U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF INSPECTOR GENERAL

Catalyst for Improving the Environment

Science Review

Office of Inspector General Scientific Analysis of Perchlorate

Report No. 10-P-0101

April 19, 2010

Author and Reviewers

Author

Michael Wilson
Toxicologist
U.S. Environmental Protection Agency
Office of Inspector General (OIG)
Office of Program Evaluation (2460T)
1200 Pennsylvania Ave, NW
Washington, DC 20460

Review

Through solicitation number RFQ261223 (dated February 8, 2008), the OIG contracted with ICF Incorporated, LLC (ICF) under contract number GS-10F-0124J, to conduct a 6-week technical review of a working draft of the *OIG Scientific Analysis of Perchlorate*. The purpose of the technical review was to provide a scientific critique of the essential features of the OIG's application of a cumulative risk assessment to this public health issue.

U.S. Environmental Protection Agency
Office of Inspector General

10-P-0101
April 19, 2010

At a Glance

Catalyst for Improving the Environment

Why We Did This Review

Stakeholders often dispute the scientific support for a particular U.S. Environmental Protection Agency (EPA) decision or program action. We believe there is a particular need for impartial reviews of EPA's regulatory toxicology. Therefore, as a prototype for this work, we conducted an independent, scientific review of the risk assessment process and procedures used by EPA to develop and derive the perchlorate reference dose (RfD).

Background

On February 18, 2005, EPA issued a perchlorate RfD that corresponds to a drinking water equivalent level of 24.5 parts per billion. A regulatory determination is pending on whether to issue a National Primary Drinking Water Regulation.

For further information, contact our Office of Congressional, Public Affairs and Management at (202) 566-2391.

To view the full report, click on the following links:

www.epa.gov/oig/reports/2010/20100419-10-P-0101.pdf

www.epa.gov/oig/reports/2010/20100419-10-P-0101 appD.pdf

www.epa.gov/oig/reports/2010/20100419-10-P-0101 appE.pdf

Office of Inspector General Scientific Analysis of Perchlorate

What We Found

EPA should conduct a cumulative risk assessment to reduce the uncertainty in characterizing the public health risk posed by perchlorate. A cumulative risk assessment is the current state-of-the-art technique for evaluating the public health risk from multiple stressors. Over the last two decades, EPA has received numerous recommendations to improve environmental risk assessments. In 1997, EPA Administrator Carol Browner issued guidance directing EPA to embrace the cumulative risk assessment approach on all future major risk assessments. Although directed to improve the environmental risk assessment process, EPA continues to rely on the outdated single chemical risk assessment approach, originally developed in 1954, to characterize the risk posed by perchlorate. The single chemical risk assessment approach fails to address known sources of scientific uncertainty, which lowers the confidence in the perchlorate RfD.

Against established EPA risk assessment procedures, EPA derived the perchlorate RfD from a nonadverse biological effect instead of an adverse effect. The perchlorate RfD protects against all human biological effects from exposure, which is a stricter public health criterion than limiting environmental exposure to protect against adverse effects in humans. This shift in risk management constitutes a significant change in environmental policy.

Based on our scientific analysis, perchlorate is only one of several chemicals that stress the thyroid's ability to uptake iodide. The other sodium iodide symporter (NIS) stressors include thiocyanate, nitrate, and the lack of iodide. All four of these NIS stressors meet EPA's risk assessment guidance for conducting a cumulative risk assessment using the dose-addition method. Our analysis implemented a cumulative risk assessment that found the following: 1) the risk from each of the four NIS stressors is not equal; 2) EPA's perchlorate RfD is conservative and protective of human health, and further reducing the perchlorate exposure below the RfD does not effectively lower risk; 3) increasing maternal total iodide intake to healthy levels will reduce the frequency and severity of permanent mental deficits in children; and 4) correcting moderate and mild iodide deficiency occurring in about 29 percent of the U.S. pregnant and nursing population is the most effective approach for reducing risk.

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

OFFICE OF
INSPECTOR GENERAL

April 19, 2010

MEMORANDUM

SUBJECT: Office of Inspector General Scientific Analysis of Perchlorate
Report No. 10-P-0101

FROM: Bill A. Roderick
Acting Inspector General

TO: Lisa P. Jackson
Administrator

The Office of the Inspector General (OIG) is providing this science report on our cumulative risk assessment of perchlorate and the other sodium (Na^+)/iodide (I^-) symporter stressors. This report represents the opinion of the OIG and does not necessarily represent the final position of the U.S. Environmental Protection Agency (EPA). EPA managers will make final determinations on matters in this report. This report contains no recommendations and no further action is required.

The estimated cost of this report is $263,907. This amount incorporates both the OIG staff cost and the contracting cost for the technical review. The OIG staff cost is $214,322 and was calculated by multiplying the project's staff days by the applicable daily full cost billing rates in effect at the time. The contracting cost for the technical review was $49,585.

If you or your staff have any questions regarding this report, please contact me at 202-566-0847 or roderick.bill@epa.gov, or Eric Lewis at 202-566-2664 or lewis.eric@epa.gov.

