UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE DMINISTRATOR
SCIENCE ADVISORY BOARD

May 29, 2013

EPA-SAB-13-004

The Honorable Bob Perciasepe
Acting Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

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

Dear Acting Administrator Perciasepe:

Perchlorate is both a naturally occurring and man-made chemical that is used to produce rocket fuel, fireworks, flares and explosives. It can be present in chlorine-based disinfection products and fertilizers. The Environmental Protection Agency identified perchlorate as a potential drinking water contaminant because it may have adverse health effects and has been detected in public drinking water systems.

In 2005, at the request of the EPA and other federal agencies, the National Research Council published a comprehensive report titled *Health Implications of Perchlorate Ingestion.* The NRC concluded that perchlorate contamination could affect thyroid function by inhibiting the transport of iodide into the thyroid, which can lead to thyroid hormone deficiency. Decreased levels of thyroid hormone can have adverse effects in sensitive populations such as people with thyroid disorders, pregnant women, fetuses, and infants.

The NRC recommended that the inhibition of iodide uptake into the thyroid, a precursor non-adverse effect, be used to derive a Reference Dose for perchlorate. The NRC recommended an RfD of 0.7 µg/kg/day 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 uncertainty factor of 10. The uncertainty factor was applied to account for differences in sensitivity between the healthy adults in the study and the most sensitive population, namely "fetuses of pregnant women who might have hypothyroidism or iodide deficiency." The NRC concluded that this RfD should be protective of the health of sensitive populations, and acknowledged that the RfD might need to be adjusted either up or down based on the results of new research. The RfD of 0.7 µg/kg/day was adopted by EPA in 2005.

In 2009, EPA identified perchlorate as a drinking water contaminant and initiated the process to develop a Maximum Contaminant 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."

The EPA developed a white paper that identified relevant perchlorate studies available since the publication of the NRC 2005 report. The agency also is evaluating the available physiologically-based pharmacokinetic models for perchlorate, as well as literature related to sensitive life stages that are likely to be at greater risk of adverse health effects. The EPA's Office of Water requested that the Science Advisory Board provide advice on how the agency should consider recent information on sensitive life stages, the agency's physiologically-based pharmacokinetic modeling efforts, epidemiological and biomonitoring studies, and approaches to use and integrate this information in deriving an MCLG. The SAB reviewed the recent information and EPA's white paper to develop advice on the four issue areas and provides its findings and recommendations in the enclosed report.

The SAB concludes it is important for the EPA to consider sensitive life stages explicitly in the development of an MCLG for perchlorate. The mode of action of perchlorate toxicity is well-understood. The mode of action involves the potential to disturb thyroid homeostasis by limiting the iodide uptake by the thyroid, which in turn can lead to production of less thyroid hormone. Interference with the thyroid and available thyroid hormones is known to produce adverse effects on neurodevelopment in humans, with fetuses and infants being most vulnerable. Although adverse neurodevelopmental effects of perchlorate in infants and children have not been reported in the literature, the risk of adverse effects can be reasonably inferred from perchlorate's mode of action and the known role of thyroid hormone on human brain development.

The NRC in 2005 concluded that the first adverse effect in the continuum of effects from perchlorate exposure would be hypothyroidism. In considering new information and health endpoints of potential concern, the SAB finds that the most sensitive life stages are the fetus, neonates and infants because these are the stages when thyroid-dependent brain development occurs. The development of the MCLG must consider the perchlorate exposure pathways relevant to each of these sensitive life stages, which for fetuses and breastfed infants includes exposure of pregnant and lactating women, respectively. The SAB further finds that hypothyroxinemia (i.e., low levels of thyroid hormone) is a more appropriate indicator of the potential adverse health effects than the more pronounced decreases in thyroid hormone associated with hypothyroidism. Thus, the sensitive populations EPA should consider for exposure to perchlorate are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women.

The SAB recommends that the 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 default MCLG approach using the RfD and specific chemical exposure parameters. Within this MOA framework, the PBPK/PD-IUI model provides a tool for integrating exposure (e.g., different drinking water consumption scenarios), perchlorate pharmacokinetics, and dose-response relationships for perchlorate effects at the different lifestages. The SAB finds that this data-driven approach represents a more rigorous way to address differences in biology and exposure between adults and sensitive life stages than is possible with the default approach for deriving an MCLG.

The SAB concludes that the epidemiological and biomonitoring data published since the NRC 2005 report are insufficient to guide causal inference with regard to the association between perchlorate exposure and thyroid dysfunction in the sensitive life stages and populations due to the inconsistent

results among the studies. As such, the current body of epidemiologic evidence cannot provide validation of a safe level of perchlorate in drinking water. Nonetheless, the SAB finds that the current epidemiology data may still be useful to support analyses to estimate perchlorate exposure of the potentially sensitive subgroups in the United States.

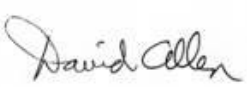
The SAB applauds the agency's efforts in developing models to better understand the adverse health effects of perchlorate in different life stages. To integrate the available information to develop a MCLG for perchlorate, the SAB urges the EPA to expand the modeling approach to account for thyroid hormone perturbations and potential adverse neurodevelopmental outcomes from perchlorate exposure. Incorporating these components into the model offers the opportunity for much greater scientific rigor in establishing quantitative relationships between perchlorate exposure and adverse effects at sensitive life stages. The SAB recognizes that full implementation of an enhanced modeling approach may take years to develop. As an interim approach, the agency could use the existing model to estimate iodide uptake inhibition and empirical observations to relate iodide uptake inhibition to thyroid hormone perturbations. Specifically, the clinical thyroid literature could be evaluated to identify the degree of iodide uptake inhibition required for onset of hypothyroxinemia in a pregnant woman. This information, together with modeling to link iodide uptake inhibition to perchlorate exposure, would provide the basis for an MCLG that addresses directly the most sensitive life stages for perchlorate effects.

The agency should incorporate the appropriate studies related to ingestion of perchlorate, pharmacokinetics of perchlorate, the effects (dynamics) of perchlorate, and dose-response relationships from all the available literature. 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 perturbations, regardless of the cause of those perturbations, to document and support parameters used in the model. Accordingly, the SAB concludes that these two streams of information — biology of iodide deficiency and perchlorate inhibition of iodide uptake — are complementary 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 on brain development due to inadequate iodide uptake or low thyroid hormone levels vary at different life stages, but are especially critical during the early formative stages of brain development, when the human brain most needs thyroid hormone.

As perchlorate research continues, studies in animals may provide important insights into neurobehavioral consequences of perchlorate exposure. A physiologically-based pharmacokinetic/pharmacodynamic framework is well suited to help place these findings in the context of human perchlorate exposure.

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

Sincerely,



Dr. David T. Allen
Chair
Science Advisory Board

Dr. Stephen M. Roberts
Chair
SAB Perchlorate Advisory Panel

Enclosure

NOTICE

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.

U.S. Environmental Protection Agency
Science Advisory Board
Perchlorate Advisory Panel

CHAIR

Dr. Stephen M. Roberts, Professor, Department of Physiological Sciences, Director, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL

MEMBERS

Dr. Grant W. Anderson, Associate Professor, Department of Pharmacy Practice and Pharmaceutical Sciences, College of Pharmacy, University of Minnesota, Duluth, MN

Dr. Hugh A. Barton, Associate Research Fellow, Modeling & Simulation, Pharmacokinetics, Dynamics, and Metabolism, Pfizer Inc, Groton, CT

Dr. Nancy Carrasco, Professor, Department of Cellular and Molecular Physiology, School of Medicine, Yale University, New Haven, CT

Dr. Claude Emond, Adjunct Clinical Professor, Department of Environmental and Occupational Health, Faculty of Medicine, University of Montreal, Montréal, QC, Canada

Dr. Jeffrey Fisher, Research Toxicologist, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR

Dr. Mary A Fox, Assistant Professor, Department of Health Policy and Management, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD

Dr. Wendy J. Heiger-Bernays, Associate Professor, Environmental Health, School of Public Health, Boston University, Boston, MA

Dr. Julie B. Herbstman, Assistant Professor, Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY

Dr. David G. Hoel, Principal Scientist, Exponent, Charleston, SC

Dr. Judy LaKind, President, LaKind Associates, LLC. Adjunct Associate Professor, Department of Epidemiology and Public Health, University of Maryland School of Medicine. Adjunct Associate Professor, Department of Pediatrics, Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center Catonsville, MD

Dr. Paul H. Lipkin, Associate Professor of Pediatrics, Division of Neurology and Developmental Medicine, The Kennedy Krieger Institute, Johns Hopkins University School of Medicine , Baltimore, MD

Dr. Jennifer Peck, Associate Professor, Department of Biostatistics and Epidemiology, Health Sciences Center, University of Oklahoma, Oklahoma City, OK

Dr. Joanne F. Rovet, Professor, Pediatrics and Psychology, University of Toronto. Senior Scientist, Neuroscience and Mental Health Program, The Hospital for Sick Children, Toronto, ON, Canada

Dr. Cheryl R. Stein, Assistant Professor, Department of Preventive Medicine, Mount Sinai School of Medicine, New York, NY

SCIENCE ADVISORY BOARD STAFF

Mr. Thomas Carpenter, Designated Federal Officer, U.S. Environmental Protection Agency, Washington, DC

U.S. Environmental Protection Agency
Science Advisory Board

CHAIR

Dr. David T. Allen, Gertz Regents Professor of Chemical Engineering and the Director of the Center for Energy and Environmental Resources, The University of Texas, Austin, TX

MEMBERS

Dr. George Alexeeff, Director, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA

Dr. Pedro Alvarez, Department Chair and George R. Brown Professor of Engineering, Department of Civil & Environmental Engineering, Rice University, Houston, TX

Dr. Joseph Arvai, Svare Chair in Applied Decision Research, Institute for Sustainable Energy, Environment, & Economy, Haskayne School of Business, University of Calgary, Calgary, Alberta, Canada

Dr. Thomas Burbacher, Professor, Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA

Dr. Ingrid Burke, Director, Haub School and Ruckelshaus Institute of Environment and Natural Resources, University of Wyoming, Laramie, WY

Dr. Thomas Burke, Professor and Jacob I. and Irene B. Fabrikant Chair in Health, Risk and Society Associate Dean for Public Health Practice, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

Dr. Edward T. Carney, Departmental Senior Science Leader and Director of Predictive Toxicology Center, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI

Dr. Terry Daniel, Professor of Psychology and Natural Resources, Department of Psychology, School of Natural Resources, University of Arizona, Tucson, AZ

Dr. George Daston, Victor Mills Society Research Fellow, Global Product Stewardship, The Procter & Gamble Company, Mason, OH

Dr. Costel Denson, Managing Member, Costech Technologies, LLC, Newark, DE

Dr. Otto C. Doering III, Professor, Department of Agricultural Economics, Purdue University, W. Lafayette, IN

Dr. Michael Dourson, President, Toxicology Excellence for Risk Assessment, Cincinnati, OH

Dr. Joel Ducoste, Professor, Department of Civil, Construction, and Environmental Engineering, College of Engineering, North Carolina State University, Raleigh, NC

Dr. David A. Dzombak, Walter J. Blenko, Sr. University Professor of Environmental Engineering, Department of Civil and Environmental Engineering, College of Engineering, Carnegie Mellon University, Pittsburgh, PA

Dr. T. Taylor Eighmy, Vice Chancellor for Research and Engagement, University of Tennessee, Knoxville, TN

Dr. Elaine Faustman, Professor and Director, Environmental and Occupational Health Sciences, University of Washington, Seattle, WA

Dr. R. William Field, Professor, Department of Occupational and Environmental Health, and Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA

Dr. H. Christopher Frey, Distinguished University Professor, Department of Civil, Construction and Environmental Engineering, College of Engineering, North Carolina State University, Raleigh, NC

Dr. John P. Giesy, Professor and Canada Research Chair, Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Dr. Barbara L. Harper, Risk Assessor and Environmental-Public Health Toxicologist, and Division Leader, Hanford Projects, and Program Manager, Environmental Health, Department of Science and Engineering, Confederated Tribes of the Umatilla Indian Reservation (CTUIR), West Richland, WA

Dr. Cynthia M. Harris, Director and Professor, Institute of Public Health, Florida A&M University, Tallahassee, FL

Dr. Robert Johnston, Director of the George Perkins Marsh Institute and Professor, Economics, Clark University, Worcester, MA

Dr. Kimberly L. Jones, Professor and Chair, Department of Civil Engineering, Howard University, Washington, DC

Dr. Bernd Kahn, Professor Emeritus and Associate Director, Environmental Radiation Center, Georgia Institute of Technology, Atlanta, GA

Catherine Karr, Associate Professor - Pediatrics and Environmental and Occupational Health Sciences and Director - NW Pediatric Environmental Health Specialty Unit, University of Washington, Seattle, WA

Dr. Madhu Khanna, Professor, Department of Agricultural and Consumer Economics, University of Illinois at Urbana-Champaign, Urbana, IL

Dr. Nancy K. Kim, Senior Executive, Health Research, Inc., Albany, NY

Dr. Francine Laden, Mark and Catherine Winkler Associate Professor of Environmental Epidemiology, Harvard School of Public Health, and Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Dr. Cecil Lue-Hing, President, Cecil Lue-Hing & Assoc. Inc., Burr Ridge, IL

Dr. Elizabeth Matsui, Associate Professor, Pediatrics, School of Medicine, Johns Hopkins University, Baltimore, MD

Dr. Surabi Menon, Director of Research, ClimateWorks Foundation, San Francisco, CA

Dr. James R. Mihelcic, Professor, Civil and Environmental Engineering, University of South Florida, Tampa, FL

Dr. Christine Moe, Eugene J. Gangarosa Professor, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA

Dr. Horace Moo-Young, Dean and Professor, College of Engineering, Computer Science, and Technology, California State University, Los Angeles, CA

Dr. Eileen Murphy, Director of Research and Grants, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ

Dr. James Opaluch, Professor and Chair, Department of Environmental and Natural Resource Economics, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

Dr. Duncan Patten, Director, Montana Water Center, and Research Professor, Hydroecology Research Program, Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT

Dr. Martin Philbert, Dean and Professor, Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI

Dr. Stephen Polasky, Fesler-Lampert Professor of Ecological/Environmental Economics, Department of Applied Economics, University of Minnesota, St. Paul, MN

Dr. C. Arden Pope, III, Professor, Department of Economics, Brigham Young University, Provo, UT

Dr. Stephen M. Roberts, Professor, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL

Dr. Amanda Rodewald, Professor of Wildlife Ecology, School of Environment and Natural Resources, The Ohio State University, Columbus, OH

Dr. James Sanders, Director and Professor, Skidaway Institute of Oceanography, Savannah, GA

Dr. William Schlesinger, President , Cary Institute of Ecosystem Studies, Millbrook, NY

Dr. Gina Solomon, Deputy Secretary for Science and Health, Office of the Secretary, California Environmental Protection Agency, Sacramento, CA

Dr. Daniel O. Stram, Professor, Department of Preventive Medicine, Division of Biostatistics, University of Southern California, Los Angeles, CA

Dr. Peter S. Thorne, Director, Environmental Health Sciences Research Center and Professor and Head, Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA

Dr. Paige Tolbert, Professor and Chair, Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA

Dr. Jeanne VanBriesen, Professor, Department of Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, PA

Dr. John Vena, University of Georgia Foundation Professor in Public Health and
Head, Department of Epidemiology and Biostatistics, Georgia Cancer Coalition Distinguished Scholar, College of Public Health , University of Georgia, Athens, GA

Dr. R. Thomas Zoeller, Professor, Department of Biology, University of Massachusetts, Amherst, MA

SCIENCE ADVISORY BOARD STAFF

Dr. Angela Nugent, Designated Federal Officer, U.S. Environmental Protection Agency, Washington, DC

TABLE OF CONTENTS

Acronyms and Abbreviations

µg	Microgram (one-millionth of a gram)
ADHD	Attention Deficit Hyperactivity Disorder
BBDR	Biologically Based Dose Response
BW	Body Weight
DAG	Directed Acyclic Graphs
DWI	Drinking Water Ingestion
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
fT4	Free thyroxine
GW	Gestational Week
HPT	Hypothalamus-Pituitary-Thyroid
HRL	Health Reference Level
I^-	Iodide
IQ	Intelligence Quotient
IUI	Iodide Uptake Inhibition
kg	Kilogram
K_m	Michaelis Constant
L	Liter
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MOA	Mode of Action
Na	Sodium
NHANES	National Health and Nutrition Examination Survey
NIS	Sodium (Na^+)/Iodide (I^-) Symporter
NOEL	No Observed Effect Level
NPDWR	National Primary Drinking Water Regulation
NRC	National Research Council
PBPK	Physiologically-Based Pharmacokinetic
PBPK/PD-IUI	Physiologically-Based Pharmacokinetic/Pharmacodynamic-Iodide Uptake Inhibition
POD	Point of Departure
PBPK/PD	Physiologically-Based Pharmacokinetic Pharmacodynamic
PWS	Public Water System
RAIU	Radioactive Iodide Uptake
RfD	Reference Dose
RSC	Relative Source Contribution
SAB	Science Advisory Board
SDWA	Safe Drinking Water Act
T3	Triiodothyronine
T4	Thyroxine or Tetraiodothyronine
TgAb	Thyroglobulin antibody
TPOAb	Thyroid Peroxidase Antibody
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone or thyrotropin
TSH-RAb	Thyroid Stimulating Hormone Receptor Antibody
THOP	Transient Hypothyroxinemia of Prematurity
UCMR	Unregulated Contaminant Monitoring Rule

UF	Uncertainty factor
μmU	Micromolar Units

1. EXECUTIVE SUMMARY

In 2005, at the request of the Environmental Protection Agency (EPA) and other federal agencies, the National Research Council (NRC) published a comprehensive report, *Health Implications of Perchlorate Ingestion.* The NRC concluded that perchlorate could affect thyroid function because it is an anion that competitively inhibits the transport of iodide into the thyroid and that a prolonged decrease of thyroid hormone can 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 recommended an RfD of 0.7 µg/kg/day, 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 (UF) of 10. The UF is intended to account for differences in sensitivity between healthy adults and the most sensitive population (i.e., fetuses of pregnant women who might have hypothyroidism or iodide deficiency). The NRC 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.

