Subject: SAB Advice on Approaches to Derive a Maximum Contaminant Level Goal for Perchlorate

Dear Administrator Jackson:

In 2005, at the request of EPA and other federal agencies, the NRC published a comprehensive report “Health Implications of Perchlorate Ingestion.” The NRC concluded that perchlorate can affect thyroid function because it is an ion that competitively inhibits the transport of iodide into the thyroid and that a prolonged decrease of thyroid hormone is potentially more likely to have adverse effects in sensitive populations (people with thyroid disorders, pregnant women, fetuses, and infants).

The NRC recommended the use of inhibition of iodide uptake, a precursor non-adverse effect, to derive a reference dose for perchlorate. The RfD was based on the no observed effect level of 7 µg/kg/day corresponding to a radioactive iodide uptake inhibition of 1.8 percent and application of an intraspecies uncertainty factor of 10 to account for differences in sensitivity between the healthy adults and the most sensitive population, fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The NRC also acknowledged that the RfD may need to be adjusted upward or downward on the basis of future research. The RfD of 0.7 µg/kg/day was adopted by EPA in 2005.

EPA has identified perchlorate as a drinking water contaminant and initiated the process to develop a Maximum Contaminants Level Goal and National Primary Drinking Water Regulation under the Safe Drinking Water Act. The MCLG is a non-enforceable goal defined under the SDWA as “the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety.”

EPA developed a white paper that identifies and summarizes recent studies and physiologically based pharmacokinetic modeling it is evaluating, in addition to the data and information used by the NRC. The Agency is evaluating these and previously published studies to consider different life stages that comprise groups within the general population that are likely to be at greater risk of adverse health effects (i.e., sensitive subgroups or sensitive life stages). EPA’s Office of Water requested the SAB to provide advice on how the Agency should consider recent information on sensitive life stages, epidemiological and biomonitoring studies and the Agency’s PBPK modeling efforts, and on approaches to use and integrate this information in deriving an MCLG.
The SAB concludes that it is important for the Agency to consider sensitive life stages explicitly in the development of a MCLG for perchlorate. The mode of action of perchlorate is well understood and involves the potential for disturbance of thyroid homeostasis. Interference with thyroid is known to produce adverse effects on neurodevelopment in humans, with the fetus and infants most vulnerable. Although adverse neurodevelopmental effects of perchlorate in infants and children have not been reported in the literature, their risk can be reasonably inferred from perchlorate’s mode of action. In considering health endpoints of potential concern, the SAB finds that hypothyroxinemia or subclinical hypothyroidism is more appropriate to consider in evaluating the potential adverse health effects for pregnant women, fetuses and infants than hypothyroidism.

The SAB recommends that EPA derive a perchlorate MCLG that addresses sensitive life stages through physiologically-based pharmacokinetic/pharmacodynamic modeling based upon its mode of action rather than the usual algebraic approach using the RfD and specific chemical exposure parameters. The SAB finds that this approach is a more facile, transparent, and rigorous way to address differences in biology and exposure between adults and sensitive life stages than is possible with the traditional approach for deriving an MCLG.

The SAB notes that Agency developed a PBPK/PD model for perchlorate that can be used to derive an MCLG. The model builds on the PBPK models reviewed by the NRC and could be used in its present form, although expansion of the model to address important aspects of vulnerability to perchlorate is strongly recommended. The SAB recommends that EPA expeditiously expand this approach to empirically relate the percent iodine uptake inhibition with thyroid hormone perturbations, specifically hypothyroxinemia. A long-term goal should be extension of the model to prediction of potential adverse neurodevelopmental outcomes from perchlorate exposure. The Agency should incorporate the appropriate studies related to ingestion of perchlorate, pharmacokinetics of perchlorate, and the effects (dynamics) of perchlorate from the entire body of literature available, including available data on potential adverse health effects due to thyroid hormone level perturbations regardless of the cause of those perturbations.

Although this approach is a departure from the usual method for MCLG calculation, it is consistent with the Agency’s increasing use of PBPK/PD modeling in support of risk assessment. The Agency will need to develop a peer reviewed technical document to provide transparency and document the additional factors used in the PBPK/PD model. The SAB has made specific recommendations on ways in which information from clinical and epidemiological studies can be used to inform the model.

The SAB notes that as perchlorate research continues, studies in animals may provide important insights into neurobehavioral consequences of perchlorate exposure. A PBPK/PD framework is well suited to help place these findings in the context of human perchlorate exposure.

The SAB appreciates the opportunity to provide EPA with advice and looks forward to the Agency’s response.

Sincerely,
This report has been written as part of the activities of the EPA Science Advisory Board (SAB), a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The SAB is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names of commercial products constitute a recommendation for use. Reports of the SAB are posted on the EPA website at http://www.epa.gov/sab.
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This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

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Acronyms and Abbreviations

1. µg  Microgram (one-millionth of a gram)
2. µmU  Micromolar Units
3. ADHD  Attention Deficit Hyperactivity Disorder
4. BBDR  Biologically Based Dose Response
5. BW  Body Weight
6. DWI  Drinking Water Ingestion Rate
7. EPA  U.S. Environmental Protection Agency
8. FDA  Food and Drug Administration
9. fT4  Free T4
10. GW  Gestational Week
11. HRL  Health Reference Level
12. IUI  Iodine Uptake Inhibition
13. kg  Kilogram
14. L  Liter
15. MCL  Maximum Contaminant Level
16. MCLG  Maximum Contaminant Goal Level
17. MOA  Mode of Action
18. NHANES  National Health and Nutrition Examination Survey
19. NIS  Sodium (Na)/iodide (I) Symporter
20. NOEL  No Observed Effect Level
21. NPDWR  National Primary Drinking Water Regulation
22. NRC  National Research Council
23. PBPK  Physiologically-Based Pharmacokinetic
24. PPBK/PD  Physiologically-Based Pharmacokinetic Pharmacodynamic
25. POD  Point of Departure
26. PWS  Public Water System
27. RAIU  Radioactive Iodide Uptake
28. RD  Reference Dose
29. RSC  Relative Source Contribution
30. SAB  Science Advisory Board
31. SDWA  Safe Drinking Water Act
32. T3  Triiodothyronine
33. T4  Thyroxine or Tetraiodothyronine
34. TDS  Total Dietary Study
35. TgAb  Thyroglobulin antibody
36. TH  Thyroid Hormones
37. TPOAb  Thyroid peroxidase antibody
38. TSH  Thyroid stimulating hormone or thyrotropin
39. UF  Uncertainty factor
40. US  United States
1. EXECUTIVE SUMMARY

In 2005, at the request of EPA and other federal agencies, the NRC published a comprehensive report “Health Implications of Perchlorate Ingestion.” The NRC concluded that perchlorate can affect thyroid function because it is an ion that competitively inhibits the transport of iodide into the thyroid and that a prolonged decrease of thyroid hormone is potentially more likely to have adverse effects in sensitive populations (people with thyroid disorders, pregnant women, fetuses, and infants).

The NRC recommended the use of a precursor, non-adverse effect (i.e., inhibition of iodide uptake) to derive a reference dose (RfD) for perchlorate. An RfD is defined by EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.” The RfD was based on the no observed effect level of 7 µg/kg/day corresponding to a radioactive iodide uptake inhibition (RAIUI) of 1.8 percent and application of an intraspecies uncertainty factor (UF) of 10 to account for differences in sensitivity between the healthy adults in the 2002 Greer et al. study and the most sensitive population, fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The NRC also acknowledged that the RfD may need to be adjusted upward or downward on the basis of future research. The RfD of 0.7 µg/kg/day was adopted by EPA in 2005.

EPA has identified perchlorate as a drinking water contaminant and initiated the process to develop a Maximum Contaminants Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) under the Safe Drinking Water Act (SDWA) (US EPA 2011). The MCLG is a non-enforceable goal defined under the SDWA as “the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety.” The SDWA specifies that the enforceable Maximum Contaminant Level be set as close to the MCLG as feasible using the best available technology, treatment techniques, and other means (taking cost into consideration). SDWA further requires that when proposing any NPDWR that includes an MCL, the Administrator must analyze “[t]he effects of the contaminant on the general population and on groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other subpopulations that are identified as likely to be at greater risk of adverse health effects due to exposure to contaminants in drinking water than the general population.”

EPA developed a white paper that identifies and summarizes recent studies and physiologically based pharmacokinetic (PBPK) modeling it is evaluating, in addition to the data and information used by the NRC. The Agency is evaluating these and previously published studies to consider different life stages that comprise groups within the general population that are likely to be at greater risk of adverse health effects (i.e., sensitive subgroups or sensitive life stages). EPA’s Office of Water requested the SAB to provide advice on how the Agency should consider recent information on sensitive life stages, epidemiological and biomonitoring studies and the Agency’s PBPK modeling efforts, and on approaches to use and integrate this information in deriving an MCLG.
The SAB finds that there is sufficient information to derive an MCLG for perchlorate. However, SAB recommends that the Agency use a mode of action approach (MOA) and physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling to integrate this information in a robust and transparent analysis. The SAB recognizes that this is a novel approach from previous MCLG derivations that formulaically use the RfD and exposure factors. However, it finds that this approach provides a more transparent tool to integrating the body of information available on perchlorate and may better present differences in life stage considerations than the standard MCLG approach. Expansion of the model to address important aspects of vulnerability to perchlorate is strongly recommended. In particular, expanding the model beyond prediction of inhibition of thyroid uptake to effects on thyroid hormone levels will be especially important to capture factors that may contribute to perchlorate sensitivity, such as iodine intake or underlying thyroid disease. Additional research could be used to extend the model to prediction of neurobehavioral effects resulting from specific concentrations of perchlorate in drinking water.

**Sensitive Life Stages**

The SAB concludes that a sensitive life stage analysis is critical to derive an MCLG for perchlorate. The specific adverse effects of low thyroid hormone levels and inadequate iodide uptake on brain development vary at different life stages. The fetus and infant are more susceptible to perchlorate exposure effects than the adult is. At different life stages, there are multiple tissue and organ targets for perchlorate and, depending on life stage, the specific targets and outcomes of perchlorate exposure on individual targets may change. Moreover, an acute exposure may be more harmful in the fetus and infant than the adult. Although no data exist on the long-term adverse neurodevelopmental effects of perchlorate per se, the data on the adverse effects of thyroid hormone perturbations (a down-stream effect from iodide uptake inhibition) on the developing brain justify the need for a life stage approach. The evidence suggests that the most sensitive life stages for the potential permanent adverse effects of perchlorate on brain development are the hypothyroxinemic pregnant woman and, specifically, her fetus and infants.

**Physiologically-based Pharmacokinetic Modeling**

EPA should utilize a MOA framework for developing the MCLG that links the different steps in the proposed mechanism leading from perchlorate exposure through iodine uptake inhibition to thyroid hormone changes and finally neurodevelopmental impacts. Within this MOA framework, the PBPK/PD iodine uptake inhibition (IUI) model provides a tool for integrating exposure (e.g., different drinking water consumption rates) with the biological changes occurring at the different lifestages to obtain predictions for perchlorate pharmacokinetics and resulting iodine uptake inhibition to address these initial steps of the MOA framework. Extension of the PBPK/PD-IUI model to describe changes in thyroid hormone levels should be expeditiously incorporated as this would provide a key tool for linking these early events with
subsequent events as reported in the literature on iodide deficiency, changes in thyroid hormone levels
and their relationship to neurodevelopmental outcomes during these sensitive early life stages.

Epidemiological Data

The SAB finds that the post-NRC epidemiology data are useful for estimating the size of potentially
sensitive subgroups in the U.S., estimating the extent to which the general U.S. population and sensitive
subgroups are exposed to perchlorate, as well as other goitrogens, and estimating the relative source
contribution of perchlorate in drinking water among sensitive subgroups not addressed in the Food and
Drug Administration’s total diet study.

The SAB concludes that the post-NRC epidemiology data are insufficient to guide causal inference with
regard to the association between perchlorate exposure and thyroid dysfunction in pregnant women,
neonates or the general population. Limitations concerning study design, exposure assessment, sample
size, and statistical modeling have led to inconsistent results. Thus, the current body of epidemiologic
evidence cannot provide validation of a safe level of perchlorate in drinking water.

Integration of Information

The SAB recommends an alternative and novel approach to integrate the body of information on
perchlorate to derive an MCLG based on the MOA previously identified for perchlorate. The
recommended approach relies on the use of a PBPK/PD model that correlates perchlorate intake via
drinking water with percent iodide uptake inhibition. The SAB recommends that EPA expeditiously
expand this approach to empirically relate the percent iodine uptake inhibition with thyroid hormone
perturbations, specifically hypothyroxinemia. A long-term goal should be extension of the model to
prediction of potential adverse neurodevelopmental outcomes from perchlorate exposure. The Agency
should incorporate the appropriate studies related to ingestion of perchlorate, pharmacokinetics of
 perchlorate, and the effects (dynamics) of perchlorate from the entire body of literature available. In
developing the pharmacodynamic aspect of this model, the EPA should take advantage of available data
on potential adverse health effects due to thyroid hormone level perturbations regardless of the cause of
those perturbations.

