonstrate that the neonate is more susceptible to perchlorate exposure. Clewell et al. (2003) developed a PBPK model to reproduce measured perchlorate distribution in the lactating and neonatal rat. This model predicts resulting effects on iodide kinetics from competitive inhibition at the NIS, using physiological and kinetic parameters obtained from literature and experiment, and systemic clearance and Michaelis-Menten (M-M) parameters estimated by fitting simulations to tissue and serum data. Neonatal iodide kinetic parameters were determined by the fit of the model to the data obtained from directly dosing the pup. Comparison of predicted dosimetrics across life-stages in the rat indicates that neonatal thyroid IUI is similar to the adult and approximately 10-fold less than the fetus.

Despite the increased dose of perchlorate to the neonate (0.07 vs. 0.01 mg/kg-day in the adult), Clewell et al. (2003) show that the postnatal day (PND) 10 pup serum average perchlorate concentrations were consistently lower than those of the adult. In fact, a comparison across life stages reveals that the serum perchlorate concentrations of the lactating dam were slightly higher than the male, pregnant, fetal or neonatal rat, suggesting that lactation may be the time period with the greatest internal perchlorate exposure. The authors surmise that while this increased serum concentration of perchlorate in the lactating rat is somewhat surprising considering the additional clearance route provided through the milk, it is likely due to increased serum binding. In spite of the multiple inhibition sites (mammary gland, milk and thyroid), inhibition in the neonatal thyroid was similar to that of the dam. This may be due to the fact that neonatal serum perchlorate levels are less than those of the dam. The neonate shows less perchlorate-induced inhibition of thyroid iodide uptake compared to the other life stages in the rat. Model estimates suggest that the fetal rat thyroid is most vulnerable to inhibition, with a 10-fold greater inhibition than the neonate at the lowest measured dose.

4.5.2 Congenital hypothyroidism and neonates

Although unrelated to perchlorate, the White Paper states that “Congenital hypothyroidism in the fetus and newborn, despite early identification and treatment, still results in language deficits, memory deficits, and visuospatial impairments that persist to adulthood.” However, NRC (2005) conclusively stated that:

> The available epidemiologic evidence is not consistent with a causal association between perchlorate exposure and congenital hypothyroidism as defined by the authors of the studies reviewed by the committee. All studies of that association were negative.

Furthermore, there are several studies that report that if thyroid hormone replacement treatment is started within the first 14 days of life, no permanent neurological damage is detectable. Based on the results of numerous clinical studies, newborns diagnosed with hypothyroidism will be, for the most part, protected from the detrimental developmental effects of this condition if treatment begins at approximately 14 days post natal.

Permanent primary CH is a serious and relatively common disease that occurs in 1 of 3,000-4,000 newborns (Macchia et al., 1998). This is the reason that children born in U.S. hospitals (and elsewhere) are screened for CH. CH is also characterized by high circulating levels of TSH as the hypothalamic-pituitary-thyroid axis tries to compensate for low thyroid hormone activity. Clinical symptoms are not always apparent in newborns as maternal thyroid hormones and iodine can pass transplacentally and through breast milk after birth, while some infants are capable of producing their own thyroid hormone albeit in sub-optimal concentrations from moderately functioning thyroid tissue. No signs or symptoms of hypothyroidism are observed in two thirds of the infants diagnosed with CH (Simpser and Rapaport, 2010). Conditions of hypothyroidism can also be transient as circulating thyroid hormone levels may be low at birth but can recover to normal levels within the first few months or years of life (Rastogi and LaFranchi, 2010). Transient hypothyroidism can occur through maternal factors such as prescription drug usage; antithyroid medications (*e.g.*, methimazole), lithium, and 4-aminosalicylic acid; iodine deficiency or excess; maternal antibodies to thyroid hormone or from premature births (Simpser and Rapaport, 2010). These factors will be persistent in newborns/young infants that are being breastfed by mothers with these conditions.

There have been several studies that have investigated the timing of neonatal supplemental thyroid hormone treatment and the prevalence of neurobehavioral abnormalities. The intellectual outcomes of infants with hypothyroidism were assessed along with the age of the initiation of treatment (before or after 30 days of age) in 62 patients. It was found that there was no difference in intelligence quotient (IQ) when tested between the ages of 3 and 6 years (Hsiao et al., 2001). Children with severe CH had developmental gaps in intellectual sequelae. A loss of 6 to 19 IQ points was thought to be closed by treating infants at 2 weeks rather than 4-5 weeks (Van Vliet, 1999).

4.5.3 Breastfed infants are not more susceptible to perchlorate-mediated effects

The White Paper states that:

> Breast-fed infants may be more vulnerable to perchlorate mediated effects compared to other formula-fed infants for two reasons—perchlorate accumulates in breast milk directly exposing the infant, and perchlorate inhibits transfer of iodide into breast milk resulting in low iodide levels in breast milk

The White Paper then cites a number of studies (Tazebay et al., 2000, Kirk et al., 2005; Kirk et al., 2007; Pearce et al., 2007; Dasgupta et al., 2008; Borjan et al., 2011) (p. 10). <u>However, the cited studies do not support the White Paper's assertions</u>. Aside from the limitations of these studies, neither Kirk et al. (2005) nor Kirk et al. (2007) demonstrate accumulation of perchlorate in breast milk, nor do they show that perchlorate inhibits transfer of iodide into breast milk. Kirk et al. (2005) measured perchlorate and iodide levels in cow and human breast milk and compared these values to corresponding levels of perchlorate in drinking water in the area. There was no correlation between levels of perchlorate in breast milk and perchlorate in drinking water, meaning even if the mother was drinking water with perchlorate, it did not define the amount of perchlorate found in breast milk. As the White Paper states, "In limited breast milk samples with perchlorate content greater than 10 μg/L [ppb], the iodide content is linearly correlated with the inverse of the perchlorate concentration (r^2 >0.9, n = 6)." However, Kirk et al. (2005) noted that this relationship only existed for the breast milk samples with the highest perchlorate levels (6 subjects out of 82), and speculated that this relationship may be coincidental. Kirk stated "If we take all the available data, there is no meaningful correlation between the perchlorate and iodide levels in breast milk."

Kirk et al. (2007) collected a total of six to 18 breast milk samples from 10 women over two to 14 days, and measured perchlorate, iodide, and thiocyanate levels in the samples. However, the authors did not measure and correlate intake levels of these ions to measured concentrations, and thus do not

demonstrate accumulation in breast milk. Further, the authors did not establish whether perchlorate inhibits transfer of iodide to breast milk, stating:

> The real role, if any, of perchlorate in reduction of milk iodide levels is as yet unknown...for a human study, at least simultaneous urinary data are needed to judge how intake affects expression. Our subjects had highly varied diets and varied timing of food intake; some used nutritional supplements and some did not. To further complicate correlations among analytes, the timing of milk sample collection was not uniform among donors or among days of individuals.

Pearce et al. (2007) does not support the statements in the White Paper. As U.S. EPA states regarding Pearce et al. (2007):

> Urinary perchlorate was significantly higher in breast milk than in formula ($p<0.0001$) but iodine was not different ($p= 1.0$)… Breast milk iodine was not significantly correlated with perchlorate levels in breast milk ($n = 49$, $r2 = 0.05$, $p = 0.1$) even at perchlorate levels > 10 µg/L ($n = 23$); or in urine ($r^2 = 0.004$; $p = 0.7$). p. 47

This study measured breast milk iodine and perchlorate concentrations as well as iodine, perchlorate, and cotinine (a surrogate for cigarette smoke which contains thiocyanate) in urine. The clinicians compared the levels of perchlorate in breast milk with 17 commercial infant formulae. Neither breast milk nor urinary perchlorate levels were significantly correlated with breast milk iodine concentrations, meaning that as levels of perchlorate in breast milk increased, iodide content in milk was not affected by perchlorate. A significant number of women in this study had iodine levels that were insufficient, but the authors did not suggest this was because of perchlorate exposure or that it represents a chronic iodine deficiency.

Borjan et al. (2011) does not support the statements in the White Paper. Borjan et al. (2011) measured perchlorate in breast milk of women in New Jersey. As U.S. EPA states regarding this study, there was "No correlation between perchlorate levels in breast milk and those in water ($r = -0.04$, $p = 0.25$)." Thus this study does not demonstrate accumulation of perchlorate in breast milk. Borjan et al. (2011) did not measure iodine levels in breast milk.

Human breast milk can contain T4 and T3, and provide breast-feeding neonates with an additional source of thyroid hormone. In a Dutch study, T4 concentrations in the breast milk ranged between 0.17 and 1.83 µg/L, resulting in a T4 supply of 0.3 µg/kg (van Wassenaer et al., 2002). In another study, it was determined that a 3 month old infant consuming about 1000 ml breast milk per day receives about 2 µg of thyroid hormones and 55 µg of iodine per day (Bohles et al., 1993). A world-wide survey of iodine concentrations in breast milk ranged from 5.4 to 2170 µg/L (median 62 µg/L)(Dorea, 2002). Serum T4 levels were significantly higher in children with CH that were breast-fed compared to formula-fed at 1 and 2 months of age but not at one year of age (Rovet, 1990). Neuropsychological functions of each group were similar, but children with ectopic glands showed "significant advantage with breast-feeding on several tasks at 3 years of age." The concentrations of T3 and T4 were analyzed in the breast milk and cord blood of full term and premature infants. As the period of lactation progresses, an increase in T3 and T4 was observed: 0.5 +/- 0.12 and 100.3 +/- 8.4 nmol/l in colostrum, 9.2 +/- 0.7 and 271.3 +/- 14.1 nmol/l in transient milk, 11.1 +/- 1.07 and 405.0 +/- 21.4 nmol/l in fully formed milk (Nabukhotnyi et al., 1987). These results suggest that breast-fed premature babies would be receiving substantially lower concentrations of T3 and T4 than from fully formed milk which was found to have T3 and T4 concentrations 3 times higher than the serum of a full term newborn. Other studies have concluded that newborn serum levels of thyrotropin, T4, free T4 and T3 were not significantly different between breast-fed and bottle-fed normal babies however,

T4 was not detected in the breast milk due to technical issues (Mizuta et al., 1983).

Clearly breast milk can be an important source of T3 and T4 to a breast-fed neonate. These factors should be taken into account when assessing any thyroidal agent associated with breast-fed infants.

4.5.4 The dose required to potentially cause a decrease in thyroid hormone is greater than environmental levels

The White Paper does not include dose-response data for perchlorate or for relevant biochemical reactions. For example, the White Paper no information on the degree of T4 reduction that would cause adverse effects. This absence is striking, because in the practice of toxicology, dose-response is a fundamental component of an assessment. Further, there is dose information on the cascade of biochemical reactions that constitute the mechanism of action, including for perchlorate and IUI. Doses are known regarding the amount of perchlorate required to affect thyroid hormone levels. Without this information, the reader may be left with the erroneous understanding that environmental doses of perchlorate are relevant to the hazards outlined in the White Paper. They are not.

The White Paper reports data on the effects of CH and untreated hypothyroidism or severe iodine deficiency during pregnancy. The White Paper does not present any evidence that perchlorate exposure has resulted in any of the effects attributed to hypothyroidism, iodine deficiency, or CH.

Regarding downstream effects, NRC (2005) states that adverse effects

> …have not been clearly demonstrated in humans exposed to perchlorate but that are biologically possible in the absence of adequate compensation. The thyroid response to increased serum TSH and an independent increase in thyroid iodide uptake would raise T3 and T4 production to normal and therefore usually prevent the later steps from occurring.

The NRC concluded that individuals with normal iodide intake would require a perchlorate dose sufficient to lower thyroid iodide uptake by at least 75% for a sustained period of time (several months or longer) to cause thyroid hormone production to decline to the point where hypothyroidism could occur. In adults, that dose is estimated as being no lower than 30 mg/d (0.4 mg/kg-d for a 70-kg person, equivalent to drinking two liters of water with a perchlorate concentration of 15,000 ppb every day).

This estimate is based on doses given therapeutically without adverse effect. In therapeutic dosing, perchlorate is administered at high doses for extended periods. Historically, if perchlorate was used to treat a person with hyperthyroidism, high initial doses (6.2 to 20.5 mg/kg-d; Crooks and Wayne, 1960) were often given, sometimes followed by lower doses to maintain normal thyroid hormone levels. The treatment lasted a minimum of several weeks (depending on how responsive a patient was) to 22 years in one documented case (Connell, 1981). Most studies of therapeutic effects followed people for several months of treatment.

Eighteen patients were treated in the 1980s for Graves' disease. Subjects were treated initially with 900 mg potassium perchlorate/d (9.2 mg/kg-d assuming a 70 kg adult[7]). As serum thyroid hormone concentrations declined (within two to four months following initiation of treatment), the dose of potassium perchlorate was reduced to between 40 and 120 mg/d (0.4 – 1.2 mg/kg-d for a 70-kg person) for the 12-month to 24-month maintenance period. The average dose for eight "late

[7] The calculation for perchlorate dose equivalence in studies which used potassium perchlorate therapeutically is as follows: Dose $KClO_4$ x MW ClO_4^- / MW $KClO_4$ = dose ClO_4^-. MW ClO_4^- = 99.45 g/mol; MW $KClO_4$ = 138.55 g/mol.

responders"[8] was 93 mg/d, while 10 "early responders" had an average dose of 76 mg/d (Wenzel and Lente, 1984). During this time, all patients had normal serum T4 and T3 levels and most had normal levels of TSH receptor stimulating antibodies. No side effects were noted and all 18 patients were able to complete the study without developing hypothyroidism (NRC, 2005 citing Wenzel and Lente, 1984). The NRC has reviewed the study and supporting data and concluded that 0.4 mg/kg-d represents an appropriate point of departure for assessing adverse effects of perchlorate exposure.

Similarly, the magnitude of T4 decrease with exposure to other antithyroid agents in animal studies is much greater than that due to perchlorate exposures in excess of the human NOEL (Appendix A, Tables A-4 and A-5).

5.0 COMMENTS TO THE SAB REGARDING PBPK MODELING

The White Paper presents the following questions regarding the PBPK modeling to the SAB:

Pertaining to PBPK Evidence

• How should [U.S. EPA] consider PBPK modeling to derive an MCLG for perchlorate?

• What are the strengths and limitations of the two PBPK model results described in this effort?

Each of the questions posed to the SAB will be addressed individually.

5.1 Regarding the use of PBPK modeling in MCLG derivation

Overall, the scientific community supports the use of PBPK modeling when all parameters are based on scientific experiments (Mager *et al.*, 2003). When scientifically validated, PBPK models can be useful to reduce uncertainty about sensitive life stages. As we have pointed out above and in Appendix B, Intertox does not agree with the quality of scientific parameters U.S. EPA has imbedded in its model, the addition of "science policy" parameters to the model, or the lack of transparency regarding revisions to the model first developed by Merrill et al. (2003). U.S. EPA should revise the model to include accurate, science-based PBPK parameters and apply the results to determine whether the threshold for regulation has been met. U.S. EPA should not offer the PBPK model as evidence that other life stages are more sensitive than the fetus of the pregnant woman or to revise UFs in the absence of empirical evidence to support such an approach.

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The White Paper infers that IUI greater than 0% is "adverse." It is not. As noted by NRC, IUI is a non-adverse event (and according to the NRC, IUI does not occur at all below the NOEL of 7 µg/kg-d). Furthermore, the PBPK has embedded policy components which we discuss in more detail in

[8] The original research paper (Wenzel and Lente, 1984) does note some difficulty in controlling hyperthyroidism in five of the patients (prior to the minimum 12 month maintenance period). The authors hypothesize that one potential cause of this was excess iodine intake by these five patients meaning diet was uncontrolled during the study period. Several chemicals found commonly in the diet, including nitrate, thiocyanate, fluoride, and iodide, also cause IUI. These other chemicals have varying potencies, but can be expressed in perchlorate dose equivalents. Changes in the intake of these chemicals could affect the results of this study. However, dietary intake (in dose equivalents) of these other chemicals is likely to be much smaller than the therapeutic doses of perchlorate given in this study. Therefore the potential confounding would be minimal.

Appendix B. Thus, the output is not a true scientific output.

5.1.1 Two uses of U.S. EPA modeling

The White Paper presents U.S. EPA's PBPK model which was first introduced in December 2010. We have provided an in-depth review of the technical limitations of U.S. EPA's model. These are attached in Appendix B. This section focuses on U.S. EPA's use of the PBPK model in the White Paper.