In 2009, EPA identified perchlorate as a drinking water contaminant and initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and a National Primary Drinking Water Regulation (NPDWR) for perchlorate under the Safe Drinking Water Act (SDWA). 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 (considering cost). The 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."

The EPA developed a white paper that identifies recent epidemiological and biomonitoring studies and physiologically-based pharmacokinetic (PBPK) models for perchlorate. The agency is evaluating these studies, in addition to the data and information used by the NRC, to consider sensitive life stages that comprise groups within the general population that are likely to be at greater risk of adverse health effects. EPA's Office of Water requested that the SAB provide advice through responses to charge questions on how the agency should consider recent information on sensitive life stages, epidemiological and biomonitoring studies and the agency's PBPK modeling efforts. The agency also sought advice on approaches to use and integrate this information in deriving an MCLG for perchlorate.

In summary, the SAB finds that there is sufficient information to derive an MCLG for perchlorate and recommends that the agency use a mode of action (MOA) approach and physiologically-based pharmacokinetic/pharmacodynamic iodide uptake inhibition (PBPK/PD-IUI) modeling to integrate this information in a robust and transparent analysis. The SAB recognizes that this is a novel approach as

compared to previous MCLG derivations that use the RfD and exposure factors. However, PBPK/PD-IUI modeling provides a more rigorous tool to integrate the totality of information available on perchlorate, and this approach may better address different life stage susceptibilities to perchlorate than the default MCLG approach.

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 inadequate iodide uptake — and the consequence of low thyroid hormone levels on brain development — vary at different life stages. The fetus[1] and infant are more susceptible to perchlorate exposure effects than is the adult given that an adequate supply of thyroid hormone is essential for normal brain development. Consequently, deficits in brain development may become permanent if thyroid hormone deprivation occurs even transiently during fetal development or early life. While the effects of transient thyroid hormone deprivation on the adult brain are measurable, most signs and symptoms are reversible upon treatment with thyroid hormones. Additionally, the tissue-specific expression patterns of the sodium/iodide symporter (NIS), the molecular target of perchlorate, vary depending on life stage. Although no data exist on the long-term adverse neurodevelopmental effects of perchlorate *per se*, the human and animal data on the adverse effects of thyroid hormone perturbations (a down-stream effect from iodide uptake inhibition) on the developing brain support the need for a life stage approach. The evidence suggests that the most sensitive life stages are the fetuses, neonates and infants because these are the stages when thyroid-dependent brain development occurs. The development of the MCLG must consider the perchlorate exposure pathways relevant to each of these sensitive life stages, which for fetuses and breastfed infants includes exposure of pregnant and lactating women, respectively. Thus, the sensitive populations EPA should consider for exposure to perchlorate are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. This would replace "the fetuses of pregnant women who might have hypothyroidism or iodide deficiency" as defined by the NRC (2005).

Physiologically-Based Pharmacokinetic Pharmacodynamic Modeling

The EPA should utilize an MOA framework for developing the MCLG that links the steps in the proposed mechanism leading from perchlorate exposure through iodide uptake 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 scenarios), perchlorate pharmacokinetics, and dose-response relationships for perchlorate effects at the different lifestages. With this model, predictions for perchlorate pharmacokinetics and resulting iodide uptake inhibition can be used to address the initial steps of the MOA framework.

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

[1] Throughout this document, the term "fetus" is used to describe both the embryonic period (less than eight weeks) and the fetal period (nine weeks to term)

Epidemiological Data

The SAB concludes that the epidemiological data published since the NRC 2005 report are insufficient to guide causal inference with regard to the association between perchlorate exposure and thyroid dysfunction in pregnant women, neonates and infants or the general population. Limitations concerning study design, exposure assessment, sample size and statistical modeling have led to inconsistent results. As such, the current body of epidemiologic evidence cannot provide validation of a safe level of perchlorate in drinking water.

Nonetheless, the SAB finds that the current epidemiology data may still be useful. The available data provide support for analyses to estimate: the size of potentially sensitive subgroups in the United States; the extent to which the general U.S. population and sensitive subgroups are exposed to perchlorate, as well as other compounds with the comparable MOA (i.e., goitrogens); and the relative source contribution of perchlorate in drinking water among sensitive subgroups not addressed in the Food and Drug Administration's Total Diet Study.

Integration of Information Using PBPK/PD Modeling

The SAB recommends integrating all of the available 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-IUI model that associates perchlorate intake via drinking water with percent iodide uptake inhibition.

The SAB notes that the EPA developed a PBPK/PD model for perchlorate that builds on the models reviewed by the NRC. The PBPK/PD model can be used in its present form to derive an MCLG based on iodide uptake inhibition. The limitation of the model in its current state, similar to the limitations of the standard MCLG approach, in that it describes a precursor event and does not explicitly predict subsequent events or adverse outcomes. Therefore, the SAB recommends that the EPA expand the PBPK/PD approach past IUI to explicitly incorporate predictions of thyroid hormone insufficiencies. This approach will then permit assessment of the predicted exposure-response relationship for perchlorate exposure and alterations in thyroid hormone levels (e.g., decreases in serum free thyroxine (fT4)). The SAB recognizes that such an effort will require resources and time, likely on the order of one to several years. To develop an MCLG in the interim, the EPA could use the existing model to estimate IUI and develop empirical relationships for each of the steps beyond perchlorate-mediated IUI using the clinical literature. The clinical thyroid literature should be evaluated to identify the degree of iodide inhibition (percentage IUI) required for the onset of hypothyroxinemia in pregnant and lactating women and to have effects on the developing brain.

The agency should incorporate the appropriate studies related to ingestion of perchlorate, pharmacokinetics of perchlorate, the effects (dynamics) of perchlorate, and dose-response relationships from all available literature. 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, to document and support parameters used in the model. The SAB notes that as perchlorate research continues, studies in animals may provide important insights into neurodevelopmental consequences of perchlorate exposure.

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, the 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 that will allow multiple sources of information to be integrated into the MCLG derivation.

2. INTRODUCTION

2.1. Background

Perchlorate is both a naturally occurring and man-made chemical that is used to produce rocket fuel, fireworks, flares, and explosives, and can be present in chlorine-based disinfection products and fertilizers. The EPA identified perchlorate as a potential drinking water contaminant because it may have an adverse health effect and has been detected in public water systems.

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 anion that competitively inhibits the transport of iodide[2] 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 biosynthesis of key thyroid hormones – triiodothyronine (T3) and thyroxine (T4) – and increased biosynthesis of thyroid stimulating hormone or thyrotropin (TSH). The NRC also concluded that a prolonged decrease of thyroid hormone can 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 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 recommended an RfD of 0.7 µg/kg/day, 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 (UF) of 10. The UF is intended to account for differences in sensitivity between healthy adults and the most sensitive population (i.e., fetuses of pregnant women who might have hypothyroidism or iodide deficiency). The NRC 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).

The EPA has initiated the process to develop a Maximum Contaminant Level Goal (MCLG) and National Primary Drinking Water Regulation (NPDWR) for perchlorate under the SDWA (U.S. 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 likely will 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 (considering cost).

[2] Molecular iodine is rapidly converted into iodide following ingestion, is efficiently absorbed throughout the gastrointestinal tract, and is prevalent in biological and physiological reactions (Welt and Blythe 1970). Trace level measurement in biological and physiological samples (e.g.,, milk, serum, urine) usually measures iodine (Shelor and Dasgupta 2011). This report uses either iodide, iodine, or the specific iodine measurement as cited in the studies to be consistent with the referenced authors' description.

EPA generally derives an MCLG using the following formula as a default:

$$\text{MCLG } (\mu g/L) = \frac{\text{RfD x BW}}{\text{DWI}} \text{ x RSC}$$

Where:

RfD is the reference dose for a contaminant (µg/kg/day).
BW is body weight in kg. A default body weight (70 kg) is typically used.
DWI is drinking water ingestion rate in L/day. A default intake (2 L/day) is typically used.
RSC is the relative source contribution. The RSC is derived as the percentage of the RfD remaining for drinking water after other sources of exposure to perchlorate (e.g., food) have been considered (U.S. EPA 2012). The EPA is relying on a Total Diet Study developed by the Food and Drug Administration (FDA) for perchlorate (Murray et al. 2008).

The regulatory schedule established by the 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. The 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."[3]

EPA developed a white paper (2012) that identifies available information published since the NRC report (2005). The white paper presents epidemiological studies, biomonitoring studies and physiologically-based pharmacokinetic (PBPK) modeling[4] that the agency is evaluating, in addition to the data and information used by the NRC, to consider sensitive life stages that are likely to be at greater risk of adverse health effects from perchlorate exposure than the general population.

EPA's Office of Water requested the Science Advisory Board's (SAB) advice on how best to consider the sensitive life stages, recent biomonitoring data, epidemiological studies, and PBPK modeling, and to 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 on the draft White Paper and discuss responses to the Charge to the SAB. The Panel held follow-up teleconferences on September 25, December 5, and December 7, in 2012 to discuss their draft responses to the EPA Charge questions The Panel's draft report was considered by the Chartered SAB on March 29, 2013. The Chartered SAB unanimously approved the report with slight modifications to provide additional citations to support infants as a sensitive

[3]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.

[4] The EPA white paper and Charge to the SAB refer to the current model as a PBPK model. The SAB notes that the current model predicts iodide uptake inhibition, which is a pharmacodynamic step in the mode of action. This report refers to the model as PBPK/PD.

population, clarify the potential use of epidemiological data, and clarify how results of PBPK-PD models could be considered to estimate adverse effects.

2.2. Charge to the Science Advisory Board

The EPA Charge to the SAB requests advice and recommendations on approaches to derive an MCLG for perchlorate. The 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 addresses iodide 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 biomonitoring and epidemiological studies. The Charge also asks the SAB how best to integrate the totality of available 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.

3. RESPONSE TO CHARGE QUESTIONS

The first three sets of specific charge questions focus on how the EPA should consider various life stage factors, PBPK modeling, and epidemiological and biomonitoring studies published since the NRC report, *Health Implications from Perchlorate Ingestion* (2005), to develop an MCLG. A fourth set of charge questions addresses the related issue of how this and other available 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.

In responses to charge questions on different life stages, the SAB identified the most sensitive life stages as the developing child — fetus[5], neonate and infant — because these are stages when thyroid-dependent brain development occurs. The development of the MCLG must consider the perchlorate exposure pathways relevant to each of these sensitive life stages, which for fetuses and breastfed infants include exposure of pregnant and lactating women, respectively. Thus, the sensitive populations that EPA should consider for exposure to perchlorate are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. Iodide deficiency, decreased thyroid hormone biosynthesis, and other key factors were identified as important considerations in addressing perchlorate health risk. The SAB also noted the agency's progress in using PBPK/PD models to better understand the potential impacts of perchlorate exposure during different life stages. The PBPK/PD models characterize the dose-response relationship between perchlorate exposure in food and water and perchlorate concentrations in plasma and tissue and resulting IUI. By selecting a POD for IUI, the current models could be used to develop an MCLG. The models could be further enhanced to encompass more of the MOA, characterizing the dose-response relationship between the dose of perchlorate and IUI and inhibition of early life thyroid hormone production, and extending finally to neurodevelopmental endpoints. At each stage of model expansion, selection of an appropriate POD would allow development of a more refined MCLG. In review of the epidemiological and biomonitoring studies, the SAB identified data of value in assessing risk of perchlorate exposure, but found that limitations and inconsistent results in the epidemiological and biomonitoring studies precluded their applicability to deriving the MCLG.

When considering how to integrate the disparate information and analyses into the derivation of an MCLG, the SAB found that the default algebraic approach provides limited ability to address the various exposure and biological factors affecting sensitivity to perchlorate at different life stages. The SAB concluded that, from a scientific standpoint, it would be more appropriate to base the MCLG derivation on the perchlorate mode of action, using PBPK/PD modeling to relate perchlorate concentrations in drinking water to biological effects rather than the default approach.

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;*

[5] Throughout this document, the term "fetus" is used to describe both the embryonic period (less than eight weeks) and the fetal period (nine weeks to term)

- *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 studies directly linking perchlorate to neurobehavioral effects in infants and children are lacking. However, the SAB notes that there are scientifically sound human clinical and rodent toxicology reports that describe the biology linking iodide deficiency, changes in thyroid hormone production and developmental and neurobehavioral effects. The mechanisms of perchlorate inhibition of NIS-mediated iodide uptake into the thyroid are also well documented (Dohan et al. 2007; Tran et al. 2008; Paroder-Belenitsky et al. 2011). Accordingly, the SAB concludes that these streams of information — neurobiology of iodide deficiency, thyroid hormone deficiency and perchlorate inhibition of iodide uptake — are complementary 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 on brain development from inadequate iodide uptake and low thyroid hormone levels vary at different life stages, but are especially critical during the early formative stages of brain development.

The thyroid hormones T3 and T4 are the only iodine-containing hormones in the body. Dietary iodide is transported from the bloodstream into the thyroid via the NIS, an intrinsic plasma membrane protein consisting 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 expressed in the salivary glands and stomach, two tissues where active iodide transport also takes place. Notably, NIS is also 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).

To synthesize these hormones, iodide is transported by NIS from the bloodstream into the interior of the thyroid cell and then oxidized and covalently incorporated into specific tyrosyl residues on a large precursor molecule called thyroglobulin in the colloid of the thyroid (Carrasco 1993). After endocytosis of iodinated thyroglobulin and proteolysis, the resulting thyroid hormones, T3 and more abundant T4, are transported from the thyroid via the bloodstream to various essential target organs. One primary target organ is the brain, which has a well-defined need for thyroid hormones for its normal development (Zoeller and Rovet 2004).

A deficit of thyroid hormones leads to inadequate brain development, which ultimately may cause intellectual and behavioral impairments in the developing child (Morreale de Escobar et al. 2000) and continue throughout life (Oerbeck et al. 2003; Kempers et al. 2006). Since the iodide needed for T3 and T4 production cannot be synthesized within the body, iodide must be obtained through the diet, and this requires a constant and sufficient supply of iodide to ensure normal thyroid function (Carrasco 1993). In addition, the need for iodide is substantially higher during pregnancy to support the increased production of maternal thyroid hormones that occurs during this period (Glinoer 2004). Children who experienced iodide or thyroid hormone insufficiency during early critical stages of brain development (viz., gestation

and infancy) are at risk of neurological, mental, and growth impairments (Glinoer and Delange 2000; Glinoer and Rovet 2009). Importantly, repletion of thyroid hormone outside these critical windows of time may be insufficient for reversal of these impairments (Porterfield and Hendrich 1993; Bernal 2005).

Perchlorate inhibits iodide uptake and therefore interferes with thyroid hormone production. Perchlorate acts by specifically inhibiting NIS-mediated transport of iodide into the thyroid, placenta, lactating breast, and all other NIS-expressing tissues in a concentration-dependent manner. 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-Belenitsky et al. 2011). Thus, in the presence of perchlorate, less iodide may be available for thyroid hormone biosynthesis. The extent of inhibition of iodide uptake is dependent upon the relative concentrations of the two anions and their respective Michaelis constants (K_m) for transport. Consequently, a primary downstream effect of perchlorate exposure is reduction in the levels of T3 and T4.

Although the critical evidence is lacking to directly link perchlorate to altered brain development in humans, animal studies show that exposing pregnant dams to perchlorate is associated with compromised brain development in their progeny (Gilbert and Sui 2008). In humans, studies of children born to mothers with either iodide or thyroid hormone insufficiencies provide complementary evidence. Specifically, the offspring of women who were iodide deficient during pregnancy show cognitive and behavioral impairments (Pharoah et al. 1984; Vermiglio et al. 2004). These impairments could be ameliorated by giving mothers iodide supplementation from the first trimester (Berbel et al. 2009; Velasco et al. 2009; Glinoer and Rovet 2009). Iodide supplementation begun in later trimesters did not show that the impairments were ameliorated, suggesting a critical and early window of iodide sufficiency for fetal brain development. Similarly, children born to women with clinical (Smit et al. 2000; Mirabella et al. 2000) or subclinical hypothyroidism (Haddow et al. 1999) show reduced intelligence quotient (IQ), selective cognitive deficits, and behavioral abnormalities compared with children whose mothers had normal pregnancy TSH levels. Haddow et al. (1999) also showed that the degree of compromised neurodevelopmental outcomes was less in the subgroup of children whose mothers reportedly took thyroid hormones exogenously in pregnancy; these findings demonstrate the importance of preventing any degree of hypothyroidism, regardless of its cause, in pregnancy.