The SAB recommendations represent an important and novel opportunity that should be implemented
carefully with attention to data quality and methodological rigor. At each step, EPA should critically
evaluate available data and describe the strengths and limitations. The SAB concludes that a stepwise
“integrated” approach is a logical way forward allowing multiple sources of information to be integrated
into the MCLG derivation.
2. INTRODUCTION

2.1. Background

In 2005, at the request of EPA and other federal agencies, the National Research Council (NRC) published a comprehensive report “Health Implications of Perchlorate Ingestion” (2005). The NRC concluded that perchlorate can affect thyroid function because it is an ion that competitively inhibits the transport of iodide into the thyroid by a protein known as the sodium /iodide symporter (NIS). Significant inhibition of iodide uptake results in intra-thyroid iodine deficiency, decreased synthesis of key thyroid hormones triiodothyronine (T3), thyroxine (T4), and increased thyroid stimulating hormone or thyrotropin (TSH). The NRC also concluded that a prolonged decrease of thyroid hormone is potentially more likely to have adverse effects in sensitive populations (e.g., people with thyroid disorders, pregnant women, fetuses, and infants).

The NRC recommended the use of a precursor, non-adverse effect (i.e., inhibition of iodide uptake) to derive a reference dose (RfD) for perchlorate. An RfD is defined by EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.” The RfD was based on the no observed effect level of 7 µg/kg/day corresponding to a radioactive iodide uptake inhibition RAIU of 1.8 percent and application of an intraspecies uncertainty factor (UF) of 10 to account for differences in sensitivity between the healthy adults in the Greer et al., (2002) study and the most sensitive population, fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The NRC also acknowledged that the RfD may need to be adjusted upward or downward based on future research. The RfD of 0.7 µg/kg/day was adopted by EPA in 2005 (U.S. EPA, 2005).

EPA has initiated the process to develop a MCLG and NPDWR for perchlorate under the SDWA (EPA 2011). The MCLG is a non-enforceable goal defined under the SDWA (§1412.b.4.B ) as “the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety.” For perchlorate, the NPDWR will likely specify an enforceable Maximum Contaminant Level (MCL) and monitoring and reporting requirements for public water systems. The SDWA (§1412.b.4.B and D) specifies that the enforceable MCL be set as close to the MCLG as feasible using the best available technology, treatment techniques, and other means (taking cost into consideration).

The regulatory schedule established by SDWA requires EPA to publish a proposed MCLG and NPDWR within 24 months of making a determination to regulate a contaminant and promulgate a final regulation within 18 months of the proposal. SDWA further requires that when proposing any NPDWR that includes an MCL, the Administrator must analyze “[i]the effects of the contaminant on the general population and on groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other subpopulations that are identified as likely
to be at greater risk of adverse health effects due to exposure to contaminants in drinking water than the
general population.\(^1\)"

EPA developed a White Paper (2012) that identifies recent information since the NRC report (2005). The White paper presents epidemiological and biomonitoring studies and physiologically based pharmacokinetic (PBPK) modeling EPA is evaluating, in addition to the data and information used by the NRC, to consider different life stages that comprise groups within the general population that are likely to be at greater risk of adverse health effects (i.e., sensitive subgroups or sensitive life stages).

EPA’s Office of Water requested the Science Advisory Board’s (SAB) advice on how best to consider the sensitive life stages, the available epidemiological studies, and PBPK modeling, and integrate this information in deriving an MCLG for perchlorate. The SAB formed an ad-hoc panel, the Perchlorate Advisory Panel, to perform this task. The Panel met on July 18-19 2012 to hear EPA technical presentations, public comments and to discuss response to the Charge to the SAB (76 FR 78256-78257). The Panel met on a follow-up teleconference on September 25, 2012 to discuss an initial draft report.

### 2.2. Charge to the Science Advisory Board

The Charge to the SAB seeks advice and recommendations on approaches to derive an MCLG for perchlorate. EPA identified recent studies on life stage information for infants and children, epidemiologic and biomonitoring data since the NRC report (2005), and physiologically based pharmacokinetic modeling that address the iodine uptake inhibition and the decreased synthesis of thyroid hormones. The Agency is seeking advice on how to consider these studies and models in terms of different life stages and adverse effects, approaches to include the information in deriving an MCLG, and what are the strengths and limitations of the studies. The Charge also asks the SAB how best to integrate the total body of information to derive a health protective MCLG. Charge questions are included at the beginning of each section of this Report and the full Charge is included as Appendix A.

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\(^1\)SDWA uses the term subpopulation to refer to groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other groups that can be identified and characterized and are likely to experience elevated health risks. In 2005 EPA started using the term life stages to refer to age-defined groups. All life stages are subpopulations but not all subpopulations are life stages. In this document, the term life stage is used predominantly because of the focus on infants and very young children.
3. RESPONSE TO CHARGE QUESTIONS

The specific charge questions focus on how various life stage factors, PBPK modeling, and post-NRC epidemiological and biomonitoring studies should be considered in MCLG development. A fourth set of charge questions address the related issue of how this and other information should be integrated into development of a health protective MCLG and how reductions in adverse health effects from lowering perchlorate concentrations in drinking water can be estimated.

The discussion of iodide deficiency, thyroid hormone inhibition, and sensitive life stages issues identified the hypothyroxinemic pregnant woman, her fetus and infants as the most sensitive portion of the population. The SAB also noted the Agency’s progress in using PBPK models to better understand the potential impacts of perchlorate exposure during different life stages. In review in the epidemiological and biomonitoring studies, the SAB identified data of value in assessing risk of perchlorate exposure, but found that limitations in the studies precluded their use in deriving the MCLG.

When considering how to integrate the disparate information and analyses into the derivation of an MCLG, the SAB found that the traditional algebraic approach provides limited ability to address the various exposure and biological factors affecting sensitivity to perchlorate at different life stages in a transparent manner. The panel concluded that an alternative approach based upon perchlorate mode of action, using PBPK/PD modeling to relate perchlorate concentrations in drinking water to biological effects, would be much better from a scientific standpoint. Some of the charge question responses describe this recommended approach for MCLG derivation directly, especially responses to some of the charge questions regarding PBPK/PD modeling and integration of information. Responses to other charge questions are also relevant, providing comments and recommendations that will be important to the Agency in implementing this novel method of MCLG development.

3.1. Sensitive Life Stages

Charge Questions:

There are currently no data available to directly link perchlorate to neurobehavioral effects in infants and children. How should 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.
3.1.1. **Rationale for Considering Life Stages in Deriving an MCLG**

The SAB finds that there is a critical need to consider sensitive life stages in deriving an MCLG for perchlorate. The SAB recognizes that the literature to directly link perchlorate to neurobehavioral effects in infants and children is lacking. However, the SAB notes that human clinical and rodent toxicology literature describing the biology linking iodine/iodide deficiency, changes in thyroid hormone production, and developmental neurobehavioral effects is robust. The mechanisms of perchlorate inhibition of iodine uptake into the thyroid are also well documented. Therefore, the SAB concludes that these two lines of information are complimentary and sufficient for the EPA to consider specific life stage factors in deriving an MCLG for perchlorate. The SAB also notes that the specific adverse effects of low thyroid hormone levels and inadequate iodide uptake on brain development vary at different life stages.

The thyroid hormones (TH) triiodothyronine (T3) and tetraiodothyronine or thyroxine (T4) are characterized by their distinctive structure of three or four iodine atoms respectively that are attached to a thyronine molecule. The incorporation of these atoms takes place on a large precursor molecule called thyroglobulin found in the colloid of the thyroid (Carrasco N 1993). The resulting T3 and the more abundant T4 are both transported from the thyroid via the bloodstream to various essential target organs. One of the primary target organs is the brain, which has a well-defined need for TH for its normal development (Zoeller and Rovet 2004). A deficit of TH leads to poor brain development that may ultimately cause intellectual and behavioral impairments in the developing child (Morreale de Escobar et al. 2000). Since the iodide needed for T3 and T4 production cannot be synthesized within the body, it must be obtained through the diet, and this requires a constant and sufficient supply of iodide to ensure normal thyroid function (Carrasco 1993). Children who experience iodide or TH insufficiency during critical stages of brain development (e.g., during gestation and infancy) are especially at risk of neurological, mental, and growth impairments. During pregnancy, the need for iodide is increased to support the higher demand for TH at this time.

Dietary iodide is transported from the bloodstream into the thyroid via the sodium/iodide symporter (NIS), an intrinsic plasma membrane protein of 643 amino acids (Dai et al. 1996, Smanik et al. 1996, Riesco-Eizaguirre and Santisteban 2006). This transport process is the first and key rate-limiting step in the biosynthesis of T3 and T4. NIS is also expressed in the salivary glands and stomach, two tissues where active iodide transport also takes place. Notably, NIS is highly expressed in the placenta and lactating breast, allowing iodide to be supplied to the fetus and the breast-feeding infant (Tazebay et al. 2000, De La Vieja et al. 2000, Dohan et al. 2003).

Perchlorate inhibits iodine uptake and therefore interferes with TH production. Perchlorate acts by specifically inhibiting NIS-mediated transport of iodide into the thyroid, as well as in the placenta and lactating breast. Although perchlorate has long been known to act as a competitive NIS inhibitor, recent studies show that perchlorate is actually an actively transported NIS substrate (Dohan et al. 2007, Tran et al. 2008, Paroder-Belenitzy et al. 2011). Consequently, a primary downstream effect of perchlorate exposure is reduction in the levels of T3 and T4 that are ultimately needed by the developing brain. Clearly, in the presence of perchlorate, less iodide is available for TH biosynthesis.
Although evidence is lacking directly linking perchlorate intake to altered human brain development, animal evidence is suggestive of perchlorate intake impacting mammalian brain development. It is important to note that any observed changes in brain development caused by perchlorate exposure should be considered adverse until otherwise shown to be non-adverse due to the difficulty in correlating changes in brain development (e.g. altered expression patterns of TH-regulated brain genes) with functional effects.

Recommendation:
The SAB recommends that the EPA consider the sensitive life stages of the fetus, neonate, infant, child, and pregnant and lactating woman in modeling target levels for perchlorate exposure.

3.1.2. Life Stage Specific Differences in Body Weight and Intakes

Considering specific differences in body weight, food intake, and drinking water consumption are important factors to understanding perchlorate induced iodide uptake inhibition (IUI) at different life stages. Although body weight would seem to be related to how much perchlorate enters the body at different life stages, the SAB recognizes that the critical evidence linking exact body size and food and drinking water intake to iodide uptake is lacking. However, evidence is available from the literature on other drug and chemical exposures showing differing absorption and metabolism rates with age and body weight of other pharmaco-active substances (Kearns et al. 2003, Bartelink et al. 2006, Anderson et al. 2009). These effects are likely identical with perchlorate intake and its inhibition of iodide uptake into the thyroid and other tissues. In addition, since NIS is expressed in tissues other than the thyroid, such as the salivary glands, stomach, lactating breast, and placenta, perchlorate exposure in fetuses, neonates and young children is distinctly different from that in the nonpregnant and non-lactating adult. Thus, pregnant and lactating women and their offspring (the fetus and breast-fed infant) are more vulnerable to perchlorate effects than are individuals at other life stages.

Recommendation:
The SAB notes that the EPA developed a PBPK model that considers life stage differences in thyroid NIS inhibition and has continued to develop this model (EPA 2009 and 2012). Using the PBPK modeling approach (see Section 3.2), life stage specific differences in body weight and food and drinking water intakes can and should be explicitly incorporated in the modeling of each life stage and documented. Additionally, differences in other parameters characterizing the biological system, such as organ weight (volumes) or NIS activity should be incorporated.

The SAB recommends that NIS expression in different tissues and at different stages be addressed using the PBPK model. The modeling effort should account for perchlorate and its movement into relevant organs that inhibit the iodide supply and may ultimately interfere with availability of TH for brain development.
3.1.3. Differences in Potential Adverse Effects to Neonates, Infants and Young Children

The SAB finds that the sensitivity and permanence of potential effects from TH inhibition to brain development of neonates, infants, and children are significantly different from the TH inhibition effects in adults. THs are essential for normal brain development (Bernal and Nunez, 1995, Anderson 2001). A broad and diverse literature, based primarily on rodents, has shown that T3 and T4 are translocated across the blood-brain barrier by specific transporters, metabolized by local deiodinases, and then transported into neural target cells by other specific transporters (Kester et al. 2004). Here, T3 serves as a transcription factor to up- or down-regulate key brain genes fundamental to neurodevelopmental processes (Bernal 2007, Anderson et al. 2003), such as neurogenesis, neuronal migration, process growth, synaptogenesis, and myelination (Chan and Rovet 2003). The timing of these processes is critical and varies during gestation and early life within different brain tissues (Zoeller and Rovet, 2004). Thus, the consequences of TH insufficiency, regardless of cause, will therefore differ depending on when the insufficiency occurs (Royland et al. 2008). Furthermore, since different brain regions vary as to when they need TH in development (Thompson and Potter 2000), the specific consequences of TH insufficiency or iodide deficiency will also differ regionally within the brain (Schweizer et al 2008).