The White Paper presents two uses for the PBPK model. First, the model is run with the dose fixed at the NOEL from Greer et al. (2002), the dose at which there was no significant difference in IUI from baseline in healthy adults. No measurable effects occur at this dose. This dose and effect should not be considered alone as the higher doses help place into context the NOEL dose. It is noteworthy that this dose is 10-fold greater than the RfD which is the starting dose for the MCLG calculation and the dose which the White Paper describes as "…the most scientifically defensible endpoint available at this time for assessing risk from perchlorate exposure."

This first model yields the greatest RAIU inhibition at 12.5% for 7-day old breast-fed infants, which is 7.8-fold greater than the average adult. However, no thyroid hormone changes were noted at this dose and the level of IUI is comparable to the next higher dose modeled, (which would still be a non-adverse effect). A 12.5% IUI is significantly below the sustained 75% inhibition that could reduce thyroid hormone to levels that could cause adverse effects (NRC, 2005).

Second, the model is used to examine RAIU inhibition at different drinking water concentrations (*i.e.*, 15, 20, and 24.5 ppb) with and without the contribution from food. This use estimates that the highest RAIU inhibition is in 7-day old bottle-fed infants, not breast-fed infants as the model predicted when the dose was fixed at the equivalent of 245 ppb. The highest RAIU inhibition was equivalent to 3.4% IUI, which is similar to normal fluctuations due to differences in diet and feeding styles. There is no credible scientific evidence that this effect has any biological significance.

In Table A-4 (second use of PBPK model), the White Paper reports estimates of RAIU inhibition at different drinking water concentrations; however, this use is misleading. Perchlorate is rarely detected in finished drinking water[9] and the average concentration when it is detected is generally less than 12 ppb (Brandhuber et al., 2009). Therefore, it is unlikely that people are exposed to 15 ppb or more of perchlorate on a daily basis. Biomonitoring studies have also reported estimated doses that are smaller than the doses depicted in table A-4 (0.234 µg/kg-d (95th percentile estimated dose from Blount et al., 2007)) or 0.066 µg/kg-d (geometric mean estimated dose from Blount et al., 2007) versus approximately 0.58 µg/kg-d). Furthermore, it appears a dose of 0.1 µg/kg-d was used to account for perchlorate dose from food for all subpopulations. It is unclear what these food sources would be for breast-fed or bottle-fed infants.

5.1.2 Sensitive populations and the PBPK model

The NRC considered all possible sensitive populations in addition to pregnant women and their fetuses; this included infants, developing children, people who have compromised thyroid function,

[9] Based on the data retrieved from U.S. EPA's UCMR1 website, the UCMR1 data includes a total of 34,728 analyses for perchlorate (U.S. EPA reports 34,221 analyses), with 647 detections of perchlorate at or above the MRL (U.S. EPA reports 637 detects). These detections include 288 detections in source water (out of 10,684 samples, or a frequency of detection of 2.7%) and 348 detections in entry point water (out of 23,319 samples, or 1.5%) (perchlorate was not detected in any samples from the midpoint of distribution systems (n = 16), the point of maximum retention (n = 19), or the location in distribution systems where disinfectant residual is lowest (n = 6); perchlorate was detected in 11 of 684 samples from "unknown" locations).

and people who are iodide-deficient. Based on its assessment, the NRC committee stated that "…fetuses and preterm newborns constitute the most sensitive populations although infants and developing children are also considered sensitive populations" (NRC, 2005). The purpose of defining an RfD based on "the most sensitive population" is that all other populations, including those that are more sensitive than average (but less sensitive than the most sensitive), would not be expected to have adverse effects, including at doses that exceed the RfD. To date—with substantial scientific justification—U.S. EPA, ATSDR, the states of California, Massachusetts, and New Jersey all regard fetuses to be the most sensitive population for health effects evaluations.

U.S. EPA's PBPK modeling appears to depict a different group as the most sensitive subpopulation. However, in U.S. EPA's first set of model runs (set at the NOEL dose), 7-day old breast-fed infants are estimated to be the most sensitive. In the second model (using water concentrations of 15, 20, or 24.5 ppb) 7-day old bottle-fed infants are depicted as the most sensitive to IUI inhibition. There is no discussion of the inconsistency in the estimated group with the highest IUI inhibition between the two models. The weight-of-evidence shows that the fetal brain is more sensitive to hypothyroidism than the neonate or infant brain (Boelaert and Franklyn, 2005). There is no scientific evidence presented in the White Paper that demonstrates that other life stages are more sensitive with regard to the development of health protective toxicity guidelines values than the fetus.

5.1.3 Use of uncertainty factors in risk assessment

The White Paper recommends increasing the UF for certain groups based on the difference in estimated RAIU at a dose that is 10 times greater than the RfD. There is no scientific evidence that this is warranted.

It is well understood that there is uncertainty in assuming that an experimental dose is applicable to all humans, including those in the population that are more sensitive than average. The calculation of toxicity guideline values accounts for this variability by using UFs to reduce the point of departure to a level that accounts for variability. The general types of uncertainty for which UFs are applied include animal-to-human (interspecies), average human-to-sensitive human (intraspecies), LOAEL-to-NOAEL conversion, lack of chronic data, and database insufficiency. Since the point of departure is based on a study in human adults, an interspecies UF was unnecessary. NRC (2005) also determined that no UF were needed for LOAEL-to-NOAEL conversion, lack of chronic data, and database insufficiency (UFs all equal to 1). Therefore, the RfD was determined by applying a UF of 10 to account for intraspecies variation to the NOEL dose reported in Greer et al. (2002), despite the fact that IUI is a non-adverse effect. However, the White Paper suggests that based on the estimates presented in Table A-3, a greater UF is warranted for the subgroups with sensitivities greater than 3 (pregnant women, fetuses, and breast-fed infants). The White Paper states:

> Therefore, in deriving an MCLG, additional PBPK sensitivity may be warranted to ensure an adequate margin of safety for all life stages. To adjust for the differences in pharmacokinetics among life stages, [U.S. EPA] could divide PBPK model predicted life stage sensitivity for RAIU inhibition by the pharmacokinetic portion (3x) of the intraspecies uncertainty factor recommended by the NRC.

Based on the two models presented in the White Paper, estimated RAIU inhibition will neither be significantly different from normal fluctuations nor biologically significant. Although the model does not consider pharmacodynamic variability, the White Paper suggests a UF of 3. However, there is no evidence that there are differences in the toxicodynamics of perchlorate that would require a UF. Rather, perchlorate has been used therapeutically in juveniles and in pregnant women (Crooks and Wayne, 1960) in doses similar to that in adults with target effects and no greater incidence of side effects (Smellie, 1957).

The PBPK model estimates that, even at a water concentration of 24.5 ppb and the addition of perchlorate in food, the greatest amount of RAIU inhibition is still similar to baseline and well within the homeostatic range. Even at a dose that is 10 times greater (equivalent to 245 ppb), the greatest inhibition is 12.5%. It is also important to recall that IUI is a non-adverse effect. The dose which is estimated to be required to potentially cause an adverse effect is equivalent to a water concentration of 14,000 ppb (NRC, 2005). No adverse health concern at any life stage would be expected based on exposure to perchlorate at the point of departure dose (7 µg/kg-d) or lower.

5.2 Regarding strengths and limitations of the two PBPK models

The use of scientifically derived PBPK models is the best scientific approach for evaluating the relative sensitivity of different life stages, consistent with the conclusions of U.S. EPA's peer reviewers and the NRC (2005). For example, it is well understood that the human HPT-axis can compensate for changing environmental stimuli and the PBPK model considers several important components. The model incorporates important parameters, is conservative in its approach, and likely overestimates IUI for a given dose. The U.S. EPA PBPK model, has scientific limitations which should be considered (and discussed below). Addressing these limitations will make the PBPK model more transparent in its construction and stronger in some key areas that are not currently science-based.

The current U.S. EPA PBPK model for perchlorate, however, uses values for some key parameters (*e.g.*, urinary clearance) that are not supported by the scientific evidence. The model also does not account for up-regulation of the NIS. As a result, the model, as applied by U.S. EPA, does not provide the best scientific estimates of IUI that would be associated with a particular perchlorate dose, and likely overestimates IUI from perchlorate exposure for various life stages. Further, input parameters are not selected from consistent points within their distributions, such that it is unclear whether the model output represents low, average, or upper-bound estimates of possible IUI. The model only considers perchlorate, leaving unexamined other goitrogens that inhibit iodide uptake. Thus the output of this model is limited to the agent that only represents, at most, 2% of the daily goitrogen load.

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Multiple chemical agents affect the uptake of iodide into the thyroid in the same way as perchlorate. Incorporation of thiocyanate and nitrate exposure into the PBPK model should provide useful information on the relative IUI caused by exposure to these agents and to perchlorate. Daily diets for children and adults including foods containing thiocyanates and nitrates would have a greater effect on IUI than would result from drinking two liters of water containing perchlorate at environmentally relevant levels. Based on these data, the contribution of perchlorate in drinking water at a concentration of 24.5 ppb (assuming consumption of 2 liters per day) to total daily IUI associated with a typical diet would be insignificant. The model does not account for exposures to other goitrogens or other thyroid active agents that have MOAs that are not NIS-dependent.

6.0 COMMENTS TO THE SAB REGARDING HUMAN STUDIES

The White Paper poses the following charge questions regarding human studies conducted since 2005 to the SAB:

How should [U.S. EPA] consider the post-NRC epidemiology data in deriving an

MCLG?

6.1 Regarding epidemiology and survey studies

The White Paper cites nine "epidemiologic" studies published since the 2005 NRC that assessed the relationship between perchlorate and thyroid function in humans (Tellez Tellez et al., 2005; Blount et al., 2006; Buffler et al., 2006; Amitai et al., 2007; Steinmaus et al., 2007; Pearce et al., 2010, 2011; Steinmaus et al., 2010; Cao et al., 2010) and one study that assessed associations between perchlorate exposure and hematocrit, hemoglobin, and serum high density lipoprotein (Schreinemachers, 2011). These studies are listed in Table 1. The cited studies are observational, as opposed to controlled, epidemiologic studies. Their value is primarily hypothesis-generating, meaning that assuming enough of the critical variables are controlled for so that the associations are not spurious, findings can lead scientists to develop more carefully designed, controlled studies to assess the relationship between exposure and effect in individuals. No studies where associations were reported between perchlorate exposure in a population and a measure of effect (*e.g.*, changes in hormone levels) were followed up with controlled studies.

Table 1. Summary of epidemiological and survey studies of perchlorate exposure published since the NRC (2005) report

Study	Type of Study/ Data Source	Measure of Perchlorate Exposure	Findings
Blount et al. (2006)	NHANES 2001-02 (survey), US	Urine (spot sample) from 2,299 subjects ≥ 12 yrs of age	In updated analytical dataset (CDC, 2011a), associations with TSH and more robust measure of IUI exposure (PEC of perchlorate, nitrate, thiocyanate) do not persist in females, males, or combined population. Negative association of total T4 (TT4) with PEC in combined population dominated by nitrate and thiocyanate.
Steinmaus et al. (2007)	NHANES 2001-02 (survey), US	Urine (spot sample) from 1,109 women ≥ 12 yrs of age	Same dataset as Blount et al. (2006), see above for comments
Schreinemachers (2011)	NHANES 2001-02 (survey), US	Urine (spot sample) from 2,094 subjects 6-85 yrs of age	Individual thyroid hormones of T4 and TSH were not well correlated with the hematological parameters and the direction of the association varied by sex
Buffler et al. (2006)	California Newborn Screening Database	Blood (spot sample, heal prick) from 50,326 exposed and 291,931 nonexposed newborns	No association between living in an area with perchlorate greater than 5 µg/L in drinking water and increased TSH (>25 µU/mL), using samples collected ≥ 24 hr of age

Study	Type of Study/ Data Source	Measure of Perchlorate Exposure	Findings
Steinmaus et al. (2010)	California Newborn Screening Database	Blood (spot sample, heal prick) from 45,750 exposed and 451,708 nonexposed newborns	Association between living in an area with perchlorate greater than 5 µg/L in drinking water and increased TSH (>25 µU/mL), only using samples collected <24 hr of age, when postnatal surge makes TSH highly variable
Cao et al. (2010)	Study of Estrogen Activity and Development, Philadelphia	Urine from diapers, serum (spot samples) from 92 infants	Positive associations between urinary perchlorate, thiocyanate, and nitrate evaluated separately (but not together) and TSH and free T4 (opposite of direction expected) measured in diaper urine in combined population but not stratified by gender
Amitai et al. (2007)	Ecological epidemiologic study, Israel	Blood (spot sample) from 1,156 newborns	No differences between neonatal T4 levels among low, high, or very high drinking water exposure groups in newborns, indicating that perchlorate up to and in excess of 340 ppb had no effect in the thyroid hormone levels
Pearce et al. (2010, 2011)	???, Cardiff, Wales, and Turin, Italy	Urine (spot sample) from 2,640 pregnant women	No associations between urine perchlorate concentrations and serum TSH or fT4
Tellez Tellez et al. (2005)	Longitudinal epidemiologic study, Chile	Urine and serum (spot samples) from 184 pregnant women and cord blood serum from 159 newborns	No increases in thyroglobulin (Tg) or TSH and no decreases in FT4 related to perchlorate in drinking water, among women during or late pregnancy

Studies assessing thyroid hormone parameters and such factors as iodine nutrition are difficult to conduct. Thyroid hormone and TSH levels fluctuate during and between days, and can be influenced by circadian rhythms and environmental stressors such as temperature. In neonates, concentrations can vary dramatically as a result of gestational age. Within individuals, changes in serum hormone concentrations can reflect short term fluctuations and be adaptive, or they may reflect impaired thyroid function. Clinically determining individual thyroid status for an individual requires repeat testing and assessment of specific thyroid measures, *e.g.*, fT4 or fT4 index (fTI). Likewise, urinary iodine values change vastly during a 24 hour period due to fluctuations in diet and urinary excretion. While the assessment of iodine status is often based on spot urine values, 24-hour collection more accurately determines iodine status.

Closer evaluation of the post-NRC (2005) human studies cited in the White Paper does not support an association between exposure to environmental levels of perchlorate and adverse health effects. The specific studies and their findings are described below.

6.1.1 The use of NHANES dataset: Blount et al. (2006), Steinmaus et al. (2007), and Schreinemachers (2011)

The White Paper cites three studies that analyzed data from the 2001-2002 NHANES (Blount et al., 2006; Steinmaus et al., 2007; and, Schreinemachers, 2011), which measured urinary perchlorate levels as well as numerous other health and nutritional endpoints (including urinary levels of nitrate, thiocyanate, and iodine), in a nationally representative sample of the U.S. population (adults and children of ≥12 years). U.S. EPA indicates that urinary levels "can be used as a robust exposure surrogate for perchlorate exposure from drinking water and food." U.S. EPA asserts that Blount et al. (2006) and Steinmaus et al. (2007) suggest an association between low environmental perchlorate exposure and TSH or T4 changes. Using linear regression statistical methods, they identified a "perchlorate-related increase in TSH and decrease in T4 in women >12 years of age with urinary iodide <100 μg/L, particularly among subjects who smoked (Blount et al., 2006; Steinmaus et al., 2007)." In addition, Steinmaus et al. (2007) reported a "stronger association observed for T4 and log perchlorate among current smokers compared to that for non-smokers; interaction between perchlorate and smoking $\beta = 1.12$, $p=0.008$." Regarding Schreinemachers (2011) they state that it:

> …evaluated indirect indicators of thyroid function and reported an inverse relationship between urinary perchlorate and the hematological parameters, hemoglobin and hematocrit, notably in men and pregnant and non-pregnant women of child-bearing ages, although individual thyroid hormones of T4 and TSH were not well correlated with the hematological parameters and the direction of the association varied by sex.

In these assessments, a number of limitations and methodological issues impact the reported findings. CDC is quite clear regarding the data in their reports:

> Research studies, separate from the Report, are required for determining whether blood or urine levels are safe or are associated with disease or adverse effects (CDC, 2009)

NHANES is a survey designed to assess the health and nutritional status of adults and children in the U.S. at a point in time, combining data from interviews and physical examinations. NHANES is not a true epidemiological or clinical study, where an effort is made to design the study to control for all variables except the variables in question. Further, urine and serum measures reflect "spot" samples, collected at a single point in time for each individual. Urine spot samples are know n to be inherently variable within individuals due to variations in urine volume and dilution and intake of exogenous compounds (*e.g.*, food, supplements) across time (Barr et al., 2005). Factors shown to influence concentrations include fasting time, time of day, nature of the last meal, sample dilution, collection method, preservation method, sample interferences, and analytical method (Rasmussen et al., 1999). In the NHANES study population, fasting times were highly variable, with a reported length of fast ranged from 0 to 63 hours, with a median of 10.3 hours and 5th and 95th percentiles of 1.7 hours and 19 hours, respectively.