Perhaps most critical are the findings from studies examining the effects of isolated maternal hypothyroxinemia, defined as a free thyroxine (fT4) value in the lower end of the normal range with normal levels of TSH. This research has involved a variety of cutoffs to signify maternal hypothyroxinemia ranging from fT4 below the 10^{th} or 5^{th} percentiles to below the 2.5^{th} percentile (Moleti et al. 2011), with the former percentiles being used to investigate neurodevelopmental outcomes and the latter the incidence and effects on pregnancy (e.g., Casey et al. 2005). Children exposed gestationally to maternal hypothyroxinemia (without hypothyroidism) show reduced levels of global and specific cognitive abilities, as well as increased rates of behavior problems including greater dysregulation in early infancy and attentional disorders in childhood (Man et al. 1991; Pop et al. 1999; Pop et al. 2003; Kooistra et al. 2006). Notably these effects are correlated with both degree (Pop et al. 1999; Henrichs et al. 2010) and duration (Pop et al. 2003) of maternal hypothyroxinemia. The Henrichs (2010) study, which stratified children into severe ($<5^{th}$ percentile) and mild ($5\text{-}10^{th}$ percentile) maternal hypothyroxinemia subgroups, showed that while effects were stronger and broader in the severe subgroup, the mild subgroup still showed delayed language development, thus suggesting that any factor that lowers maternal fT4, even slightly, can affect the offspring.

Two lines of evidence suggest that the infant also may be vulnerable to perchlorate exposure: infants born preterm who experience transient hypothyroxinemia of prematurity (THOP) and children with congenital hypothyroidism. THOP arises because the fetal thyroid system is immature if a child is born preterm and the late-gestational maternal iodine and thyroid hormone supplies are no longer available (Vulsma et al. 1989; Morreale de Escobar et al. 2008; LaGamma 2008; Simic and Rovet 2010). Follow-up studies of THOP report reduced IQ (Lucas et al. 1996), impaired visual skills (Rovet and Simic 2008) and an increased incidence of neurological dysfunction and school failure (Den Ouden et al. 1996), cognitive disabilities (Simic and Rovet 2010), cerebral palsy (Reuss et al. 1996) The effects were most severe in those with the lowest levels of thyroid hormone in the neonatal period (Simic and Rovet 2010). In an animal model of prematurity, Berbel and colleagues showed that manifestations of low thyroid hormone levels on the neural substrates of abilities are affected in THOP (Berbel et al. 2010). Congenital hypothyroidism, which arises from a defect in thyroid gland formation or function or its central regulation by the hypothalamus and pituitary (Rovet and Daneman 2003), is associated with mental retardation and severe behavior problems if untreated in the newborn period (Rovet 1992). Since the advent of newborn screening for congenital hypothyroidism, affected children undergo a far briefer period of thyroid hormone deficiency than before, showing IQ reductions of about 6-7 points (Rovet 2005), and a variety of subtle selective neurocognitive deficits (Rovet and Daneman 2003), the nature of which reflect the timing and duration of being without thyroid hormone. While children with congenital hypothyroidism demonstrate that thyroid hormone is essential throughout infancy, the exact time when the brain is no longer critically dependent on an adequate supply of thyroid hormone is unknown but estimated to be two years of age, when most essential neurodevelopment is complete.

Recommendation:

The SAB recommends that the EPA consider sensitive life stages in developing an MCLG for perchlorate. The SAB finds that the most sensitive life stages are the fetus, neonates and infants because these are the stages when thyroid-dependent brain development occurs. The development of the MCLG must consider the perchlorate exposure pathways relevant to each of these sensitive life stages, which for fetuses and breastfed infants include exposure of pregnant and lactating women, respectively. Thus, the sensitive populations that EPA should consider for exposure to perchlorate are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. This would replace “the fetuses of pregnant women who might have hypothyroidism or iodide deficiency” as defined by the NRC (2005).

3.1.2. Life Stage Specific Differences in Body Weight and Intakes

Specific differences in body weight, food intake, and drinking water consumption are important factors for the understanding of perchlorate-induced iodide uptake inhibition (IUI) at different life stages. The factors specified in this subpart of the charge question are a reflection of the default formula applied by the EPA to develop an MCLG from an RfD, which is frequently applied for chronic toxicities for which adult body weight and intake dominate exposure calculations. The challenge in the case of perchlorate is that the developing nervous system is of interest and thus, exposures during specific periods of development (e.g., *in utero* or early postnatal) need to be considered. During these periods, many biological changes occur beyond body weight and food or water intake. For example, evidence is available from the literature on other drug and chemical exposures showing differing absorption and metabolism rates with age and body weight (Kearns et al. 2003; Bartelink et al. 2006; Anderson and Lynn 2009). Since NIS is expressed in tissues other than the thyroid, such as the salivary glands, stomach, lactating breast, and placenta, one might anticipate developmental differences in pharmacokinetics and pharmacodynamics for perchlorate and iodide uptake inhibition.

Recommendation:

The SAB notes that the EPA developed a PBPK/PD model that considers life stage differences in thyroid NIS inhibition and has continued to develop this model (U.S. EPA 2009, 2012). Because the SAB recommends using the PBPK/PD modeling approach (see Sections 3.2 and 3.4), life stage specific differences in body weight, food, and drinking water intakes have been and should be explicitly incorporated in the modeling of each life stage and documented. Additionally, differences in other parameters characterizing the biological system in the model, such as organ weights (volumes), blood flows, or NIS activity have been incorporated and over time may need to be updated if more information becomes available in the scientific literature.

The SAB acknowledges that NIS expression is accounted for in different tissues and at different stages of development in the current PBPK/PD model for radioactive iodine uptake (RAIU) inhibition calculations (see Section 3.2). In addition, the current PBPK/PD model addresses the movement of perchlorate into relevant organs (i.e., lactating breast, mammary gland, placenta, and thyroid gland of the mother and the fetus) that can interfere with the availability of thyroid hormones for brain development. In the longer term, new models for the hypothalamic pituitary thyroid axis need to also include these same competitive inhibition equations for both iodide and perchlorate for NIS-bearing organs or tissues.

3.1.3. Differences in Potential Adverse Effects to Neonates, Infants and Young Children

The SAB finds that neonates, infants and children are significantly more sensitive than are adults to the potential effect of decreased thyroid hormone levels on brain development, and that these effects are significantly longer lasting in the child population.

It is well established that thyroid hormones 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 into the brain through the blood-brain barrier by specific transporters (Patel et al. 2011). From there, T4 enters glia, where it is metabolized to T3 by local deiodinases. The resulting T3 is then transported via specific transporters (Kester et al. 2004) into target brain cells, where it binds to nuclear thyroid hormone receptors and regulates expression of key brain genes fundamental to critical neurodevelopmental processes (Anderson et al. 2003; Bernal 2007). These processes include neurogenesis, neuronal migration, axon and dendritic growth, synaptogenesis, and myelination (Chan and Rovet 2003). Thyroid hormones regulate these developmental processes throughout gestation and early life (Zoeller and Rovet 2004). The temporal sensitivity of thyroid hormone deprivation differs depending on brain region. Therefore, the consequences of thyroid hormone insufficiency, regardless of cause, will vary depending on when the deficiency occurs (Royland et al. 2008). Furthermore, since different brain regions vary in development as to their timing of need for thyroid hormone (Thompson and Potter 2000; Morreale de Escobar et al. 2004), the specific consequences of thyroid hormone insufficiency or iodide deficiency will also differ regionally within the brain (Schweizer et al. 2008). Importantly, the adult brain is also sensitive to hypothyroidism with observed changes in mood and cognition, and linkage to neuropsychiatric symptoms (Bauer et al. 2008; Samuels 2008). However, in adults most signs and symptoms are reversible upon treatment with thyroid hormones, indicating that most effects of hypothyroidism on the adult brain are not permanent (Bauer et al. 2008) and are therefore less severe compared to reduced thyroid hormone levels during brain development.

Finally, as human neurodevelopment occurs along a continuum through gestation to childhood, it is also important to consider that the human thyroid develops during gestation and does not begin secreting thyroid hormones in limited amounts until the fourth month of gestation (Ballabio et al. 1989; Obregon

et al. 2007), with earlier embryonic and fetal brain development being totally reliant on the maternal thyroid hormone supply (Kempers et al. 2004).

There is diversity among the multiple markers in the developing brain that are sensitive to alterations in thyroid hormone concentrations during development, as revealed in both human and animal research (Bernal 2005; Ahmed et al. 2008; Gilbert et al. 2012). The molecular basis of thyroid hormone action is the regulation of gene transcription. Target genes can be regulated directly through receptors bound to gene regulatory regions, or indirectly through thyroid hormone-dependent changes in regulatory gene expression. Alterations in the expression of target genes in the brain may also be associated with downstream changes in, for example, brain cytoarchitecture, cellular function, morphology, physiology, and behavior (Bernal 2005; Ahmed et al. 2008; Gilbert et al. 2012). Therefore, some Perchlorate Advisory Panel members thought that a wide range of associated downstream markers could be used to indicate thyroid hormone insufficiency during development provided they are well documented as directly or indirectly regulated by thyroid hormone. The use of new neuroimaging approaches allows researchers to investigate these effects in humans (Wheeler et al. 2011, 2012). Changes in any of these validated markers could be considered evidence of a precursor event to an adverse effect when assessing the potential impact of perchlorate on iodide uptake inhibition and circulating and tissue thyroid hormone levels during brain development. Importantly, changes in these markers will vary according to the stage of development and time period over which the thyroidal perturbation occurs. Finally, observed changes may be permanent or transient depending upon the developmental time frame of thyroid hormone repletion.

The SAB recognizes that it is essential to obtain robust data in order to best assess 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 the dearth of studies of perchlorate effects on neurodevelopment, the literature on iodide deficiency, maternal hypothyroxinemia, THOP, and congenital hypothyroidism is robust and provides key data identifying the range of thyroidal perturbation attributable to reductions in iodide availability to the thyroid gland or to thyroid hormone production itself. The importance of these broad areas of research for 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, which in turn means less thyroid hormone for the developing brain. 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 while the currently available studies are insufficient to draw unequivocal conclusions regarding the impact of perchlorate exposure on human brain development, studies on iodide deficiency and maternal low thyroid hormone levels are invaluable. Indeed, recent studies based on newly available neuroimaging data show a direct impact of these deficiencies on the human brain (Willoughby 2011; Wheeler et al. 2011, 2012).

3.1.4. Thyroid Hormone Reserve Differences

It is reported that fetuses and infants have lower reserves of thyroid hormones (van De Hove et al. 1999; Savin et al. 2003) and those thyroid hormones have shorter half-lives compared to half-lives in adults (Brent 2010). However, the key evidence linking these features to perchlorate levels, iodide levels, and outcome is lacking. It is possible that gestational exposure to perchlorate can have an impact on fetal thyroid hormone production and brain development, without necessarily altering maternal thyroid hormone levels, and this effect can be compounded by iodine insufficiency (Zoeller 2004; Brent 2010). In addition, while the fetus can employ compensatory mechanisms to protect from reduced thyroid hormone levels, recent animal studies have found that while mild to moderate thyroid hormone

deficiency induces compensation, it may not be sufficient to fully protect the brain from reduced circulating thyroid hormones when exposed to goitrogens (Sharlin 2010; Bastian 2012).

A study by Blount et al. (2009) measuring perchlorate and iodine levels from multiple compartments (e.g., maternal urine, maternal serum, cord blood serum, amniotic fluid) in women undergoing cesarean section surgery showed that at time of birth, perchlorate levels were high, including in cord blood, but there was no evidence of either inhibition of iodine transport across the placenta or impact on infant growth. While the absence of effect may be due to the high levels of iodine in the study population, since most women were taking iodine-fortified prenatal vitamins, it is also possible that later developmental effects may become evident but are more subtle than those measured by Blount (Brent 2010) and that perchlorate effects will be observed in breast milk once the infant starts to feed (Blount et al. 2009). Nevertheless, the EPA should consider lower thyroid hormone reserves and shorter retention or half-lives in comparison with the non-pregnant adult.

Recommendation:

When determining safe levels of perchlorate in drinking water, the EPA should consider the shorter half-life and lower reserves of thyroid hormone and metabolic differences in each of the specific sensitive life stages evaluated. It is critical that the EPA consider these key features in making comparisons with the non-pregnant adult, based on the Greer et al. study (2002). Additionally, this issue may 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 perchlorate exposure has the potential to affect the developing embryo and fetus in several ways. First, this exposure can lead to less iodide for the fetal thyroid. In addition, gestational perchlorate exposure can mean less maternal thyroid hormone 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 thyroid hormones. Later in gestation, when the fetal thyroid is functioning and needs iodide to make its own thyroid hormones, both maternal and fetal supplies of thyroid hormone will be reduced. This hypothyroxinemia (i.e., low thyroid hormone levels) will likely have an impact on the embryonic and fetal brain, affecting those processes, structures and pathways that have the highest need for thyroid hormone at the particular time. In addition, maternal hypothyroxinemia in pregnancy can lead to adverse reproductive and pregnancy outcomes, including increased rates of preterm delivery (Casey et al. 2005).

Although the fetal thyroid develops in the first trimester of pregnancy, it does not secrete thyroid hormone until the second trimester and is not centrally regulated by the hypothalamus and pituitary (which secrete thyrotropin releasing hormone (TRH) and TSH) until the third trimester (Thorpe-Beeston et al. 1991; Obregon et al. 2007). The fetal thyroid continues to grow throughout gestation (Costa et al. 1986), as does its capacity to secrete hormone (Williams et al. 2004). Autopsy evidence indicates that the fetal brain appears to need thyroid hormone very early in gestation, including in the embryonic nervous system, given findings of thyroid hormone receptors and measurable quantities of maternally derived thyroid hormone in embryonic brain (Kilby et al. 2000; Kempers et al. 2004). Since substantial quantities of maternal thyroid hormone 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 thyroid hormone to the fetus is necessary until the end of pregnancy. The fetal thyroid T4 stores are reduced in comparison to the adult suggesting the fetal thyroid is less resilient to prolonged thyroidal perturbation (van den Hove et al 1999; Savin at al. 2003; Zoeller and Rice 2004). After birth, small amounts of thyroid hormone 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 the maternal or the child thyroid hormone supplies are inadequate.

Women with inadequate levels of thyroid hormone during pregnancy due to hypothyroidism or hypothyroxinemia are unable to provide the fetus with sufficient thyroid hormone (Moleti et al. 2011). It is well established that 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; Haddow et al. 1999; Pop et al. 1999; Smit et al. 2000; Mirabella et al. 2000; Kooistra et al. 2006; Henrichs et al. 2010). Morreale de Escobar et al. (2004) found that maternal hypothyroxinemia, when occurring during gestation, has been associated with neurological impairment. Furthermore, iodide deficiency during pregnancy and early neonatal life is also associated with impaired development of the brain and suboptimal outcomes (Pharoah et al. 1984; Vermiglio et al. 2004) since pregnant and lactating women from iodide-deficient areas provide insufficient iodide through the placenta or breast milk to their offspring (Zimmerman 2009). Finally, children who are thyroid hormone-deficient due to congenital hypothyroidism or iodide deficiency also show suboptimal to poor neurodevelopmental outcomes, which reflect directly on the severity and duration of the thyroid hormone or iodide deficiency (Rovet and Daneman 2003; Vermiglio et al. 2004). Because most thyroid hormone-mediated brain development only becomes complete by the age of two years, the fetus, infant and very young child are especially vulnerable to the effects of both thyroid hormone and iodide deficiency.

Since perchlorate inhibits iodide transport into the thyroid, exposure to perchlorate can have a direct impact on the maternal thyroid, the fetal thyroid, and the child's thyroid throughout its development. Perchlorate is likely to have a downstream effect on the developing brain similar to that observed in studies of iodide and thyroid hormone deficiency. However, no data exist in humans directly examining the relation between perchlorate exposure, its thyroidal impact, and the developing brain. Nevertheless, a recent study with perchlorate-exposed rodent dams and offspring showed specific impairments of hippocampal synaptic transmission, even at low doses that only minimally affected the dam and pup thyroid axis (Gilbert and Sui 2008).

From studies of the developing human thyroid, it is expected that in early pregnancy, when the embryo or fetus rely entirely on the maternal supply of thyroid hormone to meet the early brain needs, perchlorate exposure will lead to reduced thyroid hormone from the mother, and this will have an impact on the brain functions that are developing at this early 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 thyroid hormone. Thus, perchlorate actively transported through the placenta via NIS may block fetal iodide uptake into the thyroid and lead to lowered thyroid hormone production. This lowered fetal thyroid hormone production, along with the already reduced maternal thyroid hormone supply, will likely lead to a state of fetal hypothyroxinemia throughout pregnancy. However, the critical data on these effects do not exist.

Perchlorate exposure after birth, through either water-based formula preparations or breast milk, can reduce the infant's capacity to synthesize thyroid hormone by blocking its iodide supply and lowering its capacity to produce thyroid hormone. Notably, breast-fed infants exposed to perchlorate may also receive less thyroid hormone in the milk than non-exposed infants because their mother's thyroid hormone production has been compromised by her reduced iodide supply due to the perchlorate (Sack et al. 1981; Rovet 1990). Older infants and young children may be affected by perchlorate in dairy milk and certain foods, in addition to perchlorate in drinking water.