Table of Contents

Table of Contents (continued)

Table of Contents (continued)

Executive Summary

Background

Our science review evaluates the public health risk from the sodium (Na^+)/iodide (I^-) symporter (NIS) stressors (e.g., perchorate) that can disrupt the uptake of iodide by the thyroid. The *OIG Scientific Analysis of Perchlorate* (the OIG Analysis) documents our review. The following is background information on perchlorate and other NIS stressors:

- Perchlorate induces human toxicity by blocking the uptake of iodide into the thyroid. A protein called NIS mediates iodide transport from the blood into the thyroid. Since perchlorate acts to block the uptake of iodide, perchlorate is an NIS inhibitor. The perchlorate mechanism of toxicity is that excess perchlorate exposure lowers the total iodide uptake (TIU) of the thyroid. A prolonged low TIU decreases the supply of iodide in the thyroid and eventually results in the decreased production of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). Maintaining an adequate supply of maternal T_4 during pregnancy is critical for proper fetal brain development. A low TIU during pregnancy is associated with permanent mental deficits in the children of those mothers. The more severe the maternal TIU deficiency, the greater will be the frequency and severity of the permanent mental deficits in their children.

- In addition to perchlorate, diet exposes humans to other NIS inhibitors that also lower the amount of iodide uptake by the thyroid. The other common dietary NIS inhibitors are thiocyanate and nitrate. All NIS inhibitors share the same mechanism of toxicity as perchlorate – blocking the uptake of iodide by the thyroid.

- The risk to public health is from the combined amount of NIS inhibition acting on the body from all three NIS inhibitors (thiocyanate, nitrate, and perchlorate). This combined total NIS inhibition load acting on the thyroid determines the TIU level and the subsequent potential for an adverse outcome. The combined NIS inhibition load is known as the total goitrogen load.

- In addition to NIS inhibitors, the amount of the essential dietary nutrient iodide directly affects the amount of iodide uptake by the thyroid in humans. For an environmental pollutant, increased exposure is associated with increased frequency and severity of adverse effect(s) on the body. Iodide, however, becomes an NIS stressor when there is an insufficient amount of iodide in the diet to allow for an adequate uptake of iodide by the thyroid. Therefore, the lack of iodide in the diet (i.e., iodide deficiency) is an NIS stressor and shares the same mechanism of toxicity as the NIS inhibitors – preventing the thyroid from taking up an adequate amount of iodide. Therefore, both the combined total NIS inhibition load acting on the thyroid and the iodide nutritional level determines the TIU level and the subsequent potential for an adverse outcome.

- Diet continuously exposes all humans to all four NIS stressors (thiocyanate, nitrate, perchlorate, and iodide). Common vegetables in the diet are a significant and natural source of thiocyanate. Foods particularly rich in thiocyanate include cabbage, broccoli,

Brussels sprouts, corn (maize), turnips, rapeseed, mustard seed, cauliflower, cabbage, radishes, spinach, tomatoes, and milk. Although diet is an important source of thiocyanate, cyanide produced and absorbed through cigarette smoking is also a significant source of thiocyanate. Nitrate is also common in food. Nitrate occurs in green leafy vegetables, is added to processed meats as a preservative, and is found in milk. In addition to dietary nitrate, both surface and ground sources of drinking water commonly contain nitrate due to the agricultural use of nitrate fertilizers. According to the Food and Drug Administration (FDA) Total Dietary Study, 46% to 59% of the total estimated intake of perchlorate comes from vegetables and dairy foods. Finally, the FDA Total Dietary Study also identifies dairy and grain as the most significant sources of dietary iodine for all groups of adults. Therefore, the degree of dietary exposure to each of the four NIS stressors (not just perchlorate) is needed to determine an individual's TIU and the likelihood for an adverse outcome.

- Other potential sources of perchlorate exposure are the Department of Defense/aerospace sector (e.g., rocket propellant), commercial products (i.e., safety flares and some fireworks (up to 70% content)), agricultural use (i.e., perchlorate is a contaminant in Chilean nitrate fertilizer (CNF)), and natural atmospheric production. The Department of Defense/aerospace sector accounts for about 90% of the annual U.S. consumption of perchlorate. The burning (i.e., use) of rocket propellants, safety flares, and perchlorate-containing fireworks does not result in a significant perchlorate exposure; 0.05% or less of the original perchlorate is left after use because the fire destroys the perchlorate. However, the improper disposal of unused rocket propellants, safety flares, and perchlorate containing fireworks allows the unused perchlorate to get into the groundwater and surface water, resulting in human perchlorate exposure upon ingestion of contaminated drinking water. Although this type of improper disposal into water sources would generate "hot spots" of perchlorate exposure within the population, "hot spots" would not generate the relatively uniform background of perchlorate exposure that is observed across the entire U.S. population. Natural production of perchlorate by the oxidation of chloride by lightning or ozone is not a significant source of drinking water contamination because rainfall contains a mean concentration of perchlorate of 0.015 µg/L (max. 0.2 µg/L).