Even in 24-hour samples, urinary iodine concentrations vary up to three-fold from one day to another, suggesting that a single sample is insufficient to determine long-term iodine status (Rasmussen et al., 1999). Population iodine excretion estimates require 100 to 500 spot urine

CDC is quite clear regarding the data in their reports:

> **Research studies, separate from the Report, are required for determining whether blood or urine levels are safe or are associated with disease or adverse effects**

samples for each group or subgroup and fewer than 10 urine samples per individual may be misleading (Anderson et al., 2008).

Serum hormone measurements can also vary during the day, such that spot samples may not represent longer term averages. In humans, serum TSH concentrations are at their maximum at night, shortly before sleep, being about 50-100% greater than the morning low (Fisher, 1996). Early morning values are greater than later morning values (Surks et al., 2005). TSH is secreted in pulses, with eight to fourteen pulses occurring in 24 hours. Sleep deprivation, strenuous exercise, or working during night or evening shifts accentuate the rhythms (Surks et al., 2005). Several other important study variables, including location and time of year when samples were collected were not reported due to privacy concerns. Seasonally related changes in thyroid hormone concentrations have been shown in adults, with higher T3 and T4 values seen in winter months, and a tendency to a greater TSH response to TSH-releasing hormone (TRH) was noted at this time (Harrop et al., 1985). These changes could reflect a centrally-mediated response of the HPT axis to environmental temperature (Harrop et al., 1985). Significant annual, four-monthly, and biannual rhythms were detected in serum TSH in adults, with the lowest detected in spring. The peak-trough differences in the yearly variation expressed as a percentage of the mean were 29.1% and 8.2% for TSH and T3, respectively (Maes et al., 1997).

Further, while Blount et al. (2006) and Steinmaus et al. (2007) segregate their assessment groups based on “low” and “high” urinary iodine, a urinary iodine concentration of <100 µg/L measured in a spot urine sample is not indicative of iodine deficiency. The World Health Organization (WHO) has stated that a median urinary iodine concentration within a population, based on spot samples, of less than 100 µg/L, is indicative of iodine deficiency within the population (WHO, 2004). However, the median urinary iodine concentration in the NHANES population was much higher (172.0 µg/L; 194.5 µg/L for males and 141.0 µg/L for females), indicating this population was not iodine deficient. Combined with the variability of spot samples, classifying study subjects for iodine status based on this cutoff is inappropriate.

The initially released NHANES 2001-2002 dataset measured only total T4 and TSH. Clinically, these measures provide an initial screen for thyroid function; however, follow up testing is required to determine whether these changes reflect short term fluctuations and are adaptive or whether thyroid function is impaired. Per the American Thyroid Association (ATA; 2008), “The [fT4] fraction is the most important to determine how the thyroid is functioning…” and “Combining the TSH test with the fT4 of fTI accurately determines how the thyroid gland is functioning.” CDC added testing for more specific thyroid measures, including fT4, to NHANES in later years. In 2011, CDC released reanalyzed samples from their NHANES 2001-2002 dataset (CDC, 2011b). This new set provides updated results for the two thyroid measures (TSH and total T4 (TT4)) included in the initial release as well as additional thyroid measures, including free T3 (fT3), fT4, total T3 (TT3), thought to best reflect thyroid function.

Because perchlorate, nitrate, and thiocyanate act through the same mechanism of action on the NIS, the combined exposure to the three anions is expected to reflect the cumulative IUI impact (U.S. EPA, 2010). Further, since perchlorate, nitrate, and thiocyanate all have dietary sources, including fresh vegetables and milk, exposure levels could also be correlated due to common sources. To account for potential interaction effects, a combined measure of exposure (or perchlorate equivalent concentration, PEC) can be calculated for each subject based on their measured urinary perchlorate, nitrate, and thiocyanate concentration and the relative potency of each ion on the NIS (Tonacchera et al., 2004; U.S. EPA, 2010). In individual study subjects, perchlorate contributed an average just under 2% of the total PEC measured in urine (range, 0-24%), while nitrate contributed an average of 64% (range, 2-98%) and thiocyanate an average of 34% (range, 0-97%). If IUI is a causative agent in

decrements to thyroid function, one would expect to observe increasing decrements in measures of thyroid function (*e.g.*, decreasing thyroid hormone or increasing TSH levels) with increasing PEC. However, for a given population, consistent associations between PEC variables and thyroid measures were not apparent.

If one makes the assumption that perchlorate, thiocyanate, or nitrate can all affect the formation of thyroid hormone at sufficient levels of exposure, one would expect to see a consistent and increasing response on thyroid parameters with increasing exposure to IUI agents, rather than an association with isolated measures for a single subgroup. The new data do not support a consistent relationship between exposure to IUI agents and thyroid function in women or men with urinary iodine less than or greater than the population median. No consistent relationship (*e.g.*, low thyroid hormone coupled with high TSH) is seen in the enhanced dataset despite the availability of more thorough measures of exposure to IUI agents and enhanced estimates of thyroid function.

As the mechanism of action of perchlorate is well understood, and exposures to NHANES 2001-2002 participants were below the reported NOEL for perchlorate, no effects would be expected to occur. Despite this, and despite the criticisms of the study, it is important to note that changes in thyroid hormone levels that the study authors claimed to observe were all within normal ranges—further substantiating that there are no adverse effects from environmental levels of perchlorate.

6.1.2 Data from Buffler et al. (2006) and Steinmaus et al. (2010) do not support an association between increased TSH in neonates and perchlorate in drinking water

U.S. EPA states that Buffler et al. (2006) and Steinmaus et al. (2010) evaluated the relationship between TSH levels and perchlorate in drinking water in newborns using data from the California Newborn Screening Database. U.S. EPA (2010) reports that:

> Associations were reported between higher perchlorate concentration associated with TSH levels above the 95th and 99th percentile in neonates with monitoring within 24 hours of birth and TSH levels above the 95th percentile, but not TSH levels above the 99th percentile, in neonates with monitoring after 24 hours of birth (Buffler et al., 2006; Steinmaus et al., 2010).

However, closer examination of the data from these studies does not support the White Paper's assertions. The most important consideration with this work is that there is a natural surge of TSH. The screening test for CH determines whether there is or is not a surge. Its purpose is not to diagnose, with any degree of accuracy, the exact TSH value. A further complication is that the timing of the surge occurs largely during the first 24 hours after birth (sometimes longer). However, when a hospital takes a blood sample, it is variable and it is not possible to test the exact height of the TSH surge with any degree of reliability. Thus, using data sets with these analytical measurements and poor ability to standardize the sample timing result in a dataset that is not scientifically reliable.

Buffler et al. (2006) and Steinmaus et al. (2010) evaluated data on neonatal TSH from the California Newborn Screening Database of 342,257 newborns born in 1998, collected to screen for primary CH, and perchlorate in municipal drinking water analyzed during 1997-1998 based on the address of the mothers. There was no attempt to even ensure exposure to perchlorate at the levels analyzed. Data were collected from 50,326 newborns who were "exposed" to perchlorate (living in California communities with estimated average perchlorate in the municipal drinking water supply of greater than 5 µg/L) and 291,931 who were "unexposed" (living in communities with average perchlorate less than or equal to 5 µg/L). The authors identified a "high" TSH level as one greater than 25 µU/mL, the cutoff point used for primary CH screening. Buffler et al. (2006) evaluated the same dataset that Steinmaus et al. (2010) later reanalyzed, and concluded that there was no association

between living in an area with perchlorate greater than 5 µg/L and increased TSH. Buffler et al. (2006) were very clear that "Given the documented physiologic postnatal surge of TSH before 24 hrs of age, we restricted the TSH analyses to those newborns with an age at specimen collection ≥ 24 hr."

Steinmaus et al. (2010) reanalyzed the same dataset used in a previous study by Buffler et al. (2006), but included infants sampled within 24 hours of birth and included adjustments for several different variables (including mother's age and income). Steinmaus et al. (2010) report a statistically significant association with "high" TSH (greater than 25 µU/mL) only for data collected before 24 hours. They report that in TSH samples collected within 24 hours of birth, the odds ratio for TSH greater than 25 µU/mL in exposed communities was 1.53 ($P < 0.0001$), and after 24 hours, the odds ratio for TSH greater than 25 µU/mL in exposed communities was 0.72. In infants for whom data was collected after 24 hours, the association was only significant if the TSH level that was considered "high" was dropped from the standard 25 µU/mL to 8 µU/mL (in this group, the odds ratio for a TSH more than the 95th percentile (8 µU/mL) was 1.27 ($P < 0.0001$)). Based on these results, they conclude, "These findings suggest that perchlorate is associated with increased neonatal TSH levels."

There are additional concerns with using this dataset for the purposes outlined by Steinmaus et al. (2010). These include:

- TSH measurements in primary CH screenings are not diagnostic values. Elevated screening values should be retested for confirmation. If the second value is consistent, then other thyroid variables should be measured to confirm CH.
- There was no attempt to confirm exposure to the "exposed" population. Rather, addresses of the mothers were used to establish an assumption of exposure. However, an address is not sufficient to reliably confirm exposure to perchlorate.
- Even if it was confirmed that a woman was exposed to concentrations more than 5 ppb, no IUI, let alone changes in pituitary hormone changes, have been shown to occur at these low exposure levels.

Other issues exist with this type of study. For example, to understand thyroid status TSH measurement should be assessed along with other parameters including gestational age, body weight, T3, T4, free T4, etc. Although the difference in geometric means of TSH levels (4.03 for unexposed and 4.35 µU/mL for exposed) is statistically significant, the TSH levels are unlikely to have clinical significance.

6.1.3 Limitations in Cao et al. (2010) impact the ability to draw conclusions about perchlorate exposure and thyroid effects

As described in the White Paper,

> Cao et al. (2010) found an association between perchlorate exposure and increased TSH levels under iodide deficiency conditions. Based on the exposure findings using the same cohort as Cao et al. (2010), Valentin-Blasini et al. (2011) estimated the geometric mean perchlorate dose as 0.03-0.22 µg/kg/day depending upon the type of formula consumed.

However, methodological issues and biologically inconsistent associations with TSH and thyroid hormone levels affect the ability to draw conclusions.

Cao and colleagues report the results from a subset of samples and data already collected from infants in a study designed to evaluate estrogen activity and development (SEAD). The purpose of

the SEAD study was to evaluate associations between sex hormone levels and development in infants fed breast milk, cow-milk formula, or soy formula. It was not designed to assess perchlorate.

In a subset of 92 infants, however, the authors analyzed perchlorate, iodide, nitrate, and thiocyanate in stored urine collected from diapers or in urine bags. The standard approach to reliably measuring these variables is from a urine sample not from urine extracted from diapers. The authors note they are the first to use this method of collecting urine.

There are a number of methodological problems with extractions from diapers, including that perchlorate was detected in control diapers. TSH and fT4 were also measured in both blood serum and urine samples. Since perchlorate was also detected in water extracted from control cloth diapers (mean 1.24 ± 0.23 µg/L), a concentration of 1.24 µg/L was subtracted from all diaper urine samples. Using a mixed linear statistical model, the authors analyzed associations between urinary levels of the anions and urinary or serum levels of TSH and fT4. The authors did not find any association between urinary perchlorate, nitrate, or thiocyanate and serum TSH or fT4. They report positive associations between urinary perchlorate, thiocyanate, and nitrate evaluated separately and TSH and fT4 measured in diaper urine (not a standard medium for measurement). This means that as diaper urine levels of TSH increased, diaper urine levels of fT4 increased. This, however, is opposite of what is known about the normal function of the thyroid gland and the pituitary gland, as well as the known mechanism of action of perchlorate. When perchlorate, thiocyanate, and nitrate were evaluated simultaneously in the model, thiocyanate and nitrate, but not perchlorate, were associated with TSH and fT4. There were no associations between perchlorate and TSH or fT4 when stratified by gender. When the authors looked at children with urinary iodine less than 100 µg/L (n = 48 children), "…those with higher perchlorate had statistically significantly higher TSH…, but not statistically significantly higher [fT4]…" The authors also remark, "In general, associations between TSH and thiocyanate were larger than those between TSH and perchlorate or nitrate."

6.1.4 Several other studies, as cited by the White Paper, demonstrate no association between environmental perchlorate exposure and thyroid effects in neonates and pregnant women

As stated by the White Paper

> Other studies of pregnant women or neonates and thyroid function did not report associations between residence in city with perchlorate in drinking water supplies or between urinary perchlorate at similar or higher exposure levels than those estimated for Blount et al. (2006), Steinmaus et al. (2007), or Cao et al. (2010) (Tellez Tellez et al., 2005; Amitai et al., 2007; Pearce et al., 2010, 2011).

Tellez Tellez et al. (2005) conducted a longitudinal epidemiologic study of the effects of environmental perchlorate exposure, which is found naturally at high levels in Chilean soils and water supplies, on the thyroidal status of pregnant women and neonates in 184 women in three cities with low (Antofagasta, <4 ug/L), medium (Chanaral, mean 5.82 ug/L), and high (Taltal, mean 113.9 ug/L) perchlorate in residential tap water. These exposure levels yielded dramatically higher urinary perchlorate levels than Blount et al. (2006). Urinary perchlorate was measured during two prenatal visits and one postnatal visit, and TSH, T3, and fT4 were measured in maternal prenatal and postpartum serum and in cord blood. The authors also measured neonatal birth weight, length, and head circumference. Some have questioned the relevance of this study to the U.S. population, since Chile has historically had high levels of dietary iodide supplementations. However, this supplementation had been decreased to levels that are similar to U.S. levels by the time of the study. In the study, the levels of maternal iodine in urine were intermediate to those found in NHANES I

and NHANES III and consistent with the WHO recommendations.

No increases in thyroglobulin (Tg) or TSH and no decreases in T4 among either the women during early pregnancy (16.1 ± 4.1 weeks) or late pregnancy (32.4 ± 3.0 weeks) were seen. Further, the authors found that "…perchlorate in drinking water at 114 µg/L did not cause changes in neonatal thyroid function or fetal growth retardation" and the levels of iodine in breast milk were not associated with perchlorate exposure.

Regarding Tellez Tellez et al. (2005), the White Paper states,

> Although the study in Chile (Tellez Tellez et al., 2005) reported urinary and serum perchlorate levels in women during pregnancy and during postpartum (a longitudinal cohort study), perchlorate assignment to subjects was based solely on geographical location.

Of note, other studies cited by U.S. EPA (*e.g.*, Steinmaus et al., 2010) also classify perchlorate exposure based on location rather than direct measurement of individual exposure levels. The Agency states that there is "Likely some exposure misclassification due to incomplete perchlorate testing or perchlorate from food," although it is acknowledged that perchlorate was measured in urine (like Blount et al., 2006; Steinmaus et al., 2007; and Schreinemachers et al., 2011) and that "Urinary perchlorate represents integrated proxy for all exposure routes." While, as U.S. EPA (2012) states, "Dietary perchlorate exposure in Chile (22-34 µg/day) appears higher than that for U.S.," these exposure levels (0.31-0.49 µg/kg-day, assuming a 70 kg body weight) are less than the NOEL for IUI from Greer et al. (2002) (7 µg/kg-day), and thus still do not exceed the expected threshold for IUI.

Amitai and colleagues conducted an ecological epidemiological study of a population in Israel to "…assess the effect of gestational perchlorate exposure through drinking water on neonatal thyroxine (T4)" by comparing T4 levels among newborns whose mothers lived in areas with drinking water perchlorate levels associated with "very high exposure" (≥340 ppb, or 10 to 100-fold greater than levels in the U.S), "high exposure" (42-94 ppb), or "low exposure" (<3 ppb). T4 levels were measured within 36 to 48 hours after birth, but there was no comment on whether the infants were breast fed or formula fed during the postnatal period, which could affect T4 measures in infants. The authors report no statistical differences between neonatal T4 levels among the groups, indicating that perchlorate in drinking water at up to and in excess of 340 ppb had no effect in the thyroid hormone levels of newborns exposed in utero.

Amitai et al. provided a follow-up to their previously reviewed 2007 publication at a satellite meeting of the Society of Toxicology (SOT) (Seattle 2008). They located a subset of the original study population and evaluated the children using the Bayley Scales of Infant Development to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants ages 0 to 3. They reported no difference between groups of children.