Overall, these findings signify that perchlorate exposure at different sensitive life stages may lead to reduced thyroid hormone, which in turn can adversely affect brain development in gestation and infancy. Moreover, the effects may be particularly profound if exposure occurs during a critical window of development. Although some literature examining perchlorate levels in relation to maternal and neonatal thyroid hormone levels does exist, the findings are contradictory. Furthermore, the evidence is often limited methodologically and/or the statistical approach is inadequate (see Section 3.3.2). Nevertheless, the findings show that the fetus and infant are definitely more susceptible to effects of perchlorate exposure than is the adult. Exposure may be more harmful for fetuses and infants given that their brains are undergoing rapid thyroid hormone-dependent development, in contrast to the fully developed adult brain. Although no data exist on the long-term adverse neurodevelopmental effects of perchlorate *per se*, the data on the adverse effects of iodide deficiency and thyroid hormone perturbations (a downstream target) on the developing brain justify the need for a life stage approach to setting an MCLG.

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 measure fetal integrity directly by obtaining measurements such as fetal heart rate, ultrasound measures of fetal thyroid, fetal movement, growth and response to stimulation (Allen and Lipkin 2005). Additionally, in light of advances in neuroimaging of the fetus and neonate, future research could obtain direct measurements of the fetal brain in relation to perchlorate exposure at different levels.

3.2. Physiologically Based Pharmacokinetic Modeling

Charge Question:
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

Charge Question:
How should EPA consider 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 this effect of perchlorate 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 of 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 RAIU studies. The continuum of events in the MOA 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 as discussed in the previous section. 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 in sensitive populations. This MOA framework allows determination of the MCLG using the percent IUI as a surrogate for the adverse effect.

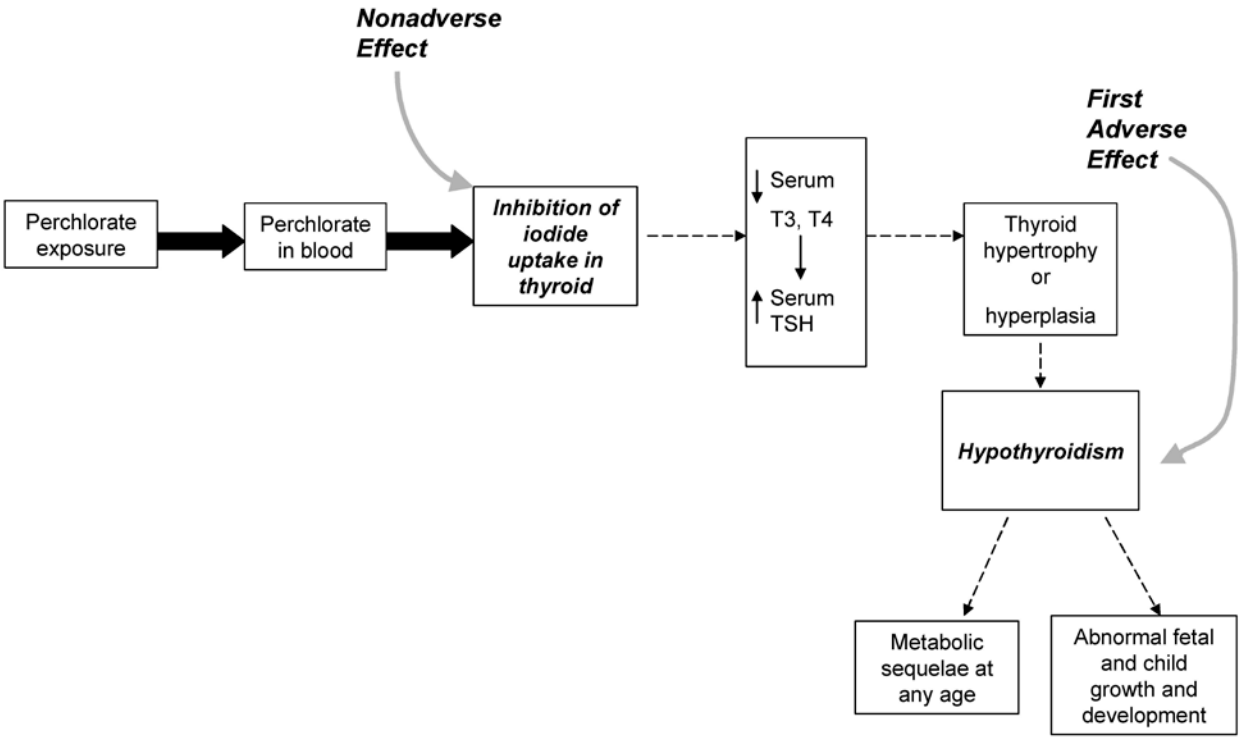


Figure 1. NRC suggested mode of action for perchlorate toxicity in humans indicating the first adverse effect in the continuum. (Reprinted with permission from Health Implication of Perchlorate Ingestion, 2005 by the NAS. Courtesy National Academy Press.)

Research scientists at the toxicology laboratory at Wright-Patterson Air Force Base 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, 2003b; Merrill et al. 2003, 2005). These models included the adult rat, pregnant rat and fetus, 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 expressing the NIS protein, such as the thyroid gland, small intestine, placenta, and lactating mammary tissue (Merrill et al. 2005). Both anions—perchlorate and iodide—compete for active uptake by NIS-expressing tissues. The inhibition of thyroidal uptake of radiotracer iodide by perchlorate is recognized as the primary mode of action for perchlorate leading to potential disruption of the hypothalamic-pituitary-thyroid (HPT) axis by depleting the thyroid gland of iodide used in synthesizing thyroid hormones. 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 sensitive life stages (i.e., the fetus, infant and child). The human PBPK/PD-IUI life stage model (U.S. EPA 2008) was the subject of an EPA-sponsored peer review and underwent modest revisions in response to the reviewers' comments (U.S. EPA 2009). This peer-reviewed model was used for the predictions of RAIU inhibition presented in the EPA white paper (2012) provided to the SAB. This modeling approach starts to answer questions about sensitivity of life stages to RAIU inhibition that otherwise are only qualitative justifications for the UF of 10 used in the RfD to protect sensitive populations.

Future mathematical modeling development should describe HPT axis events after RAIU inhibition in human life stages. The model would need to describe a range of status for thyroid hormones (e.g., hypothyroxinemia), with consideration of the appropriate reference ranges during different lifestages (e.g., trimesters of pregnancy), and recognition of variations among measurement assays for thyroid hormones. An expanded model should describe dietary iodide intake that is the source of iodide for thyroid hormone synthesis; the current model does not describe thyroid hormone levels or the dietary iodide intake. Expansion of the model to incorporate these aspects has been accomplished in the adult rat (McLanahan et al. 2008, 2009) and ongoing efforts to model humans were reported for the pregnant mother and fetus (Lumen et al. 2013).

Lumen and coworkers described the serum pharmacokinetics of perchlorate and dietary iodide in the near-term pregnant mother and fetus, thyroid iodide stores, iodide, and total serum T4 (from which fT4 is calculated) 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 maternal fT4 levels associated with hypothyroxinemia (decrease in serum T4 and changes in serum TSH within normal reference ranges) and for the onset of hypothyroidism (increase in serum TSH and decrease in serum fT4 levels). This biologically 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 an 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. (2013) would be to perform Monte Carlo analysis to address variability in the human population. The contributions to NIS inhibition from other NIS inhibitors (e.g., thiocyanate, nitrate) could also 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 both modelers and non-modelers will be an important challenge for the 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 detailed scientific information. The model documentation should describe model structure, data used to establish that structure and to 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; Chiu et al. 2007; Thompson et al. 2008) and the World Health Organization International Programme on Chemical Safety PBPK Guidance (WHO 2010) provide useful approaches for developing documentation. This documentation should also reference the published literature on the model and the 2009 peer review of the 2008 EPA PBPK/PD-IUI model and its subsequent revisions.

Recommendations:

The SAB recommends that the EPA utilize an MOA framework for developing the MCLG that links the different steps in the proposed mechanism from perchlorate exposure through NIS inhibition to thyroid hormone changes and finally to 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 symporter inhibition to address these initial steps of the MOA framework. (See section 3.4.1)

The EPA should extend the PBPK/PD-IUI model expeditiously to describe changes in thyroid hormone levels. This would provide a key tool for linking early events with subsequent events as reported in the scientific and clinical literature on iodide deficiency, changes in thyroid hormone levels, and their relationship to neurodevelopmental outcomes during sensitive early life stages.

Development of a clear communications strategy, including documentation of the MOA framework and the PBPK/PD-IUI model, will facilitate stakeholder and public understanding of the approach used to develop the MCLG.

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

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

The two analyses that the EPA presented in the white paper address different aspects of the model and its use in developing an MCLG (U.S. EPA 2012). The first analysis (Table A3 in the EPA white paper) 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 lifestages. This analysis helps support the use of the UF in deriving the RfD as it predicts greater inhibition at fetal and neonatal/infant lifestages as compared to the adult. The second analysis (Table A4 in the EPA white paper (2012)) 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 exposure concentrations.

The SAB identified some strengths and limitations of the first analysis of life stage-dependent biological variability. 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. In the absence of a full population analysis, it is important for the EPA to document and justify when model parameter values are selected that either represent an upper or lower bound rather than the average (e.g., using upper bound drinking water intake) or, when given uncertainty in the experimental literature, they select a specific value (e.g., the highest or lowest urinary clearance rate) rather than using an average value. Sensitivity analyses for PBPK model predictions could be useful for identifying key parameters to make such population analyses more tractable or to evaluate and demonstrate the impact of selection of particular parameter values. 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, if they occur; the model does not currently include thyroid hormones to permit such a feedback description nor potential effects of chronic perchlorate exposure. A strength of the analysis is that the EPA evaluated the model's capability to describe both perchlorate transport into breast milk and assessed the expected impact of NIS 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.

The major strength and limitation of the current model as noted above is that it provides a tool to link perchlorate exposure with impacts on iodide uptake, but goes no further in the MOA at this time. Nevertheless, this early step can usefully be extended to represent the consequences of those changes on thyroid hormone levels at different life stages under varied conditions of basal iodide intake and thyroid hormone status.

Recommendation:

The SAB finds the second analysis is the more valuable for asking what extent of NIS inhibition would be predicted for different potential MCLG concentrations; the analysis provides perspective on the protection offered by different perchlorate concentrations. Since it uses 90th percentile drinking water consumption rates, it starts to address population issues in exposure, although most of the biological aspects of the model are for an average individual. As noted above, the EPA needs to document and justify when selecting values other than average values in the absence of a full population analysis in order to be transparent about scientific, science policy or regulatory policy choices involved.

Limited data have been available for perchlorate in plasma and breast milk so checking the availability of new data in the literature would inform alternative parameterization or characterization of the uncertainty in the current model parameters. 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.

The choices for urinary clearance values for perchlorate and iodide at the different life stages should be reviewed and the current or revised values documented and justified as appropriate for a model of the average individual at each life stage in light of uncertainties in the scientific literature.

3.3. Epidemiological Studies

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

The SAB finds that the epidemiological data published since the NRC 2005 report are useful for estimating the size of potentially sensitive populations in the United States, estimating the extent to which the United States general population and sensitive populations are exposed to perchlorate and other goitrogens, and estimating the relative source contribution of perchlorate in drinking water among sensitive populations not included in the Food and Drug Administration (FDA) Total Diet Study (Murray et al. 2008).

The SAB concludes that these epidemiological data are insufficient to guide causal inference of an 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 resulted in inconsistent findings. 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 others should consider when planning analyses based on existing data or when developing new studies to improve the agency's understanding of the effect of perchlorate exposure in hypothyroxinemic women. The SAB also provides specific comments in Appendix B on the strengths and weaknesses of recent epidemiologic studies identified by EPA and others.

3.3.1. Using Exposure and Biomonitoring Studies

Manuscripts published since the 2005 NRC report are informative for providing an estimate of the size of potentially sensitive populations in the United States, for estimating exposure to perchlorate and other goitrogens among sensitive populations and for estimating the relative source contribution of perchlorate in drinking water among sensitive populations.

Prevalence of Sensitive Populations

Epidemiologic studies can be used to identify sensitive populations. However, methodological considerations (see review of epidemiologic literature in Appendix B) limit the scientific 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 subgroups to produce a relatively representative sample of the U.S. population (CDC 2004). NHANES can be used to estimate the prevalence of potentially sensitive populations, including pregnant women who are iodide insufficient.

Iodide is critical for the formation of thyroid hormone. Iodide deficiency occurs when iodide falls below recommended levels. According to the WHO guidelines, urinary iodine levels > 100 μg/L (representing an iodine daily intake of 150 μg) are considered "adequate" among the general population (WHO 2001). 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. (2005) used iodine measured in spot urine samples from NHANES 2001-2002 to characterize iodine levels in the U.S. population. Among women ages 15 to 44, 37.2% have iodine levels <100 μg/L. Using the 2005-2006 and 2007-2008 NHANES samples, Caldwell et al. (2011) reported that the proportion of women ages 15 to 44 with urinary iodine <100 μg/L remains relatively constant at 38.1%. Among pregnant women, however, 56.7% have urinary iodine concentrations less than the recommended 150 μg/L.

Estimating Perchlorate Exposure and Exposure to Other Goitrogens

Biomonitoring and exposure studies published since the 2005 NRC report can be used to identify subgroups with the highest exposures to perchlorate. NHANES studies can produce population estimates of perchlorate exposure, including among potentially sensitive subgroups.

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

Huber et al. (2010) provides information for estimating perchlorate exposure in pregnant women. The authors used data from a random subset of NHANES 2001-2002 that measured perchlorate in n=2708 spot urine samples (creatinine adjusted), including 116 pregnant women. Compared to non-pregnant women aged 15 to 44 years, pregnant women had significantly higher average daily perchlorate intake (geometric mean: 0.060 μgkg/day vs. 0.051 μgkg/day). These data, however, may be imprecise because

they are estimated from a single spot urine sample (Mendez et al. 2010) and because during pregnancy, creatinine adjustment for urinary dilution is less effective as pregnancy alters creatinine excretion (Blackburn 2007). Huber et al. (2010) also examined the EPA Unregulated Contaminant Monitoring Regulation (UCMR) data, which provide data on perchlorate levels in public drinking water sources. In the UCMR data, the estimated perchlorate contribution from food was 86% and from drinking water was 14%.

Some potentially sensitive populations, such as infants, are not represented in NHANES. Exposure information for these missing subgroups can be inferred from exposure and biomonitoring studies that specifically targeted these groups. While these studies are often comprised of highly selected study subjects and may not be representative of the U.S. population, the paucity of epidemiologic data on potentially sensitive populations makes these targeted studies useful nevertheless. Some of the studies published since the NRC report may inform parameters for PBPK/PD models.

Four studies provide information for estimating perchlorate exposure among infants less than 6 months of age (Kirk et al. 2005; Dasgupta et al. 2008; Schier et al. 2010; Valentin-Blasini et al. 2011). Kirk et al. (2005) reported average perchlorate concentrations of 2.0 μg/L (range: 0.0 to 11.0 μg/L) and 10.5 μg/L (range: 1.4 to 92.2 μg/L) in 47 samples of dairy milk from 11 states and 36 breast milk samples from lactating volunteers in 18 states, respectively. Using these data, the authors estimate that the majority of breast-fed infants would exceed the NRC RfD (0.7 μg kg/day). Dasgupta et al. (2008) measured perchlorate in repeated milk and urine samples from a small number of lactating women (n=13). Based on these data, the authors estimated that 9 of 13 infants exceeded the NRC perchlorate RfD. Schier et al. (2010) estimated perchlorate intake from four varieties of infant formula: bovine-based with lactose, bovine-based without lactose, soy-based, and elemental. The authors reported that bovine formula with lactose had the highest concentrations of perchlorate (geometric mean: 1.72 μg/L), which could lead to estimated daily doses at 1 and 6 months of age that exceeded the perchlorate RfD. Valentin-Blasini et al. (2011) directly measured perchlorate exposure in the urine of breast- and formula-fed infants age 1 to 377 days by collecting up to four samples per infant (n=205 samples from 92 infants). The highest average perchlorate concentrations were among breast-fed infants (geometric mean: 2.65 μg/L vs. 1.3 μg/L for bovine-based formula and 0.35 μg/L for soy-based formula). Correspondingly, the highest average estimated perchlorate intake (geometric means for breast-fed, bovine-based formula fed, and soy-based formula fed, respectively: 0.922 μgkg/day, 0.103 μgkg/day, and 0.027 μgkg/day) were among breastfed infants. Based on these estimates, 16% of all infants (and 31% of breast-fed infants) had at least one feeding with perchlorate exposure exceeding the RfD. There was, however, a great deal of intra-individual variability of perchlorate concentrations across repeated samples (intraclass correlation coefficient (ICC) = 0.07). These authors also reported concurrent urinary levels of nitrate, thiocyanate, and iodide concentrations.

In addition to perchlorate, NHANES provides an opportunity to evaluate the extent to which the U.S. population, including sensitive populations, may be co-exposed to other goitrogens with comparable MOAs, such as 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 provides simultaneous measurement of nitrate, thiocyanate and iodide (Valentin-Blasini et al. 2007). While the geometric mean concentrations of all four compounds 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). Ultimately, 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 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 perchlorate reference dose remaining for drinking water after considering exposure from other sources. For perchlorate, food is the only other important exposure pathway. The EPA used the FDA Total Diet Study by Murray et al. (2008) to estimate the drinking water RSC (Table A-2, U.S. EPA 2012) based on estimated perchlorate intake from food among 14 age/sex subgroups of the U.S. population. RSC estimates ranged from 44% to 89%, although the Total Diet Study did not provide intake estimates for all potentially sensitive populations (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 an association between perchlorate exposure and thyroid dysfunction, or to support a derived MCLG. Methodological and statistical issues limiting the applicability of these studies to the Charge question include: (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 that do not properly assess confounding and effect measure modification or explore potential non-linear associations; 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

Existing exposure and biomonitoring studies are useful for understanding the prevalence of sensitive populations. Additional analyses of NHANES data can be undertaken to estimate the prevalence of sensitive populations not previously described. The typically small number of pregnant women in NHANES, however, may limit the precision of these analyses. In addition to perchlorate, urinary concentrations of other goitrogens are also available in NHANES data.