In 2002, the U.S. Environmental Protection Agency (EPA) issued a draft perchlorate risk assessment titled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization* (NCEA-1-1053) that proposed a perchlorate reference dose (RfD) of 0.00003 milligram/kilogram-day (mg/kg-day). EPA's proposed RfD corresponds to a drinking water equivalent level (DWEL) of 1 part per billion (ppb). EPA's draft risk assessment relied primarily on a rat study conducted by Argus Research Laboratories in 2001. Due to the scientific controversy surrounding the concentration at which perchlorate should be regulated, EPA and others[1] charged the National Academy of Sciences (NAS) Committee to Assess the Health Implications of Perchlorate Ingestion (the NAS Committee) with assessing the current state of the science regarding potential adverse effects of disruption of thyroid function by perchlorate in humans and laboratory animals at various stages of life. Part of the NAS

[1] The U.S. Department of Defense (DOD), U.S. Department of Energy (DOE), and the National Aeronautics and Space Administration (NASA).

Committee's charge was to determine whether EPA's 2002 draft single chemical risk assessment of perchlorate was consistent with the current scientific evidence. In 2005, the NAS Committee rejected EPA's approach of using the results from the Argus rat studies to derive the perchlorate RfD.

In January 2005, the NAS Committee recommended an RfD of 0.0007 mg/kg-day based on the results observed in the Greer human perchlorate dose response study.[2] The NAS Committee included a 10-fold uncertainty factor (UF) in the RfD to protect the most sensitive population, the fetuses of pregnant women who have hypothyroidism or iodide deficiency. On February 18, 2005, EPA established an RfD of 0.0007 mg/kg-day, which corresponds to a DWEL of 24.5 ppb. EPA is currently in the process of making a regulatory determination for perchlorate, which is a formal decision on whether to issue a National Primary Drinking Water Regulation.

In the May 1, 2007, Federal Register (72 FR 24016), EPA indicated that it needed additional information on perchlorate exposure to determine a value for the relative source contribution of perchlorate. EPA must set a perchlorate relative source contribution value before it can make a regulatory determination on whether regulating perchlorate in drinking water presents a meaningful opportunity for health risk reduction. The relative source contribution will determine how much perchlorate exposure comes from food and water.

Purpose of Science Review

The OIG is frequently requested to examine the scientific support for a particular EPA decision or program action. Stakeholders often want an entity without a political or scientific bias to independently evaluate the scientific process and procedures used by the Agency. We believe there is a particular need for this type of independent review of regulatory toxicology. Generally, we have been unable to provide this service because we have a limited capacity to do this kind of work. Additionally, cutting edge science is many times a difficult subject to address. For this project, we had on staff a toxicologist with the requisite experience and training to deliver this service.

In conducting this scientific analysis, we sought to use the state of the art techniques and to comply with EPA's risk assessment requirements so that the validity of our evaluation could be defended. We think we have done that. We expected to be challenged and we were. We believe we have responded fairly to all scientific comments and reached a reasonable conclusion. However, we know that other scientific entities will want to contest our findings. In our response to those organizations and people we explain why we think the data we used was valid and we identified the short comings of other data for use in an environmental risk assessment. Stakeholders should carefully consider all points before making a value judgment on the merits of this report.

[2] The Greer study is a human perchlorate dose response study in which 37 male and female volunteers were given perchlorate in drinking water at 0.007, 0.02, 0.1, or 0.5 mg/kg-day for 14 days. The study results identified a no-observed-effect-level (NOEL) of 0.007 mg/kg-day. The NAS Committee used this NOEL value to derive its recommend perchlorate RfD.

The potential regulation of perchlorate in drinking water by EPA has been a contentious and divisive environmental issue. The congressional bills H.R. 1747 and S. 150, which would amend the Safe Drinking Water Act to require EPA to regulate perchlorate in drinking water, reflect the interest in addressing this public health issue. We analzyed the science supporting EPA's perchlorate RfD. The completion of a human health risk assessment that derives a perchlorate RfD is a key element in the process of making a regulatory determination. Of particular concern is whether EPA's perchlorate RfD is protective of human health at all life stages. Since EPA's final perchlorate RfD is taken directly from the NAS Committee's recommended perchlorate RfD of 0.0007mg/kg-day, we analyzed whether the NAS Committee's recommended RfD is protective of human health at all life stages. Further, we evaluated the following scientific issues to assess the effectiveness of EPA's risk assessment procedures:

1. The NAS Committee used an unconventional approach to derive its recommended perchlorate RfD by using a no-effect-level, and asserted that this was a conservative, health-protective approach to the perchlorate risk assessment.

2. The NAS Committee used a single UF of 10 for intraspecies variation to derive its recommended perchlorate RfD. The NAS Committee stated that its recommended perchlorate RfD needed no additional UFs.

3. The NAS Committee stated that the first adverse effect of perchlorate is hypothyroidism. The Committee stated, "Defining the adverse effect is important because it influences how the RfD is derived and ultimately the value of the drinking-water standard."

4. EPA's charge directed the NAS Committee to assess the potential adverse effects from perchlorate at various stages of life. The NAS Committee derived its recommended perchlorate RfD based on the TIU in healthy adults observed in the Greer study. However, since the scientific literature reports that the fetal thyroid is more sensitive to a deficiency in TIU. Therefore, an uncertainty to consider in addressing this public health issue is how well the adult thyroid response to perchlorate measured in the Greer study represents the fetal thyroid response to lack of an adequate TIU.

5. The NAS Committee indicated that its recommended RfD is protective of the most sensitive population – "the fetuses of pregnant women who might have . . . iodide deficiency."

6. EPA's charge to NAS is written in a single chemical risk assessment approach, which prevents the evaluation of the impact that the other dietary NIS inhibitors (i.e., thiocyanate and nitrate) have on this public health issue.