The SAB also notes that neurodevelopment occurs along a continuum through gestation to childhood. It is important to note that the human thyroid and its regulation have a protracted development (Ballabio et al. 1989).

It is essential to obtain robust data assessing the long-term effects of perchlorate exposure on thyroidal iodide uptake, and resultant impact on thyroid function, as measured by TSH and free T4 levels, in both human and animal models. In contrast to studies of perchlorate effects on neurodevelopment, the iodide deficiency literature is robust and provides key data identifying the range of thyroidal perturbation attributable to reductions in iodide availability to the thyroid gland. The importance of this broad area of research to interpreting the results of perchlorate studies is that the ultimate mechanism of perchlorate toxicity is known: perchlorate limits the access of iodide to the thyroid. Iodide insufficiency results in the same limited access of iodide to the thyroid. These data can be compared to the known neurodevelopmental effects of mild, moderate and severe iodide deficiency on human and animal brain development. The SAB finds that the currently available studies are insufficient to draw unequivocal conclusions regarding the impact of perchlorate exposure on human brain development.

3.1.4. Thyroid Hormone Reserve Differences

It is known that infants have lower reserves of TH than adults do and that the infant TH has a shorter half-life than adult TH. However, the key evidence linking these features to perchlorate levels, iodide levels, and outcome is lacking. Nevertheless, it is established that during pregnancy and lactation, the maternal need for TH and iodide is increased by as much as 50%. Thus, the EPA should consider these two key features in comparison with the non-pregnant adult, based on the Greer et al study (2002). It should also be noted that this study was limited for various methodological reasons.
Recommendation:

The EPA should consider the shorter half-life and lower reserves for thyroid hormone and metabolic differences between sensitive life stages in determining safe levels of perchlorate in drinking water. This issue may also be studied in animals using appropriate experimental designs.

3.1.5. Intrauterine Exposure to Perchlorate and Thyroid Status Impact in Fetuses

The SAB finds that intrauterine exposure to perchlorate has the potential to affect the developing fetus in several ways. First, it can lead to less iodide for the fetal thyroid. In addition, it can mean less maternal TH because her iodide supply has been reduced. In early pregnancy, prior to the onset of fetal thyroid function, the main disruption will be less maternal TH, whereas later in gestation, when the fetal thyroid needs iodide to make her/his own TH, both maternal and fetal supplies of TH will be reduced. This hypothyroxinemia will likely have an impact on the fetal brain, affecting those pathways that have the highest need for TH at the time. In addition, maternal hypothyroxinemia in pregnancy can have adverse reproductive as well as pregnancy outcomes, including preterm delivery.

Although the fetal thyroid develops in the first trimester of pregnancy, it does not secrete TH until the second trimester, and is not centrally regulated by the hypothalamus and pituitary (which secrete TRH and TSH) until the third trimester. Furthermore, the fetal thyroid continues to grow throughout gestation. Nevertheless, autopsy evidence indicates that the brain appears to need TH very early in gestation, given that TH receptors and measurable quantities of maternally derived TH are found in the fetal brain in the first trimester (Kilby et al. 2000). Since substantial quantities of maternal TH are also observed both in fetal compartments throughout gestation (Calvo et al. 2002) and in neonatal serum at term (Vulsma et al. 1989), an adequate maternal supply of TH to the fetus is necessary until the end of pregnancy. After birth, small amounts of TH may be transferred from the mother to the infant via breast milk (Rovet 1990). This dual maternal–fetal/child system typically allows for normal brain development, unless either maternal or child TH supplies are inadequate.

Women with inadequate levels of TH during pregnancy due to clinical or subclinical hypothyroidism or hypothyroxinemia are unable to provide the fetus with sufficient TH (Moleti et al. 2011). It is well established that the offspring of these women are at risk for poor outcomes, including mild to severe IQ reductions, specific cognitive and motor deficits, learning disabilities, and behavioral problems (Man et al. 1991, Smit et al 2000, Mirabella et al 2000; Haddow et al 1999; Pop et al 1999; Heinrichs et al. 2010, Kooistra et al. 2006). Even the least severe TH inadequacy, maternal hypothyroxinemia, when occurring during gestation, has been associated with neurological impairment (Morreale de Escobar et al. 2004).

Furthermore, iodide deficiency during pregnancy and early neonatal life is also associated with impaired development of the brain and suboptimal outcome since pregnant and lactating women from iodide deficient areas provide insufficient iodide to their offspring through the placenta or breast milk (Zimmerman 2009). Finally, children who are TH-deficient due to congenital hypothyroidism or iodide deficiency also show suboptimal to poor neurodevelopmental outcome depending on the severity and duration of the TH or iodide deficiency (Rovet and Daneman 2003, Viermiglio et al. 2004). Because most TH-mediated brain development is only complete by the age of two years, the fetus, infant, and young child are especially vulnerable to the effects of both TH and iodide deficiency.
Since perchlorate inhibits iodide transport to the thyroid, exposure to perchlorate will have a direct impact on the maternal thyroid, the fetal thyroid, and the child’s thyroid throughout his/her development. Perchlorate is likely to have downstream effects on the developing brain that are similar to those observed in studies of iodide and TH deficiency. To date, however, no studies exist that have directly examined the relation between perchlorate exposure, its thyroidal impact, and the developing human brain. Nevertheless, a recent study with perchlorate-exposed rodents showed subtle and specific brain and learning impairments that directly reflect the perchlorate dosing regimen (Axelstad et al. 2008).

From studies of the developing human thyroid, it is expected that in early pregnancy, when the fetus relies entirely on the maternal supply of TH to meet its brain needs, perchlorate exposure will lead to reduced TH from the mother, and this will have an impact on developing brain functions at this time. Once the fetal thyroid starts to function in the second trimester, the fetus will require its own supply of iodide in order to make TH. Thus, perchlorate actively transported through the placenta via NIS may block fetal iodide uptake into the thyroid, leading to a lowered production of TH. This, along with the already reduced maternal TH supply, will likely lead to a state of fetal hypothyroxinemia throughout pregnancy. After birth, perchlorate exposure may reduce the infant’s capacity to synthesize TH by blocking iodide supply in two possible ways: through the water added to formula preparations or through breast milk. Notably, breast-fed infants exposed to perchlorate will also receive less TH in the milk than non-exposed infants because their mother’s TH production has been compromised by her reduced iodide supply caused by perchlorate; the infants’ own capacity to produce TH will be reduced. Older infants and young children may be affected by perchlorate in dairy milk and certain foods. Overall, these findings signify that perchlorate exposure at these different sensitive life stages can lead to less available TH, which in turn can adversely affect brain development in gestation and infancy.

Although some literature does exist examining perchlorate levels in relation to maternal and neonatal TH levels, the findings are contradictory; furthermore, the evidence is limited methodologically and/or the statistical approach is inadequate.

The fetus and infant are more susceptible to effects from perchlorate exposure than the adult (as above) is. Moreover, an acute exposure to perchlorate may be more harmful in the fetus/infant than in the adult. Although no data exist on the long-term adverse neurodevelopmental effects of perchlorate per se, the data on the adverse effects of TH perturbations (a downstream target) on the developing brain justify the need for a life stage approach.

**Recommendation:**

It is important that future studies monitor maternal iodide and thyroid hormone levels throughout pregnancy in relation to perchlorate exposure and reproductive/pregnancy outcomes. Future studies may also consider measuring fetal integrity directly using measurements of heart rate, ultrasound measures of fetal thyroid, fetal movement, growth, and response to stimulation (Allen and Lipkin 2005). In light of advances in neuroimaging of the fetus and neonate, future research may also consider conducting such measurements on perchlorate exposure at different levels.
Strengthening Future Research

These recommendations are made to guide both the interpretation of the strengths and weaknesses of previously published studies and provide advice for the design of new research studies. Future research in humans needs to improve on methodological and statistical techniques as well as capitalize on repositories containing early blood samples and those where subsequent cohorts have also been assessed. Several suggestions include:

a) correlating the perchlorate levels found by Pearce in the Lazarus cohort with later outcome in children (Lazarus et al. 2012),

b) measuring perchlorate levels in the Generation R study samples and correlating these with child outcome data (Gassabian et al. 2012),

c) measuring perchlorate levels in the ongoing I2S2 study of iodide therapy for preterm infants in relation to child outcome in Scotland,

d) measuring perchlorate levels in the stored samples of the CDC study of maternal hypothyroidism and child outcome, and

e) measuring perchlorate levels along with other endocrine disruptors in long-term follow-up studies of the Children’s Environmental Health Centres funded by NIEHS/EPA. Studies examining child outcome following measurement of perchlorate in breast milk versus formula also need to be conducted.

In addition, outcome measures, including child developmental and behavioral questionnaires, may be provided to participants in ongoing NHANES studies. Furthermore, any study proposing to measure outcome in relation to early TH levels should also include serum measurements of perchlorate and iodide levels. Finally, thyroid function tests should include repeated measurements of both TSH and free T4 to better correlate perchlorate-induced thyroidal perturbation with possible neurodevelopmental effects. This recommendation is made so we can better assess the impact of perchlorate exposure on TH levels, particularly the impact of mild to severe TH deficits on brain development.

3.2. Physiologically-Based Pharmacokinetic Modeling

Charge Questions:

How should 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?

3.2.1. Considering PBPK Modeling to Derive an MCLG for Perchlorate

The NRC committee made a recommendation to use inhibition of iodide uptake by the thyroid arising from competitive inhibition of the NIS by perchlorate as the first step in the MOA for perchlorate leading to all subsequent events (See Figure 1) (NRC 2005). The NRC indicated it was relevant for perchlorate risk assessment and provided a health-protective and scientifically valid approach, which has been incorporated by EPA in the derivation of the perchlorate RfD (0.7 μg/Kg/day). The physiologically
based pharmacokinetic/pharmacodynamic-iodide uptake inhibition (PBPK/PD-IUI) model links perchlorate exposure in food and water with perchlorate concentrations in plasma and tissue and resulting NIS inhibition assessed by radioactive iodide uptake (RAIU) studies. The continuum of events in the mode of action after NIS inhibition would include possible changes in serum thyroid hormone levels, which have been linked with neurodevelopmental changes in iodine-deficient individuals during early life stages. Using the MOA framework, the model provides a key tool for assessing the potential for the upstream step (iodide uptake inhibition) at different lifestages or sensitive populations. This MOA framework should be a good way to determine the MCLG using the percent IUI as a surrogate for the adverse effect.

Figure 1. NRC suggested mode of action for perchlorate toxicity in humans indicating the first adverse effect in the continuum. Reprinted with permission from Health Implication of Perchlorate Ingestion, 2005 by the NAS. Courtesy National Academy Press.

Research scientists at the toxicology laboratory at Wright-Patterson AFB developed a series of physiological models to describe the effect of perchlorate on the inhibition of thyroidal uptake of radiotracer iodide (Fisher et al. 2000, Clewell et al. 2003a and b, Merrill et al. 2003 and 2005). These models included the adult rat, pregnant rat and fetus, and the lactating rat and rat pup, and the adult human. The PBPK/PD-IUI models described the uptake, distribution and urinary elimination of both perchlorate and radiotracer iodide anions. Serum levels of perchlorate and radiotracer iodide are predicted to describe active transport of perchlorate and radiotracer iodide into cells containing the NIS protein, such as the thyroid gland, small intestine, placenta, and mammary tissue. Both anions compete for active uptake by NIS containing tissues. The inhibition of thyroidal uptake of radiotracer iodide by
perchlorate is recognized as the primary mode of action for perchlorate leading to possible disruption of
the hypothalamic-pituitary-thyroid (HPT) axis by depleting the thyroid gland of iodide used in
synthesizing thyroid hormones. Radioactive iodide uptake (RAIU) inhibition for the thyroid gland is
measured for different doses of perchlorate. Later the PBPK/PD-IUI human model for perchlorate and
radiotracer iodide was extended to human life stages (Clewell et al. 2007) to make RAIU inhibition
predictions in the fetus and infant and child, the sensitive sub-population. Model predictions of RAIU
inhibition are presented in the EPA White Paper. This modeling approach starts to answer questions
about sensitivity of life stages to RAIU inhibition that otherwise are only qualitative justifications for the
uncertainty factor (UF) of 10 used in the RfD to protect sensitive populations.