It may be possible to pool data from existing studies with similar design and analytic measures to alleviate some of the methodological and statistical issues discussed in Appendix B. However, *post-hoc* pooled analyses should be undertaken with caution and with careful consideration of potential sources of heterogeneity across studies.

Recommendations:

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 the role of perchlorate as a contributor to meaningful health outcomes in susceptible populations, specifically endpoints directly related to neurodevelopment.

Additionally, future studies may benefit from improved statistical methods. Investigating non-linear patterns of effect across low, moderate and high exposure categories may be informative for identifying potential associations at the extremes of the exposure distribution. Careful and thorough consideration of

appropriate control variables may reduce bias and improve the precision of estimated perchlorate effects. For instance, directed acyclic graphs (DAGs) are useful tools that apply systematic rules to graphically depict assumptions about causal relations among variables (Greenland et al. 1999). DAGs can inform statistical modeling strategies by helping to determine which covariates should be controlled to reduce confounding and avoid bias. Rather than adjusting models for characteristics of potentially vulnerable populations, it may be more informative to stratify the analysis by the characteristic. For instance, iodide-deficient pregnant women may be more susceptible to the effect of perchlorate than iodide-sufficient pregnant women. Stratification highlights this differential susceptibility instead of providing an average effect over all iodide levels. Such studies, however, would require large sample sizes to observe these divergent effects.

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 to compounds with the same (or different) modes of action. Studies of the temporal variability of perchlorate, iodide, nitrate, and thiocyanate in spot urine samples also should inform methods for minimizing measurement error.

3.4. Integration of Information

3.4.1. Integrating Information to Derive a MCLG

Charge Question:
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?

The EPA white paper describes a process for deriving an MCLG for perchlorate that incorporates an RfD and RSC (U.S. EPA 2012). The SAB recommends that the EPA integrate the available information on perchlorate to derive an MCLG using the MOA previously identified for perchlorate rather than the default algebraic approach. The MOA approach relies on the use of a PBPK/PD model that relates perchlorate intake via drinking water with percent IUI. The SAB recommends that EPA use a PBPK/PD- IUI approach and where possible expand this approach to relate the percent IUI with thyroid hormone perturbations and potential adverse neurodevelopmental outcomes.

The SAB recommendation represents an important and novel opportunity that should be implemented carefully with attention to data quality and methodological rigor. At each step, the 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 recommends that the EPA undertake the necessary literature review and critical analysis to fully test the feasibility and utility of the approach. Further, the SAB recommends that the EPA incorporate into the MCLG development the recent recommendations from the National Academy of Sciences to improve the scientific basis and clarity of assessment documents (NRC 2009, 2011).

This SAB advisory report presents specific recommendations for considering sensitive life stages, PBPK-PD 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 consider the entire literature related to ingestion of perchlorate, pharmacokinetics of perchlorate and the effects (dynamics) of perchlorate (such

as Clewell et al. 2001, 2003a, 2003b). In addition, the SAB recommends that EPA should also consider available data on potential adverse health effects (neurodevelopmental outcomes) due to thyroid hormone level perturbations regardless of the cause of those perturbations.

The three previous sections provide the foundation for an approach to derive the MCLG for perchlorate using the entire body of available information.

- *Sensitive Life Stages*: The most important SAB recommendations are the focus on *subtle* changes in thyroid hormone levels. The SAB finds the most sensitive life stages are the fetuses, neonates and infants because these are the stages when thyroid-dependent brain development occurs. The development of the MCLG must consider the perchlorate exposure pathways relevant to each of these sensitive life stages, which for fetuses and breastfed infants includes exposure of pregnant and lactating women, respectively. Thus, the sensitive populations that the EPA should consider for exposure to perchlorate are the fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. This delineation of sensitive subpopulations would replace "the fetuses of pregnant women who might have hypothyroidism or iodide deficiency" as defined by the NRC (2005).

- *PBPK/PD Modeling:* The current PBPK/PD-IUI model can link perchlorate exposure in food and water with perchlorate concentrations in plasma and tissue and resulting NIS inhibition assessed by 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. Work to extend the PBPK/PD-IUI model with links to serum thyroid hormone levels is presented in Lumen et al. (2013).

- *Epidemiology and Biomonitoring Data:* The SAB concluded that the data in the scientific literature since the 2005 NRC report were insufficient to provide the basis for an MCLG. However, a consideration of the full literature 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 hypothyroxinemic pregnant and lactating women, their fetuses and infants, and bottle fed infants as the sensitive subpopulation.

The SAB recognizes that an MOA has been determined that links the different steps in the proposed mechanism leading from perchlorate exposure through NIS inhibition to thyroid hormone changes and finally neurodevelopmental impacts. The SAB finds that this framework provides a strong foundation for the EPA to develop the MCLG. 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.

In order to ensure that the model is predictive of actual adverse health outcomes, the 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 (i.e., iodide deficiency, thyroid hormone levels, hypothyroxinemia).

The SAB recognizes the existence of a large amount of scientific research on perchlorate and also thyroid hormone perturbations and potential adverse health outcomes (unrelated specifically to perchlorate). As a result, the SAB recommends that the EPA explore the use of the literature beyond that which focuses solely on perchlorate.

The SAB notes that the recommendation to use the MOA and PBPK/PD 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.

Regarding using epidemiological and biomonitoring data to establish the bounds on a potential MCLG of perchlorate, the SAB was not provided the full extent of data on 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 the 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 95^{th} percentiles and ranges of values rather than point estimates representing average population values (see Section 3.2).

The SAB notes that in applying the framework to the epidemiological data, the agency should consider the available evaluation tools such as Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklists (ISPM 2012) or Grading of Recommendations Assessment, Development and Evaluation (GRADE 2012). The SAB recommends that as the EPA integrates information, the agency should consider the general frameworks for evaluating quality of studies used to support the MCLG derivation (as discussed briefly in Appendix C).

Steps In A Mode of Action Modeling Approach

The SAB recommends the following MOA-based approach for using PBPK/PD modeling and additional clinical and toxicological data to inform the derivation of a health-protective MCLG recognizing that the sensitive populations for perchlorate exposure are the fetuses of hypothyroxinemic pregnant women, and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. The effects of concern are neurodevelopmental outcomes in the offspring. The SAB presents this approach as a series of steps to progressively improve the scientific rigor in the evaluation of different life stages considered for the MCLG and recognizes that the steps described here may require an increased level of effort and additional data. As part of this approach, the EPA would obtain a point of departure (POD) from which the MCLG would then be derived. The POD selection would be dependent upon the MOA-based endpoint used in EPA's analysis (e.g., NIS inhibition, thyroid hormone changes, neurodevelopmental effects). The approach is discussed below and summarized in Figure 2. The SAB's recommended approach follows the solid arrows in the diagram and an alternative approach follows the dashed arrows in the figure. As shown, there are three proposed approaches (2, 3a, or 3b) available to the agency that vary in terms of data, resource, and time requirements.

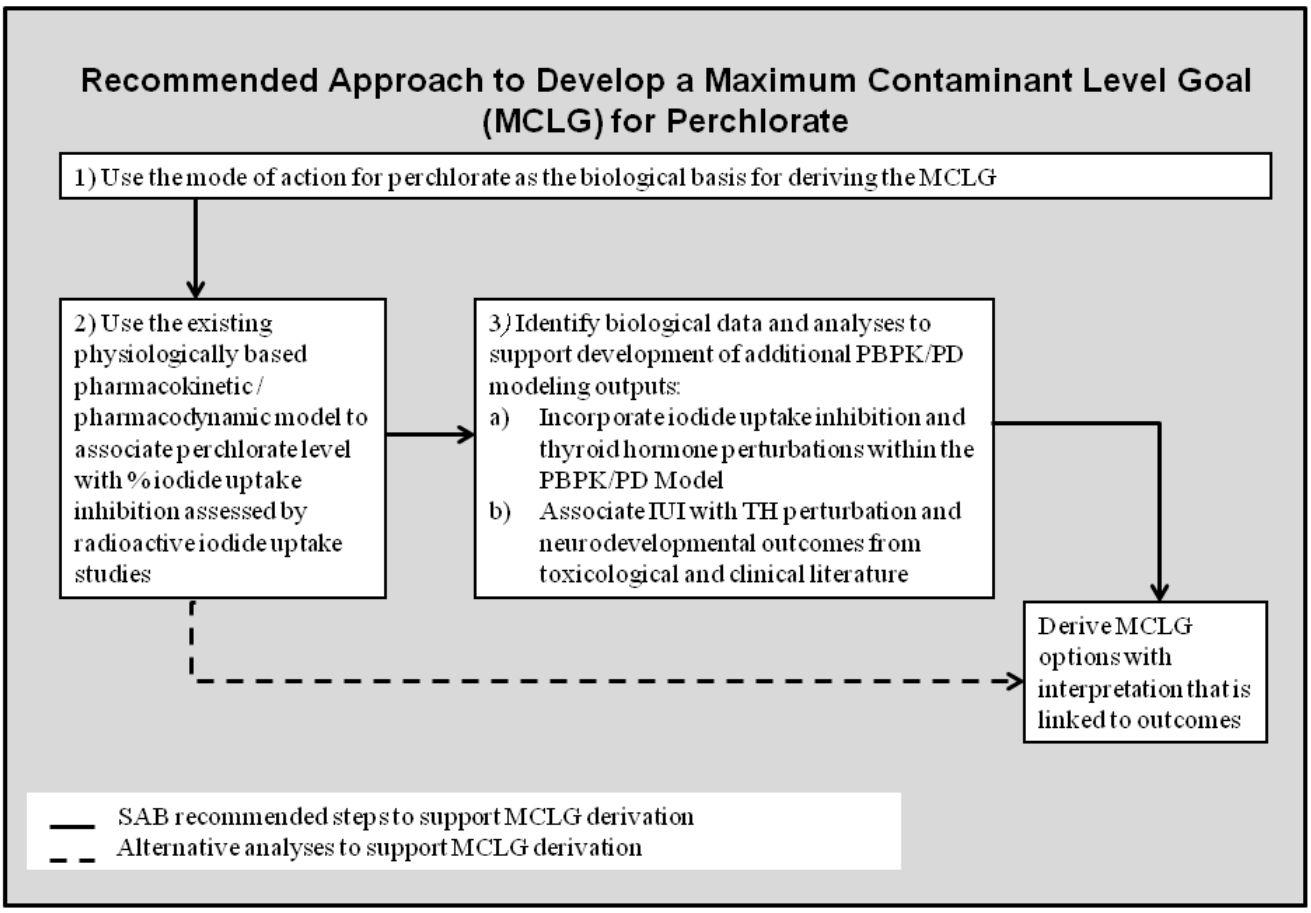


Figure 2. Steps in a mode of action and modeling approach to derive an MCLG for perchlorate.

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

Step 2. Use the existing PBPK/PD-IUI model to link perchlorate exposure from drinking water with perchlorate concentrations in plasma and tissue and resulting NIS inhibition assessed by RAIU studies. The model in its current form addresses important aspects of biological life stage sensitivities, but limitations should be clearly stated or the model should be adjusted (e.g., iodide and perchlorate clearance in the early postnatal period as noted in Section 3.2). While the preferred MOA approach would link IUI with subsequent events (e.g., thyroid hormone perturbations), using predictions of IUI from the current PBPK/PD-IUI model is consistent with the derivation of the RfD. This would be the most rapid analysis for EPA to implement since the model predicts percent IUI for the relevant life stages and has already been subject to peer review. The NRC report proposed that by minimizing IUI, one would minimize subsequent events and adverse health consequences. The limitation of using either the RfD in the default algebraic equation or IUI predicted by the model is that both describe a precursor event and neither explicitly provides predictions for subsequent events and adverse outcomes. The advantage of the PBPK/PD-IUI model approach over the algebraic calculation is that it explicitly predicts IUI at the relevant lifestages that the SAB considers important.

Step 3. The SAB urges the EPA to expand the PBPK/PD model to address as many of the downstream MOA outcomes as possible. The agency should identify literature and conduct analyses to support the

model outputs for the downstream steps. While incorporating these subsequent steps into the PBPK/PD-IUI model is the preferred approach, the SAB recognizes the additional effort required. An interim approach is to obtain data from the clinical and toxicological literature to describe empirical relationships to the downstream effects not provided by the model outputs. Benefits and limitations to both approaches are described below.

a) The SAB recommends that the EPA extend the PBPK/PD-IUI model to incorporate predictions of thyroid hormone perturbations. Such an extension of the model would need to explicitly address dietary iodide intake (both adequate and insufficient intake) and thyroid hormone production at different life stages for women and children with adequate and insufficient iodide intakes. This approach would permit assessment of the predicted exposure-response relationship for perchlorate exposure and alterations in thyroid hormone levels (e.g., decreases in serum fT4). To establish what magnitude of decrease in T4 would be relevant and establish a point of departure, EPA would need to document the relationship between the levels of maternal serum biomarkers, (e.g., 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. The assumption of this approach is that regardless of the cause of decreased iodide for thyroid hormone synthesis (e.g., lack of dietary intake or competition by perchlorate) the subsequent events are driven by the decrease of thyroid hormone levels. Such an effort will require resources and time, likely up to a couple of years. The SAB notes that similar modeling efforts are underway at other federal agencies and collaboration with these researchers could facilitate development thereby reducing the level of effort.

b) An interim approach is to use the existing PBPK/PD-IUI model to estimate IUI and then develop empirical relationships for each of the steps beyond perchlorate-mediated IUI. The thyroid clinical literature would be used 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 (see section 3.1). If one could establish equivalence between perchlorate-mediated IUI and reduced iodide intake as observed by measured urinary iodide, one could utilize the relationship between urinary iodide and thyroid hormones levels described in Silva and Silva (1981) for varying levels of iodide intake in pregnant women. Again, the relationship between changes in thyroid hormone levels and neurodevelopmental outcomes just discussed would be required to complete the linkages. This approach will require resources and time, perhaps less than required for explicitly expanding the PBPK/PD-IUI model to include thyroid hormone levels, but that depends upon being able to identify data to provide the needed empirical relationships for steps between IUI and neurodevelopment.

As a check on the predictions from either of these approaches, the agency could 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-IUI model outputs. However, it may be possible to gain a better understanding of the effect of perchlorate exposure on 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, and 2012) studies as one potential data source given the common set of investigators. A pooled analysis, however, addresses only some of the existing limitations and would still require cautious interpretation regarding causal inference because these data are cross-sectional.

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). The improved statistical methods described in the recommendations under Section 3.3.3 also would be relevant for any pooled analyses. (Further information on model misspecification in the epidemiological literature the SAB reviewed is found in Appendix B).

The SAB identified a number of potential options to identify and apply biological data in support of the PBPK/PD-IUI modeling to derive an MCLG for perchlorate. The SAB provides rough estimates of the time requirements for each potential option below.

Short-term option (estimated up to one year)

- Use existing clinical literature to identify empirical linkages between existing PBPK/PD-IUI model to downstream changes (i.e., thyroid hormones, neurodevelopment)

Medium-term option (estimated one to two years)

- Extend PBPK/PD-IUI model to incorporate the prediction of thyroid hormone perturbations

Long-term options (estimated more than two years)

- Pooled analysis of existing epidemiological data
- New longitudinal epidemiological studies

This MOA-based approach is consistent in some ways with the concept of Adverse Outcome Pathways that is being used increasingly by the agency to understand and describe the linkages between initiating molecular events and adverse outcomes. Going forward, the agency should consider whether it would be beneficial to present perchlorate and the PBPK/PD-IUI modeling in the context of an adverse outcome pathways framework.

3.4.2. Estimating Reductions In Adverse Health Effects

Charge Question:
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?

The SAB finds that the epidemiological studies provided to the panel are inadequate for quantitatively estimating reduction in adverse health effects that would result from regulating perchlorate in drinking water. Specifically, the epidemiological studies provided are not adequate to support 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 neurodevelopmental effects arising from exposures during the sensitive lifestages as the potential adverse effects of perchlorate. These effects may range from changes in brain development and structure to impaired behavior, learning and memory, among others (Rovet and Willoughby 2010). These effects have been observed in studies of iodine deficiency or altered thyroid hormone function – conditions consistent with the MOA for perchlorate. Changes in brain development and structure have been observed in studies of animals where maternal hypothyroxinemia or thyroid hormone deficiency were modeled (for

example, Lavado-Autric et al. 2000; Auso et al. 2004). Impaired learning, cognition and motor development have been observed in studies of children whose mothers were iodine deficient or hypothyroxinemic (for example, Zoeller and Rovet 2004; Henrichs et al. 2010; Li et al. 2010; Suarez-Rodriguez et al. 2012). For the purposes of deriving an MCLG for perchlorate, the SAB recommends that the EPA focus on measurements relevant to these adverse effects including iodine deficiency and hypothyroxinemia.

- The sensitive population. The sensitive populations for perchlorate exposure are the fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women. This would replace "the fetuses of pregnant women who might have hypothyroidism or iodide deficiency" as defined by the NRC (2005).