The White Paper does not elaborate on Amitai et al. (2007, 2008), U.S. EPA (2012) cites in Table A-5, that there were "No individual exposure data. Likely some exposure misclassification due to incomplete perchlorate testing or perchlorate from food." However, the findings are important in that the study evaluates exposures to pregnant women and their developing fetuses. These are the highest concentrations of perchlorate ever reported in a study in which the public, including the most sensitive subpopulation, was exposed. For comparison, exposure to drinking water with 340 ppb, assuming consumption of 2 liters per day by a 70 kg individual, yields a dose of 9.7 µg/kg-d, which is greater than the NOEL dose from Greer et al. (2002), a drinking water equivalent of 245 ppb. This dose would likely cause some small degree of IUI even without contribution from nitrates and thiocyanates in food. Finally, the endpoints assessed are measures of neurological development—the

endpoints of greatest concern when assessing thyroidal influence. The results presented thus far point to no adverse effect on neurological development from exposures to levels of perchlorate up to and in excess of 340 ppb.

Pearce and her colleagues published the results from a large study of 22,000 pregnant women living in Cardiff, Wales and Turin, Italy. Subsequently, the same authors have presented similar studies conducted in Los Angeles and Cordoba, Argentina (Pearce et al., 2010; Athens, 2012). The authors measured iodine, thiocyanate, and perchlorate in spot urine samples and TSH, free T4, and thyroperoxidase (TPO) in blood in a subset of 2,640 participants. They further stratified the study population to look at women with urinary iodine less than 100 μg/L (999 women) to determine if perchlorate effects "are limited to individuals with low dietary iodine intake, as was seen in the NHANES data set" (cites Blount et al., 2006). The cause of low urinary iodine was not determined, but it was noted that only 5% of households use iodized salt in the United Kingdom and that Italian legislation requiring iodized salt (unless specifically requested) went into effect after a large number of these samples were collected. The study concluded that for pregnant women, the median urinary iodide was low in all groups compared with WHO guidelines. The authors say "There were no associations between urine perchlorate concentrations and serum TSH or T4 in the individual euthyroid or hypothyroid/hypothyroxinemic cohorts." This study addresses the concerns about whether environmental levels of perchlorate might affect the thyroid or pituitary gland function of iodine deficient individuals. These data demonstrate that perchlorate does not and provides results that are different than those reported by Blount et al. (2006). This could be due to the experimental design of the Pearce et al. (2010, 2011) studies which were conducted in a larger population.

Regarding this study, the White Paper states,

> Pearce et al. (2010, 2011) studies were cross–sectional designs and evaluated the association between urinary perchlorate and thyroid function using correlation analyses and linear regression statistical methods with covariates. Subjects in Pearce et al. (2010) were a combined group of hypothyroid/hypothyroxinemic pregnant women or a group of pregnant women with normal thyroid function (euthyroid). The ranges of thyroid hormones overlapped between these two groups suggesting a possible disease misclassification.

The White Paper also states that

> Information on mother's ages or assay urinary smoking biomarkers not reported. Urinary perchlorate represents integrated proxy for all exposure routes. Small numbers of subjects, low statistical power.

These criticisms would also apply in limiting conclusions that can be drawn in Blount et al. (2006) or Steinmaus et al. (2007).

6.2 Regarding biomonitoring and exposure studies

The White Paper summarizes a number of biomonitoring studies of perchlorate published after the NRC (2005) review (Blount et al., 2007, 2010; English et al., 2011; Huber et al., 2011; Kirk et al., 2005, 2007; Leung et al., 2009; Mendez et al., 2010; Schier et al., 2010; Valentin-Blasini et al., 2011; Woodruff et al., 2011). These studies demonstrate population exposure in the U.S. to perchlorate, based primarily on measurements in urine and in some cases in breast milk. Exposure likely occurs through drinking water and/or milk.

Overall, these estimates demonstrate that while exposure occurs, maximum estimated exposures do not exceed the NOEL for IUI demonstrated by Greer et al. (2002)

The maximum reported geometric mean concentration in urine of adults was 6.44 µg/L (English et al., 2011), corresponding to an estimated geome tric mean perchlorate dose of 0.11 µg/kg-d, for residents of Imperial Valley, California, ages 18-88. Mendez et al. (2010) estimated an upper bound 95th percentile of perchlorate from food and water for reproductive age women of 0.15 µg/kg-d. Overall, these estimates demonstrate that while exposure occurs, maximum estimated exposures do not exceed the NOEL for IUI demonstrated by Greer et al. (2002).

6.3 Regarding weight-of-evidence

Since the 2005 NRC report, no reliable scientific evidence has been developed that reliably reports doses lower than the Greer et al. (2002) NOEL cause any effect on any subpopulation. Most of the studies published since 2005 focus on characterizing rates of exposure to perchlorate by men, women, and infants. For example, some studies estimate exposures to infants through breast milk or from food. These studies are useful for conducting risk assessments; however, while these studies demonstrate that exposure has occurred, they do not change the foundational science regarding perchlorate's mechanism of action or toxicity nor do they demonstrate that adverse effects occur at doses below the NOEL for IUI of perchlorate.

Examining the entirety of the human studies using a weight-of-evidence approach, it is clear that the data demonstrate that perchlorate will not cause adverse health effects at environmental doses. The White Paper appears to place greater weight on the few studies that report an association without equal use of the criticisms applied to similar studies.

7.0 COMMENTS TO THE SAB REGARDING INTEGRATION OF INFORMATION

The White Paper concludes by requesting the SAB suggest methods for integration of the life stage considerations, PBPK modeling, and human study data. The White Paper poses the following charge questions to the SAB:

> **Pertaining to Integration of Information**
>
> **• How can [U.S. EPA] best use the total body of information to derive a health protective MCLG, while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values?**
>
> **• How can [U.S. EPA] use the available data to estimate reductions in adverse health effects (*i.e.*, dose response) that are likely to result from reducing perchlorate levels in drinking water?**

7.1 Regarding the use of the total body of information to derive a health protective MCLG, while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values

7.1.1 Use weight-of-evidence approach in including epidemiological and biomonitoring literature

U.S. EPA has stated that it uses a weight-of-evidence approach to the available dataset, yet the White Paper gives more weight a very few recent biomonitoring or ecological studies that suggest an association between environmental perchlorate and thyroid effects. <u>This approach ignores the vast amount of data that reports no effect from environmental levels of perchlorate</u>. Further, three of these studies that U.S. EPA has reported as "positive" studies (Blount et al., 2006; Steinmaus et al., 2007; Schreinemachers, 2011) are based on the same dataset. These studies and one other (Steinmaus et al., 2010) have been criticized for lack of scientific rigor (Tarone et al., 2010). None of these studies was

designed to determine a causal relationship and all used data that were collected for purposes other than how these authors used them. Similar studies based on other datasets that are more relevant for sensitive populations did not find associations.

7.1.2 Sensitive populations

The NRC considered all possible sensitive populations in addition to pregnant women and their fetuses. This included infants, developing children, people who have compromised thyroid function, and people who are iodide-deficient. Based on its assessment, the NRC (2005) states "…fetuses and preterm newborns constitute the most sensitive populations although infants and developing children are also considered sensitive populations." The White Paper dismisses the determinations of authoritative bodies and posits that other life stages may be more sensitive to perchlorate exposure, yet fails to present data that empirically demonstrate this greater sensitivity.

7.1.3 Transparent presentation of materials

The White Paper is inconsistent in what it describes as the most sensitive population. This inconsistency is confusing and undermines U.S. EPA's stated goal of protection of the most sensitive populations.

Figure 1 (White Paper) presents a comparison of the calculated doses for a person who is exposed to perchlorate in drinking water at a concentration of 15 ppb compared to the RfD. As discussed in Section 5.1, it is unlikely that a person would be exposed to a concentration of 15 ppb on a regular basis. Additionally, the RfD already incorporates a UF to account for variability between the average adult and sensitive populations and is based on a dose which causes no effect. The RfD is not a "bright line" above which adverse effects will occur. The RfD is developed to consider daily exposure for a lifetime. Even if a person were exposed to a dose slightly in excess of the RfD, any effect, adverse or otherwise, is unlikely to occur. When the White Paper presents a table that depicts up to 60-day old breast- and bottle-fed infants exceeding the RfD by up to 5-fold, it suggests that the RfD is not protective. However, even a 5-fold increase is well within the UF of 10 — meaning the hypothetical exposure is still below the NOEL; exposure only occurs up to 60 days (by the White Paper's definition), and exposure to 15 ppb is unlikely.

7.2 Regarding the use of the available data to estimate reductions in adverse health effects (*i.e.*, dose-response) that are likely to result from reducing perchlorate levels in drinking water

The Agency has presented no data reporting that adverse effects are occurring at current environmental concentrations. At doses below the NOEL, there is no effect, adverse or otherwise. The RfD is based on a dose that is 10-fold lower than the dose which causes no effect. Decreasing the dose from a point where there are no adverse effects cannot decrease the number of adverse effects — a fraction of zero is still zero.

8.0 REFERENCES

American Thyroid Association, 2008. Thyroid Function Tests. American Thyroid Association, Falls Church, VA, from www.thyroid.org/patients/patient_brochures/function_tests.html#tests.

Amitai, Y., B. Blount, *et al.*, 2008. *Perchlorate Exposures, Iodine Molulation of Effect and Epidemiologic Associations: Implications for Risk Assessment*, Ancillary Program of the Annual Meeting of the Society of Toxicology, Seattle, WA.

Amitai, Y., G. Winston, *et al.*, 2007. Gestational Exposure to High Perchlorate Concentrations in Drinking Water and Neonatal Thyroxine Levels. *Thyroid* (9): 843-850.

Andersen, S., J. Karmisholt, *et al.*, 2008. Reliability of Studies of Iodine Intake and Recommendations for Number of Samples in Groups and in Individuals. *Br J Nutr* (4): 813-818.

ATSDR, 2008. *Toxicological Profile for Perchlorates,* Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

Barr, D.B., L.C. Wilder, *et al.*, 2005. Urinary Creatinine Concentrations in the U.S. Population: Implications for Urinary Biologic Monitoring Measurements. *Environ Health Perspect* (2): 192-200.

Bekkedal MY, Carpenter T, *et al.*, 2000. A Neurodevelopmental Study of the Effects of Oral Ammonium Perchlorate Exposure on the Motor Activity of Pre-Weanling Rat Pups.

Belzer, R.B., G.M. Bruce, *et al.*, 2004. Using Comparative Exposure Analysis to Validate Low-Dose Human Health Risk Assessment: The Case of Perchlorate. *Comparative Risk Assessment and Environmental Decision Making*. R.A. Linkov I.Kluwer New York**:** 57-74

Blount, B.C., K.U. Alwis, *et al.*, 2010. Perchlorate, Nitrate, and Iodide Intake through Tap Water. *Environ Sci Technol* (24): 9564-9570.

Blount, B.C., J.L. Pirkle, *et al.*, 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. *Environ Health Perspect* (12): 1865-1871.

Blount, B.C., and L. Valentin-Blasini, 2007. Biomonitoring as a Method for Assessing Exposure to Perchlorate. *Thyroid* (9): 837-841.

Bohles, H., M. Aschenbrenner, *et al.*, 1993. Development of Thyroid Gland Volume During the First 3 Months of Life in Breast-Fed Versus Iodine-Supplemented and Iodine-Free Formula-Fed Infants. *Clin Investig* (1): 13-20.

Borjan, M., S. Marcella, *et al.*, 2011. Perchlorate Exposure in Lactating Women in an Urban Community in New Jersey. *Sci Total Environ* (3): 460-464.

Brandhuber, P., S. Clark, *et al.*, 2009. A Review of Perchlorate Occurrence in Public Drinking Water Systems. *Journal AWWA* (11): 63-73.

Buffler, P.A., M.A. Kelsh, *et al.*, 2006. Thyroid Function and Perchlorate in Drinking Water: An Evaluation among California Newborns, 1998. *Environ Health Perspect* (5): 798-804.

Cao, Y., B.C. Blount, *et al.*, 2010. Goitrogenic Anions, Thyroid-Stimulating Hormone, and Thyroid Hormone in Infants. *Environ Health Perspect* (9): 1332-1337.

CDC, 2009. *Fourth National Report on Human Exposure to Environmental Chemicals*, Department of Health and Human Services, Centers for Disease Control and Prevention, Washington, D.C.

CDC, 2004. National Health and Nutrition Examination Survey 2001-2002 Public Data General Release File Documentation. Atlanta, GA, Centers for Disease Control.

CDC, 2011a. National Health and Nutrition Examination Survey 2001 - 2002 Data Documentation, Codebook, and Frequencies Nitrate, Thiocyanate, Perchlorate (Surplus Urine) Atlanta, GA, Centers for Disease Control.

CDC, 2011b. National Health and Nutrition Examination Survey 2001 - 2002 Data Documentation, Codebook, and Frequencies Surplus Sera Thyroid Profile. Atlanta, GA, Centers for Disease Control.

Clark, J., 2000. Toxicology of Perchlorate. *Perchlorate in the Environment*. E. Urbansky.Plenum Publishers New York,

Connell, J.M., 1981. Long-Term Use of Potassium Perchlorate. *Postgrad Med J* (670): 516-517.

Crooks, J., and E.J. Wayne, 1960. A Comparison of Potassium Perchlorate, Methylthiouracil, and Carbimazole in the Treatment of Thyrotoxicosis. *Lancet* (7121): 401-404.

Crump, C., P. Michaud, *et al.*, 2000. Does Perchlorate in Drinking Water Affect Thyroid Function in Newborns or School-Age Children? *J Occup Environ Med* (6): 603-612.

Dallman, P., K. Shannon, *et al.*, 2003. Blood and Blood-Forming Tissues. *Rudolph's Pediatrics*.McGraw-Hill Medical Publishing, New York, NY.,

Dasgupta, P.K., A.B. Kirk, *et al.*, 2008. Intake of Iodine and Perchlorate and Excretion in Human Milk. *Environ Sci Technol* (21): 8115-8121.

De Groef, B., B.R. Decallonne, *et al.*, 2006. Perchlorate Versus Other Environmental Sodium/Iodide Symporter Inhibitors: Potential Thyroid-Related Health Effects. *Eur J Endocrinol* (1): 17-25.

Delange, F., 1998. Screening for Congenital Hypothyroidism Used as an Indicator of the Degree of Iodine Deficiency and of Its Control. *Thyroid* (12): 1185-1192.

Denton, J.E., 2005. Responses to Recent Comments on the Perchlorate PHG (Public Health Goal). to Alan C Lloyd. Sacramento, CA, California EPA.

Dorea, J.G., 2002. Iodine Nutrition and Breast Feeding. *J Trace Elem Med Biol* (4): 207-220.

English, P., B. Blount, *et al.*, 2011. Direct Measurement of Perchlorate Exposure Biomarkers in a Highly Exposed Population: A Pilot Study. *PLoS One* (3): e17015.

Fisher, D.A., 1996. Physiological Variations in Thyroid Hormones: Physiological and Pathophysiological Considerations. *Clin Chem* (1): 135-139.

Fomon, S.J., L.J. Filer, Jr., *et al.*, 1969. Relationship between Formula Concentration and Rate of Growth of Normal Infants. *J Nutr* (2): 241-254.

Fomon, S.J., L.J. Filer, *et al.*, 1977. Skim Milk in Infant Feeding. *Acta Paediatr Scand* (1): 17-30.

Fomon, S.J., L.J. Filmer, Jr., *et al.*, 1975. Influence of Formula Concentration on Caloric Intake and Growth of Normal Infants. *Acta Paediatr Scand* (2): 172-181.

Fomon, S.J., L.N. Thomas, *et al.*, 1971. Food Consumption and Growth of Normal Infants Fed Milk-Based Formulas. *Acta Paediatr Scand Suppl*: 1-36.

Fomon, S.J., E.E. Ziegler, *et al.*, 1983. Sweetness of Diet and Food Consumption by Infants. *Proc Soc Exp Biol Med* (2): 190-193.

Fomon, S.J., E.E. Ziegler, *et al.*, 1995. What Is the Safe Protein-Energy Ratio for Infant Formulas? *Am J Clin Nutr* (2): 358-363.

Fomon, S.J., E.E. Ziegler, *et al.*, 1999. Infant Formula with Protein-Energy Ratio of 1.7 G/100 Kcal Is Adequate but May Not Be Safe. *J Pediatr Gastroenterol Nutr* (5): 495-501.

Glinoer, D., F. Delange, *et al.*, 1992. Maternal and Neonatal Thyroid Function at Birth in an Area of Marginally Low Iodine Intake. *J Clin Endocrinol Metab* (3): 800-805.