7. EPA's charge to NAS is written in a single chemical risk assessment approach, which prevents the evaluation of the impact that other NIS stressors, such as the lack of iodide, have on this public health issue.

Results of Review

The OIG Analysis indicates that although EPA used a single chemical risk assessment for perchlorate, a cumulative risk assessment that assesses and characterizes the combined human health risk from all NIS stressors would better describe the nature and sources of risk affecting this public health issue. The major findings, which directly address each of seven scientific issues identified in the "Purpose" section of this report, are as follows.

1. *The NAS Committee's Unconventional RfD Approach Is Conservative and Protective*

By applying the Tonacchera *in vitro* NIS Model of Competitive Inhibition in the OIG Analysis, we analyzed the NAS Committee's statement that its unconventional approach of using a no-effect-level to derive its recommended RfD is both conservative and protective of human health. As with the NAS Committee, we could not utilize the conventional approach of deriving a perchlorate RfD because no perchlorate study has observed adverse effects in any human population. Therefore, we used the principles of cumulative risk and identified in the OIG Analysis that the other common dietary NIS inhibitors, thiocyanate and nitrate, act through the same mechanism of toxicity. The toxic levels observed in humans from excessive thiocyanate exposure allowed us to estimate a perchlorate RfD by converting the observed toxic NIS inhibition level for thiocyanate into a perchlorate equivalent concentration through the use of a relative potency factor. This allowed us to use the conventional risk assessment approach of deriving an RfD from a lowest-observed-adverse-effect-level by utilizing the adverse effect of hypothyroxinemia observed in women exposed to an excess NIS inhibition load caused by excessive thiocyanate exposure. When this decreased production of T_4 observed in hypothyroxinemia occurs during pregnancy, subtle mental deficits occur in the offspring. Therefore, the decreased production of T_4 during pregnancy is the same mechanism by which perchlorate's NIS inhibition potentially causes mental damage during gestation and lactation.

The OIG Analysis confirmed that the NAS-recommended perchlorate RfD is conservative by a factor of 6.6 times. In other words, a conventionally derived perchlorate RfD is 6.6 times higher than the NAS Committee's recommended perchlorate RfD of 0.0007 mg/kg-day. This finding supports the NAS Committee's statement that its unconventional approach of deriving the perchlorate RfD represents a conservative value. However, the perchlorate RfD derived from a single chemical risk assessment approach is accurate only for those individuals with a typical background exposure to the other three NIS stressors. In other words, the perchlorate RfD is not a static value, but changes depending on the exposure level to the other three NIS stressors (thiocyanate, nitrate, and lack of iodide).

2. *Increasing Uncertainty Factors Do Not Effectively Protect Public Health*

The NAS Committee used a single UF of 10 for intraspecies variation to derive its recommended perchlorate RfD. EPA defines an RfD as a daily oral exposure likely to be without an appreciable risk of adverse health effects over a lifetime with uncertainty/variability factors applied to reflect limitations of the data used. In other words, the purpose of an RfD is to allow the setting of a regulatory exposure level that avoids the occurrence of adverse effect in

humans. The OIG Analysis identified that the use of UFs in a single chemical risk assessment is effective only if the chemical that the risk assessors are evaluating (i.e., perchlorate) is one of the principal agents inducing the adverse effect. We specifically evaluated the effectiveness of increasing the application of UFs in deriving the perchlorate RfD and measured the effect on preventing the TIU from becoming too low in pregnant women, fetuses, and nursing infants. We found that the scientific data indicate that perchlorate has the weakest impact of the four NIS stressors on the TIU. Lowering the perchlorate RfD has only a minimal effect on maintaining a TIU within a healthy range. The OIG Analysis identified that:

- No increase in UF value derives a perchlorate RfD that is low enough to prevent mental damage in children, by itself.
- In contrast, the only way to maintain a maternal TIU within a healthy range is to prevent the excessive exposure to each one of the four NIS stressors.
- Limiting the exposure to perchlorate alone at or below the RfD cannot compensate for an excessive exposure to one or more of the other NIS stressors.

3. *Hypothyroxinemia Occurs Before Hypothyroidism*

As part of the OIG Analysis, we evaluated the NAS Committee statement that the first adverse effect of perchlorate is hypothyroidism. Hypothyroidism is a stressed thyroid condition characterized by a low free thyroxine (fT_4) level with an elevated thyroid-stimulating hormone (TSH) level. The OIG Analysis of the scientific data found that the maternal thyroid's initial response to a diminished uptake of iodide is not hypothyroidism but a less stressful thyroid condition called hypothyroxinemia (a low fT_4 level with normal TSH level). Hypothyroxinemia is a common condition in pregnant women. Hypothyroxinemia reflects a thyroid condition in which the pregnant mother has difficulty meeting her own T_4 needs and is unable to meet the fetal demand for T_4, needed for proper brain development. We determined that hypothyroxinemia occurs in pregnant women when the TIU becomes less than or equal to 25% of normal. The OIG Analysis of the scientific data indicates that a maternal TIU equal to or less than 25% is associated with these effects in their children:

- Attention-deficit/hyperactivity disorder (ADHD)
- Lower verbal intelligence quotient (IQ)
- Lower overall IQ
- Lower motor performance

The OIG Analysis of the scientific data estimated that up to 6.9% of infants (i.e., 276,000 infants per year) are born to U.S. mothers with maternal TIU of less than 25%. These children have a higher risk of suffering from these permanent subtle neurological, psychological, and intellectual deficits. This frequency of fetal exposure to maternal TIU of less than 25% is consistent with the estimated occurrence of attention-deficit/hyperactivity disorder (ADHD) at 19 years of age of 7.5% (6.5% to 8.4% at a 95% confidence interval).