As a first step in beginning to understand reductions in adverse health effects, EPA should examine shifts in the distribution of exposure to perchlorate to the sensitive subpopulation if relevant data are available.

Any further effort to gain insight on reductions in adverse health effects depends on the availability of data as EPA proceeds along the steps of the recommended integrated approach, as shown in Figure 2. For example, if EPA can proceed by making empirical linkages of perchlorate levels in water and associated PBPK/PD model output (IUI and TH changes) with neurodevelopmental effects from literature sources (Figure 2, Step 3b), the EPA may have a means to assess how a particular perchlorate level relates to a specific outcome. If the health effects literature contains ranges of IUI or TH and a range of effects are described, the EPA may be able to analyze these three distributions (perchlorate in water, modeled output of IUI and TH, literature on IUI or TH linked to a range of effects) empirically connected in series to support statements about reductions in adverse effects.

REFERENCES

Allen, M.C. and P.H. Lipkin. 2005. Editors. Neurodevelopmental Assessment of the Fetus and Infant. *Mental Retardation and Developmental Disabilities Research Reviews.* 11:1.

Ahmed, O.M., A.W. El-Gareib, A.M. El-Bakry,, S.M. Abd El-Tawab, and R.G. Ahmed. 2008. Thyroid hormones states and brain development interactions. *Int J Dev Neurosci* 26:147-209.

Anderson, G.D. and A.M. Lynn. 2009. Optimizing Pediatric Dosing: A Developmental Pharmacologic Approach. *Pharmacotherapy.* Jun: 29(6):680-90.

Anderson, G.W. 2001. Thyroid Hormones and The Brain. *Front Neuroendocrinol.* 2001: 22:1-17.

Anderson, G.W., C.M. Schoonover, S.A. Jones. 2003. Control of Thyroid Hormone Action in the Developing Rat Brain. *Thyroid*: 13: 1039-1056.

Andersson, M., B. de Benoist, F. DeLange, J. Zupan, WHO Secretariat. 2007. Prevention and Control of Iodine Deficiency In Pregnant and Lactating Women and in Children Less Than 2-Years-Old: Conclusions and Recommendations of the Technical Consultation. *Public Health Nutr*. 10(12A): 1606-11.

Auso, E., R. Lavado-Autric, E. Cuevas, F. Escobar del Rey, G. de Morreale Escobar, P. Berbel. 2004. A Moderate and Transient Deficiency of Maternal Thyroid Function at the Beginning of Fetal Neocorticogenesis Alters Neuronal Migration. *Endocrinology.* 145:4037-4044.

Ballabio, M., U. Nicolini, T. Jowett, M.C. Ruiz de Elvira, R.P. Ekins, C.H. Rodeck. 1989. Maturation of Thyroid Function in Normal Human Foetuses. *Clin Endocrinol* 31: 564-571.

Bastian, T.W., J.A. Anderson , S.J. Fretham, J.R. Prohaska, M.K. Georgieff, G.W. Anderson. 2012. Fetal and neonatal iron deficiency reduces thyroid hormone-responsive gene mRNA levels in the neonatal rat hippocampus and cerebral cortex. *Endocrinology* 153(11):5668-80. doi: 10.1210/en.2012-1067. Epub 2012 Oct 9.

Bauer M,T. Goetz, T.Glenn, P.C.Whybrow. 2008. The thyroid-brain interaction in thyroid disorders and mood disorders. *J Neuroendocrinol*. (10):1101-14.

Bartelink, I.H., C.M. Rademaker, A.F. Schobben, J.N.van den Anker. 2006. Guidelines on Paediatric Dosing on the Basis of Developmental Physiology and Pharmacokinetic Considerations. *Clin Pharmacokinet.* 45(11):1077-97.

Berbel, P, J.L. Mestre, A. Santamaría, I. Palazón, A. Franco, M. Graells, A. González-Torga, G.M. de Escobar. 2009. Delayed Neurobehavioral Development in Children Born to Pregnant Women With Mild Hypothyroxinemia During the First Month Of Gestation: The Importance of Early Iodine Supplementation. *Thyroid* 19(5):511-9.

Berbel, P., Navarro, D., Auso, E., Varea, E., Rodriguez, A.E., Ballesta, J.J., Salinas, M., Flores, E., Faura, C.C., and G. Morreale de Escobar. 2010. Role of Late Maternal Thyroid Hormones in Cerebral Cortex Development: An Experimental Model for Human Prematurity. *Cerebral Cortex* 20:1462-75.

Bernal, J. 2005. Thyroid hormones and brain development. *Vitam Horm* 71:95-122. http://www.ncbi.nlm.nih.gov/pubmed/16112266

Bernal, J. 2007. Thyroid Hormone Receptors in Brain Development and Function. *Nat Clin Pract Endocrinol Metab.* 3: 249-259.

Bernal, J., J. Nunez. 1995. Thyroid Hormones and Brain Development. *Eur J Endocrinol* 133(4):390-8.

Blackburn, S.T. 2007. *Maternal, fetal, & neonatal physiology: a clinical perspective*. 3rd edition. Saunders Elsevier. St. Louis. 379p.

Blount, B.C., J.L. Pirkle, J.D. Osterloh, L. Valentín-Blasini, And K.L. Caldwell. 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. *Environmental Health Perspectives*. 114(12):1865-71.

Blount, B.C., D. Q. Rich, L.Valentin-Blasini,S. Lashley,C.V. Ananth, E. Murphy, J.C. Smulian, B.J.Spain, D.B. Barr, T. Ledoux,P. Hore,M. Robson. 2009. Perinatal Exposure to Perchlorate, Thiocyanate, and Nitrate in New Jersey Mothers and Newborns. *Environmental Science and Technology* 43:7543-7549.

Brent, G.A. 2010. The Impact of Perchlorate Exposure in Early Pregnancy: Is it Safe to Drink the Water? *Journal of Clinical Endocrinology & Metabolism* 95:3254-3157.

Caldwell, K.L., R. Jones, and J.G. Hollowell. 2005. Urinary Iodine Concentration: United States National Health and Nutrition Examination Survey 2001-2002. *Thyroid* 15(7): 692-9.

Caldwell, K.L., A. Makhmudov, E. Ely, R.L. Jones, R.Y. Wang. 2011. Iodine Status of the U.S. Population, National Health And Nutrition Examination Survey, 2005-2006 and 2007-2008. *Thyroid* 21(4):419-27.

Calvo R.M., E. Jauniaux, B. Glubis, M. Asuncion, C. Gerby, B. Contempre, G. Morreale de Escobar. 2002. Fetal Tissues Are Exposed to Biologically Relevant Free Thyroxine Concentrations During Early Phases Of Development. *J Clin Endocrinol Metab:* 87: 1768-1777.

Carrasco, N. 1993. Iodide Transport In the Thyroid Gland. *Biochim Biophys Acta* 1154:65-82.

Casey, B.M., J.S. Dash, C.E. Wells, D.D. Mcintire, W. Byrd, K.J. Leveno, F.G. Cunningham. 2005. Subclinical Hypothyroidism and Pregnancy Outcomes. *Obstet Gynecol*, 105:239-245.

CDC (Center For Disease Control). 2004. *National Health and Nutrition Examination Survey*. 30 July 2012; Available From: Http://Www.Cdc.Gov/Nchs/Nhanes.Htm.

Chan S. and J. Rovet. 2003. Thyroid Hormones in Fetal Central Nervous System Development. *Fetal Matern Med Rev.* 13: 177-208.

Chiu, W. A., H.A. Barton, R. S. Dewoskin, P. Schlosser, C.M. Thompson, B. Sonawane, J. Lipscomb, and K. Krishnan. 2007. Evaluation of Physiologically-Based Pharmacokinetic Models for Use in Risk Assessment. *J Appl. Toxicol* 27(3):218-237.

Clark, L. H., R. Woodrow Setzer, and H.A. Barton. 2004. Framework for Evaluation of Physiologically-Based Pharmacokinetic Models for Use in Safety or Risk Assessment. *Risk Analysis.* 24(6): 1697-1717.

Clewell R.A., E.A. Merrill, P.J. Robinson. 2001. The Use Of Physiologically-Based Models to Integrate Diverse Data Sets and Reduce Uncertainty in the Prediction of Perchlorate and Iodide Kinetics Across Life Stages and Species. *Toxicology and Industrial Health*. 17(5-10):210-222.

Clewell, R.A., E. A. Merrill, K. O. Yu, D. A. Mahle, T.R. Sterner, J. W. Fisher, And J.M. Gearhart. 2003a. Predicting Neonatal Perchlorate Dose and Inhibition of Iodide Uptake in the Rat During Lactation Using Physiologically-Based Pharmacokinetic Modeling. *Toxicol. Sci*. 109:416-436.

Clewell, R.A., E.A. Merrill, K.O. Yu, D.A. Mahle, T.R. Sterner, D.R. Mattie, P.J. Robinson, J.W. Fisher, and J.M. Gearhart. 2003b. Predicting Fetal Perchlorate Dose and Inhibition of Iodide

Kinetics During Gestation: A Physiologically-Based Pharmacokinetic Analysis Of Perchlorate And Iodide Kinetics in the Rat. *Toxicol. Sci*. 73:235-255.

Clewell, R.A., E.A. Merrill, J.M. Gearhart, P.J. Robinson, T.R. Sterner, D.R. Mattie, And H.J. Clewell 3rd. 2007. Perchlorate and Radioiodide Kinetics Across Life Stages in the Human: Using PBPK Models to Predict Dosimetry and Thyroid Inhibition and Sensitive Subpopulations Based on Developmental Stage. *J Toxicol Environ Health* 70(5):408-28.

Costa, A, V. Filippis, M. Panizzo, G. Giraudi, E. Bertino, R. Arisio, M. Mostert, G. Trapani, C. Fabris. 1986. Development of thyroid function between VI-IX month of fetal life in humans. *Journal of Endocrinological Investigation* 9(4):273-280.

Dai, G., O. Levy And N. Carrasco. 1996. Cloning and Characterization of the Thyroid Iodide Transporter. *Nature* 379:458-460.

Dasgupta, P.K., A.B. Kirk, J.V. Dyke and S. Ohira. 2008. Intake of Iodine and Perchlorate and Excretion in Human Milk. *Environmental Science & Technology* 42(21):8115-21.

De La Vieja, A.,O. Dohan, O. Levy, N. Carrasco. 2000. Molecular Analysis of the Sodium/Iodide Symporter: Impact on Thyroid and Extrathyroid Pathophysiology. *Physiol Rev.* 80:1083-1105.

Den Ouden, A.L., J.H. Kok, P.H. Verkerk, R. Brand, and S.P. Verloove-Vanhorick. 1996. The Relation between Neonatal Thyroxine Levels and Neurodevelopmental Outcome at Age 5 and 9 Years in a National Cohort of Very Preterm and/or Very Low Birth Weight Infants. *Pediatr Res.* 39:142-5.

Dohan, O., A. De La Vieja, V. Paroder, C. Riedel, M. Artani, M. Reed, C.S. Ginter and N. Carrasco. 2003. The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical *Significance. Endocr Rev* 24:48-77

Dohan O, C. Portulano, C. Basquin, A. Reyna-Neyra, L.M. Amzel and N. Carrasco. 2007. The Na+/I Symporter (NIS) Mediates Electroneutral Active Transport of the Environmental Pollutant Perchlorate. *Proc Natl Acad Sci U S A* 104:20250-20255

English, P., B. Blount, M. Wong, L. Copan, L. Olmedo, S. Patton, R. Haas, R. Atencio, J. Xu, And L. Valentin-Blasini. 2007. Direct Measurement of Perchlorate Exposure Biomarkers in a Highly Exposed Population: A Pilot Study. *Plos One*. 6(3): P. E17015.

Fisher, J. W., P. Todd, D. Mattie, D. Godfrey, L. Narayanan, and K. Yu. 2000. Preliminary Development of a Physiological Model for Perchlorate in the Adult Rat: A Framework for Further Studies. *Drug Chem. Toxicol*. 23:243-258.

Gilbert, M.E. and L. Sui. 2008. Developmental Exposure to Perchlorate Alters Synaptic Transmission in Hippocampus of the Adult Rat. *Environmental Health Perspectives* 116(6):752-60.

Gilbert, M.E., J. Rovet, Z. Chen, and N. Koibuchi. 2012. Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. *Neurotoxicology* 33:842-852. Accessed December 10, 2012. http://www.sciencedirect.com/science/article/pii/S0161813X11002051

Glinoer, D., 2004. The Regulation of Thyroid Function During Normal Pregnancy: Importance of the Iodine Nutrition Status. *Best Practice & Research Clinical Endocrinology & Metabolism*. 18:133-152.

Glinoer, D., and F. Delange. 2000. The Potential Repercussions of Maternal, Fetal, and Neonatal Hypothyroxinemia on the Progency. *Thyroid*. 10:871-887.

Glinoer, D. and J. Rovet. 2009. Gestational Hypothyroxinemia and the Beneficial Effects of Early Dietary Iodine Fortification. *Thyroid.* 19(5): 431-434.

Grade Working Group. 2012. Grading of Recommendations Assessment, Development and Evaluation (GRADE). http://www.gradeworkinggroup.org/index.htm (Accessed July 30, 2012).

Greenland S., J.Pear, J.M.Robins. 1999. Causal Diagrams for Epidemiologic Research. *Epidemiology.* 10(1):37–48.

Greer, M.A., G. Goodman, R.C. Pleuss, and S.E. Greer. 2002. Health Effect Assessment for Environmental Perchlorate Contamination: The Dose Response for Inhibition of Thyroidal Radioiodide Uptake In Humans. *Environ. Health Perspect*. 110:927-937.

Haddow, J.E., G.E. Palomaki, W.C. Allan, J.R. Williams, G.J. Knight, J. Ganon, C.E. O'Heir, M.L. Mitchell, R.J. Hermos, S.E. Waisbren, J.D. Faix, and R.Z. Klein. 1999. Maternal Thyroid Deficiency During Pregnancy and Subsequent Neuropsychological Development of the Child. *N. Engl. J. Med*. 341:549-555.

Henrichs J, J.J. Bongers-Schokking, J.J. Schenk, A. Ghassabian H.G. Schmdit, T.J. Visser, H. Hooijkaas, S. M.P.F. De Muinck Keizer-Schrama, A. Hofman, V.V.W. Jassoe, W. Visser, E.A.P. Steegers, F.C. Verhulst, Y.B. De Rijke, H.J. Tiemeier. 2010. Maternal Thyroid Function During Early Pregnancy and Cognitive Functioning in Early Childhood: The Generation R Study. *J. Clinical Endocrinol. Metab*. 95(9)227-34.

Huber, D.R., B.C. Blount, D.T. Mage, F.J. Letkiewicz, A. Kumar and R.H.Allen. 2010. Estimating Perchlorate Exposure from Food and Tap Water Based on US Biomonitoring and Occurrence Data. *Journal of Exposure Science & Environmental Epidemiology*. 21(4): P. 395-407.

Institute Of Social And Preventive Medicine. 2012. Strengthening The Reporting Of Observational Studies In Epidemiology (STROBE). http://www.strobe-statement.org/index.php?id=available-checklists (Accessed July 30, 2012).

Kearns GL, S.M. Abdel-Rahman, S.W. Alander, D.L. Blowey, J.S. Leeder and R.E. Kauffman. 2003. Developmental Pharmacology--Drug Disposition, Action and Therapy in Infants and Children. *N Engl J Med*. 2003 Sep 18;349(12):1157-67.

Kempers, M.J.E., van der Sluijs Veer, L., Nijhuis-van der Sanden, M.W.G., Kooistra, L., Wiedijk, B.M. Faber, I., Last, B.F. de Vijlder, J.J.M., Grootenhuis, M.A., Vulsma, T. 2004. Intellectual and motor development of young adults with congenital hypothyroidism diagnosed by neonatal screening. *J. Clin Endocrin Metab*, 91: 418-424.

Kester M.H., M.R. De Martinez, M.J. Obregon, D. Marinkovic, A. Howatson, T.J. Visser, R. Hume, G. Morreale De Escobar. 2004. Iodothyronine Levels in the Human Developing Brain: Major Regulatory Roles of Iodothyronine Deiodinases in Different Areas. *J Clin Endocrinol Metab* 89: 3117-3128.

Kilby M.D., N. Gittoes, C. Mccabe, J. Verhaeg, J.A. Franklyn. 2000. Expression of Thyroid Receptor Isoforms in the Human Fetal Central Nervous System and the Effects of Intrauterine Growth Restriction. *Clin Endocrinol*: 53: 469-477.

Kirk, A.B., P.K. Martinelango, K. Tian, A. Dutta, E.E. Smith, and P.K. Dasgupta. 2005. Perchlorate and Iodide in Dairy and Breast Milk. *Environmental Science & Technology*. 39(7): P. 2011-7.

Kooistra, L., S. Crawford, A.L. Van Baar, E.P. Brouwers, and V.J. Pop . 2006. Neonatal Effects of Maternal Hypothyroxinemia During Early Pregnancy. *Pediatrics* 117: 161-167.

Krassas, G. E, K. Poppe and D. Glinoer. 2010. Thyroid Function and Human Reproductive Health. *Endocrine Reviews* 31 (5): 702.

LaGamma, E.F. 2008. Introduction to Special Issue on Transient Hypothyroxinemia of Prematurity. *Sem Perinatol* 6:377-9.