Greer, M.A., G. Goodman, *et al.*, 2002. Health Effects Assessment for Environmental Perchlorate Contamination: The Dose Response for Inhibition of Thyroidal Radioiodine Uptake in Humans. *Environ Health Perspect* (9): 927-937.

Greer, M.A., A.K. Stott, *et al.*, 1966. Effects of Thiocyanate, Perchlorate and Other Anions on Thyroidal Iodine Metabolism. *Endocrinology* (2): 237-247.

Haddow, J.E., G.E. Palomaki, *et al.*, 1999. Maternal Thyroid Deficiency During Pregnancy and Subsequent Neuropsychological Development of the Child. *N Engl J Med* (8): 549-555.

Harrop, J.S., K. Ashwell, *et al.*, 1985. Circannual and within-Individual Variation of Thyroid Function Tests in Normal Subjects. *Ann Clin Biochem*: 371-375.

Hsiao, P.H., Y.N. Chiu, *et al.*, 2001. Intellectual Outcome of Patients with Congenital Hypothyroidism Detected by Neonatal Screening. *J Formos Med Assoc* (1): 40-44.

Huber, D.R., B.C. Blount, *et al.*, 2011. Estimating Perchlorate Exposure from Food and Tap Water Based on Us Biomonitoring and Occurrence Data. *J Expo Sci Environ Epidemiol* (4): 395-407.

Integrated Risk Information System (IRIS), 2005. Perchlorate and Perchlorate Salts. *IRIS Glossary/Acronyms & Abbreviations*. Washington, D.C., U.S. EPA.

Integrated Risk Information System (IRIS), 2011. Reference Dose (RfD). *IRIS Glossary/Acronyms & Abbreviations*. Washington, D.C., U.S. EPA.

Kahn, H.D., and K. Stralka, 2009. Estimated Daily Average Per Capita Water Ingestion by Child and Adult Age Categories Based on Usda's 1994-1996 and 1998 Continuing Survey of Food Intakes by Individuals. *J Expo Sci Environ Epidemiol* (4): 396-404.

Kirk, A.B., J.V. Dyke, *et al.*, 2007. Temporal Patterns in Perchlorate, Thiocyanate, and Iodide Excretion in Human Milk. *Environ Health Perspect* (2): 182-186.

Kirk, A.B., P.K. Martinelango, *et al.*, 2005. Perchlorate and Iodide in Dairy and Breast Milk. *Environ Sci Technol* (7): 2011-2017.

Leung, A.M., E.N. Pearce, *et al.*, 2009. Colostrum Iodine and Perchlorate Concentrations in Boston-Area Women: A Cross-Sectional Study. *Clin Endocrinol (Oxf)* (2): 326-330.

Levoxyl, 2010. Levoxyl (Levothyroxine Sodium) Tablet Bethesda, MD, from http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=5915b2bb-ae53-4781-91ed-ff90188fe1d8

Lewander, W.J., P.G. Lacouture, *et al.*, 1989. Acute Thyroxine Ingestion in Pediatric Patients. *Pediatrics* (2): 262-265.

Li, Z., F.X. Li, *et al.*, 2000. Neonatal Thyroxine Level and Perchlorate in Drinking Water. *J Occup Environ Med* (2): 200-205.

Macchia, P.E., P. Lapi, *et al.*, 1998. Pax8 Mutations Associated with Congenital Hypothyroidism Caused by Thyroid Dysgenesis. *Nat Genet* (1): 83-86.

Maes, M., K. Mommen, *et al.*, 1997. Components of Biological Variation, Including Seasonality, in Blood Concentrations of Tsh, TT3, fT4, Prl, Cortisol and Testosterone in Healthy Volunteers. *Clin Endocrinol (Oxf)* (5): 587-598.

Mager, D., E. Wyska, *et al.*, 2003. Diversity of Mechanism-Based Pharmacodynamic Models. *Drug Metab Dispos* (5): 510-518.

Martino, E., S. Mariotti, *et al.*, 1986. Short Term Administration of Potassium Perchlorate Restores Euthyroidism in Amiodarone Iodine-Induced Hypothyroidism. *J Clin Endocrinol Metab* (5): 1233-1236.

Mendez, W., E. Dederick, *et al.*, 2010. Drinking Water Contribution to Aggregate Perchlorate Intake of Reproductive-Age Women in the United States Estimated by Dietary Intake Simulation and Analysis of Urinary Excretion Data. *J Expo Sci Environ Epidemiol* (3): 288-297.

Merrill, E.A., R.A. Clewell, *et al.*, 2003. PBPK Predictions of Perchlorate Distribution and Its Effect on Thyroid Uptake of Radioiodide in the Male Rat. *Toxicol Sci* (2): 256-269.

Mizuta, H., N. Amino, *et al.*, 1983. Thyroid Hormones in Human Milk and Their Influence on Thyroid Function of Breast-Fed Babies. *Pediatr Res* (6): 468-471.

Morgans, M., and W. Trotter, 1960. Potassium Perchlorate in Thyrotoxicosis. *British Medical Journal*: 1086-1087.

Nabukhotnyi, T.K., V.P. Pavliuk, *et al.*, 1987. [Role of Breast Feeding in Providing the Newborn Infant with Thyroid Hormones]. *Vopr Pitan* (1): 29-31.

Nasir, A.M., I.D. Mahmoud, *et al.*, 2010. Urine Iodine Concentration in Neonates with Congenital Hypothyroidism. *Curr Pediatr Res* (1): 47-49.

Newnham, H.H., D.J. Topliss, *et al.*, 1988. Amiodarone-Induced Hyperthyroidism: Assessment of the Predictive Value of Biochemical Testing and Response to Combined Therapy Using Propylthiouracil and Potassium Perchlorate. *Aust N Z J Med* (1): 37-44.

NRC, 2005. *Health Implications of Perchlorate Ingestion*. The National Academies Press, Washington, DC.

OMB, 2002. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies. *Fed. Reg.*: 8452-8460.

Pearce, E.N., J.H. Lazarus, *et al.*, 2010. Perchlorate and Thiocyanate Exposure and Thyroid Function in First-Trimester Pregnant Women. *J Clin Endocrinol Metab* (7): 3207-3215.

Pearce, E.N., A.M. Leung, *et al.*, 2007. Breast Milk Iodine and Perchlorate Concentrations in Lactating Boston-Area Women. *J Clin Endocrinol Metab* (5): 1673-1677.

Pearce, E.N., C.A. Spencer, *et al.*, 2011. Effect of Environmental Perchlorate on Thyroid Function in Pregnant Women from Cordoba, Argentina, and Los Angeles, California. *Endocr Pract* (3): 412-417.

Pop, V.J., E.P. Brouwers, *et al.*, 2003. Maternal Hypothyroxinaemia During Early Pregnancy and Subsequent Child Development: A 3-Year Follow-up Study. *Clin Endocrinol (Oxf)* (3): 282-288.

Pop, V.J., J.L. Kuijpens, *et al.*, 1999. Low Maternal Free Thyroxine Concentrations During Early Pregnancy Are Associated with Impaired Psychomotor Development in Infancy. *Clin Endocrinol (Oxf)* (2): 149-155.

Rasmussen, L.B., L. Ovesen, *et al.*, 1999. Day-to-Day and within-Day Variation in Urinary Iodine Excretion. *Eur J Clin Nutr* (5): 401-407.

Rastogi, M.V., and S.H. LaFranchi, 2010. Congenital Hypothyroidism. *Orphanet J Rare Dis*: 17.

Rovet, J.F., 1990. Does Breast-Feeding Protect the Hypothyroid Infant Whose Condition Is Diagnosed by Newborn Screening? *Am J Dis Child* (3): 319-323.

Schier, J.G., A.F. Wolkin, *et al.*, 2010. Perchlorate Exposure from Infant Formula and Comparisons with the Perchlorate Reference Dose. *J Expo Sci Environ Epidemiol* (3): 281-287.

Schreinemachers, D.M., 2011. Association between Perchlorate and Indirect Indicators of Thyroid Dysfunction in NHANES 2001-2002, a Cross-Sectional, Hypothesis-Generating Study. *Biomark Insights*: 135-146.

Shepherd, R.W., D.B. Oxborough, *et al.*, 1988. Longitudinal Study of the Body Composition of Weight Gain in Exclusively Breast-Fed and Intake-Measured Whey-Based Formula-Fed Infants to Age 3 Months. *J Pediatr Gastroenterol Nutr* (5): 732-739.

Simpser, T., and R. Rapaport, 2010. Update on Some Aspects of Neonatal Thyroid Disease. *J Clin Res Pediatr Endocrinol* (3): 95-99.

Smellie, J.M., 1957. The Treatment of Juvenile Thyrotoxicosis with Potassium Perchlorate. *Proc R Soc Med* (12): 1026.

Soldin, O.P., L.E. Braverman, *et al.*, 2001. Perchlorate Clinical Pharmacology and Human Health: A Review. *Ther Drug Monit* (4): 316-331.

Specker, B.L., A. Beck, *et al.*, 1997. Randomized Trial of Varying Mineral Intake on Total Body Bone Mineral Accretion During the First Year of Life. *Pediatrics* (6): E12.

Steinmaus, C., M.D. Miller, *et al.*, 2007. Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001-2002 National Health and Nutrition Examination Survey. *Environ Health Perspect* (9): 1333-1338.

Steinmaus, C., M.D. Miller, *et al.*, 2010. Perchlorate in Drinking Water During Pregnancy and Neonatal Thyroid Hormone Levels in California. *J Occup Environ Med* (12): 1217-1524.

Surks, M.I., G. Goswami, *et al.*, 2005. The Thyrotropin Reference Range Should Remain Unchanged. *J Clin Endocrinol Metab* (9): 5489-5496.

Tarone, R.E., L. Lipworth, *et al.*, 2010. The Epidemiology of Environmental Perchlorate Exposure and Thyroid Function: A Comprehensive Review. *J Occup Environ Med* (6): 653-660.

Tazebay, U.H., I.L. Wapnir, *et al.*, 2000. The Mammary Gland Iodide Transporter Is Expressed During Lactation and in Breast Cancer. *Nat Med* (8): 871-878.

Tellez Tellez, R., P. Michaud Chacon, *et al.*, 2005. Long-Term Environmental Exposure to Perchlorate through Drinking Water and Thyroid Function During Pregnancy and the Neonatal Period. *Thyroid* (9): 963-975.

Tonacchera, M., A. Pinchera, *et al.*, 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* (12): 1012-1019.

U.S. EPA, 1996. Proposed Guidelines for Carcinogen Risk Assessment. Washington, D.C., Office of Research and Development.

U.S. EPA, 2002. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity, of Information Disseminated by the Environmental Protection Agency. EPA/260R-02-008.

U.S. EPA, 2004. Estimated Per Capita Water Ingestion and Body Weight in the United States–an Update Based on Data Collected by the United States Department of Agriculture‟S 1994–1996 and 1998 Continuing Survey of Food Intakes by Individuals. EPA-822-R-00-001.

U.S. EPA, 2008a. Drinking Water: Preliminary Regulatory Determination on Perchlorate. *Fed. Reg.*: 60262-60282.

U.S. EPA, 2008b. Child-Specific Exposure Factors Handbook. EPA/600/R-06/096F.

U.S. EPA, 2009. Interim Drinking Water Health Advisory for Perchlorate. EPA 822-R-08-025.

U.S. EPA, 2010. Office of Inspector General Scientific Analysis of Perchlorate. 10-P-0101.

U.S. EPA, 2011. Exposure Factors Handbook. EPA/600/R-09/052F.

U.S. EPA, 2012a. Life Stage Considerations and Interpretations of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate, Office of Science and Technology, Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2012b. Notification of a Public Meeting and Public Teleconference of the Science Advisory Board (SAB); Perchlorate Advisory Panel. *Fed. Reg.*: 31847-31848.

Valentin-Blasini, L., B.C. Blount, *et al.*, 2011. Perchlorate Exposure and Dose Estimates in Infants. *Environ Sci Technol* (9): 4127-4132.

van den Hove, M.F., C. Beckers, *et al.*, 1999. Hormone Synthesis and Storage in the Thyroid of Human Preterm and Term Newborns: Effect of Thyroxine Treatment. *Biochimie* (5): 563-570.

Van Vliet, G., 1999. Neonatal Hypothyroidism: Treatment and Outcome. *Thyroid* (1): 79-84.

van Wassenaer, A.G., M.R. Stulp, *et al.*, 2002. The Quantity of Thyroid Hormone in Human Milk Is Too Low to Influence Plasma Thyroid Hormone Levels in the Very Preterm Infant. *Clin Endocrinol (Oxf)* (5): 621-627.

Vulsma, T., M.H. Gons, *et al.*, 1989. Maternal-Fetal Transfer of Thyroxine in Congenital Hypothyroidism Due to a Total Organification Defect or Thyroid Agenesis. *N Engl J Med* (1): 13-16.

Wenzel, K.W., and J.R. Lente, 1984. Similar Effects of Thionamide Drugs and Perchlorate on Thyroid-Stimulating Immunoglobulins in Graves' Disease: Evidence against an Immunosuppressive Action of Thionamide Drugs. *Journal of Clinical Endocrinology & Metabolism* (1): 62-69.

WHO, and FAO, 2004. *Vitamin and Mineral Requirements in Human Nutrition*. World Health Association and Food and Agriculture Organization, Geneva, Switzerland.

Wolff, J., 1998. Perchlorate and the Thyroid Gland. *Pharmacol Rev* (1): 89-105.

Woodruff, T.J., A.R. Zota, *et al.*, 2011. Environmental Chemicals in Pregnant Women in the United States: NHANES 2003-2004. *Environ Health Perspect* (6): 878-885.

Wyngaarden, J.B., J.B. Stanbury, *et al.*, 1953. The Effects of Iodine, Perchlorate, Thiocyanate, and Nitrate Administration Upon the Iodide Concentrating Mechanism of the Rat Thyroid. *Endocrinology* (5): 568-574.

Wyngaarden, J.B., B.M. Wright, *et al.*, 1952. The Effect of Certain Anions Upon the Accumulation and Retention of Iodide by the Thyroid Gland. *Endocrinology* (5): 537-549.

York, R.G., J. Barnett, Jr., et al., 2004. A Rat Neurodevelopmental Evaluation of Offspring, Including Evaluation of Adult and Neonatal Thyroid, from Mothers Treated with Ammonium Perchlorate in Drinking Water. Int J Toxicol (3): 191-214.

Zimmermann, M.B., 2011. The Role of Iodine in Human Growth and Development. *Semin Cell Dev Biol* (6): 645-652.

APPENDIX A

SUPPLEMENTAL TABLES

Supplemental Tables

Table 1. Summary of clearances rates for renally excreted, non-metabolized drugs in adults and children approximately two years old: one compartment model

Drug	Age	Ratio of CL_{child} to CL_{adult} (child rate/ adult rate)	% of adult Css with same ADD	Reference[10]
Gabapentin	3 – 6 y (n=8)	1.2 (4.8 ml/kg/m)/(3.9 ml/kg/m)	83%	Gatti et al., 2003
Gentamicin	2.25 y (n=17)	1.4 (.138 L/h/kg)/(.0966 L/h/kg)	71%	Johnson et al., 2006 citing Bass et al., 1998, Matzke et al., 1989/ Wallace et al., 2002
Gentamicin	0.08 – 7.2 y 1 – 5 y	1.3 (1.57 ml/kg/m)/(1.25 ml/kg/m) 2.2 (2.74 ml/kg/m) /(1.25 ml/kg/m)	77% 45%	Edginton et al., 2006 citing Assael et al., 1980, Ho et al., 1995, Kirkpatrick et al., 1999
Isepamicin	0.4 – 5.9 y	1.9 (2.64 ml/kg/m)/(1.4 ml/kg/m)	53%	Edginton et al., 2006 citing Scaglione et al., 1995, Radwanski et al., 1997
Ticarcillin	0.08 – 2 y (n=16) 2 – 12 y (n=23)	1.6 (3 ml/min-kg)/(1.9 ml/min-kg) 1.5 (2.8 ml/min-kg) /(1.9 ml/minkg)	63% 67%	Ginsberg et al., 2002 citing Reed, 1998
Vancomycin	1 – 5 y (n=12)	1.9 (.153 L/h/kg) /(.0786 L/h/kg)	53%	Johnson et al., 2006 citing Rodvold et al., 1997, Matzke et al., 1989/ Wallace et al., 2002
Geometric Mean (Range)	**1.6** (1.2 – 2.2)	**63%** (45 – 83%)		
Geometric Mean w/o gabapentin[11]	**1.7**	**59%**		

y: year kg: kilogram CL: clearance
m: minute L: liter Css: steady-state serum concentration
ml: milliliter h: hour ADD: average daily dose

[10] Values not verified from primary literature.
[11] Analyzed without gabapentin, as the age range does not include our range of 1-3 year old.