Future mathematical models should describe HPT axis events after RAIU inhibition in human life
stages. This has been accomplished in the adult rat (McLanahan et al. 2008, 2009) and ongoing efforts
were reported for the pregnant mother and fetus (Lumen et al. 2012). Lumen and coworkers described
the serum pharmacokinetics of perchlorate and dietary iodide in the near term pregnant mother and
fetus, thyroid stores of thyroid hormones, and serum bound and fT4 and total T3. The competitive
inhibition of each anion (perchlorate and dietary iodide) on the other for uptake by the NIS is described
for the thyroid gland and placenta. Serum fT4 levels in the mother and fetus were predicted at steady
state for a range of dietary iodide intakes ranging from mild iodide deficiency (75 µg/day) to sufficient
iodide intake (250 µg/day) with no perchlorate intake (exposure) and for a range of perchlorate intakes
(0.00001 to 1.0 mg/kg/d). The authors predicted the exposure conditions for perchlorate, under varying
dietary iodide diets, that would result in serum fT4 levels associated with hypothyroxinemia (decrease in
serum T4 and no change in serum TSH) and for the onset of hypothyroidism (increase in serum TSH
and decrease in serum fT4 levels). This biological based dose response (BBDR) model for the HPT axis
in the pregnant woman and fetus provides a quantitative approach to better understand the adverse
health consequences (hypothyroxinemia and hypothyroidism) using a MOA-based analysis of
perchlorate exposure for a range of dietary iodide intakes. A substantial enhancement in this modeling
effort reported by Lumen et al. would be to perform Monte Carlo analysis to address variability in the
human population (2012). Other NIS inhibitors (e.g., thiocyanate, nitrate) contributions to NIS inhibition
also could be incorporated in the modeling, but may be addressed as qualitative uncertainties at this
time.

Documenting the MOA framework and the PBPK/PD-IUI model to make them accessible to non-
modelers will be an important challenge for EPA. By comparison with the simple algebraic default
equation, describing an MCLG as a function of a few terms (e.g. RfD, body weight, water intake, and
source contribution) the proposed analysis could appear opaque despite the fact that it captures much
scientific information. The model documentation would describe model structure, data used to establish
that structure and estimate parameter values, sensitivity of model outputs such as NIS inhibition to
parameters, and characterization of the model strengths and limitations. Publications on model
evaluation and documentation (Clark et al. 2004, Chu et al. 2007, Thompson et al. 2008) as well as the
World Health Organization International Programme on Chemical Safety PBPK Guidance (IPCS, 2010)
provide useful approaches for developing documentation. This documentation would also reference the
published literature on the model and the 2008 EPA peer-review of the PBPK/PD-IUI model and its
subsequent revisions.
Recommendations:

The SAB recommends that EPA should utilize a MOA framework for developing the MCLG that links the different steps in the proposed mechanism leading from perchlorate exposure through NIS inhibition to thyroid hormone changes and finally neurodevelopmental impacts. Within this MOA framework, the PBPK/PD-IUI model provides a tool for integrating exposure (e.g., different drinking water consumption rates) with the biological changes occurring at the different life stages to obtain predictions for perchlorate pharmacokinetics and resulting symporter inhibition to address these initial steps of the MOA framework.

Extension of the PBPK/PD-IUI model to describe changes in thyroid hormone levels should be expeditiously incorporated as this would provide a key tool for linking these early events with subsequent events as reported in the literature on iodide deficiency, changes in thyroid hormone levels, and their relationship to neurodevelopmental outcomes during these sensitive early life stages.

Development of a clear communications strategy, including documentation of the MOA framework and the PBPK model, will facilitate stakeholder and public understanding of methods used in the development of the MCLG.

3.2.2. Strengths and Limitations of EPA’s PBPK Model Results

The two analyses EPA presented in the White Paper address different aspects of the model and its use in developing an MCLG (US EPA 2012). The first analysis (Table A3) evaluates the predicted RAIU inhibition for the same perchlorate dose (7 μg/kg/day) that arises from biological variations captured in the PBPK model for different life stages. This analysis helps support the use of the UF in deriving the RfD as it predicts greater inhibition at fetal and neonatal/infant life stages as compared to the adult. The second analysis (Table A4) evaluates the combined effects of life stage-dependent differences in exposure (e.g., drinking water consumption) with the biological variability by assessing the predicted RAIU inhibition at fixed drinking water concentrations.

Some strengths and limitations of the first analysis of life stage dependent biological variability were identified. A limitation of the first analysis is the selection of the urinary excretion rate for perchlorate. Literature for iodide excretion indicates the rate is faster in neonate/infants than at later ages, which might then be expected to be the case for perchlorate (Malvaux et al. 1965, Oddie et al. 1966, Ponchon et al. 1966). The values in the model need to be reassessed and justified. While the model addresses life-stage variations, it is a model of the average human at each life stage. Extension of the model to a full population description would be useful, but it is recognized that this would be a major effort. Sensitivity analyses for model predictions could be useful for identifying key parameters to make such analyses more tractable. The human biological modeling uses life-stage specific uptake rates mediated by NIS levels but does not reflect changes in NIS in response to TSH regulation or potential effects of chronic perchlorate exposure. A strength of the analysis is that EPA evaluated the model’s capability to describe both perchlorate transport into breast milk as well as assessing the expected impact of transporter
inhibition on iodide transfer to breast milk, so that predictions for inhibition in breast-fed infants account
for both these aspects.

The second analysis would share these same strengths and limitations because it combines the biological
variability with life-stage dependent differences in exposures. Data for water and diet consumption at
the different lifestages that inform the exposure modeling appear somewhat variable in extent across the
lifestages.

Recommendation:

The SAB finds the second analysis is the most valuable for asking what extent of NIS inhibition would
be predicted for different potential MCLG concentrations; it provides perspective on the protection
offered by different concentrations. Since it uses 90th percentile drinking water consumption rates, it
starts to address population issues in exposure, although the biological aspects of the model are for an
average individual.

Limited data have been available for perchlorate in plasma and breast milk/ so checking the availability
of new data in the literature would usefully inform alternative parameterization or characterization of the
uncertainty in the current model parameters. It is recognized that there is widespread sensitivity to
information on potential impacts of breast and bottle-feeding for infants so care in communications
about these topics will be beneficial.

3.3. Epidemiological Studies

Charge Question:

How should EPA consider the post-NRC epidemiology data in deriving an MCLG?

The SAB finds that the post-NRC (2005) epidemiology data are useful for estimating the size of
potentially sensitive subgroups in the U.S., estimating the extent to which the general U.S. population
and sensitive subgroups are exposed to perchlorate as well as other goitrogens, and estimating the
relative source contribution of perchlorate in drinking water among sensitive subgroups not addressed in
the FDA’s total diet study (Murray et al. 2008).

The SAB concludes that the post-NRC epidemiology data are insufficient to guide causal inference with
regard to the association between perchlorate exposure and thyroid dysfunction in pregnant women,
neonates or the general population. Limitations concerning study design, exposure assessment, sample
size, and statistical modeling have led to inconsistent results. Thus, the current body of epidemiologic
evidence cannot provide validation of a safe level of perchlorate in drinking water.

The SAB provides specific comments on how the Agency could use the exposure and biomonitoring
studies published since the NRC report (2005). The SAB identifies research components that the EPA
and researchers should consider in developing future analyses based on available data or new studies to
improve the Agency’s understanding of hypothyroxinemia and exposed populations. The SAB also
provides specific comments on the strengths and weaknesses of the studies identified by EPA and other recent studies in Appendix B.

3.3.1. Using Exposure and Biomonitoring Studies

New manuscripts published since the 2005 NRC report are informative for providing an estimate of the size of potentially sensitive subgroups in the U.S., estimating exposure to perchlorate and other goitrogens in the U.S. population, including among sensitive subgroups and estimating the relative source contribution of perchlorate in drinking water among sensitive subgroups.

Prevalence of Sensitive Subgroups

Ideally, epidemiologic studies would be used to identify sensitive subgroups. However, methodological considerations (see review of epidemiologic literature in Appendix B) limit the conclusions that can be drawn from the studies published to date. The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional, population-based survey that over-sampled some populations to produce a representative sample of the U.S. population in almost all age groups (CDC 2004). Studies in NHANES can be used to estimate the population prevalence of potentially sensitive subgroups, including pregnant women who are iodide insufficient and pregnant women with detectable thyroid antibodies.

Iodine is critical for the formation of TH. Iodine deficiency occurs when iodine falls below recommended levels. According to the WHO guidelines, urinary iodine levels > 100 µg/L are considered “adequate” among the general population (WHO 1994). However, among pregnant women, the demand for iodine is greater; therefore, in this population group, urinary iodine levels <150 µg/L are considered “insufficient” (Andersson et al. 2007). Caldwell et al. used iodine measured in spot urine samples from NHANES 2001-2002 to characterize iodine levels in the U.S. population (2005). Among women age 15-44, 37.2% have iodine levels <100 µg/L. Using the 2005-6 and 2007-8 NHANES samples, Caldwell et al. reported that the proportion of women ages 15-44 with urinary iodine <100 µg/L remains relatively constant at 38.1%(2011). Among pregnant women, however, 56.7% have urinary iodine concentrations less than the recommended 150 µg/L.

Thyroid antibodies, thyroglobulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb) can interfere with TH synthesis (Sinclair 2006). Hollowell et al. estimated the prevalence of thyroid antibodies in the NHANES 1988-1994 sample (2002). In the total U.S. population, 13.0% and 11.5% had detectable TPOAb and TgAb, respectively. Approximately 18% of participants without thyroid disease had detectable TgAb or TPOAb. Those who tested positive were more likely to be female and among females, antibody prevalence significantly increased with age. Using NHANES data, it would be possible to estimate the proportion of women of childbearing age and the proportion of pregnant women (with some imprecision due to the small number of pregnant women typically represented in NHANES) that have detectable thyroid antibodies.
Estimating Perchlorate Exposure and Exposure to Other Goitrogens

Biomonitoring and exposure studies published since the NRC report can be used to identify subgroups with the highest exposures to perchlorate. Studies that use data from NHANES can reliably produce population estimates of exposure to perchlorate, particularly among some of the potentially sensitive subgroups.

Blount et al. provides information for estimating perchlorate exposure and dose using spot urines among a representative sample of n=2820 men and women ≥ 6 years of age in NHANES 2001-2002 (2006). Perchlorate was detectable in all samples, indicating widespread exposure in the U.S. population. Children ages 6-11 had the highest concentrations of urinary perchlorate (geometric mean: 5.40 ug/L, adjusted for race/ethnicity, sex, age, fasting time and urinary creatinine).

Huber et al. provides information for estimating perchlorate exposure and dose in pregnant women (2011). The authors used data from a random subset of NHANES 2001-2002 that measured perchlorate dose in n=2708 spot urine samples (creatinine adjusted). Among these individuals, n=116 were pregnant. Compared to non-pregnant women of childbearing age (15-44 years), pregnant women had significantly higher average daily perchlorate doses (geometric mean: 0.06 ug/kg/day vs. 0.051 ug/kg/day). However, these data are likely imprecise, particularly at the population distribution extremes, because they are estimated from spot urine samples and during pregnancy, adjustment for urinary dilution using creatinine excretion is less precise (Mendez et al. 2010). The authors also merged the biomonitoring data from NHANES with the EPA Unregulated Contaminant Monitoring Regulation (UCMR) data, which indicates the potential for perchlorate exposure from public drinking water sources. In the total population and among women of childbearing age, the perchlorate contribution from food was 86% and from drinking water 14%; this ratio could not be estimated for pregnant women specifically due to small numbers.

There are some potentially sensitive subgroups that are not represented in NHANES, including infants. Therefore, exposure information must be inferred from exposure and biomonitoring studies that specifically target these populations. However, these studies are often comprised of highly selected study subjects that may not be representative of the U.S. population. Given the paucity of epidemiologic data on potentially sensitive subgroups, the following studies published since the NRC report can inform dose parameters for PBPK models.