Lavado-Autric R., E. Ausó, J.V. García-Velasco, MC. Arufe, F. Escobar Del Rey, P. Berbel, G. Morreale De Escobar. 2000. Early Maternal Hypothyroxinemia Alters Histogenesis and Cerebral Cortex Cytoarchitecture of the Progeny. *Journal Clinical Investigation*. 111(7):1073-82.

Li Y., Z. Shan, W .Teng, X. Yu, Y. Li, C. Fan, X. Teng, R. Guo, H. Wang, J Li, Y.Chen, W. Wang, M Chawinga, L. Zhang, L. Yang, Y. Zhao, and T. Hua. 2010. Abnormalities of Maternal Thyroid Function During Pregnancy Affect Neuropsychological Development of Their Children at 25-30 Months. *Clin Endocrinol*. 72(6):825-9.

Lucas, A., Morley, R., and M. Fewtrell. 1996. Low Triiodothyronine Concentration in Preterm Infants and Subsequent Intelligence Quotient at 8-Year Follow Up. *Brit Med J*. 312:1132-3.

Lumen, A, D.R. Matti and J.W. Fisher, JW. 2013. Evaluation of Perturbations in Serum Thyroid Hormones During Human Pregnancy Due to Dietary Iodie and Perchlorate Exposure Using a Biologically Based Dose Response Model *Toxicological Sciences*. In press.

Malvaux P., C. Beckers, and M. Devisscher. 1965. Dynamic Studies on the Inorganic Iodine Compartment and its Exchanges During Adolescence. *J Clin Endocrinol Metab*. 25:817-22.

Man E.B., J.F. Brown, S.A. Serunian. 1991. Maternal Hypothyroxinemia: Psychoneurological Deficits of Progeny. *Ann Clin Lab Sci* 21: 227-239.

McLanahan, E., M. Andersen, and J. Fisher. 2008. A Biologically Based Dose-Response Model for Dietary Iodide and the Hypothalamic-Pituitary-Thyroid Axis in the Adult Rat: Evaluation Of Iodide Deficiency. *Toxicol. Sci*. 102: 241-253.

McLanahan, E.D., M.E. Andersen, J.L. Campbell, and J.W. Fisher. 2009. Competitive Inhibition of Thyroidal Uptake of Dietary Iodide by Perchlorate Does Not Describe Perturbations in Rat Serum Total T4 and TSH. *Environ. Health Perspect*. 117: 731-738.

Mendez, W., E. Dederick, and J. Cohen, 2010. Drinking Water Contribution to Aggregate Perchlorate Intake of Reproductive-Age Women in the United States Estimated by Dietary Intake Simulation and Analysis of Urinary Excretion Data. *Journal of Exposure Science & Environmental Epidemiology*. 20(3):288-97.

Mendez, W. and S.E. Eftim. 2012. Biomarkers of Perchlorate Exposure are Correlated With Circulating Thyroid Hormone Levels in the 2007-2008 NHANES. *Environmental Research*. 118:137-144.

Merrill, E.A., R.A. Clewell, J.M. Gearhart, P.J. Robinson, T.R. Sterner, K..O. Yu, D..R. Mattie and J.W. Fisher. 2003. PBPK Predictions of Perchlorate Distribution and its Effect on Thyroid Uptake of Radioiodide in the Male Rat. *Toxicol. Sci*. 73:256-269.

Merrill, E.A., R.A. Clewell, P.J. Robinson, A.M. Jarabek, J.M. Gearhart, T.A. Sterner, and J.W. Fisher. 2005. PBPK Model for Radioactive Iodide and Perchlorate Kinetics and Perchlorate-Induced Inhibition of Iodide Uptake in Humans. *Toxicol. Sci*. 83:25-43.

Mirabella G., D. Feig, E. Asztalos, K. Perlman, and J.F.Rovet .2000. The Effect of Abnormal Intrauterine Thyroid Hormone Economies on Infant Cognitive Abilities. *J Pediatr Endocrinol Metab.* 13:191-194.

Moleti M, F. Trimarch, and F. Vermiglio. 2011. Doubts and Concerns About Isolated Maternal Hypothyroxinemia. *J Thyroid Res* : 1-7. doi:10.4061/2011/463029

Morreale de Escobar G, M.J. Obregon, and F. Escobar Del Rey. 2000. Is Neurodevelopment Related to Maternal Hypothyroidism or to Maternal Hypothyroxinemia? *J Clin Endocrol Metab*. 85:3975-3987.

Morreale de Escobar G., M.J. Obregon, and F. Escobar Del Rey 2004. Maternal Thyroid Hormones Early in Pregnancy and Fetal Brain Development. *Best Pract Res Clin Endocrinol Metab.* 18: 225-248.

Morreale de Escobar, G., S. Ares, P. Berbel, M.J. Obregon, and F.F. Escobar del Rey. 2008. The Changing Role of Maternal Thyroid Hormone in Fetal Brain Development. *Sem Perinatol* 32:380-6.

Murray, C.W., S.K. Egan, H. Kim, N. Beru, and P.M. Bolger. 2008. U.S. Food snd Drug Administration's Total Diet Study: Dietary Intake of Perchlorate and Iodine. *Journal of Exposure Science & Environmental Epidemiology* 18(6):571-80.

NRC (National Research Council). 2005. Committee To Assess The Health Implications Of Perchlorate Ingestion, *Health Implications Of Perchlorate Ingestion*. National Academy Press. Washington, D.C. http://www.nap.edu/catalog.php?record_id=11202. (Accessed July 26, 2012)

NRC (National Research Council). 2009. Committee On Improving Risk Analysis Approaches Used By The U.S. EPA. Science And Decisions: Advancing Risk Assessment. Washington, D.C.: National Academy Of Sciences, 2009. Http://Www.Nap.Edu/Catalog.Php?Record_Id=12209. (Accessed July 31, 2012.)

NRC (National Research Council). 2011. Committee To Review EPA's Draft IRIS Assessment Of Formaldehyde. *Review Of The Environmental Protection Agency's Draft IRIS Assessment Of Formaldehyde.* Washington, D.C.:National Academy Of Sciences. http://www.nap.edu/openbook.php?record_id=13142 (Accessed July 31, 2012)

Obregon, M.J., R.M. Calvo, F.E. Del Rey, and G.M. de Escobar. 2007. Ontogenesis of thyroid function and interactions with maternal function. *Endocrine Development.* **10** 868. doi:10.1159/000106821.

Oerbeck, B., K. Sundet, B.F. Kase, S. Heyerdahl. Congenital hypothyroidism: Influence of disease severity and L-thyroxine treatment on intellectual, motor, and school-associated outcomes in young adults. *Pediatrics.* 112: 923-930.

Oddie, T.H., J.H. Meade Jr., J. Myhill, and D.A. Fisher. 1966. Dependence of Renal Clearance of Radioiodide on Sex, Age and Thyroidal Status. *J Clin Endocrinol Metab*. 1966 .26(12):1293-6.

Patel, J., K. Landers, H. Li H,R.H. MortimerK. Richard. 2011. Thyroid hormones and fetal neurological development. .*J Endocrinol*. 209(1):1-8.

Paroder-Belenitsky M, M.J. Maestas, O. Dohan, J.P. Nicola, A. Reyna-Neyra, A. Follenzi, E. Dadachova, S. Eskandari, L.M. Amzel and N. Carrasco. 2011. Mechanism of Anion Selectivity and Stoichiometry of The Na+/I- Symporter (NIS). *Proc Natl Acad Sci USA* 108:17933-17938.

Pearce, E.N., J.H. Lazarus, P.P.A. Smyth, X. He, D. Dall'Amico, A.B. Parkes, R. Burns, D.F. Smith, A. Maina, J.P. Bestwick, M. Jooman, A. M. Leung, and L.E. Braverman. 2010. Perchlorate and Thiocyanate Exposure and Thyroid Function in First-Trimester Pregnant Women. *The Journal of Clinical Endocrinology and Metabolism* 95(7):3207-15.

Pearce, E.N., C.A. Spencer, J.H. Mestman, R.H. Lee, L.M. Bergoglio, P. Mereshian, X. He, A.M. Leung, and L.E. Braverman. 2011. Effect of Environmental Perchlorate on Thyroid Function in Pregnant Women from Cordoba, Argentina and Los Angeles, California. *Endocrine Practice : Official Journal of the American College Of Endocrinology and the American Association of Clinical Endocrinologist* 17(3): 412-417.

Pearce, E.N., M. Alexiou, E. Koukkou,L.E., Braverman, X.·He,·I. Ilias, M. Alevizaki, and K.B. Markou. 2012. Perchlorate and Thiocyanate Exposure and Thyroid Function in First Trimester Pregnant Women from Greece. *Clinical Endocrinology*. In Press 77(3)471-474. (Accessed August 9, 2012)

Pharoah, P.O., K.J. Connolly, R.P. Ekins, and A.G. Harding. 1984. Maternal Thyroid Hormone Levels in Pregnancy and the Subsequent Cognitive and Motor Performance of the Children. *Clinical Endocrinology.* 21:265-270.

Ponchon, G., C. Beckers and M. de Visscher. 1966. Iodide Kinetic Studies in Newborns and Infants. *J Clin Endocrinol Metab*. 26(12):1392-4.

Pop, V.J., J.L. Kuijpens, A.L. Van Baar, G. Verkerk, M.M. Van Son, J.J. De Vijlder, T. Vulsma, W.M. Wiersinga, H.A. Drexhage, and H.L. Vader. 1999. Low Maternal Free Thyroxine Concentrations During Early Pregnancy Are Associated With Impaired Psychomotor Development in Infancy. *Clin. Endocrinol*. 50(2):149-155.

Pop, V.J., E.P. Browers,,H.L. Vader,T. Bulsma,,A.L. Van Baar,J.J. de Vijlder. 2003. Maternal Hypothyroxinaemia During Early Pregnancy and Subsequent Child Development: A 3-Year Follow-Up Study. *Clinical Endocrinology* 59:282-288.

Porterfield, S.P., and C.E. Hendrich. 1993. The role of thyroid hormones in prenatal and neonatal neurological development--current perspectives. *Endocr Rev* 14:94-106.

Reuss ,M.L., Paneth, N., Pinto-Martin, J.A., Lorena, J.M., Susser, M. 1996. The Relation of Transient Hypothyroxinemia in Preterm Infants to Neurologic Development at Two Years of Age. *New Engl J Med*. 334:821-7.

Riesco-Eizaguirre G. and P. Santisteban. 2006. A Perspective View of Sodium Iodide Symporter Research sand its Clinical Implications. *Eur J Endocrinol.* 155(4):495-512.

Rovet, J.F. 1990a. Does Breast Feeding Protect the Hypothyroid Infant Diagnosed by Newborn Screening? *American Journal of Diseases in Childhood.* 144:319-323.

Rovet, J.F. 1990b. *Hypothyroidism. Intellectual and Neuropsychological Functioning*. In C. Holmes (ed): Psychoneuroendocrinology: Brain, Behavior, and Hormonal Interactions. New York: Springer Verlag.

Rovet, J.F. 1992. Neurodevelopment in infants and preschool children with congenital hypothyroidism: Etiological and Treatment Factors affecting Outcome. 17:187-213.

Rovet, J. 2005. Congenital Hypothyroidism: Treatment and Outcome. *Curr Opin Endocrinol Metab.* 12:42-52.

Rovet, J. and D. Daneman. 2003. Congenital Hypothyroidism: A Review of Current Diagnostic Procedures and Treatment. *Pediatric Drugs* 5:141-149.

Rovet, J. and N. Simic. 2008. The Role of Transient Hypothyroxinemia of Prematurity in Development of Visual Abilities. *Sem Perinatol.* 32: 431-7.

Rovet, J.F. and K.A. Willoughby. 2010. Maternal Thyroid Function During Pregnancy: Effects on the Developing Fetal Brain. In *Maternal Influences On Fetal Neurodevelopment: Clinical And Research Aspects.* Zimmermann and Connors (Eds). Springer Science Business Media. New York, New York. pp 55-77.

Royland J.E., J.S. Parker, M. E. Gilbert. 2008. A Genomic Analysis of Subclinical Hypothyroidism In Hippocampus and Neocortex of the Developing Rat Brain. *Journal of Neuroendocrinology*. 20(12):1319-38.

Sack, J, H. Frucht, and O. Amadeo. 1981. Breast milk thyroxine and not cow's milk may mitigate and delay the clinical picture of neonatal hypothyroidism. *Acta Paediatr Scand*. 277:54–56.

Samuels, M.H. 2008. Cognitive function in untreated hypothyroidism and hyperthyroidism. Current Opinion. *Endocrinol Diabetes Obes*. 15(5):429-33. doi: 10.1097/MED.0b013e32830eb84c.

Savin, S., D. Cveejic, O. Nedic, and R. Radosavljevic. 2003. Thyroid Hormone Synthesis and Storage in the Thyroid Gland of Human Neonates. *Journal of Pediatric Endocrinology and Metabolism* 16:521-528.

Schier, J.G., A.F. Wolkin, L. Valentin-Blasini, M.G. Belson, S.M. Kieszak, C.S. Rubin, and B.C. Blount. 2010. Perchlorate Exposure from Infant Formula and Comparisons with the Perchlorate Reference Dose. *Journal of Exposure Science & Environmental Epidemiology*. 20(3):281-7.

Schweizer, U., J.M. Weitzel And L. Schomburg. 2008. Think Globally – Act Locally. New Insights Into the Local Regulation of Thyroid Hormone Availability Challenge Long Accepted Dogmas. *Molecular And Cellular Endocrinology*. 289(1-2):1-9.

Sharlin, D.S., M.E. Gilbert, M.A. Taylor, D.C. Ferguson, R.T. Zoeller**,** 2010. The nature of the compensatory response to low thyroid hormone in the developing brain. *J. Neuroendocrinology*, 22**:** 153-165.

Shelor C.P., and P.K. Dasgupta. 2011. Review of analytical methods for the quantification of iodine in complex matrices. *Anal Chim Acta*. Sep 19;702(1):16-36. doi: 10.1016/j.aca.2011.05.039. Epub 2011 Jun 23.

Silva J.E. and Silva S. 1981. Interrelationships Among Serum Thyroxine, Triiodothyronine, Reverse Triiodothyronine, and Thyroid-Stimulating Hormone in Iodine-Deficient Pregnant Women and Their Offspring: Effects of Iodine Supplementation. *J Clin Endocrinol Metab*. 52(4):671-7.

Simic, N. and J. Rovet. 2010. Transient Hypothyroxinemia of Prematurity: Current State of Knowledge. *Thyroid Intern.* 1-13.

Smanik P.A., Q.Liu , T.L. Furminger, K. Ryu, S. Xing, E.L. Mazzaferri, and S.M. Jhiang 1996. Cloning of the Human Sodium Iodide Symporter. *Biochem Biophys Res Commun.* 226:339-345.

Smit, B.J., J.H. Kok, T. Vulsma, J.M. Briet, K. Boerk, W.M. Wiersinga. 2000. Neurologic Development of the Newborn and Young Child in Relation to Maternal Thyroid Function. *Acta Paediatrica.* 89:291-295.

Suárez-Rodríguez M., C. Azcona-San Julián, and V. Alzina de Aguilar. 2012. Hypothyroxinemia During Pregnancy: The Effect on Neurodevelopment in the Child. *Int J Dev Neurosci.* 30(6):435-8.

Taioli, E., and S. Bonassi. 2002. Methodological Issues in Pooled Analysis of Biomarker Studies. *Mutation Research*.512: 85-92.

Tazebay U.H., I.L. Wapnir, O. Levy, O. Dohan, L.S. Zuckier, Q.H. Zhao, H.F. Deng, P.S. Amenta, S. Fineberg, R.G. Pestell, and N. Carrasco. 2000. The Mammary Gland Iodide Transporter Is Expressed During Lactation and In Breast Cancer. *Nature Medicine* 6:871-878.

Thompson, C.C., and G.B. Potter. 2000. Thyroid Hormone Action in Neural Development. *Cereb Cortex.* 10: 939-945.

Thompson, C. M., B. Sonawane, H.A. Barton, R.S. Dewoskin, J.C. Lipscomb, P. Schlosser, W.A. Chiu, and K. Krishnan. 2008. Approaches for Applications of Physiologically-Based Pharmacokinetic Models in Risk Assessment. *Journal of Toxicology and Environmental Health* Part B 11(7), 519-547.

Thorpe-Beeston, J.G., K.H. Nicolaides. C.V. Felton, J. Butler, and A.M. McGregor, 1991. Maturation of the Secretion of Thyroid Hormones and Thyroid-Stimulating Hormone in the Fetus. *New England Journal of Medicine* 324:532-536.

Tran N., L. Valentin-Blasini, B.C. Blount, C.G. McCuistion, M.S. Fenton, E. Gin, A. Salem and J.M. Hershman. 2008. Thyroid-Stimulating Hormone Increases Active Transport of Perchlorate Into Thyroid Cells. *Am J Physiol Endocrinol Metab* 294:E802-806.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Integrated Risk Information System For Perchlorate And Perchlorate Salts. Available From: http://www.epa.gov/iris/subst/1007.htm

U.S. EPA (U.S. Environmental Protection Agency). 2008. Inhibition Of The Sodium-Iodide Symporter by Perchlorate: An Evaluation of Life Stage Sensitivity Using Physiologically-Based Pharmacokinetic (PBPK) Modeling. EPA/600/R-08/106A. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=212508. (Accessed August 1, 2012.)