Table 2. Summary of clearance rates for renally excreted, non-metabolized drugs in adults and neonates: one compartment model

Drug	Age	Ratio of CL_{child} to CL_{adult} (child rate/adult rate)	% of adult Css with same ADD	Reference[12]
Gentamicin	0 – 1 d (n=27) 8 – 28 d (n=14)	0.87 (0.084 L/h/kg)/(0.0966 L/h/kg) 1.2 (0.118 L/h/kg)/(0.0966 L/h/kg)	115% 83%	Johnson et al., 2006 citing Bass et al., 1998, Matzke et al., 1989/Wallace et al., 2002
Gentamicin	1 – 84 d	1.0 (1.04 ml/kg/m)/(1.06 ml/kg/m)	100%	Ginsberg et al., 2002 citing Rodvold, 1993, Kirkpatrick, 1999
Isepamicin	2 d 2 m	0.79 (1.1 ml/kg/m)/(1.4 ml/kg/m) 1.5 (2.14 ml/kg/m)/(1.4 ml/kg/m)	127% 67%	Edginton et al., 2006 citing Scaglione et al., 1995, Radwanski et al., 1997
Ticarcillin	0.08 – 2 y (n=16)	1.6 (3 ml/min-kg)/(1.9 ml/min-kg)	63%	Ginsberg et al., 2002 citing Reed, 1998
Vancomycin	Premature, 4 – 17 d post-natal (n=11)	0.8 (0.74 ml/kg/m) /(0.96 ml/kg/m)	125%	Ginsberg et al., 2002 citing Jarret, 1993 and Cuttler, 1994
Geometric Mean (Range)	**1.1** (0.79 – 1.6)	**91%** (63 – 127%)		

y: year kg: kilogram CL: clearance ADD: average daily dose
m: minute L: liter Css: steady-state serum concentration
ml: milliliter h: hour d: day

[12] Values not verified from primary literature.

Table A-3. Summary of neurodevelopmental tests in offspring of rat dams treated with anti-thyroid agents

Study	Strain	Agent and Dose	Maternal Dosing Regimen	Tests in Offspring	Statistically Significant Effects in Treated Group(s)
Albee *et al*. (1989)	Fischer 344	10, 30, 100 ppm MMI	GD17 – PND10	Brain weight and histopathology (PND 11); Eye opening (PND 18); Motor activity (PND 20); Auditory brainstem responses (PND 90)	100 ppm: Increased latency, decreased power, and altered shape of auditory brainstem response
Ahmed *et al*. (2010)	White albino	200 ppm MMI; exogenous T4 20 ppm	GD10-PND10	Monoamine levels; FT4, FT3 levels, AchE activity, GABA content and APTase activity	Decrease in FT4 and FT3 levels; decrease monoamine levels and AchE activity; increase in GABA content associated with suppression of Na^+, K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+} ATPase activity in different brain regions
Ahmed *et al*. (2012)	White albino	200 ppm MMI, and exogenous T4 20 ppm	GD1-PND21	Neuronal development of cerebellar and cerebral cortex, ROS/antioxidant defense system in cerebrum, cerebellum and MO at the end of first, second and third PN weeks	Decrease levels in FT3, FT4 and growth hormone, and significant increase in TSH level; reversed pattern observed in hyperthyroid group; both hypo- and hyper-thyroidism caused severe growth retardation in cerebellar and cerebral cortex neurons from the 1st to 3rd weeks; oxidative damage by increase in LPO and inhibition of enzymatic and non-enzymatic antioxidant defense systems
Axelstad *et al*. (2008)	Wistar	400, 800, 1200 ppm PTU*	GD7-PND17	Motor activity (PND14); learning and memory; auditory function	Decrease T4 levels in high-dose group, and weight and histology of the thyroid glands were affected; decrease motor activity levels on PND14, and increase on PND23 and in adulthood; learning and memory was impaired in adult offspring; auditory function was impaired in the highest dose group
Bekkedal et al. (2000)	Sprague-Dawley	0.71, 7.1, 21, 71 ppm AP#	GD2-PND10	Motor activity in male and female pups (PNDs 14, 18, and 22);	No significant differences in general locomotor activity
Comer and Norton (1982)	Sprague-Dawley	100 ppm MMI	GD17 – PND10	Righting response (PND 1 to criterion); Reflex suspension (PND 7-14); Auditory startle (PND 10 to appearance); Eye opening; Open field activity (PND 21)	Increased righting times on PND 2-13; Decreased suspension times; Mean 6-day delay in acquisition of acoustic startle; Mean ~2-day delay in eye opening; Reduced open field activity
Comer and Norton (1985)	Sprague-Dawley	100 ppm MMI	GD17 – PND10	Spatial maze (PND35); Motor activity (PND 42, 4 mths, 6 mths)	Differences in spatial maze exploration; Increased motor activity in males at PND42, and in both males and females at 4 and 6 mths
Darbra *et al*. (1995)	Wistar	200 ppm MMI	GD9 – PND20	Motor activity, Elevated maze (for anxiety), and Open field activity (PND 75-90)	Increased motor activity, anxiety-related behaviors, and open field activity

Study	Strain	Agent and Dose	Maternal Dosing Regimen	Tests in Offspring	Statistically Significant Effects in Treated Group(s)
El-Bakry *et al*. (2010)	White albino	200 ppm MMI; exogenous T4 (20 ppm)	GD1-PND21	Developmental effects in cerebellar cortex, and cerebellum and skeletal features during PND	Malformation and developmental defects in the cerebellar and cerebral cortex more prominent after the third PN week; degeneration, deformation and severe growth retardation in neurons of these regions; skeletal features delayed partially in hypotryroid group
Gilbert *et al*. (2006)	Long-Evans	3 or 10 ppm PTU	GD6-PND30	Spatial learning and synaptic function in the dentate gyrus of the hippocampus	T4 levels in the low-dose group at weaning were reduced by 70% and the majority of high-dose animals fell below the MDC of the assay
Goldey *et al*. (1995)	Long-Evans	1, 5, 25 ppm PTU	GD18 – PND21	Motor activity within automated figure eight mazes for a 30-minute period on PND13, 15, 17, 19, and 21, and for a 60-minute period on PND30, 40, 50, 75, and 120	5 ppm: reduction in motor activity at PND 15, delayed acoustic startle ; 25 ppm ~4 day delay in eye opening, reduction in motor activity, delayed acoustic startle
Knipper *et al*. (2000)	Wistar	200 ppm MMI	GD17 – PND3, PND5, PND8, PND9, PND10, PND12, PND13, PND14, or PND40	Auditory brainstem responses; Distortion product otoacoustic emissions (DPOAEs)	Elevated hearing thresholds in all groups; Impaired DPOAEs in all groups treated through PND5 or later
Saegusa *et al*. (2010)	Rats (Japan)	200 ppm MMI or 3 ppm PTU or 12 ppm PTU	GD10-PND20	Reeling expressing cells in the dentate hilus	Growth retardation lasting into adult stage with MMI and 12 ppm; sustained increases of immature GABAergic interneurons synthetizing Reeling in the hilus
Schaefer *et al*. (2002)	Sprague-Dawley	10, 30, 100 ppm MMI	GD6 – PND21	Motor activity (PND 13, 17, 21, 61); Auditory startle response (PND20 and 60); Learning and memory (water maze) (PND22 and 62)	30 and 100 ppm: Increased motor activity on PND13 and increased motor activity on PND 21; effect on auditory startle on PND20 and 60; decreased swimming ability at PND22 and increased latency to escape water maze on PND22 and PND62
Shibutani *et al*. (2009)	Rats (Japan)	3 or 12 ppm PTU or 200 ppm MMI	GD10-PND20	Systemic effect including sexual differentiation; neuronal migration	Growth suppression at weaning to PTU at 12 ppm; stunted growth and delayed maturation continued in offspring to the adult stage in groups exposed to 12 ppm PTU or MMI; thyroid follicular cell hypertrophy; delayed onset of puberty and an adult stage gonadal enlargement
Schneider and Golden (1985)	Sprague-Dawley	500 ppm MMI	GD17 – PND10	Acoustic startle response (PND10 to appearance)	Delay in acquisition of acoustic startle response (Mean PND 15.2 in treated v. PND 11.9 in control)
Sui *et al*. (2005)	Long-Evans	3 or 10 ppm PTU	GD6-PND30	Examined synaptic transmission and plasticity in the hippocampus; measured T4, T3 levels in dams and offsprings	T4 in the low-dose group was reduced by 70% at weaning and majority of high dose animals fell below level of detection; perturbations in hipoccampal synaptic function persist despite return to normal tyroid status

Study	Strain	Agent and Dose	Maternal Dosing Regimen	Tests in Offspring	Statistically Significant Effects in Treated Group(s)
Weller *et al.* (1996)	Sprague-Dawley	200 ppm MMI	GD17 – PND0	Eye opening, ear opening, reflex tests on random days from PND5-14 (bar suspension, cliff aversion, turning up an incline, air-righting, surface-righting, startle, hot-plate paw lift)	Delay in eye and ear opening; Developmental delay in four of seven reflexes
York *et al.* (2004)	Sprague-Dawley	0.71, 7.1, 21, 71 ppm AP#	GD0-PND10	Neurobehavioral testing included passive avoidance, watermaze, motor activity, and auditory startle habituation evaluations; adult regional brain weights, morphometry, and neuropathology	No behavioral effects in exposed groups at high-dose

Notes: DPOAE = distortion product otoacoustic emission; GD = gestational day; KI = potassium iodide; MMI = methimazole; PND = postnatal day; PTU = propylthiouracil; MDC = minimal detectable concentration; LPO- lipid peroxidation; AP = Ammonium perchlorate; *Authors state that dose given in mg/kg/day was converted to ppm by dividing the given dose by .002 L of H_20; #Based on a study by the Health Effects Research Program, Environmental Health Risk Assessment Program, female rats consumed 28 ml H_20/d. Conversion from dose to water concentration was performed for a 200g rat and the doses reported in the paper usaphcapps.amedd.army.mil/erawg/tox/files/WTAPerchlorate%28Final%2907v2.pdf.

Table 4. Thyroid hormone decreases in dams treated with different doses of anti-thyroid agents in drinking water

Study	Strain	Regimen	Dose	Time of Thyroid Hormone Measurement	Mean T_4 (Dams-Treated) (µg/dL)	Mean T_4 (Dams-Control) (µg/dL)	T_4 Decrease	Mean T_3 (Dams-Treated) (ng/dL)	Mean T_3 (Dams-Control) (ng/dL)	T_3 Decrease
Calvo *et al*., 1992	Wistar	GD14 to concl.	200 ppm MMI	GD21	0.4 ± 0.1	1.7 ± 0.1	-76%	NA	NA	NA
Calvo *et al*., 1992 (A)	Wistar	GD14 to concl.	200 ppm MMI	GD21	0.28 ± 0.038	1.53 ± 0.092	-82%	NA	NA	-62%
Calvo *et al*., 1992 (B)	Wistar	GD14 to concl.	200 ppm MMI	GD21	0.31	0.69	-55%	NA	NA	-50%
El-Zaheri *et al*., 1981	Sprague-Dawley	GD8 to concl.	500 ppm MMI	GD21	<0.3	1.8 ± 0.3	>-83%	12.6 ± 1.0	25.5 ± 1.8	-51%
Lilienthal *et al*., 1997	Wistar	GD7 to PND21	5 ppm PTU	GD19	1.02± 0.10	4.14 ± 0.56	-75%	100 ± 10	100 ± 10	-0%
Morreale de Escobar *et al*., 1988	Wistar	GD16 to concl.	200 ppm MMI	GD21	0.3 ± 0.1	1.3 ± 0.2	-77%	22 ±2	30 ± 5	-27%
Morreale de Escobar *et al*., 1989	Wistar	GD14 to concl.	200 ppm MMI	GD21	0.46 ± 0.15	1.62 ± 0.23	-72%	NA	NA	-31%
Pedraza *et al*., 1996	Wistar	GD11 to concl.	200 ppm MMI	GD21	0.25 ± 0.03	0.81 ± 0.09	-69%	3 ± 0.3	14.7 ± 3.1	-93%
Sack *et al*., 1995	Sprague-Dawley	GD10 to birth	200 ppm MMI	GD21	0.53± 0.17	2.79 ± 0.25	-81%	NA	NA	NA
Schwartz *et al*., 1997	NA	GD14 to concl.	250 ppm MMI	GD21	<1.0	2.7 ± 0.3	>-63%	7 ± 3	42 ± 13	-83%
Weller *et al*., 1996	Sprague-Dawley	GD10 to birth	200 ppm MMI	"Postpartum"	0.53 ± 0.17	2.79 ± 0.25	-81%	NA	NA	NA
York *et al*., 2004	Sprague-Dawley	2 wks before conception to concl.	0.01 mg/kg-day perchlorate	GD21	2.06 ±	2.31 ± 0.13	-11%	97.3 ± 9.7	99.7 ± 6	-2%
			0.1 mg/kg-day perchlorate	GD21	1.28 ± 0.13	2.31 ± 0.13	-45%	95.8 ± 6.3	99.7 ± 6	-4%
			1 mg/kg-day perchlorate	GD21	1.19 ± 0.12	2.31 ± 0.13	-48%	93.01 ± 9	99.7 ± 6	-7%
			30 mg/kg-day perchlorate	GD21	1.06 ± 0.11	2.31 ± 0.13	-54%	87.4 ± 8.5	99.7 ± 6	-12%
York *et al*., 2001	Sprague-Dawley	before conception to concl.	0.3 mg/kg-day perchlorate	Postpartum	2.9 ± 0.04	2.13 ± 0.68	+136%	64.8 ± 29.3	57.8 ± 28.2	+112%
			3.0 mg/kg-day perchlorate	Postpartum	2.92 ± 0.84	2.13 ± 0.68	+137%	56.4 ± 14	57.8 ± 28.2	-3%
			30 mg/kg-day perchlorate	Postpartum	2.42 ± 0.79	2.13 ± 0.68	+114%	60.4 ± 22	57.8 ± 28.2	+104%

Table A-5. Thyroid hormone decreases in pups of dams treated with different doses of anti-thyroid agents in drinking water

Study	Strain	Regimen	Dose	Time of Thyroid Hormone Measurement	Mean T_4 (Pups-Treated) (µg/dL)	Mean T_4 (Pups-Control) (µg/dL)	T_4 Decrease	Mean T_3 (Pups-Treated) (ng/dL)	Mean T_3 (Pups-Control) (ng/dL)	T_3 Decrease
Goldey *et al*. (1995)	Longs-Evans	GD18 to PND 1, 7, 14	1 ppm PTU	PND 1, 7, or 14	0.3 ± 0.05 to 5.7 ± 0.3	0.4 ± 0.1 to 5.6 ± 0.3	0 to -25%	NA	NA	NA
		GD18 to PND21	1 ppm PTU	PND 1, 7, or 14	5.0 ± 0.2	4.1 ± 0.3	0%	NA	NA	NA
		GD18 to PND 1, 7, 14	5 ppm PTU	PND 1, 7, or 14	0.2 ± 0.05 to 0.8 ± 0.1	0.4 ± 0.1 to 5.6 ± 0.3	-50 to -89%	NA	NA	NA
		GD18 to PND21	5 ppm PTU	PND21	1.2 ± 0.2	4.1 ± 0.3	-71%	NA	NA	NA
		GD18 to PND 1, 7, 14	25 ppm PTU	PND 1, 7, or 14	<0.1	0.4 ± 0.1 to 5.6 ± 0.3	-75 to -98%	NA	NA	NA
		GD18 to PND21	25 ppm PTU	PND21	<0.1	4.1 ± 0.3	>-98%	NA	NA	NA
Lilienthal *et al*. (1997)	Wistar	GD7 to PND21	5 ppm PTU	PND21	1.82 ± 0.38	6.59 ± 0.64	-72%	110 ± 20	100 ± 10	0%
Sack *et al*. (1995)	Sprague-Dawley	GD10 to birth	200 ppm MMI	PND1	0.54 ± 0.08	1.08 ± 0.07	-50%	NA	NA	NA
Weller *et al*. (1996)	Sprague-Dawley	GD10 to birth	200 ppm MMI	"Newborn"	0.54 ± 0.08	1.08 ± 0.07	-50%	NA	NA	NA
Argus (2001)	Sprague-Dawley	2 wks before conception to concl.	0.01 mg/kg-day perchlorate	PND10	3.73 ± 0.36	3.73 ± 0.41	0%	75.2 ± 8.1	79.5 ± 6.6	-5%
			0.1 mg/kg-day perchlorate	PND10	3.5 ± 0.42	3.73 ± 0.41	-6%	74.4 ± 8	79.5 ± 6.6	-6%
			1 mg/kg-day perchlorate	PND10	3.37 ± 0.35	3.73 ± 0.41	-10%	66.2 ± 6.5	79.5 ± 6.6	-17%
			30 mg/kg-day perchlorate	PND10	3.35 ± 0.38	3.73 ± 0.41	-10%	64.4 ± 7.6	79.5 ± 6.6	-19%

References

Ahmed, O.M., S.M. Abd El-Tawab, et al., 2010. Effects of Experimentally Induced Maternal Hypothyroidism and Hyperthyroidism on the Development of Rat Offspring: I. The Development of the Thyroid Hormones-Neurotransmitters and Adenosinergic System Interactions. Int J Dev Neurosci (6): 437-454.