Information for estimating perchlorate exposure and dose among infants less than 6 months of age is available in four studies (Kirk et al. 2005, Dasgupta et al. 2008, Schier et al. 2010, and Valentin-Blasini et al. 2011). Kirk et al. reported average perchlorate concentrations of 2.0 ug/L (range: 0.0 to 11.0 ug/L) and 10.5 ug/L (range: 1.4 to 92.2 ug/L) in dairy milk and breast milk, respectively (2005). Using these data, the authors estimate that the majority of breast-fed infants would exceed the NRC RfD (0.7 ug/Kg/day). The authors also measured iodide concentrations in a small number of samples (n=23) and find that there are no samples with “high” concentrations of both perchlorate and iodide (“high” exposure = greater than the sample median). However, a study with this size and cross-sectional design is not able to provide evidence about whether this observed relationship is causal. Dasgupta et al. measured perchlorate in repeated milk and urine samples from a small number of lactating women.
Based on these data, the authors estimate that 9 of 13 infants exceed the NRC perchlorate RfD. Schier et al. estimated perchlorate intake from four varieties of infant formula; bovine-based with lactose, bovine-based without lactose, soy-based, and elemental formulas (2010). The authors report that bovine milk with lactose has the highest concentrations of perchlorate (geometric mean: 1.72 μg/L), which could lead to estimated daily doses that exceed the perchlorate RfD (0.7 μg/Kg/day) at 1 and 6 months of age. Valentin-Blasini et al. directly measured perchlorate exposure in the urine of breast and formula-fed infants age 1-377 days old (n=205) and report that the highest average perchlorate concentrations are among breast-fed infants (geometric mean: 2.65 μg/L vs. 1.3 μg/L for cow milk-based formula and 0.35 μg/L for soy-based formula) (2011). Correspondingly, the highest average estimated perchlorate doses (geometric means for breast-fed, cow milk-based formula fed, and soy-based formula fed, respectively: 0.922 μg/kg/day, 0.103 μg/kg/day, and 0.027 μg/kg/day) are found among breastfed infants. Based on these estimates, 16% of all infants (and 31% of breast-fed infants) had at least 1 perchlorate exposure dose exceeding the RfD. These authors also report concurrent urinary levels of nitrate, thiocyanate, and iodide concentrations.

In addition to perchlorate, analyses within NHANES provide an opportunity to evaluate the extent to which the U.S. population (including sensitive subgroups) may also be co-exposed to other goitrogens including thiocyanate and nitrate. The ion chromatography coupled with tandem mass spectrometry method used to measure perchlorate in urine in the NHANES sample from 2001-2002 offers the simultaneous measurement of nitrate, thiocyanate, and iodide (Valentin-Blasini et al. 2007). While the geometric mean concentrations are reported in Blount et al (2006) and Mendez and Eftim (2012), these data have not yet been described in detail in a peer-reviewed publication (English et al 2011). Finally, it should be noted that while data from epidemiologic studies are insufficient for evaluating the causal association between perchlorate exposure and thyroid dysfunction (because of the methodological issues described in Appendix B), these and other epidemiological studies may be useful for understanding perchlorate exposure and co-exposure to other goitrogens among pregnant women and infants.

**Estimating the relative source contribution**

The relative source contribution (RSC) is the proportion of an individual’s daily exposure to a contaminant attributable to drinking water after considering perchlorate exposure from food or air. For perchlorate, the food exposure is the only other significant exposure pathway than drinking water. The EPA used a report of the Food and Drug Administration’s (FDA) Total Diet Study (TDS) by Murray et al. to estimate the drinking water RSC (Table A-2, US EPA 2012) based on estimated perchlorate intake from food among n=14 age/sex subgroups of the U.S. population (2008). However, the TDS does not include intake estimates for some potentially sensitive subgroups (e.g., pregnant or lactating women, infants less than 6 months of age). Studies outlined above provide information for estimating perchlorate dose for drinking water and food intake levels within sensitive subgroups.
3.3.2. Epidemiologic Studies of Associations between Perchlorate Exposure and Thyroid Dysfunction

The SAB finds that epidemiologic studies published since the 2005 NRC report are insufficient to guide causal inference concerning the association between perchlorate exposure and thyroid dysfunction. In a review of recent studies, several issues were identified that account for the lack of concordance among the epidemiological studies. The ecological study designs of environmental perchlorate exposure are insufficient to guide causal inference even for the interim question of whether exposure to perchlorate results in thyroid dysfunction. The inconsistency in the study design, methods, or conclusions of the cross-sectional studies also limits their use in deriving an MCLG. Additionally, the differences in the treatment of parameters (e.g., urinary creatinine, iodide status, and presence of thyroid antibodies) among the studies limits their use. The review of the studies identifies methodological issues of concern that relate to: 1) use of ecological measures of perchlorate exposure based on community drinking water concentrations, 2) cross-sectional study designs, 3) small sample size, 4) misspecified statistical models and 5) inconsistent treatment of creatinine, iodide status, thyroid antibodies and co-exposures to other goitrogens. These issues are discussed in detail in Appendix B.

3.3.3. Recommendations for Future Analyses and Studies

Exposure and biomonitoring studies are useful for understanding the prevalence of sensitive subgroups. Additional analyses within NHANES can be undertaken to estimate the prevalence of sensitive subgroups not previously described, including the proportion of pregnant women who have detectable thyroid antibodies. In addition to perchlorate, urinary concentrations of other goitrogens are also available in NHANES data. Prospective studies of individual urinary biomarkers of perchlorate exposure and thyroid function and child neurobehavioral development are recommended. Studies that evaluate hypothyroxinemia endpoints during pregnancy may offer a better picture of perchlorate’s role as a contributor to meaningful health outcomes in susceptible populations, specifically endpoints directly related to neurodevelopment. Statistical models that investigate non-linear patterns of exposure effects across low, moderate and high exposures categories may also be informative. Careful and thorough consideration of appropriate control variables, possibly by using directed acyclic graphs to identify relevant confounders that are neither intermediates nor colliders, can reduce bias and improve the precision of estimated perchlorate effects. Additionally, assessing characteristics of potentially vulnerable populations as effect measure modifiers rather than confounders will allow for the identification of populations more susceptible to the potential effects of perchlorate. Such studies, however, would require large sample sizes to detect interactions. It may be possible to pool data from studies with similar design and analytic measures to try to address these issues. However, post-hoc pooled analyses should be undertaken with caution and with careful consideration of potential sources of heterogeneity across studies. Finally, co-exposures to other goitrogens should be consistently measured in future studies and consideration should be given to conducting sensitivity analyses to address uncertainties of modeling co-exposures with the same (or different) modes of action. Studies of the variability of perchlorate, iodide, nitrate, thiocyanate in spot urine samples should also inform methods for minimizing measurement error.
3.4. Integration of Information

**Charge Questions:**

*How can 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 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?*

3.4.1. Integrating Information to Derive a MCLG

The EPA White Paper describes a process for deriving an MCLG for perchlorate that incorporates a RfD and RSC (US EPA 2012). The SAB recommends an alternative and novel approach to integrate the body of information on perchlorate to derive an MCLG based on the MOA previously identified for perchlorate. This approach relies on the use of a PBPK/PD model that correlates perchlorate intake via drinking water with percent iodide uptake inhibition. The SAB recommends that EPA use a PBPK/PD IUI approach and where possible expand this approach to empirically relate the percent IUI with TH perturbations and potential adverse neurodevelopmental outcomes.

The SAB recommendations represent an important and novel opportunity that should be implemented carefully with attention to data quality and methodological rigor. At each step, EPA should critically evaluate available data and describe the strengths and limitations. The SAB concludes that a stepwise “integrated” approach is a logical way forward allowing multiple sources of information to be integrated into the MCLG derivation. The SAB also recommends that EPA undertake the necessary literature review or critical analysis to fully test the feasibility or utility of the approach. The SAB also recommends that EPA incorporate into the MCGL development the recent recommendation from the National Academy of Sciences to improve the scientific basis and clarity of assessment documents and the move towards an assessment of costs and benefits of the regulatory proposals (NRC 2009 and 2011).

This advisory report presents specific recommendations for considering sensitive life stages, PBPK modeling, and the epidemiological and biomonitoring data that were presented to the SAB to derive an MCLG. While the Charge to the SAB focused on scientific literature published since the release of NRC’s 2005 report, clearly the Agency needs to incorporate the entire body of literature related to ingestion of perchlorate, pharmacokinetics of perchlorate, and the effects (dynamics) of perchlorate. In addition, the SAB finds that EPA should also consider available data on potential adverse health effects due to thyroid hormone level perturbations regardless of the cause of those perturbations.

A summary of key findings in the three previous sections provides the foundation for an approach to derive the MCLG for perchlorate using the entire body of information.

*Sensitive Life Stages:* The most important result related to SAB deliberations regarding sensitive life stages is the focus on subtle changes in thyroid hormone levels in pregnant
women—specifically, hypothyroxinemia (defined as “fT4 levels below the 10th percentile and concomitant TSH values <2.0 μmU/liter”). Hypothyroxinemic pregnant women should be considered the sensitive life stage; this would replace pregnant women with clinical hypothyroidism as the sensitive life stage as defined by the NRC (2005). As discussed in section 3.1, the SAB finds that the sensitive subpopulation of concern is pregnant hypothyroxinemic women and the adverse effect is neurodevelopment of the fetus and young child born to these women.

Epidemiology and Biomonitoring Data: The key conclusion was that the data in the scientific literature post-2005 were insufficient to provide the basis for an MCLG. However, it is possible that a consideration of the full literature (pre and post-2005) and/or other combined analyses (such as meta-analysis or pooled analysis) might provide important information that could be used to support an MCLG based on pregnant hypothyroxinemic women as the sensitive life stage.

PBPK Modeling: The physiologically-based pharmacokinetic/pharmacodynamic-iodide uptake inhibition (PBPK/PD-IUI) model links perchlorate exposure in food and water with perchlorate concentrations in plasma and tissue and resulting NIS inhibition assessed by radioactive iodide uptake (RAIU) studies. The continuum of events in the MOA after NIS inhibition would include possible changes in serum thyroid hormone levels, which have been associated with neurodevelopmental changes in offspring of iodine-deficient women. Using the MOA framework, the model provides a key tool for assessing the potential for the downstream step at different lifestages or sensitive populations.

EPA should utilize an MOA framework for developing the MCLG that links the different steps in the proposed mechanism leading from perchlorate exposure through NIS inhibition to thyroid hormone changes and finally neurodevelopmental impacts. Within this MOA framework, the PBPK/PD-IUI model provides a tool for integrating exposure (e.g., different drinking water consumption rates) with the biological changes occurring at the different lifestages to obtain predictions for perchlorate pharmacokinetics and resulting NIS inhibition to address these initial steps of the MOA framework. The SAB recognizes that an MOA has been determined with confidence and is supported clinical evidence; this is key to the derivation of the MCLG.

In order to ensure that the model is predictive of actual adverse health outcomes, EPA will need to examine the literature on the associations between reduced iodide uptake, subtle changes in thyroid hormone levels as defined by hypothyroxinemia, and adverse neurodevelopmental outcomes in children, including literature not specifically designed to include perchlorate in its study design.

The SAB recognizes the existence of a large scientific body of work on perchlorate and also thyroid hormone perturbations and potential adverse health outcomes (unrelated specifically to perchlorate). As a result, the SAB recommends that EPA explore the use of the literature beyond that which focuses on perchlorate.
The SAB notes that the recommendation to use the MOA and PBPK mathematical model is a novel and alternative approach to developing the MCLG. The SAB emphasizes the need for transparency in approaches for identifying and/or excluding model input data, compiling datasets for purposes of identifying and bounding numerical estimates needed for the MCLG and transparency and robust explanation of the approach and modeling used for the derivation of the MCLG.

In response to establishing the bounds on potential MCLG values considering the results of the epidemiological and biomonitoring data, the SAB was not provided the full extent of the epidemiologic, biomonitoring, water concentration, or physiologic data related to perchlorate, nor asked to complete each step in the new approach to developing an MCLG. Therefore, the SAB finds that it is premature to provide specific guidance on bounding estimates. The SAB recommends that EPA fully evaluate the breadth and depth of the data, data variability and uncertainty, and the utility of the data. The SAB further notes the importance of incorporating metrics and statistics, such as 95th percentiles and ranges of values rather than point estimates.

The SAB notes that in applying the framework to the epidemiological data, there are available evaluation tools such as Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) or Grading of Recommendations Assessment, Development and Evaluation (GRADE). The SAB recommends that as the EPA integrates information they consider the general frameworks for evaluating quality of studies used to support the MCLG derivation (as discussed briefly in Appendix C).

**Steps In An Integrated Approach**

The SAB recommends the following approach for using the total body of available data to inform a health protective MCLG, recognizing the sensitive population as hypothyroxinemic pregnant women (and their fetuses and infants). The effects of concern are neurodevelopment outcomes in children.

The SAB recommends that EPA explore the use of a MOA-based PBPK model for estimation of the percent inhibition of iodine uptake in the sensitive population, as well as empirical comparisons with serum free T4 and TSH (as biomarkers of thyroid status) and adverse effects associated with levels of these biomarkers from the non-perchlorate clinical literature. The SAB recognizes that the steps described here may require additional data and information than are currently available; these steps are presented as an ideal approach. The approach is discussed below and summarized in Figure 2.

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1) Use the MOA for perchlorate (Figure 1 See section 3.2.) as the biological basis for deriving the MCLG. This MOA links perchlorate exposure to NIS inhibition to thyroid hormone changes and ideally neurodevelopmental impacts.

2) Use the PBPK/PD-IUI model to link perchlorate exposure in water with perchlorate concentrations in plasma and tissue and resulting NIS inhibition assessed by radioactive iodide uptake (RAIU) studies. If the PBPK model in its current form does not completely address important life stage sensitivities, these limitations must be clearly stated and either the model should be adjusted or other adjustments to the MCLG should be made.