4. *Low TIU Adversely Affects the Fetus First*

The EPA and others charged the NAS Committee to assess the potential adverse effects from perchlorate at various stages of life. The NAS Committee derived its recommended perchlorate RfD based on the TIU of healthy adults observed in Greer study. However, since the scientific literature indicates that the fetal thyroid is more sensitive to a deficiency in TIU, a scientific uncertainty to consider in addressing this public health issue is how well the adult thyroid response to perchlorate in the Greer study represents the fetal thyroid's response to lack of an adequate TIU. The OIG Analysis of the scientific literature identified that the fetal thyroid is less able to adapt to a low TIU than the mother's thyroid. Therefore, the first adverse effect occurs to the fetus before the mother's thyroid performance shows any signs of stress (i.e., changes in thyroid hormone levels). Thus, risk assessors should use neither maternal hypothyroidism nor hypothyroxinemia as the key biological event in a risk assessment. By contrast, the first adverse effects in the fetus occur when the maternal TIU is between 25% and 50% of normal. The OIG Analysis of the scientific data indicates that a maternal TIU between 25% and 50% of normal is associated with these effects in their children:

- Permanent delayed reaction time to stimuli
- A higher frequency of elevated TSH in newborns (i.e., the cognitive impact of elevated TSH in newborns has not been studied)
- An increased frequency of mild thyroid dysfunction (subclinical hypothyroidism) later in childhood (if born with elevated TSH levels)

The OIG Analysis estimated that up to an additional 22% of infants (i.e., 880,000 infants per year) are being born to U.S. mothers with maternal TIU between 25% and 50%.

5. *Perchlorate RfD Alone Does Not Protect Most Sensitive Populations*

As part of the OIG Analysis, we evaluated the NAS Committee's statement that its recommended perchlorate RfD is protective of the most sensitive population – "the fetuses of pregnant women who might have . . . iodide deficiency." The OIG Analysis found that mild iodide deficiency decreases maternal TIU by 50% to 75%, while moderate iodide deficiency decreases maternal TIU by more than 75%. By contrast, the OIG Analysis calculated that decreasing the perchlorate exposure in drinking water from 24.5 ppb to 6.1 ppb would result in only a 1.0% increase in the maternal TIU. Clearly, a 1.0% increase in maternal TIU is insufficient to compensate for a 50% or more decrease in maternal TIU caused by mild to moderate iodide deficiency. Further, the OIG Analysis found that no amount of reduction in the body's total NIS inhibition load (i.e., reducing the exposure to all three NIS inhibitors, not just perchlorate) is capable of compensating for mild or moderate iodide deficiency in humans. Iodide deficiency has a much stronger effect on lowering the maternal TIU than can be compensated for by reducing the environmental exposure to perchlorate and/or the other NIS inhibitors. Thus, to reduce the risk from this public health issue, mild and moderate iodide deficiency during pregnancy and nursing must be addressed.

6. *Thiocyanate and Nitrate Must Be Included in the Risk Assessment*

The NAS charge prevented the NAS Committee from considering the impact of the other NIS inhibitors (i.e., thiocyanate and nitrate) on this public health issue. The OIG Analysis of the scientific literature identified that thiocyanate and nitrate have the same mechanism of toxicity as perchlorate. Therefore, thiocyanate and nitrate must be considered together in the risk assessment because their exclusion seriously distorts the portion of the total risk attributed to the principal stressor of concern, perchlorate. Although perchlorate is a strong NIS inhibitor, human exposure to perchlorate is relatively low so the contribution to the body's total NIS inhibition load is relatively small. By contrast, although thiocyanate and nitrate are weak NIS inhibitors, their human exposure levels are much greater than perchlorate. Therefore, thiocyanate and nitrate account for the vast majority of the body's typical total NIS inhibition load in adults, fetuses, and nursing infants.

The OIG Analysis of the scientific data identified that the consumption of 2 liters of drinking water contaminated with nitrate at EPA's maximum contaminant level induces 12 times the amount of NIS inhibition that perchlorate exposure at the RfD in an adult would cause. This indicates that lowering the nitrate exposure in drinking water is a more effective approach to increasing maternal TIU than lowering the perchlorate DWEL below 24.5 ppb.

7. *Lack of Iodide Is the Dominant NIS Stressor Affecting this Public Health Issue*

The NAS charge also prevented the NAS Committee from fully considering the role that iodide has on this public health issue. The OIG Analysis of the scientific literature identified that the lack of iodide is a nonchemical stressor that acts through the same mechanism of toxicity as perchlorate – limiting the TIU. The lack of iodide (a NIS stressor) has the same mechanism of toxicity as perchlorate and, therefore, must be included in the risk assessment because its exclusion seriously distorts the portion of the total risk attributed to the principal stressor of concern, perchlorate. The OIG Analysis found that the lack of iodide is the dominant and principal NIS stressor that determines a TIU level.