U.S. EPA (U.S. Environmental Protection Agency). 2009. Summary of External Peer Review Comments and Disposition for the 2008 External Review of the Report "Inhibition of the Sodium-Iodide Symporter By Perchlorate: An Evaluation of Lifestage Sensitivity Using Physiologically-Based Pharmacokinetic (PBPK) Modeling (External Review Draft). Accessed July 17, 2012. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199347#Download

U.S. EPA (U.S. Environmental Protection Agency). 2011. Drinking Water: Regulatory Determination on Perchlorate. Federal Register Notice. 76 FR No. 29. Pages 7762 -7767. https://www.federalregister.gov/articles/2011/02/11/2011-2603/drinking-water-regulatory-determination-on-perchlorate (Accessed on August 1, 2012).

U.S. EPA (U.S. Environmental Protection Agency). 2012. Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal For Perchlorate. 2012, U.S. Environmental Protection Agency: Washington, DC. http://yosemite.epa.gov/sab/sabproduct.nsf/0/d3bb75d4297ca4698525794300522ace/$file/final+perchlorate+white+paper+05.29.12.pdf (Accessed July 27, 2012).

Valentin-Blasini, L., B.C. Blount, and A. Delinsky. 2007. Quantification of Iodide and Sodium-Iodide Symporter Inhibitors in Human Urine Using Ion Chromatography Tandem Mass Spectrometry. *Journal of Chromatography* 1155(1): P. 40-6.

Valentin-Blasini, L., B.C. Blount, S. Otero-Santos, Y. Cao, J.C. Bernbaum, and W.J. Rogan., 2011. Perchlorate Exposure and Dose Estimates in Infants. *Environmental Science & Technology* 45(9): P. 4127-32.

van den Hove M. F, C. Beckersb, H. Devlieger, F. de Zegher and P. De Nayerb. 1999. Hormone synthesis and storage in the thyroid of human preterm and term newborns: Effect of thyroxine treatment. *Biochimie* 81 (1999) 563-570.

Vermiglio F., V.P. Lo Presti, M. Moleti, M. Sidoti, G. Tororell, G. Scaffidi, M.G. Castagna, F. Mattina, M.A. Violi, A. Crisa, A. Artemisia, and F. Trimarchi. 2004. Attention Deficit and Hyperactivity Disorders in the Offspring of Mothers Exposed to Mild-Moderate Iodine Deficiency: A Possible Novel Iodine Deficiency Disorder in Developed Countries. *J Clin Endorinol Metab* 89: 6054-6060.

Velasco, I., M. Carreira, P. Santiago, J. A. Muela, E. García-Fuentes, B Sánchez-Muñoz, M. J. Garriga, M. C. González-Fernández, Á. Rodríguez, F. F. Caballero, A Machado, S. González-Romero, M. T. Anarte and F. Soriguer. 2009. Effect of Iodine Prophylaxis during Pregnancy on Neurocognitive Development of Children during the First Two Years of Life. *Endocrine Care* 94(9) 3234.

Vulsma T., M.H. Gons, and J.J. De Vijlder. 1989. Maternal-Fetal Transfer Of Thyroxine In Congenital Hypothyroidism Due to a Total Organification Defect or Thyroid Agenesis. *N Engl J Med* 321:13-16.

Welt L.G. and W.B. Blythe. 1970. Anions: phosphate, iodide, fluoride and other anions. In: Goodman LD, Gilman A, eds. *The pharmacological basis of therapeutics*, 4th ed. New York,Macmillan.

Wheeler, S.M., K.A., Willoughby, M.P. McAndrews, and J.F Rovet. 2011. Hippocampal Size and Memory Functioning in Children and Adolescents with Congenital Hypothyroidism. *Journal of Clinical Endocrinology and Metabolism*. 96: E1427-E1434.

Wheeler, S.M., M.P McAndrews, E. Sheard, and J. Rovet. 2012. Visuospatial Associative Memory and Hippocampal Functioning in Congenital Hypothyroidism. *Journal of the International Neuropsychological Society* 18:49-56.

Williams, F.L.R., J. Simpson J, C. Delahunty, S. Ogston , C. Bongers, H. van Toor, S.Y. Wu, T.J. Visser, and R. Hume, with collaboration from the Scottish Preterm Thyroid Group. 2004. Developmental trends in cord and postpartum serum thyroid hormones in preterm infants. *J Clin Endocrinol Metab* 89:5314–5320.

Willoughby, K.A. 2011. Effects of Early Thyroid Hormone Deficiency on Autobiographical Memory and Hippocampal Structure and Function During Late Childhood and Early Adolescence. PhD Thesis. University Of Toronto. http://hdl.handle.net/1807/31973 (Accessed October 14, 2012.)

WHO (World Health Organization). 2001. *Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination. A Guide for Programme Managers*. WHO/NUT, Editor. World Health Organization/United Nations Children's Fund/International Council For The Control Of Iodine Deficiency Disorders: Geneva. http://www.who.int/nut/documents/assessment_idd_monitoring_elimininationpdf. (Accessed December 5, 2012)

WHO (World Health Organization). 2010. *Characterization and Application of Physiologically-Based Pharmacokinetic Model In Risk Assessment. Harmonization Project Document No. 9, 1-72*. International Programme On Chemical Safety. World Health Organization. Http://Www.Inchem.Org/Documents/Harmproj/Harmproj/Harmproj9.Pdf (Accessed July 27, 2012)

Zimmerman, M.B. 2009. Iodine Deficiency. *Endocrine Review*. 30(4):376-408.

Zoeller, R. T. and D.C. Rice. 2004. Critical effect of perchlorate on neonates is iodide uptake inhibition. *Regulatory, Toxicology and Pharmacology* 40(3):376-7.

Zoeller T. and J . Rovet. 2004. Timing of Thyroid Hormone Action in the Developing Brain – Clinical Observations And Experimental Findings. *J Neuroendocrinol.* 16:809-818.

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*[6]*."*

[6]*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 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 their efforts to address perchlorate contamination while EPA was continuing to review scientific issues (U.S. EPA, 2009a).

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, *Health Implication of Perchlorate Ingestion*, 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 these issues is discussed in detail in 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 at least 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 contributions 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 assessed 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 2010). Nine studies present individual measures of urinary perchlorate exposure (Cao 2010; Pearce et al. 2010, 2011, 2012; Leung 2012; Blount 2006; Steinmaus 2007; Schreinemachers 2011; Mendez 2012). 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 ones' 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 may use 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 2010). Consequently, estimating total perchlorate exposure solely by drinking water source may be 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). In water supply systems reliant on Colorado River water, the level of perchlorate in the drinking 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 insufficient to determine a causal association between perchlorate in drinking water and thyroid function nor are they useful for determining direct inputs for deriving an MCLG for perchlorate in drinking water. Two of these studies, however, may provide complementary evidence to assess the broad-based public health impact of regulating perchlorate in municipal water supplies. These two studies (Buffler 2006; Steinmaus 2010) linked data on perchlorate in municipal drinking water measured by the California Drinking Water Program to thyroid hormone levels and primary congenital hypothyroidism, as assessed through the California Newborn Screening Program. Studies using a similar design can provide population-level disease (primary congenital hypothyroidism) prevalence in relation to the concentration of perchlorate in municipal water. Using Buffler 2006 and/or Steinmaus 2010 to describe the "pre-regulation" rates of disease in exposed and unexposed communities, future studies using a similar design may broadly inform the public health implications of regulating perchlorate in drinking water. These two studies have been noted because of the broad geographic area represented (California) and the large sample size (>300,000 newborns).

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, three use NHANES data from 2001-2002 (Blount et al. 2006; Steinmaus 2010; Schreinemachers 2011). Mendez and Eftim used 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 adjusted for in the model although their role may be better treated as stratification variables (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 et al. 2010, 2011, 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 and Eftim 2012; Pearce et al. 2010, 2011, 2012; 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 like thyroid antibodies. 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 even hypothyroxinemia may result in irreversible neurodevelopmental deficits in the offspring (Delahunty 2010). However, existing studies have not incorporated this endpoint.

Some studies have considered thyroid antibodies in their analyses. The thyroid antibodies thyroglobulin antibody (TgAb), thyroid stimulating hormone receptor antibody (TSH-RAb), and thyroid peroxidase antibody (TPOAb) can interfere with thyroid hormone synthesis via humoral and cell-mediated mechanisms leading to clinical or subclinical hypothyroidism (Sinclair 2006). Individuals with hypothyroidism may be more susceptible to additional thyroid disruption, such as that occurring when exposed to perchlorate. Hollowell et al. (2002) estimated the prevalence of thyroid antibodies in the NHANES 1988-1994 sample. In the overall study population, 13.0% and 11.5% had detectable TPOAb and TgAb, respectively. Among the disease-free population, 11.3% (TPOAb) and 10.4% (TgAB) were antibody-positive. Antibody-positive participants were more likely to be female and among females, antibody prevalence increased significantly with age. If the effect of perchlorate on thyroid function differs among people with thyroid antibodies, antibody status should be measured in studies of perchlorate effects and evaluated as a potential effect modifier in the statistical modeling (see detailed discussion below).

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 et al. 2010, 2011, 2012), infants at 1-3 months of age (Leung 2012), and the general U.S. population as represented by NHANES (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 them 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 (Morreale 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 et al. 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; 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 based on 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. Unnecessarily adjusting for factors that are associated only with thyroid function (and, therefore are not acting as confounders) can result in loss of precision; however, gains in precision can sometimes occur depending on the type of statistical model and strength of association with the outcome variable (Schisterman et al. 2009).

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 unnecessary adjusting 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 et al. 2010, 2012; Leung et al. 2012), nitrate (Blount 2006; Steinmaus 2007), cotinine (Pearce et al. 2010) or self-reported smoking (Leung 2012). Some studies, however, addressed the question by evaluating interactions between perchlorate and thiocyanate (Steinmaus 2007; Pearce et al. 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.

REFERENCES

Amitai, Y., G. Winston, J. Sack, J. Wasser, M. Lewis, B.C. Blount, L. Valenti-Blasini, N. Fisher, A. Israeli, and A. Leventhal. 2007. Gestational Exposure to High Perchlorate Concentrations In Drinking Water and Neonatal Thyroxine Levels. *Thyroid.* 17(9):843-50.

Blount, B.C., J.L. Pirkle, J.D. Osterloh, L. Valentín-Blasini, and K.L. Caldwell. 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. *Environmental Health Perspectives*. 114(12): 1865-71.

Buffler, P.A., M.A. Kelsh, E.C. Lau, C.H. Edinboro, J.C. Barnard, G.W. Rutherford, J.J. Daaboul, L. Palmer, and F.W. Lorey. 2006. Thyroid Function and Perchlorate in Drinking Water: An Evaluation Among California Newborns. 1998. *Environmental Health Perspectives*, 2006. 114(5): 798-804.

Cao, Y., B.C. Blount, L. Valentin-Blasini, J.C. Bernbaum, T.M. Phillips, and W.J. Rogan. 2010. Goitrogenic Anions, Thyroid-Stimulating Hormone, and Thyroid Hormone in Infants. *Environmental Health Perspectives*. 118(9): 1332-7.

Delahunty, C., S. Falconer, R. Hume, L. Jackson, P. Midgley, M. Mirfield, S. Ogston, O. Perra, J. Simpson, J. Watson, P Willatts, F. Williams. 2010. Levels of Neonatal Thyroid Hormone In Preterm Infants and Neurodevelopmental Outcome at 5 1/2 Years: Millennium Cohort Study. *The Journal of Clinical Endocrinology And Metabolism*. 95(11): 4898-908.

Hollowell, J.G., N.W. Staehling, W. D. Flanders, W. H. Hannon, E. W. Gunter, C.A. Spencer and L.E. Braverman. 2002. Serum TSH, T(4), and Thyroid Antibodies in the United States Population (1988 To 1994): National Health and Nutrition Examination Survey (NHANES III). *The Journal of Clinical Endocrinology and Metabolism.* 87(2): 489-99.

Huber, D.R., B.C. Blount, D.T. Mage, F.J. Letkiewicz, A. Kumar and R.H.Allen. 2010. Estimating Perchlorate Exposure from Food and Tap Water Based on US Biomonitoring and Occurrence Data. *Journal of Exposure Science & Environmental Epidemiology*. 21(4): 395-407.

Leung, A., L. E. Braverman, X. He, K.E. Schuller, A. Roussilhes, K.A. Jahreis, and E.N. Pearce. 2012. Environmental Perchlorate and Thiocyanate Exposures and Infant Serum Thyroid Function. *Thyroid* 22: 9:938-943.

Mendez, W. and S.E. Eftim. 2012. Biomarkers of Perchlorate Exposure are Correlated with Circulating Thyroid Hormone Levels in the 2007-2008 NHANES. *Environmental Research*. 118:137-144

Morreale de Escobar, G.M., S. Ares , P. Berbel, M.J. Obregón, F.E. Del Rey. 2008. The Changing Role of Maternal Thyroid Hormone in Fetal Brain Development. *Semin Perinatol*. 32(6):380-6.

NRC (National Research Council). 2005. Committee To Assess The Health Implications Of Perchlorate Ingestion, *Health Implications Of Perchlorate Ingestion*. National Academy Press. Washington, D.C. Http://Www.Nap.Edu/Catalog.Php?Record_Id=11202. (Accessed July 26, 2012)

Pearce, E.N., J.H. Lazarus, P.P.A. Smyth, X. He, D. Dall'Amico, A.B. Parkes, R. Burns, D.F. Smith, A. Maina, J.P. Bestwick, M. Jooman, A. M. Leung, and L.E. Braverman. 2010. Perchlorate and Thiocyanate Exposure and Thyroid Function in First-Trimester Pregnant Women. *The Journal oOf Clinical Endocrinology and Metabolism*. 95(7):3207-15.

Pearce, E.N., C.A. Spencer, J.H. Mestman, R.H. Lee, L.M. Bergoglio, P. Mereshian, X. He, A.M. Leung, and L.E. Braverman. 2011. Effect of Environmental Perchlorate on Thyroid Function in Pregnant Women from Cordoba, Argentina and Los Angeles, California. *Endocrine Practice :*

Official Journal of The American College of Endocrinology and The American Association of Clinical Endocrinologist. 17(3): 412-417.

Pearce, E.N., M. Alexiou, E.·Koukkou,L.E.·Braverman, X.·He, I. Ilias, M.·Alevizaki, and K.B. Markou. Perchlorate and Thiocyanate Exposure and Thyroid Function in First Trimester Pregnant Women from Greece. *Clinical Endocrinology*. 77(3)471-474. Schisterman, E.F., S.R. Cole, and R.W. Platt. 2009. Over Adjustment Bias and Unnecessary Adjustment in Epidemiologic Studies. *Epidemiology.* 20(4):488-95.

Schreinemachers, D.M., 2011. Association Between Perchlorate and Indirect Indicators of Thyroid Dysfunction in NHANES 2001-2002, A Cross-Sectional, Hypothesis-Generating Study. *Biomark Insights*. 6:135-46.

Sinclair, D. 2006. Clinical And Laboratory Aspects Of Thyroid Autoantibodies. *Ann Clin Biochem*. 43(Pt 3):173-83.

Steinmaus, C., M.D. Miller, and A.H. Smith. 2010. Perchlorate in Drinking Water During Pregnancy and Neonatal Thyroid Hormone Levels in California. *Journal of Occupational And Environmental Medicine*. 52(12): 1217-524.

Steinmaus, C., M.D. Miller, and R. Howd. 2007. Impact of Smoking and Thiocyanate on Perchlorate and Thyroid Hormone Associations in the 2001-2002 National Health and Nutrition Examination Survey. *Environmental Health Perspectives.* 115(9): 1333-8.

Tellez Tellez, R., P. M. Chacon, C. R. Abarca, B.C. Blount, C.B. Van Landingham, K.S. Crump, and J.P. Gibbs. 2005. Long-Term Environmental Exposure to Perchlorate Through Drinking Water and Thyroid Function During Pregnancy and the Neonatal Period. *Thyroid* 15(9):963-75.

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.

Framework to Summarize Data Evaluation and Application

1) Critically evaluate the quality and content of each type of information in a transparent manner (may need to address each study or component of the larger 'dataset', e.g., life-stage specific intake estimates). Document:
    a. Strengths
    b. Limitations
    c. Information on variability
    d. Key uncertainties of the information
2) Define or describe the contribution of the information towards qualitative or quantitative understanding of perchlorate exposure, biological sensitivity, variability, toxicity and ultimately risk. Include discussion of how specific characteristics limit or support the contribution.

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)[7] or Grading of Recommendations Assessment, Development and Evaluation (GRADE)[8], as appropriate.

[7] Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) http://www.strobe-statement.org/index.php?id=available-checklists [accessed July 30, 2012].

[8] Grading of Recommendations Assessment, Development and Evaluation (GRADE) http://www.gradeworkinggroup.org/index.htm [accessed July 30, 2012].

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.

REFERENCES

National Research Council (NRC). 2009. Committee On Improving Risk Analysis Approaches Used By The U.S. EPA. Science And Decisions: Advancing Risk Assessment. Washington, D.C.: National Academy Of Sciences, 2009. Http://Www.Nap.Edu/Catalog.Php?Record_Id=12209. (Accessed July 31, 2012.)

National Research Council (NRC). 2011. Committee To Review EPA's Draft IRIS Assessment Of Formaldehyde. *Review Of The Environmental Protection Agency's Draft IRIS Assessment Of Formaldehyde.* Washington, D.C.:National Academy Of Sciences. Http://Www.Nap.Edu/Openbook.Php?Record_Id=13142 . (Accessed July 31, 2012)