3) EPA should identify and use empirical data to document and confirm the associations between water perchlorate level, IUI, thyroid hormone perturbations in the mother, and neurodevelopmental outcomes in the child as predicted by the MOA to ensure that the model results are consistent with human studies where possible. The current PBPK model could be used to derive the MCLG by giving perchlorate water concentrations associated with the percentage IUI leading to hypothyroxinemia. However, the SAB urges EPA to expand on this framework by addressing as many of the downstream MOA components as possible. The Agency should identify literature and conduct analyses to support the model outputs for the downstream steps (as described in steps 3a, 3b and 3c).

   a. Use the thyroid clinical literature to identify the degree of symporter inhibition (percentage IUI) required for onset of hypothyroxinemia in the pregnant woman. The relevant literature for this step may include the clinical literature on iodine deficiency as well as other literature on hypothyroxinemia.

   b. Document the relationship between the levels of maternal serum biomarkers fT4 and TSH associated with adverse effects on neurodevelopment of infants. Examples of useful literature to support this step may include the Haddow et al. (1999) and Pop et al. (1999) studies.
c. Compare model predictions with epidemiological data. As previously discussed, the post-2005 epidemiological studies have significant limitations for the purposes of MCLG derivation and have limited utility for evaluating the PBPK-PD model outputs. However, it may be possible to gain information on perchlorate exposure and thyroid hormone perturbations from an examination of the raw data, i.e., a pooled analysis. If a pooled analysis is pursued, the SAB advises exploring the recent Pearce et al. (2010, 2011, 2012) studies as the source material, although there may be other relevant studies as well. Pooled analyses are challenging and the data to be combined must be carefully evaluated to ensure that such an analysis is appropriate. Methodological issues particular to pooled analysis of biomarkers studies are presented by Taioli and Bonassi (2002). Guidance on the preferred statistical approaches (model specification, etc) for a possible pooled analysis is outlined in section 3.3.3.

3.4.2. Estimating Reductions In Adverse Health Effects

The SAB finds that existing data are inadequate for quantitatively estimating reduction in adverse health effects realized in regulating perchlorate in drinking water. Specifically, the available data are not adequate to support fully quantitative dose-response modeling and related adverse health effects reduction analyses. To move toward the goal of quantitative dose-response and reduction in adverse health effects assessment for perchlorate, the Agency must first define:

- The adverse effect. The SAB recognizes a range of neurodevelopmental impairments in the infant as the “adverse effects.” However, measurements relevant to these adverse effects may range from iodine deficiency, hypothyroxinemia, changes in expression of genes involved in brain development and function, neuropsychology, and impaired behavior, learning and memory, among others (Rovet and Willoughby 2010); and

- The sensitive population. The SAB identified the sensitive population as hypothyroxinemic pregnant women, their fetuses and infants.

EPA may be able to begin to estimate reduction in adverse health effects from of reducing perchlorate levels in drinking water by examining shifts in the distribution of exposure to the sensitive population - hypothyroxinemic pregnant women – if relevant data are available.
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APPENDIX A

Charge to EPA Science Advisory Board

LIFE STAGE CONSIDERATIONS AND INTERPRETATION OF RECENT EPIDEMIOLOGICAL EVIDENCE TO DEVELOP A MAXIMUM CONTAMINANT LEVEL GOAL FOR PERCHLORATE

Background

On February 11, 2011 (U.S. EPA, 2011a), EPA published a determination to regulate perchlorate under the Safe Drinking Water Act (SDWA) because:

- perchlorate may have an adverse effect on the health of persons;
- perchlorate is known to occur or there is a substantial likelihood that it will occur in public water systems with a frequency and at levels of public health concern; and,
- in the sole judgment of the Administrator, regulation of perchlorate presents a meaningful opportunity for health risk reduction for persons served by public water systems.

EPA has initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) for perchlorate. The MCLG is a non-enforceable goal defined under the SDWA (§1412.b.4.B ) as “the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety.” For perchlorate, the NPDWR will likely specify an enforceable Maximum Contaminant Level (MCL) and monitoring and reporting requirements for public water systems. The SDWA (§1412.b.4.B and D) specifies that the enforceable MCL be set as close to the MCLG as feasible using the best available technology, treatment techniques, and other means (taking cost into consideration).

The regulatory schedule established by SDWA requires EPA to publish a proposed MCLG and NPDWR within 24 months of making a determination to regulate a contaminant and promulgate a final regulation within 18 months of the proposal. As part of this proposed rulemaking, EPA also must develop a Health Risk Reduction and Cost Analysis that includes an assessment of the quantifiable and non-quantifiable health risk reduction benefits likely to occur as a result of treatment to remove the perchlorate. SDWA further requires that when proposing any NPDWR that includes an MCL, the Administrator must analyze “[t]he effects of the contaminant on the general population and on groups within the general population such as infants, children, pregnant women, the elderly, individuals with a
history of serious illness, or other subpopulations that are identified as likely to be at greater risk of adverse health effects due to exposure to contaminants in drinking water than the general population.”

In 2005, at the request of EPA and other federal agencies, the NRC published a comprehensive report “Health Implications of Perchlorate Ingestion” (NRC, 2005). The NRC concluded that perchlorate can affect thyroid function because it is an ion that competitively inhibits the transport of iodide into the thyroid by a protein known as the sodium (Na)/iodide (I) symporter (NIS). Significant inhibition of iodide uptake results in intra-thyroid iodine deficiency, decreased synthesis of key thyroid hormones (Triiodothyronine, T3 and Thyroxine, T4), and increased thyroid stimulating hormone or thyrotropin (TSH). The NRC also concluded that a prolonged decrease of thyroid hormone is potentially more likely to have adverse effects in sensitive populations (people with thyroid disorders, pregnant women, fetuses, and infants).

The NRC recommended the use of a precursor, non-adverse effect (i.e., inhibition of iodide uptake) to derive a reference dose (RfD) for perchlorate. An RfD is defined by EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.” The NRC identified a clinical study involving 37 healthy men and women by Greer et al. (2002) as the critical study and determined an RfD of 0.7 µg/kg/day for perchlorate. The RfD was based on the No Observed Effect Level (NOEL) of 7 µg/kg/day corresponding to a radioactive iodide uptake (RAIU) inhibition of 1.8 percent and application of an intraspecies uncertainty factor (UF) of 10 to account for differences in sensitivity between the healthy adults in the Greer et al., (2002) study and the most sensitive population, fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The NRC also acknowledged that the RfD may need to be adjusted upward or downward on the basis of future research. The RfD of 0.7 µg/kg/day was adopted by EPA in 2005 (U.S. EPA, 2005a). EPA believes that this RfD is the most scientifically defensible endpoint available at this time for assessing risk from perchlorate exposure.

In October 2008, EPA published a preliminary determination not to regulate perchlorate in drinking water using a health reference level (HRL) of 15 µg/L, which was derived from the RfD of 0.7 µg/kg/day, using a default body weight (70 kg), a default drinking water consumption rate (2 L/day), and a perchlorate-specific relative source contribution (RSC) of 62% for a pregnant woman (U.S. EPA, 2008). The RSC is the percentage of the RfD remaining for drinking water after the other sources of exposure to perchlorate (e.g., food) have been considered. In January 2009, EPA issued an interim health advisory (15 µg/L perchlorate in drinking water) to provide guidance to state and local officials in

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1 SDWA uses the term subpopulation to refer to groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other groups that can be identified and characterized and are likely to experience elevated health risks. In 2005 EPA started using the term life stages to refer to age-defined groups. All life stages are subpopulations but not all subpopulations are life stages. In this document, the term life stage is used predominantly because of the focus on infants and very young children.
In August 2009, EPA published a supplemental request for comment with a new analysis that derived potential alternative HRLs for 14 life stages, including infants and children. The analysis used the RfD of 0.7 µg/kg/day and life stage-specific body weight and exposure information (i.e., drinking water intake, RSC) (U.S. EPA, 2009b). The HRLs ranged from 1 µg/L to 47 µg/L. In February 2011, EPA published the Final Regulatory Determination to regulate perchlorate under SDWA. The Final Regulatory Determination stated that EPA was evaluating the potential alternative HRLs and considered them to be levels of public health concern for the purposes of final determination (U.S. EPA, 2011a).

**Charge to the SAB**

The purpose of this white paper is to seek guidance from the SAB on how best to consider and interpret the life stage information, the epidemiologic and biomonitoring data since the NRC report, physiologically-based pharmacokinetic (PBPK) analyses, and the totality of perchlorate health information to derive an MCLG for perchlorate.

**Specific Charge Questions**

**Issue I - Sensitive Life Stages**

While studies directly demonstrating the adverse effects of perchlorate in humans are not available, potential effects can be inferred from the mode of action for perchlorate and the literature on thyroid hormone decrements and neurological deficits in various life stages. Perchlorate blocks the transport of iodide into the thyroid gland leading to iodide deficiency and decreased synthesis of thyroid hormones, T3 and T4. Transfer of iodide from blood into the thyroid gland is essential for the synthesis of the thyroid hormones. In its deliberations on the health effects of perchlorate in drinking water, the NRC committee considered pregnant women who might have hypothyroidism or iodide deficiency and their fetuses to be particularly sensitive populations to perchlorate mediated health effects (NRC, 2005).

Based on the discussion in Section IV of the white paper, pregnant women and their fetuses, neonates, infants (breast-fed and bottle-fed) and young children have been identified as life stages of concern for adverse effects due to perchlorate. Significant thyroid perturbations *in utero* are well known to cause neurological deficits in infants and children (NRC, 2005). High turnover rate of thyroid hormones, and low storage capacity in the fetus and neonate make these in particular, sensitive life stages for thyroid hormone perturbations. Furthermore, infants and children, in general, are more susceptible to xenobiotics effects because of low urinary clearance of contaminants, and higher food consumption and drinking water intake per body weight relative to adults (USEPA, 2011b). As in the thyroid gland, perchlorate is actively taken up into mammary tissue via NIS. Perchlorate also competitively inhibits the uptake of iodide into the mammary gland, reducing the amount of available iodide in breast milk. Therefore, breast-fed infants also represent a population of particular concern as they experience a double hit – exposure to perchlorate accumulated in breast milk in addition to a
deficiency of iodine in the breast milk. (Kirk et al., 2005; Dasgupta et al., 2008; Valentin-Blasini et al., 2011).

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

Issue II - Physiologically-Based Pharmacokinetic Evidence

The NRC relied on information on inhibition of RAIU in a small group of healthy, iodine sufficient, adults, similar data are not available for other life stages. With the development of the PBPK model (U.S. EPA, 2009b), it is now possible to provide estimates of the effect of perchlorate on RAIU in different life stages as outlined in white paper Section VI.

The PBPK model predictions can be evaluated in two different ways. The first application is based on a comparison of the relative RAIU inhibition sensitivity at a fixed dose (point of departure, POD of 7 µg/kg/day identified by NRC) for different life stages. One exception in the first application scenario with regard to dosing is that the breast-fed infants received a dose higher than the POD, but lactating mothers received a dose equivalent to the POD. The second application involves comparing RAIU inhibition at a fixed drinking water exposure level (15, 20 and 24.5 ppb) with and without perchlorate contribution via food for various life stages. Thus, the doses for different life stages varied in the second application scenario.

The findings from the first application indicate a greater sensitivity for RAIU inhibition for fetuses and breast-fed infants compared to other life stages/sub populations (Table A-3 of the White Paper). The findings from the second application indicate a RAIU inhibition of 2.2% or less for all life stages when they are exposed to drinking water containing 15 µg/L perchlorate in addition to perchlorate in food (Table A-4 of the White Paper). In the context of significance of RAIU inhibition, NRC determined 1.8% RAIU inhibition was not significant at the POD/NOEL of 7 µg/kg/day for healthy adults, but recommended that a 10-fold uncertainty factor be applied to the POD to protect the fetus of the pregnant woman who might have hypothyroidism or iodine deficiency. However, the doses infants receive when exposed to 15 µg/L perchlorate in water and perchlorate in food are up to 5 times higher than the RfD.
• How should 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?

Issue III – Epidemiological Evidence

Since the NRC report (2005), a number of epidemiological studies have investigated the association between perchlorate exposure and thyroid hormone perturbations. None evaluated the neurodevelopmental outcomes. The studies reported findings for sensitive life stages of concern: pregnant women, neonates and infants. Several of these studies investigated the association between perchlorate exposure in drinking water and thyroid hormone levels in the US, Israel and Chile (Tellez et al., 2005, Amitai et al., 2007, Steinmaus et al., 2010). The study in Chile (Tellez et al., 2005) reported urinary and serum perchlorate levels in women during pregnancy and post partum (a longitudinal cohort study). However, perchlorate assignment to subjects was based solely on geographical location. Other studies that examined the association between perchlorate and thyroid hormone levels included urinary perchlorate concentrations as biomarkers of exposure (Blount et al., 2006; Pearce et al., 2010, 2011).