Ahmed, O.M., R.G. Ahmed, et al., 2012. Effects of Experimentally Induced Maternal Hypothyroidism and Hyperthyroidism on the Development of Rat Offspring: Ii-the Developmental Pattern of Neurons in Relation to Oxidative Stress and Antioxidant Defense System. Int J Dev Neurosci.

Albee, R.R., J.L. Mattsson, et al., 1989. Neurological Consequences of Congenital Hypothyroidism in Fischer 344 Rats. Neurotoxicol Teratol (2): 171-183.

Argus Research Laboratories, 1998. Neurobehaviorial Development Study of Ammonium Perchlorate Administered Orally in Drinking Water to Rats, Horsham, PA.

Argus Research Laboratories, 2001. Two-Generation Reproduction Study of Ammonium Perchlorate in Drinking Water in Rats Evaluates Thyroid Toxicity. Int J Toxicol (4): 183-197.

Axelstad, M., P.R. Hansen, et al., 2008. Developmental Neurotoxicity of Propylthiouracil (Ptu) in Rats: Relationship between Transient Hypothyroxinemia During Development and Long-Lasting Behavioural and Functional Changes. Toxicology and Applied Pharmacology 232(1): 1-13.

Behnam-Rassoli, M., L.C. Herbert, et al., 1991. Effect of Propylthiouracil Treatment During Prenatal and Early Postnatal Development on the Neocortex of Rat Pups. Neuroendocrinology (4): 321-327.

Calvo, R., M.J. Obregon, et al., 1992. The Rat Placenta and the Transfer of Thyroid Hormones from the Mother to the Fetus. Effects of Maternal Thyroid Status. Endocrinology (1): 357-365.

Comer, C.P. and S. Norton, 1982. Effects of Perinatal Methimazole Exposure on a Developmental Test Battery for Neurobehavioral Toxicity in Rats. Toxicol Appl Pharmacol (1): 133-141.

Comer, C.P. and S. Norton, 1985. Behavioral Consequences of Perinatal Hypothyroidism in Postnatal and Adult Rats. Pharmacol Biochem Behav (4): 605-611.

Darbra, S., F. Balada, et al., 1995. Perinatal Alterations of Thyroid Hormones and Behaviour in Adult Rats. Behav Brain Res (2): 159-164.

Edginton AN, Schmitt W, Voith B, Willmann S. 2006. A Mechanistic Approach for the Scaling of Clearance in Children. Clin Pharmacokinet 45(7): 683-704.

El-Bakry, A.M., A.W. El-Gareib, et al., 2010. Comparative Study of the Effects of Experimentally Induced Hypothyroidism and Hyperthyroidism in Some Brain Regions in Albino Rats. Int J Dev Neurosci (5): 371-389.

El-Zaheri, M.M., A.G. Vagenakis, et al., 1981. Maternal Thyroid Function Is the Major Determinant of Amniotic Fluid 3,3',5'-Triiodothyronine in the Rat. J Clin Invest (4): 1126-1133.

Gatti G, Ferrari AR, Guerrini R, Bonanni P, Bonomi I, Perucca E. 2003. Plasma Gabapentin Concentrations in Children With Epilepsy: Influence of Age, Relationship With Dosage, and Preliminary Observations on Correlation With Clinical Response. Therapeutic Drug Monitoring 25(1):54-60.

Gilbert, M.E. and L. Sui, 2006. Dose-Dependent Reductions in Spatial Learning and Synaptic Function in the Dentate Gyrus of Adult Rats Following Developmental Thyroid Hormone Insufficiency. Brain Res (1): 10-22.

Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S, Goble R.2002. Evaluation of child/adult pharmacokinetic differences from a database derived from thetherapeutic drug literature. Toxicological Sciences 66:185-200.

Goldey, E.S., L.S. Kehn, et al., 1995. Effects of Developmental Hypothyroidism on Auditory and Motor Function in the Rat. Toxicol Appl Pharmacol (1): 67-76.

Johnson TN, Rostami-Hodjegan A, Tucker GT. 2006. Prediction of the Clearance of ElevenDrugs and Associated Variability in Neonates, Infants and Children. Clin Pharmacokinet45(9): 931-956.

Kirkpatrick, C.M., S.B. Duffull, et al., 1999. Pharmacokinetics of Gentamicin in 957 Patients with Varying Renal Function Dosed Once Daily. Br J Clin Pharmacol (6): 637-643.

Knipper, M., C. Zinn, et al., 2000. Thyroid Hormone Deficiency before the Onset of Hearing Causes Irreversible Damage to Peripheral and Central Auditory Systems. J Neurophysiol (5): 3101-3112.

Lilienthal, H., A. Weinand-Harer, et al., 1997. Effects of Maternal Exposure to 3,3',4,4'-Tetrachlorobiphenyl or Propylthiouracil in Rats Trained to Discriminate Apomorphine from Saline. Toxicol Appl Pharmacol (1): 162-169.

Morreale de Escobar, G., M.J. Obregon, et al., 1988. Transfer of Thyroxine from the Mother to the Rat Fetus near Term: Effects on Brain 3,5,3'-Triiodothyronine Deficiency. Endocrinology (4): 1521-1531.

Pedraza, P., R. Calvo, et al., 1996. Displacement of T4 from Transthyretin by the Synthetic Flavonoid Emd 21388 Results in Increased Production of T3 from T4 in Rat Dams and Fetuses. Endocrinology (11): 4902-4914.

Radwanski, E., V. Batra, et al., 1997. Pharmacokinetics of Isepamicin Following a Single Administration by Intravenous Infusion or Intramuscular Injections. Antimicrob Agents Chemother (8): 1794-1796.

Sack, J., A. Weller, et al., 1995. A Simple Model for Studying the Correction of in Utero Hypothyroidism in the Rat. Pediatr Res (4 Pt 1): 497-501.

Schaefer, G., M. Nemec, et al., 2002. Validation of Developmental Neurotoxicity Endpoints in Rats Administered Methimazole in Drinking Water.

Schneider, B.F. and W.L. Golden, 1986. Acquisition of Acoustic Startle Shows a Dose-Response to Serum Free T4. Int J Dev Neurosci (4): 397-400.

Schwartz, H.L., M.E. Ross, et al., 1997. Lack of Effect of Thyroid Hormone on Late Fetal Rat Brain Development. Endocrinology (8): 3119-3124.

Shibutani, M., G.H. Woo, et al., 2009. Assessment of Developmental Effects of Hypothyroidism in Rats from in Utero and Lactation Exposure to Anti-Thyroid Agents. Reprod Toxicol (3): 297-307.

Sui, L., W.L. Anderson, et al., 2005. Impairment in Short-Term but Enhanced Long-Term Synaptic Potentiation and Erk Activation in Adult Hippocampal Area Ca1 Following Developmental Thyroid Hormone Insufficiency. Toxicol Sci (1): 647-656.

Wallace, A.W., M. Jones, et al., 2002. Evaluation of Four Once-Daily Aminoglycoside Dosing Nomograms. Pharmacotherapy (9): 1077-1083.

Weller, A., A. Rozin, et al., 1996. Neurobehavioral Development of Neonatal Rats after in-Utero Hypothyroxinemia: Efficacy of Prenatal Thyroxine Treatment. Early Hum Dev (1-2): 63-76.

APPENDIX B

PBPK CRITIQUE FROM INTERTOX (2009)

Interpretation of the Physiologically Based Pharmacokinetic (PBPK) Modeling

Properly conducted, PBPK models provide accurate, peer reviewed scientific information to reduce uncertainty about sensitive life stages. PBPK models for perchlorate have been under development for a number of years. In its 2005 assessment of perchlorate, the NRC identified several subpopulations that are potentially more sensitive than average adults to thyroid effects from perchlorate exposure, including pregnant women, fetuses, and infants, based in part on perchlorate PBPK models for pregnant and lactating rats and rat pups and adult humans. After the NRC evaluation, the perchlorate PBPK model was improved to include pregnant women, the fetus, lactating women, and the neonate, and was published in the peer reviewed literature (Merrill *et al.*, 2005; Clewell *et al.*, 2007).

Regarding the preliminary regulatory determination for perchlorate, US EPA evaluated published PBPK models for perchlorate "based on their ability to provide additional information surrounding this critical effect for potentially sensitive subgroups and reduce some of the uncertainty regarding the relative sensitivities of these subgroups" (US EPA, 2008a). US EPA used public comments on the preliminary regulatory determination, as well as comments from an external peer review (Eastern Research Group, 2008), to further refine the model which is based on both pharmacokinetics and pharmacodynamics (US EPA, 2009b) and used it to predict the relative RAIU inhibition for 17 distinct populations or life stages, including pregnant and lactating women, the fetus, and the neonate, assumed to be exposed to 0.007 mg/kg-d in drinking water. The results of this evaluation are presented in Table 1 of the Notice. From these results, US EPA concluded that "by protecting the fetus of the hypothyroid or iodide-deficient woman from the effects of perchlorate on the thyroid, all other life stages and subgroups would be protected."

US EPA requested comments on three general questions regarding its use of the PBPK model as an alternative approach to assessing the relative sensitivity of different exposure populations (US EPA, 2009a). Responses to these questions follow. Note that the responses to Questions 1 and 2 are combined, as the questions are not substantively different.

1. [US EPA] requests comment on using the PBPK model to evaluate the relative sensitivity of the various life stages to perchlorate exposure in drinking water.

2. [US EPA] requests comment on the utility of the PBPK model for predicting the impact of different perchlorate drinking water concentrations on sensitive life stages to inform HRL selection.

PBPK modeling is valuable as it can integrate dozens of experimentally derived variables drawn from peer reviewed scientific studies to characterize chemical absorption, distribution, metabolism, excretion, and toxicity/effect. PBPK modeling is well established in the pharmacological and toxicological sciences, where it has been used for more than 80 years to understand the action of chemicals in the body. More recently, PBPK models have been used in filling scientific gaps in human health data, especially when conducting animal studies is infeasible, unethical, or impractical.

As stated by Mager *et al.* (2003) in a review of the use of PBPK models:

> The resolution of PK/PD [pharmacokinetic/pharmacodynamic] models and parameters is best achieved by having relevant pharmacokinetics…, an understanding of the mechanism of action of the drug, appreciating the determinants of any time dependence in responses, and collecting a suitable array of experimental measurements as a function of dose and time. When possible, such measurements should be sensitive, gradual, quantitative, reproducible,

and meaningful (Mager *et al.*, 2003).

This statement reflects scientific consensus about how scientific information should be used in a PBPK model. The US EPA PBPK model is for the most part based on this philosophy and can be useful for informing risk management decisions. Not all parameters in this model, however, meet the criteria required for robust and predictive PBPK modeling. To be useful, the PBPK model inputs should be transparent and scientifically based. This would advance the overarching science policy articulated by the current US EPA Administrator, Hon. Lisa Jackson.[13]

While supportive of a science based PPBK model, an evaluation of the US EPA PBPK model reveals that model does not produce the best and most scientific estimates of IUI. For example, some components of US EPA's PBPK model for perchlorate are not supported by the scientific literature and result in IUI predictions that do not reflect best scientific estimates of IUI associated with a particular perchlorate dose. In particular, US EPA's uses values for one of the key parameters in the PBPK model, the urinary clearance of perchlorate, that are not supported by the scientific literature.[14]

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[13] US EPA Administrator Lisa Jackson, in her May 9, 2009 memorandum on scientific integrity stated, "While the laws that EPA implements leave room for policy judgments, the scientific findings on which these judgments are based should be arrived at independently using well-established scientific methods, including peer review, to assure rigor, accuracy, and impartiality. This means that policymakers must respect the expertise and independence of the Agency's career scientists and independent advisors while insisting that the Agency's scientific processes meet the highest standards of rigor, quality, and integrity (Jackson 2009)."

[14] Per US EPA's evaluations, perchlorate PBPK model predictions are highly sensitive to urinary clearance rate, such that changes in it significantly impact predicted RAIU inhibition for a particular life stage and perchlorate dose. For each of the 17 distinct populations or life stages evaluated in the model, US EPA used one of two different urinary clearance values: 0.05 L/h-kg for the pregnant woman at gestational weeks 13, 20, and 40 (which also impacts the RAIU prediction for the fetus at gestational week 40), and 0.13 L/h-kg for all other populations/life stages.

If one calculates urinary clearance in L/h by multiplying these values by the corresponding scaled body weight, the resulting urinary clearance rates for the pregnant woman are much lower than for all other adults evaluated by the model: urinary clearance rates range from 1.20 to 1.32 L/h for the pregnant woman, 3.21 to 3.28 L/h for the lactating woman, 3.08 L/h for the woman of child-bearing age, and 3.15 L/hr for the average adult. The assumption of reduced urinary excretion during pregnancy, however, is not supported by the bulk of the scientific literature.

The perchlorate urinary clearance rates used in the model are based on data collected in Greer *et al*. (2002). These values were originally described in the Merrill *et al.* (2005) model for adult humans, and were incorporated into the Clewell *et al.* models (Clewell *et al.*, 2003a; Clewell *et al.*, 2003b; Clewell *et al.*, 2007). Per Merrill *et al.* (2005), the 0.13 L/h-kg clearance rate is based on the mean "fit" urinary clearance rate for perchlorate for adult males and females (nonpregnant) in the Greer *et al*. (2002) study; the values for the 23 individuals in that study ranged from 0.06 to 0.24 L/h-kg. According to Clewell *et al*. (2007), the 0.05 L/h-kg urinary clearance value applied to the pregnant woman (and fetus) was selected from the lower bound of this range, in part because this lower value was consistent with data from a study of perchlorate-exposed pregnant women in Chile (Tellez Tellez *et al.*, 2005). Clewell *et al.* (2007) describes their rationale as follows:

> In the nonpregnant adult model, Merrill and coauthors (2005a) fitted the perchlorate urinary clearance value to each individual for which data were available and noted a marked variation between subjects. Values for urinary clearance (ClUcp) ranged from 0.05 to 0.24 ng/L-h [*sic*], with an average of 0.13 ng/L-h [*sic*]. Using the lower bound of these values (0.05 ng/L) [*sic*], the gestation model predicted serum levels were within the range of the experimental values of Tellez *et al.* (2005)… Since the lower clearance value lies within the range of the normal adult, improves the perchlorate prediction, and yields a more conservative estimate of risk, it was used for all subsequent maternal simulations and dose metric calculations.

Note that the actual lower bound of the perchlorate urinary clearance range from Greer *et al.* (2002) (reported in Merrill *et al*. (2005)) is 0.06 L/h-kg, not 0.05 L/h-kg. Further, this value of 0.06 L/h-kg is for an adult male; no pregnant women were included in the study population, and the lowest reported value for any woman is 0.09 L/h-kg.

Clewell *et al.* (2007) indicated the lower-bound value from Greer *et al*. (2002) is consistent with data collected by Tellez *et al*. (2005). Tellez Tellez *et al.* (2005) evaluated perchlorate exposure in pregnant women in three cities in Chile exposed to perchlorate in water at average concentrations ranging from 0.5 to 114 µg/L. This paper says that urinary perchlorate was measured during two prenatal visits and one postnatal visit, and reported excretion in units of µg/L and µg/g creatinine. Unfortunately, no data are provided in the paper on time course of excretion, so it is unclear how Clewell *et al*. (2007) established that the lower bound of the urinary clearance rate for these populations was 0.05 L/h-kg.