The OIG Analysis of the scientific literature indicates that to reduce the frequency and severity of permanent mental deficits in children, the excessive exposure to each of the four NIS stressors is required to prevent low maternal TIU during pregnancy and nursing. Since thiocyanate, nitrate, and perchlorate are all chemicals, managing their exposure is within EPA's legislative mandate. However, what would be the most effective action, overseeing the iodide nutrition level during pregnancy and lactation, is not in EPA's legislative mandate. EPA's *Framework for Cumulative Risk Assessment* directs EPA to seek expertise outside of the Agency to address aspects outside EPA authority. Therefore, EPA would have to work cooperatively with other federal agencies, such as the FDA and the National Institutes of Health, to safely correct low maternal TIU. To reduce the frequency and severity of permanent mental deficits in children from low maternal TIU, our cumulative risk assessment has identified adding iodide to prenatal vitamins and implementing their use before and during pregnancy and nursing as an approach to addressing this public health issue.

Conclusion

The OIG Analysis of the scientific literature identified that the risk from perchlorate exposure is only part of a larger public health issue that is defined by the subtle mental deficits occurring in children born to mothers with low maternal TIU during pregnancy and nursing. The TIU results from the combined biological effect of four NIS stressors acting on the thyroid: thiocyanate, nitrate, perchlorate, and lack of iodide. Diet constantly exposes everyone to each of the four NIS stressors, and an individual's TIU level is the result of the combined effect of all four NIS stressors, not just perchlorate exposure. The OIG Analysis concludes that a single chemical risk assessment of perchlorate is not sufficient to assess and characterize the combined human health risk from all four NIS stressors. However, both EPA's draft perchlorate RfD from the Argus rat study and the NAS Committee's recommended perchlorate RfD from the Greer study used a single chemical risk assessment approach. Only a cumulative risk assessment can fully characterize the nature and sources of risk affecting this public health issue. Furthermore, a cumulative risk assessment allows an informed environmental decision to be made on how to mitigate the risk effectively

All four NIS stressors meet EPA's risk assessment guidance requirements for conducting a cumulative risk assessment using the dose-addition method. In the OIG Analysis, we conducted a cumulative risk assessment and determined that the risk from each of the four NIS stressors is not equal. The OIG Analysis also confirmed that EPA's perchlorate RfD is conservative and protective of human health, but limiting perchlorate exposure does not effectively address this public health issue. Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk. By contrast, addressing moderate and mild iodide deficiency occurring in about 29% of the U.S. pregnant and nursing population appears to be the most effective approach to increase TIU to healthy levels during pregnancy and nursing, thereby reducing the frequency and severity of permanent mental deficits in children.

1. Defining the Public Health Issue

Background

Perchlorate induces human toxicity by blocking the uptake of iodide into the thyroid. A protein called the sodium (Na^+)/iodide (I^-) symporter (NIS) mediates iodide transport from the blood into the thyroid. Since perchlorate acts to block the uptake of iodide, perchlorate is an NIS inhibitor. The perchlorate mechanism of toxicity is that excess perchlorate exposure lowers the total iodide uptake (TIU) of the thyroid. A prolonged low TIU decreases the supply of iodide in the thyroid and eventually results in decreased production of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). Maintaining an adequate supply of maternal T_4 during pregnancy is critical for proper fetal brain development. A low TIU during pregnancy is associated with permanent mental deficits in the children of those mothers. The more severe the maternal TIU deficiency, the greater will be the frequency and severity of the permanent mental deficits in their children.

In addition to perchlorate, diet exposes humans to other NIS inhibitors that also lower the amount of iodide uptake by the thyroid in humans. The other common dietary NIS inhibitors are thiocyanate and nitrate. All NIS inhibitors share the same mechanism of toxicity as perchlorate – blocking the uptake of iodide by the thyroid. The risk to public health is from the combined amount of NIS inhibition acting on the body from all three NIS inhibitors (thiocyanate, nitrate, and perchlorate). This combined total NIS inhibition load acting on the thyroid determines the TIU level and the subsequent potential for an adverse outcome. The combined NIS inhibition load is known as the total goitrogen load.

In addition to NIS inhibitors, the amount of the essential nutrient iodide in the diet directly affects the amount of iodide uptake by the thyroid in humans. Unlike an environmental pollutant where increased exposure is associated with increased frequency and severity of adverse effect(s) on the body, iodide becomes an NIS stressor when there is an insufficient amount of iodide in the diet to allow for an adequate uptake of iodide by the thyroid. Therefore, the lack of iodide in the diet (i.e., an iodide deficiency) is an NIS stressor and shares the same mechanism of toxicity as the NIS inhibitors – preventing the NIS from taking up an adequate amount of iodide. Therefore, both the combined total NIS inhibition load acting on the thyroid and the iodide nutritional level determines the TIU level and the subsequent potential for an adverse outcome.