Using NHANES 2001-2002 data, Blount et al. (2006) demonstrated a perchlorate-related increase in TSH and decrease in T4 in women >12 years of age with urinary iodide <100 µg/L. Pearce et al. (2010, 2011) did not find an association between urinary perchlorate and thyroid hormone perturbations in first trimester pregnant women. Differences in study designs, numbers and age of subjects, exposure assessment approaches, and statistical methods may explain the mixed findings among these studies. The studies published in the literature since the NRC (2005) review are described in Section VII and Table A-5 of the white paper. The new epidemiological evidence may inform bounding of the possible life stage-specific MCLG estimates derived in the White Paper (Table-1).

• How should EPA consider the post-NRC epidemiology data in deriving an MCLG?

Issue IV – Integration of Information

The primary action of perchlorate exposure is on the thyroid gland, where perchlorate inhibits the transport of iodide from the blood into the thyroid gland which in turn can lead to perturbations in the synthesis of thyroid hormones. Perturbations in thyroid hormones during critical stages of development lead to permanent neurological deficits in children (NRC, 2005). EPA generally derives an MCLG on the basis of the RfD. EPA believes that the NRC derived RfD of 0.0007 mg/kg/day (0.7 µg/kg/day) for perchlorate is the most scientifically defensible endpoint available at this time for deriving an MCLG. In deriving the RfD, the NRC applied an intraspecies factor of 10x to protect the fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The UF 10 can be further subdivided into a UF_{TK} = 10^{1/2} = 3.16 (generally rounded to 3) to account for differences in internal dosimetry due to toxicokinetic differences, and a UF_{TD} = 10^{1/2} = 3.16 (generally rounded to 3) to account for differences in toxicodynamics. This convention is used by EPA in the absence of compound-specific data as is the case with perchlorate.
At a fixed dose of 7 µg/kg/day, the first application of PBPK model findings indicate 6.7x, 2.6x, 7.8x, and 1.1x greater sensitivity for RAIU inhibition for GW 40 fetuses, 7 day breast-fed infants, 7-day bottle-fed infants and children from 6 months to 2-years, respectively, as compared to adults (Table A-3 of the White Paper). It was not possible to estimate sensitivity in younger than term fetus. The second use of PBPK modeling indicates a RAIU inhibition of 2.2% or less for all life stages when they are exposed to drinking water containing 15 µg/L perchlorate in addition to perchlorate in food (Table A-4 of the White Paper). In the context of significance of RAIU inhibition, NRC determined 1.8% RAIU inhibition not significant for healthy adults. However, the doses infants receive when exposed to 15 µg/L perchlorate in water and perchlorate in food are up to about 5 times higher than the RfD.

As discussed previously the mixed pattern of observations in the epidemiologic studies which investigated the association between perchlorate exposure and thyroid perturbations since the 2005 NRC review is not surprising in light of their different study designs, numbers and age of subjects, exposure assessment approaches, and statistical methods. In an ecological study, Steinmaus et al. (2010) found increased TSH levels in neonates when the mothers were exposed to perchlorate concentrations above 5 µg/L in drinking water. Using 2001-2002 NHANES data, perchlorate-related increases in TSH and decreases in T4 were demonstrated in women >12 years of age with urinary iodide <100 µg/L (Blount et al., 2006). The changes in thyroid hormone levels in the NHANES analyses were observed at a mean perchlorate intake level of approximately 0.1 µg/kg/day (including food and drinking water) reported by Huber et al. (2011) for the NHANES populations, suggesting thyroid hormone perturbations at a perchlorate intake level less than the RfD determined by NRC (2005). The perchlorate dose estimated from Huber et al. (2011) is consistent with that reported from other biomonitoring studies and analyses reported in Section VIII and Table A-6 of the White Paper. Other studies of pregnant women or neonates did not report associations between residence in a 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) (Tellez et al., 2005; Amitai et al., 2007; Pearce et al., 2010, 2011). Together the results of these studies may serve as a means to bound the drinking water exposure range of concern, and assist in determining where within the range of potential MCLGs an appropriate regulatory value can be set.

- How can 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 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?
APPENDIX B

Critique of Recent Epidemiological Data for Deriving a Perchlorate MCLG

Epidemiologic studies published since the 2005 NRC report are insufficient to guide causal inference with regard to the association between perchlorate exposure and thyroid dysfunction. This conclusion is based on methodological inconsistencies and limitations pertaining to study design, exposure assessment, samples size, and statistical modeling. Each of the issues are discussed in detail this Appendix.

Study design

The prototypical epidemiologic study is a randomized controlled trial. When the primary study question is whether perinatal exposure to an environmental chemical adversely affects child cognitive and behavioral development, observational studies must suffice. The ideal observational study to identify potential effects of perinatal perchlorate exposure on child health is not difficult to conceive, although it would be large, expensive, logistically challenging, and take approximately 10 years to complete. Ideally, the study would, from the first trimester of pregnancy, prospectively collect serial urinary biomarkers of maternal prenatal perchlorate exposure, serial serum biomarkers of maternal prenatal thyroid function, including TSH, fT4, and thyroid antibodies, and serial urinary maternal prenatal biomarkers of the related compounds iodide, nitrate, and thiocyanate. To determine the relative source contribution of perchlorate in drinking water and perchlorate from other sources, such as food or prenatal vitamins, serial drinking water and dietary measures like a food frequency questionnaire, 24-hour dietary recall, or duplicate plate, must be included and coincide with the collection of exposure biomarkers. Once the child is born, perchlorate, iodide, nitrate, thiocyanate, and thyroid function must be serially monitored in the child. Breast milk, formula, and eventually early solid foods should be assayed for goitrogens. Beginning at birth the child’s development must be tested and then monitored every 2 to 3 years by performance on standardized neurobehavioral assessments. The home environment should be evaluated by trained research personnel, the mother’s IQ should be measured, and other known predictors of child IQ and behavior, for instance lead exposure, should be obtained. The study can conclude with a final round of cognitive and behavioral testing when the child reaches 7 – 9 years of age.

When even an observational study of perinatal perchlorate exposure and child development is such a massive undertaking, researchers look to other study designs, data collected for other purposes, and interim outcomes (e.g., maternal prenatal thyroid dysfunction rather than impaired child cognitive skills) to address the study question. Unfortunately, the epidemiologic studies of health effects of environmental perchlorate exposure are insufficient to guide causal inference even for the interim question of whether exposure to perchlorate results in thyroid dysfunction.

Thirteen epidemiological studies published since the monograph Health Implications of Perchlorate Ingestion (NRC 2005) and assessing thyroid function can be divided into 2 groups based on the level of measurement of the exposure. Four ecological studies present environmental measures of perchlorate in drinking water based on residential location (Tellez 2005, Buffler 2006, Amitai 2007, and Steinmaus...
Ecological studies compare groups, not individuals. Defining exposure based on group level characteristics, such as water district, is a variation on the ecological study design. These types of studies are often the first investigative hypothesis-testing tool. They can lend credence to a new hypothesis and provide important preliminary data for planning future studies, but the ecological fallacy precludes any causal interpretation. The ecological fallacy occurs when population level associations are also assumed to occur at the individual level. For these studies, specifically, the fallacy occurs with the assignment of exposure: someone with a residence in a city with high levels of perchlorate in drinking water (person A) is assumed to be exposed to more perchlorate than someone with a residence in a city with low levels of perchlorate in drinking water (person B). There are several reasons why this scenario may be untrue. While one’s official residence at the time of exposure is defined for the study is located in the high-exposure city, this may be a new residence (i.e., the subject may have moved during pregnancy so the address listed on a birth certificate is not the address where the majority of the pregnancy occurred). The subject may have an official residence, but actually spend the majority of time at a different location. The subject may not drink tap water or used filtered tap water (i.e., under the counter reverse osmosis filters remove perchlorate) or use a private well. Conversely, for the same reasons why person A may not actually be exposed to high levels of perchlorate through drinking water, person B may be exposed to higher than expected levels for someone with a residence in a city with low levels of perchlorate in drinking water.

For perchlorate studies where exposure is an ecological measure based on drinking water source, there are additional concerns that may lead to further exposure misclassification. First, drinking water typically accounts for an estimated 20% of total perchlorate dose Huber et al. 2009. Consequently, estimating total perchlorate exposure solely by drinking water source may be grossly inaccurate. Second, perchlorate levels in drinking water may not be constant even though studies using ecological exposure measures define them as such (e.g., person A either does or does not reside in a high exposure location). Buffler et al. notes that in southern California, the proportion of Colorado River water used for drinking water varies seasonally (2006). For example, the Colorado River is a drinking water source and detected perchlorate concentrations. Consequently, the level of perchlorate in water supply systems reliant on Colorado River water may change as more or less river water is diverted into the drinking water system. Categorical assignment of high/medium/low exposure water districts may not be true over time and season.

Overall, the four studies examining ecological measures of perchlorate exposure in drinking water in relation to thyroid function, regardless of whether or not they show an association, are of little value for guiding decisions regarding a maximum contaminant level goal for perchlorate in drinking water.

Cross-sectional studies using individual level measures of both exposure and outcome are often the next investigative tool for examining an association. With cross-sectional studies, there is an individual measure of exposure and an individual measure of the outcome, but the exposure and outcome are assessed at the same point in time so causality cannot be inferred. With a cross-sectional study, there is
no way to know whether the exposure preceded the outcome and consequently no way to determine whether the exposure is a causal factor in development of the outcome. Nonetheless, cross-sectional studies may be useful for elucidating relationships.

Of the nine cross-sectional studies, four use NHANES data from 2001-2002 (Blount et al. 2006, Steinmaus 2010, and Schreinemachers 2011). Mendez and Eftim uses NHANES 2007 – 2008 (2012). Blount observed biologically plausible and consistent associations between increased urinary perchlorate concentration and increased TSH and decreased T4 among women with low urinary iodide concentration. Steinmaus carried these analyses forward and observed that this relationship appeared to be strengthened as urinary thiocyanate concentration increased. Mendez also showed inverse associations between levels of perchlorate and T3 and T4. In these analyses, however, TSH, thyroid antibodies, and iodine were included as covariates although their role may be better treated as effect measure modifiers (see Statistical Model Misspecifications below). Schreinemachers used indirect measures of thyroid function (HDL cholesterol, hemoglobin, hematocrit), which may be more relevant to the thyroid’s role in metabolic pathways rather than neurobehavioral development.

Only one of the five non-NHANES cross-sectional studies replicated the association between higher urinary perchlorate concentration and higher TSH among infants with lower urinary iodide levels (Cao 2010). This study, however, measured thyroid hormones in urine, not serum and the correlation between thyroid hormones in urine and serum is low (Cao 2010). Unexpectedly, higher urinary perchlorate was also associated with higher T4. None of the remaining four cross-sectional studies observed associations between urinary perchlorate levels and thyroid function in pregnant women (Pearce 2010, Pearce 2011, Pearce 2012) or in infants (Leung 2012).

Overall, there is little consistency in the study design, methods, or conclusions of the 9 cross-sectional studies. Many of the studies suffer from a small sample size, several have poorly specified statistical models (see discussion below), and there is inconsistent treatment of urinary creatinine, iodide status, and presence of thyroid antibodies. Given these methodological concerns, the lack of concordance in results is not surprising. A prospective study using individual level measures of both exposure and outcome is needed to truly determine a causal link between perchlorate exposure and either thyroid function or child neurobehavioral development. There are no prospective studies examining the association between individual urinary biomarkers of perchlorate exposure and individual serum biomarkers of thyroid function.

One final piece needed to fully interpret studies using spot urine specimens for determination of perchlorate and iodide is an improved understanding of the temporal variability of urinary measures of perchlorate, iodide, nitrate, and thiocyanate. Variability incorporates both daily variation in urine excretion and variation in exposure due to a variable diet. A thorough review and synthesis of the literature examining how well a single spot urinary measure of these compounds reflects long term exposure patterns is advised.
Misspecification of Statistical Models in Epidemiologic Studies

Potential statistical model misspecification is an important consideration when interpreting the results of seven studies published since the 2005 NRC report that have incorporated individual-level measures of perchlorate exposure and serum thyroid hormone concentrations (Blount et al. 2006, Steinmaus et al. 2007, Mendez 2012, Pearce 2010, 2011, 2012, and Leung 2012). Concerns relate to: 1) modeling perchlorate exposure as a linear term when the relationship with health outcomes may not be linear, 2) proper assessment of suspected effect measure modifiers, 3) inappropriately controlling for causal intermediates, 4) inadequate assessment of confounders leading to over-adjustment for factors suspected to be associated with the thyroid hormone outcomes but not with perchlorate exposure, and 5) suitable methods for modeling co-exposures to other goitrogens or thyroid hormone disrupters. These elements are addressed in more detail as they relate to specific studies.