In selecting a urinary clearance value to use for the pregnant woman, US EPA considered two additional values: clearance during pregnancy in the average adult based on Aboul-Khair *et al.* (1964); and clearance during pregnancy equal to nonpregnant average values. Aboul-Khair *et al*. (1964) evaluated urinary clearance of iodide in pregnant women in the United States and reported that

"renal (urinary) clearance for iodide is elevated to as much as two times control (nonpregnant) values during pregnancy, and, while clearance then declines fairly rapidly towards control after birth, it is still elevated in the first couple of months" (Aboul-Khair *et al*., 1964). US EPA assumed that urinary clearance of perchlorate would have the same pregnant to nonpregnant ratio reported by Aboul-Khair *et al*. (1964).

US EPA ran the model using all three values. Of these values, the clearance value used by Clewell *et al*. (2007) resulted in the highest predicted RAIU inhibition. For example, at a dose of 7 µg/kg-d (a dose ten times the RfD of 0.7 mg/kg-d), US EPA (2009a) reports a RAIU inhibition for the fetus of 11% using the Clewell *et al*. (2007) clearance value, 5.2% using the average non-pregnant adult clearance value, and 3.4% using the Aboul-Khair *et al.* (1964) clearance value. Regarding final selection of this value, US EPA says:

> Since there are no conclusive human pregnancy data to distinguish which of these alternatives is more likely, US EPA selected option 1, the lower clearance value reported in the peer-reviewed paper by Clewell *et al*. (2007) for relative response estimation (life stage sensitivity analysis), since this value leads to the most sensitive predictions… While this analysis uses the lowest urinary clearance value among the alternatives evaluated, it does not provide an overall upper-bound effect estimate because the impact of uncertainty and variability in parameters other than those examined here (*e.g.*, uncertainty in thyroid NIS parameters and interindividual variability in urinary excretion) was not evaluated (US EPA, 2009b).

Thus, US EPA selected a urinary clearance value for this population based on a nonscience consideration that lacked corroborating data in the face of other data supporting a higher value. US EPA states that selection of this value is not meant to reflect uncertainty or a bounded estimate. This is inconsistent with the statement in the Notice regarding the use of conservatism in the model, that:

> The PBPK model for each life stage is presumed to represent a typical or average individual from that life stage. Thus, in evaluating specific PBPK parameter choices, especially for urinary clearance, US EPA's objective was to ensure that the values appropriately represented an average or central value for a given life stage and age (US EPA, 2009b).

US EPA further elaborates:

> In the case where more than one alternative clearance value appeared to be equally likely (*i.e*., had a similar quality and amount of supporting data), then the more "sensitive" option was selected (*i.e*., the option that leads to predictions of the highest sensitivity to perchlorate-induced RAIU). The overall analysis did not seek to select parameters associated with highest predicted effect and is not intended to represent an upper-bound estimate of the average effect of perchlorate in these populations. Modeling choices are assumed to represent the best or most likely (central) estimate for an average individual within the population. There is uncertainty about the average, and US EPA has selected from within the range of uncertainty, but there is still expected to be population variability around the model predictions based on that choice, with the model prediction being near the center of the distribution (US EPA, 2009b).

The Notice states that the urinary clearance value was selected to "represent the best or most likely (central) estimate for an average individual within the population." Selecting the lowest urinary excretion value—one that produces the highest estimate of RAIU inhibition—is not the "average." The Notice declares that all three clearance values considered had a similar quality and amount of supporting data; in fact, this cannot be true based on the information presented. The 0.05 L/h-kg value was of low scientific quality (it is not supported by either source US EPA cites as its primary basis, *i.e*., Greer *et al*. (2002) or Tellez Tellez *et al*. (2005)) and higher values are better supported by the scientific literature, as discussed below.

The scientific literature shows that, although minimal, data are available on perchlorate urinary clearance or excretion rates during pregnancy, but do not suggest lower excretion of perchlorate during pregnancy. In the study of Chilean women, Tellez *et al.* (2005) reported higher urinary perchlorate excretion (measured as µg/g creatinine) during pregnancy relative to postpartum for women in two of the three cities evaluated (Chanaral and Taltal, which had mean concentrations of perchlorate in tap water of 5.8 and 114 µg/L). In the "low perchlorate" city (Antofagasta, with a mean tap water concentration of 0.5 µg/L), excretion was somewhat higher postpartum. Based on urine samples collected during the NHANES 2001–2002 study, Blount et al. (2007) reported higher urinary perchlorate in pregnant women (mean 3.27 µg/g creatinine, n = 115), relative to all women of reproductive age (mean 2.97 µg/g creatinine, n = 662). Interpretation of data on urinary clearance or excretion of iodide is complicated by the iodide status of populations; however, Smyth *et al.* (1997) reported that in an area of moderate iodine intake (median urinary iodide 82 µg/g creatinine), urinary iodide excretion was increased during all three trimesters of pregnancy relative to nonpregnant controls.

US EPA's selection of the lowest identified urinary clearance value for the pregnant woman and fetus is inconsistent with the statement in the Notice about the Agency's use of conservatism in the model (US EPA, 2009a):

> The PBPK model for each life stage is presumed to represent a typical or average individual from that life stage. Thus, in evaluating specific PBPK parameter choices, especially for urinary clearance, EPA's objective was to ensure that the values appropriately represented an average or central value for a given life stage and age.

US EPA further states, "there is still expected to be population variability around the model predictions based on that choice, with the model prediction being near the center of the distribution," and:

> While this analysis uses the lowest urinary clearance value among the alternatives evaluated, it does not provide an overall upper-bound effect estimate because the impact of uncertainty and variability in parameters other than those examined here (e.g., uncertainty in thyroid NIS parameters and interindividual variability in urinary excretion) was not evaluated.

Nonetheless, in practice, selection of the lowest urinary clearance value for two of the subpopulations renders the RAIU

In addition to using the best science to support PBPK values, other parameters could be added, such as up-regulation of the NIS. Up-regulation of the NIS represents an adaptive response to iodine deficiency in the thyroid, but US EPA (2009b) did not incorporate a mechanism of up-regulation in the US EPA model. Incorporation of NIS regulation into the model would more accurately reflect the probable response to perchlorate exposure in the environment.[15]

Parameters used in the PBPK model were selected from inconsistent points within their distributions. For example, as described above, US EPA selected a lower bound value to describe the urinary clearance of perchlorate for the pregnant woman and fetus (US EPA, 2009b). In contrast, US EPA reports that the urinary clearance values it selected for all other population subgroups fall in the middle of the range of possible values (US EPA, 2009b). As a result, it is difficult to interpret the model output because it is unclear whether the output represents lower-, middle-, or upper- bound predictions of possible IUI caused by perchlorate exposure.[16]

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inhibition predictions for these populations more conservative than for other populations, making application of model results to risk assessment difficult to interpret from a purely scientific basis.

[15] Over the past decade, research regarding thyroid autoregulation 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 causes the number of NIS membrane proteins to increase; 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 Draft US EPA Office of the Inspector General (OIG) report appears to consider up-regulation of NIS expression as well (US EPA, 2008b).

Earlier versions of the model using the rat did consider up-regulation, and showed no RAIU inhibition in the rat thyroid after 18 days of exposure at perchlorate doses up to 10 mg/kg-d (350,000 ppb assuming default body weight and water intake) (Clewell *et al*., 2003a). Up-regulation was not included in the human model, "based on the fact that up-regulation was not seen in the nonpregnant adult after 2 wk of doses as high as 1 mg ClO_4^- (Merrill *et al*., 2005)" and although "the current simulations cover lifetime exposures rather than a few weeks, the accompanying data on thyroid hormones (TSH, T4) still do not show evidence of thyroidal up-regulation (Crump *et al*., 2000; Tellez *et al*., 2005)" (Clewell *et al*., 2007). The NRC report, however, acknowledges the up-regulation of NIS expression, but did not incorporate it into their risk assessment. NRC (2005) states:

> …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) provide perhaps the most comprehensive review of the NIS regulation at the time of the NRC report. Dohán *et al*. reported that "up-regulation of thyroid NIS expression and iodide uptake activity by TSH 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 (Dohan *et al.*, 2003). TSH up-regulates iodide uptake activity by an increase in NIS transcription. They say:

> 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 (Dohan *et al.*, 2003). Other studies support up-regulation of NIS during changing levels of iodine (Eng *et al.*, 1999; Wagner *et al.*, 2002; Merrill *et al.*, 2003; Pedraza *et al.*, 2006; Norden *et al.*, 2007; Bizhanova and Kopp, 2009).

[16] When conducting calculations to reflect exposure within a population, each parameter can have a range of possible values. In order for PBPK model output to be meaningful, the basis for selection of specific values within the range of possible values should be described and the relationship to the overall distribution for that parameter should be characterized (*e.g.*, lower-bound, average, median, upper-bound). Further, in the absence of probabilistic assessment, if point estimates are selected for incorporation in the model, a consistent rationale should be applied in choosing values for the various parameters so that the location of the output within the overall distribution can be approximated. For example, when conducting baseline human health risk assessments for a reasonable maximum exposure (RME) scenario, US EPA recommends estimating "a conservative exposure case (*i.e*., well above the average case) that is still within the range of possible exposures," and intake variable values for a given pathway should be selected so that the combination of all intake variables results in an estimate of the reasonable maximum exposure for that pathway (US EPA, 1998). The objective of evaluating conservative exposures is to generate an upper-bound estimate of exposure below which adverse health effects are unlikely. Note that although US EPA presents the impact of alternative urinary clearance assumptions on model-predicted RAIU inhibition in its discussion document on the PBPK model (Table 4-2, US EPA 2009b), it does not present this information in the Notice.

3. [US EPA] requests suggestions for ways to use the PBPK modeling analysis to inform the regulatory determination for perchlorate that are different from those described in this notice or the October 10, 2008 notice.

The PBPK model can provide useful information to characterize the relative sensitivity of this life stage if model parameters are scientifically defensible and selected from comparable points within their parameter distributions, and if nonscientific considerations do not introduce unexplained bias into the selection of parameter values. To be most useful, US EPA should use probabilistic methods to characterize the uncertainty and variability around parameter distributions and the range and likelihood associated with model-predicted RAIU inhibition for each population or life stage. Use of probabilistic methods is consistent with the Agency's current recommendations for use of probabilistic methods in risk assessment to help inform risk management decisions (US EPA 2009c).

Additional considerations would provide perspective on the significance of the IUI associated with low dose perchlorate exposure. For example, US EPA should present the change in RAIU inhibition when the RfD is modeled rather than the Greer *et al*. (2002) POD, which is tenfold higher. Also, multiple chemical agents affect the uptake of iodide into the thyroid in the same way as perchlorate. These goitrogens, which include thiocyanate and nitrate, can be found naturally in common foods, including produce, milk, and cured meats. Incorporation of thiocyanate and nitrate exposure into the PBPK model should provide useful information on the relative IUI caused by exposure to these agents and to perchlorate. Thus, daily diets for children and adults including these foods are predicted to have a greater effect on IUI than would result from drinking two liters of water containing perchlorate at environmentally relevant levels (*e.g.*, estimated perchlorate drinking water equivalent concentrations associated with the thiocyanate and nitrate present in these diets are estimated to be at least 1,500 to 29,000 ppb). Based on these data, the contribution of perchlorate in drinking water at a concentration of 20 ppb (assuming consumption of 2 liters per day) to total daily IUI associated with a typical diet would be a fraction of these values.

Aboul-Khair, S.A., J. Crooks, A.C. Turnbull, and F.E. Hytten, 1964. The Physiological Changes in Thyroid Function during Pregnancy. *Clin Sci*: 195-207.

Bizhanova, A., and P. Kopp, 2009. Minireview: The sodium-iodide symporter NIS and pendrin in iodide homeostasis of the thyroid. *Endocrinology* (3): 1084-1090.

Clewell, R.A., E.A. Merrill, J.M. Gearhart, P.J. Robinson, T.R. Sterner, D.R. Mattie, and H.J. Clewell, 3rd, 2007. 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. *J Toxicol Environ Health A* (5): 408-428.

Clewell, R.A., E.A. Merrill, K.O. Yu, D.A. Mahle, T.R. Sterner, J.W. Fisher, and J.M. Gearhart, 2003a. Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicol Sci* (2): 416-436.

Clewell, R.A., E.A. Merrill, K.O. Yu, D.A. Mahle, T.R. Sterner, D.R. Mattie, P.J. Robinson, J.W. Fisher, and J.M. Gearhart, 2003b. Predicting fetal perchlorate dose and inhibition of iodide kinetics during gestation: a physiologically-based pharmacokinetic analysis of perchlorate and iodide kinetics in the rat. *Toxicol Sci* (2): 235-255.

Dohan, O., A. De la Vieja, V. Paroder, C. Riedel, M. Artani, M. Reed, C.S. Ginter, and N. Carrasco, 2003. The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance. *Endocr Rev* (1): 48-77.

Eastern Research Group. 2008. *External Letter Peer Review of EPA's Draft Report, Inhibition of the Sodium-Iodide Symporter by Perchlorate: An Evaluation of Lifestage Sensitivity Using Physiologically-based Pharmacokinetic (PBPK) Modeling*. Submitted to the United States Environmental Protection Agency, Lexington, MA.

Eng, P.H., G.R. Cardona, S.L. Fang, M. Previti, S. Alex, N. Carrasco, W.W. Chin, and L.E. Braverman, 1999. Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. *Endocrinology* (8): 3404-3410.

Jackson L, 2009. *Memo to EPA Employees – Scientific Integrity: Our Compassd for Environmental Protection*, Environmental Protection Agency, Washington, DC.

Mager, D., E. Wyska, and W. Jusko, 2003. Diversity of mechanism-based pharmacodynamic models. *Drug Metab Dispos* (5): 510-518.

Merrill, E.A., R.A. Clewell, J.M. Gearhart, P.J. Robinson, T.R. Sterner, K.O. Yu, D.R. Mattie, and J.W. Fisher, 2003. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat. *Toxicol Sci* (2): 256-269.

Merrill, E.A., R.A. Clewell, P.J. Robinson, A.M. Jarabek, J.M. Gearhart, T.R. Sterner, and J.W. Fisher, 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol Sci* (1): 25-43.

Norden, M.M., F. Larsson, S. Tedelind, T. Carlsson, C. Lundh, E. Forssell-Aronsson, and M. Nilsson, 2007. Down-regulation of the sodium/iodide symporter explains 131I-induced thyroid stunning. *Cancer Res* (15): 7512-7517.

Pedraza, P.E., M.-J. Obregon, H.F. Escobar-Morreale, F. Escobar del Rey, and G.M. de Escobar, 2006. Mechanisms of Adaptation to Iodine Deficiency in Rats: Thyroid Status Is Tissue Specific. Its Relevance for Man. *Endocrinology* (5): 2098-2108.

Tellez Tellez, R., P. Michaud Chacon, C. Reyes Abarca, B.C. Blount, C.B. Van Landingham, K.S. Crump, and J.P. Gibbs, 2005. Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid* (9): 963-975.

U.S. EPA, 1998. *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information, Review Draft (NCEA-1-0503)*. Office of Research and Development , Washington, D.C.

US EPA, 2008a. Drinking Water: Preliminary Regulatory Determination on Perchlorate. *Fed. Reg.*: 60262-60282.

US EPA, 2009a. Drinking Water Perchlorate Supplemental Request for Comments. *Fed. Reg.*: 41883-41893, Government Printing Office, Washington, D.C.

US EPA, 2009b. Inhibition of the Sodium-Iodide Symporter By Perchlorate: An Evaluation of Life Stage Sensitivity Using Physiologically Based Pharmacokinetic (PBPK) Modeling. EPA/600/R-08/106A.

US EPA, 2009c. Availability of the External Peer Review Draft of Using Probabilistic Methods to Enhance the Role of Risk Analysis in Decision-Making with Case Study Examples, *Fed. Reg.:* 41695-41696, Government Printing Office, Washington, D.C.

US EPA, 2008b. *Scientific Analysis of Perchlorate: External Review Draft*, Office of Inspector General, environmental Protection Agency, Washington, D.C.

Wagner, S., G. Aust, M. Schott, W.A. Scherbaum, J. Feldkamp, J. Seissler, C. Puppin, F. Arturi, E. Ferretti, D. Russo, R. Sacco, G. Tell, G. Damante, and S. Filetti, 2002. Regulation of sodium-iodide-symporter gene expression in human thyrocytes measured by real-time polymerase chain reaction

Transcriptional regulation of human sodium/iodide symporter gene: a role for redox factor-1. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association* (8): 398-402.