Diet continually exposes all humans to all four NIS stressors (thiocyanate, nitrate, perchlorate, and iodide). Vegetables are a significant and natural source of thiocyanate. Foods particularly rich in thiocyanate include cabbage, broccoli, Brussels sprouts, corn (maize), turnips, rapeseed, mustard seed, cauliflower, cabbage, radishes, spinach, tomatoes, and milk. Although diet is an important source of thiocyanate, cyanide produced and absorbed through cigarette smoking is also a significant source of thiocyanate. Nitrate is also common in food. Nitrate occurs in green leafy vegetables, is added to processed meats as a preservative, and is found in milk. In addition to dietary nitrate, both surface and ground sources of drinking water commonly contain nitrate due to the agricultural use of nitrate fertilizers. According to the Food and Drug Administration (FDA) Total Dietary Study (TDS), 46% to 59% of the total estimated intake of

perchlorate comes from vegetables and dairy foods. Finally, the FDA TDS also identifies dairy and grain as the most significant sources of dietary iodine for all groups of adults. Therefore, the degree of dietary exposure to each of the four NIS stressors (not just perchlorate) is needed to determine an individual's TIU and the likelihood for an adverse outcome.

In 2002, the U.S. Environmental Protection Agency (EPA) issued a draft perchlorate risk assessment titled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization* (NCEA-1-1053) that proposed a perchlorate reference dose (RfD) of 0.00003 milligram/kilogram-day (mg/kg-day). EPA's proposed RfD corresponds to a drinking water equivalent level (DWEL) of 1 ppb. EPA's draft risk assessment relied primarily on a rat study conducted by Argus Research Laboratories in 2001. Due to the scientific controversy surrounding the concentration at which perchlorate should be regulated, the National Academy of Science (NAS) Committee to Assess the Health Implications of Perchlorate Ingestion (NAS Committee) was charged to assess the current state of the science regarding potential adverse effects of thyroid function disruption by perchlorate in humans and laboratory animals at various stages of life. Part of the NAS Committee's charge was to determine whether EPA's 2002 draft single chemical risk assessment of perchlorate was consistent with the current scientific evidence. In 2005, the NAS Committee rejected EPA's approach of using the Argus rat studies to derive the perchlorate RfD.

In January 2005, the NAS Committee recommended an RfD of 0.0007 mg/kg-day based on the results observed in the Greer human perchlorate exposure study. The NAS Committee included a 10-fold uncertainty (UF) in the RfD to protect the most sensitive population, the fetuses of pregnant women who have hypothyroidism or iodide deficiency. On February 18, 2005, EPA established an RfD of 0.0007 mg/kg-day, which corresponds to a DWEL of 24.5 ppb. EPA is currently in the process of making a regulatory determination for perchlorate, which is a formal decision on whether to issue a National Primary Drinking Water Regulation.

In the May 1, 2007, Federal Register (72 FR 24016), EPA indicated that it needed additional information on perchlorate exposure to determine a value for the Relative Source Contribution of perchlorate. EPA must set a perchlorate Relative Source Contribution value before it can make a regulatory determination on whether regulating perchlorate in drinking water presents a meaningful opportunity for health risk reduction. The Relative Source Contribution will determine how much perchlorate exposure comes from food and water.

Utilizing Cumulative Risk Assessment. In 2005, the NAS Committee reviewed the current state of the science regarding the potential adverse health effects of perchlorate exposure (NAS 2005). The charge to the NAS Committee limited the context of its assessment to the potential adverse effects from perchlorate only. This approach to assess the toxicity of a single chemical is an outdated, conventional approach to characterizing the risk to the public. By contrast, the state-of-the-art technique to assess risk is to perform a cumulative risk assessment when multiple chemicals or stressors act through the same mechanism of toxicity. As indicated by former EPA Administrator Browner in 1997, cumulative risk assessment "provides a platform for significant advances in our scientific approach to assessing environmental risks" (EPA 1997b).

Recent Perchlorate Studies Have Different Conclusions. Various published perchlorate studies draw substantially different conclusions on the toxicity of perchlorate.

- The Argus 2001 rat study and the Blount 2006 urinary perchlorate study suggest the need for a lower RfD. Based principally on the observations made in the Argus 2001 rat study (Argus 2001), in 2002 the EPA proposed a perchlorate RfD of 0.00003 mg/kg-day, which corresponds to a DWEL of 1 ppb (EPA 2002a).
- The Blount 2006 urinary perchlorate study observed that increased urinary perchlorate was associated with increased thyroid-stimulating hormone (TSH) and decreased T_4 levels in women with iodide deficiency (i.e., urinary iodide levels less than 100 µg/L) (Blount 2006b).
- By contrast, based on the perchlorate dosing of humans in the Greer study (Greer 2002), the NAS Committee proposed a perchlorate RfD of 0.007 mg/kg-day, which corresponds to a DWEL of 24.5 ppb (NAS 2005).
- The Tellez 2005 study of pregnant women and neonates in Taltal, Chile, which are exposed to drinking water at 114 ppb, are observed to have no statistically significant changes in neonatal thyroid function or birth weight (Tellez 2005).

The lack of agreement in the perceived toxicity of perchlorate among these studies indicates that there is still an unacceptable level of uncertainty in the science of perchlorate to instill confidence that the current EPA perchlorate RfD is protective of human health.

Cumulative Risk Assessment is Possible. Perchlorate is not the only anion acting on the thyroid to inhibit the uptake of iodide by the NIS. Humans are exposed nutritionally and environmentally to NIS inhibitors other than perchlorate that also inhibit the uptake of iodide by the NIS.