All epidemiologic studies of urinary perchlorate concentrations and thyroid function published after the 2005 NRC report have reported results of linear regression models or generalized additive mixed models (GAMM) specifying perchlorate exposure as a linear term predicting continuous measures of thyroid function (Mendez 2012). Approaches that assume a monotonic linear relationship between perchlorate and thyroid hormone concentrations may fail to reveal other plausible patterns of association such as effects that occur only after some exposure threshold is reached, low dose effects that plateau at some point along the exposure continuum, or other possible U-shaped or inverted U-shaped patterns. Evidence for non-linear associations with perchlorate was examined by adding a square of the log of perchlorate to the linear regression models (Blount et al. 2006) and by using GAMM to determine whether smoothing of the perchlorate term provided a better model fit (Mendez 2012). However, the extent to which other patterns of association were explored in these and other studies is not evident. Furthermore, hypothyroxinemia during the first trimester of pregnancy rather than overt thyroid disease is increasingly of interest because hypothyroxinemia may result in irreversible neurodevelopmental deficits in the offspring (Delahunty 2010). However, existing studies have not incorporated this endpoint.

The seven studies that use individual-level biomarkers of exposure can be grouped according to their target populations which include women during the first trimester of pregnancy (Pearce 2010, 2011, 2012), infants at 1-3 months of age (Leung 2012), and the general U.S. population as represented by NHANES data (Blount 2006, Steinmaus 2007, Mendez 2012). The three cross-sectional studies of pregnant women by Pearce and colleagues (2010, 2011, 2012) have reported no observed associations between urinary perchlorate concentrations and first-trimester thyroid hormone levels in populations from California, Argentina, Wales, Italy, and Greece. While the studies were generally similar, the outcome assessment in one of the differed from the others in that fT4 and TSH levels were assessed as multiples of the median (Pearce et al. 2010). All of these studies used linear regression models adjusted for urinary iodine and TPOAb as well as other factors selected for their suspected associations with thyroid hormone status. Adjustment for iodine concentrations, TPOAb status and other indicators of potential susceptibility, however, deserves careful consideration. The rationale provided for controlling for both iodine and TPOAb titers is that women with low iodine or TPOAb may be more susceptible to the effects of perchlorate exposure on thyroid function. If the effect of perchlorate is anticipated to
differ across defined subgroups, it is appropriate to examine the factor as a potential effect measure modifier by using stratification or interaction terms rather than adjusting for the factor as a control variable. Otherwise, associations that may be present in defined subgroups could be obscured when these subgroups are combined for analysis. While these studies examined correlations between urinary perchlorate and thyroid hormones among women with urinary iodine concentrations < 100 µg/L, multivariable regression analyses of perchlorate exposure were not examined for interactions with iodine status. This evaluation was presumably limited by small sample sizes in the defined strata. The Pearce et al. study of 134 pregnant women from California and 107 pregnant women from Argentina reported examining a multivariable analysis restricted to TPOAb negative women from the combined study populations(2011). Results were not shown but were reportedly similar to results obtained from the unrestricted analyses of all women combined. Analyses among the potentially susceptible population of TPOAb positive women were likely limited due to small numbers. The study of 134 pregnant women from Greece reported examining and observing no interaction between urinary perchlorate and TPOAb positivity, although the statistical power to detect such interactions was again limited by the small sample size (Pearce et al. 2012).

It is noteworthy that Pearce et al. (2010) also controlled for smoking status defined as cotinine >500 ng/ml or thiocyanate concentrations (in separate models). The selected cotinine cutpoint of >500 ng/ml would represent relatively heavy smoking and would not successfully control for more modest levels of active smoking commonly indicated by urinary cotinine concentration of 15 ng/ml or 50 ng/ml. However, if the effect of perchlorate on thyroid function is suspected to be greater among smokers than non-smokers as reported by Steinmaus et al., then evaluation of potential interactions with smoking would precede assessment of confounding (2007). Other potential confounders such as age, race, body mass index (BMI), or creatinine concentrations were not considered in these models. Of particular note, there was no evaluation of confounding or effect measure modification by gestational age to consider the potential impact of changes in increasing fT4 and decreasing TSH concentrations that occur during the first trimester due to increased circulating concentrations of human chorionic gonadotropin and estrogen (de Escobar 2008). While the explanation for a potential association between perchlorate and gestational age remains unclear, gestational age was identified as a confounding factor of the perchlorate and thyroid hormone association among pregnant women in Greece (Pearce et al. 2012).

Another consideration is the potential bias that could be introduced by controlling for covariates that lie on the causal pathway between perchlorate exposure and thyroid function. The mechanism by which perchlorate may alter thyroid hormone status is by competitively inhibiting iodide uptake. This leads to the question of whether urinary iodide concentrations would be a proxy for intra-thyroid iodine deficiency, which lies on the causal pathway between perchlorate and thyroid hormone alterations. Inappropriately controlling for a causal intermediate can distort results by underestimating the true exposure effect, a result of partial or complete control of effects that occur through this pathway. Pearce et al. 2010 controlled for urinary iodide concentrations in fT4 models, but reported that urinary iodide concentrations were removed from the TSH models because iodide concentrations were not a significant predictor of TSH and the model was not significant when urinary iodide was included (Pearce 2011). All linear regression models in the remaining two Pearce et al. studies (2011, 2012) controlled for urinary iodide. Results were not available to compare multivariable models with and without control for these factors to determine if adjustment for iodide altered point estimates.
According to power analyses provided in the Pearce et al. publications, the studies of first trimester thyroid function were powered to detect stronger correlations than those observed; thus, the sample sizes were not sufficient to confirm the absence of more modest associations (2010, 2010, 2012).

Three studies have evaluated urinary perchlorate associations with thyroid function in NHANES study populations (Blount et al. 2006, Steinmaus et al. 2007, and Mendez 2012). The analysis by Blount et al. is considered one of the most definitive studies to date, due to the large nationally representative sample size and use of individual measures of urinary perchlorate concentrations. In the analysis of NHANES 2001-2002 data, Blount et al. observed no associations between perchlorate exposure and thyroid function in men. However, in women with urinary iodine <100 µg/L, log-transformed urinary perchlorate concentrations were positively associated with TSH concentrations and negatively associated with T4 concentrations. In women with urinary iodine ≥ 100 µg/L, perchlorate remained positively associated with TSH, but was not statistically associated with T4 concentrations. This was the first study to separately evaluate associations among women with insufficient iodine intake (urinary iodine <100 µg/L). The analysis by Blount et al. evaluated an extensive list of covariates selected on the basis of known or suspected associations with T4 or TSH concentration. These included age, race/ethnicity, BMI, estrogen use, menopausal status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate and selected medication groups. Models were also controlled for log creatinine to adjust for variability in urine dilution. The authors aimed to assess effects of perchlorate that were independent of other factors known to alter thyroid function. However, when the aim is to estimate causal associations, the goal is to control for those factors that may distort the true exposure-disease association due to mutual associations with the perchlorate exposure and thyroid hormone function outcome. The impact of unnecessarily adjusting for factors that are associated only with thyroid function (and, therefore are not acting as confounders) is potential loss of precision.

Steinmaus et al. extended the NHANES 2001-2002 analyses reported by Blount et al. in 2006 to examine interactions between perchlorate and smoking and between perchlorate and thiocyanate on thyroid function(2007). In women with urinary iodine concentrations < 100 µg/L, the negative association between log perchlorate and T4 was stronger in self-reported smokers, those with high serum cotinine concentrations, and those with higher urinary thiocyanate levels than in those without these characteristics. Similar interactions were not observed for log TSH. Although the T4 models were adjusted for fasting time, kilocalories, BMI, c-reactive protein, nitrate, race, estrogen use, pregnancy and menopause status, the authors reported that in most of the regression models only modest differences were observed between the adjusted and unadjusted coefficients. As in the Blount et al. study, it is unclear how some of the covariates may also be related to perchlorate exposure such as c-reactive protein, estrogen use, and menopause status, but controlling for extraneous covariates that are not confounders and not intermediates on the causal pathway would likely impact model precision but not bias results.

While the previous NHANES analyses were limited to assessments of total T4 and TSH, Mendez and Eftim’s (2012) analysis of NHANES 2007-2008 data incorporated total and free T4 and T3 concentrations. The results of generalized additive mixed models (GAMM) indicated log-transformed...
perchlorate concentrations were negatively associated with total T4 and free T3 in both males and females. In acknowledgment of the mutual effects of TSH, T3 and T4 levels on one another due to the negative feedback loop in the hypothalamic-pituitary-thyroid axis, the regression models in this study were controlled for TSH concentrations. However, TSH alterations may be a common effect of both the exposure (perchlorate) as well as the outcome (T4 concentrations); thus, the observed associations adjusted for TSH concentrations could be the result of collider-stratification bias, which is a form of selection bias that can produce spurious associations when controlling for a shared effects (Schisterman et al. 2009). Other covariates controlled in the analysis included thyroid antibodies and creatinine-adjusted urinary iodine, thiocyanate and nitrate and other environmental contaminants such as phthalate metabolites and bisphenol A. The covariates retained in final models were selected on the basis of statistical significance of associations with thyroid hormone levels; thus, confounding of the perchlorate-thyroid hormone association was not assessed directly, as in other studies, and overadjusting for non-confounders could reduce the precision of the point estimates (Schisterman et al. 2009). Of note, urinary iodine and thyroid antibodies were controlled in the analyses and were not assessed for potential effect measure modification.

Uncertainties exist regarding the optimal method for considering co-exposures to other goitrogens such as thiocyanate (including exposure occurring through tobacco exposure) and nitrate, which share the same mode of action as perchlorate. Studies have predominantly addressed this concern by controlling for urinary concentrations of other contaminants in multivariable models when the data are available for thiocyanate (Blount 2006, Mendez 2012, Pearce 2012, Pearce 2010, Leung et al. 2012), nitrate (Blount 2006, Steinmaus 2007), cotinine (Pearce 2010) or self-reported smoking (Leung 2012). Some studies, however, addressed the question by evaluating interactions between perchlorate and thiocyanate (Steinmaus 2007, Pearce 2012) and between perchlorate and smoking (Steinmaus 2007). These inconsistencies emphasize the need for more in-depth evaluation of co-exposures, including consideration of assessment of cumulative exposure.

The only study of infant thyroid function to incorporate individual measures of perchlorate exposure was conducted by Leung et al. (2012). This cross-sectional study of 64 (partially or exclusively breast-fed) infants ages 1-3 months reported no association between serum TSH or fT4 in infants and perchlorate concentrations in breast milk, maternal urine, and infant urine. The multivariable linear regression models controlled for thiocyanate (presumably measured in the same medium), maternal age, ethnicity, smoking status, iodine-containing prenatal multivitamin use and supplemental infant formula use. The effects of infant urinary perchlorate on infant serum fT4 and TSH were not statistically significant and the small effect sizes were interpreted by the authors as clinically insignificant changes. The small sample size, however, limits statistical power as well as precision of the point estimates.
APPENDIX C

General Comments on Integration of Information

Risk-based regulation that rests on quantitative analyses is designed to integrate disparate types of data and information for hazard, exposure and risk. For any given assessment, some of the available data will be of poor or lesser quality or of limited relevance, precluding their use for quantitative analyses. Therefore the Agency must employ transparent, rigorous review criteria and clear presentation of information to justify the data and methods selected for use in developing risk-based values such as MCLGs (NRC, 2011). The SAB considered the topic of ‘integration of information’ in this more general sense and offers the following recommendations for integration of the available data and information to guide its development of the perchlorate MCLG.

As EPA builds on the analyses presented in the White Paper and incorporates the panel’s recommendations, the Agency should consider the advice of the NRC Committee in its Review of the Draft IRIS Assessment on Formaldehyde (NRC 2011) to improve the clarity of assessment documents. The Agency needs an a priori approach for inclusion or exclusion and weighting of studies. Specifically the panel recommends that EPA develop a structured framework to capture the key points of the evaluation and application of each type of data or model used in the development of the perchlorate MCLG, as well as the strengths, limitations and uncertainties associated with each. This framework should be incorporated into the text, at the end of each relevant section. The text box below describes the elements of such a framework discussed by the panel. These elements can be supplemented with additional elements from the Agency’s guidance documents and current practices of data and weight of evidence evaluation. In applying the framework to the epidemiological data, the panel recommends that EPA take advantage of available evaluation tools such as Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)\(^5\) or Grading of Recommendations Assessment, Development and Evaluation (GRADE)\(^6\), as appropriate.

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\(^5\) Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

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Science Advisory Board (SAB) Draft Advisory Report (September 5, 2012)
For Discussion to Assist Meeting Deliberations
-- Do Not Cite or Quote --

This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

The draft framework also reflects the recommendations of the NRC as presented in Science and Decisions: Advancing Risk Assessment (NRC 2009), specifically the necessity to estimate and document the uncertainties in all aspects of an assessment including doses, exposures and outcomes.

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6 Grading of Recommendations Assessment, Development and Evaluation (GRADE)