- The other principal NIS inhibitors of concern to humans are thiocyanate (SCN^-) and nitrate (NO_3^-).
- Furthermore, since iodine is the signature element in the molecular structure of the two thyroid hormones, T_4 and T_3, a sufficient supply of iodide in the diet is essential for the thyroid to be able to synthesize an adequate supply of thyroid hormones to the body.

These three additional factors involved in the uptake of iodide are known and often cited in the scientific literature, but their relative contribution to the uptake of iodide has not been quantified or used in either EPA's or the NAS Committee's risk characterization. However, to reduce the uncertainties in the science, the relative contributions of each of the four factors (i.e., thiocyanate, nitrate, perchlorate, and the lack of iodide in the diet) to the uptake of iodide must be quantified and included in the risk assessment before making any effective environmental risk management decisions. The Tonacchera *in vitro* NIS Model of Competitive Inhibition (Tonacchera Model) quantified the relationship to the uptake of iodide from each of these four factors (Tonacchera 2004). The Tonacchera Model provides the critical information upon which to conduct a state-of-the-art cumulative risk assessment for this public health issue.

Summary. The EPA and NAS Committee approach to characterizing the risk from perchlorate suggests that the public health issue is driven by perchlorate only. This approach implies that the proposed remedy of lowering the exposure to perchlorate also directly lowers the risk to the public. In other words, if the perchlorate level is lowered to a "safe" level, the public health issue is eliminated. However, this single chemical approach and remedy underestimates the complexity of the public health issue. The actual occurrence of an adverse outcome is determined by the combined interaction of all four factors on the uptake of iodide by the NIS. For EPA and the NAS Committee to determine the risk from just one factor without knowing or managing the risk from the other three factors inadequately captures the amount of risk to the public. Further, managing only the risk from perchlorate will not effectively address the public health issue because the OIG Analysis shows that perchlorate contributes the least amount of risk of the four factors. Therefore, the public health issue must be defined beyond just perchlorate. The public health issue must consider and address all factors that prevent an adequate supply of thyroid hormones during gestation and lactation to ensure proper brain development in both fetuses and nursing infants. All the factors that impact the adequate supply of thyroid hormones must be quantitatively evaluated in a cumulative risk assessment. The use of cumulative risk assessment allows the relative contribution of risk from each factor to be identified and an effective and cost-efficient solution to be determined.

2. OIG's Approach to the Scientific Analysis of Perchlorate

2.1 Introduction

Our science review identified and focused on scientific issues associated with the setting of the perchlorate RfD, which is a key element in the process of making a regulatory determination for perchlorate. We focused on the scientific process from EPA's issuance of the 2002 draft perchlorate risk assessment, *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization*, to July 2008. We conducted field work from April 2007 to July 2008. The *OIG Scientific Analysis of Perchlorate (External Review Draft)* (the OIG Analysis) documents our science review. The OIG Analysis cites approximately 145 scientific journals as well as technical references and EPA risk assessment guidance.

The OIG Analysis identified that a single chemical risk assessment determines the risk to a defined population to the exposure of a single chemical from all routes of exposure (e.g., air, water, soil, food, etc.). By contrast, a cumulative risk assessment is an analysis, characterization, and quantification of the combined risks to health from exposure to multiple chemicals or stressors. Since 1992, experts in the scientific community, the National Academy of Sciences, the President, Congress, and former EPA Administrator Browner have either identified the need or directed EPA to use a cumulative risk assessment approach to assess the risk to public health from exposure to environmental contaminations. Therefore, our OIG Analysis applied a cumulative risk assessment approach to evaluate the public health risk from exposure to perchlorate and the other NIS stressors.

We implemented a cumulative risk assessment by following the concepts in EPA's *Framework for Cumulative Risk Assessment* (EPA/600/P-02/001F) and the principle of dose addition described in EPA's risk assessment guidance for chemical mixtures. Further, we used the NAS Committee on Toxicity Testing and Assessment of Environmental Agents (NAS Toxicity Testing Committee) recommendation for the development of a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by the environmental agent. We implemented the NAS Toxicity Testing Committee's vision and strategy by applying the Tonacchera Model to evaluate the cumulative public health risk from perchlorate and the other NIS stressors. Therefore, we conducted a cumulative risk assessment on this public health issue defined as low TIU during pregnancy and lactation.

The Agency's Peer Review policy encourages and expects all scientific and technical information intended to inform or support Agency decision making (i.e., rulemaking) to be peer reviewed. Specifically, "influential scientific information" and "highly influential scientific assessments" should be peer reviewed in accordance with the Agency's Peer Review Handbook (EPA 2006). Since the OIG does not conduct rulemaking, the OIG Analysis did not undergo a formal peer review. However, the OIG Analysis did undergo an independent, third-party technical review. Four senior scientists from ICF International with direct knowledge and experience in environmental risk assessment reviewed and provided comments on a working draft of the *OIG Scientific Analysis of Perchlorate.* The purpose of this technical review was for the OIG to get an independent scientific critique of our application of a cumulative risk assessment to this public health issue induced by a low TIU during pregnancy and lactation. In

short, ICF International supported the implementation of a cumulative risk assessment, but recommended using the whole mixture cumulative risk assessment approach as opposed to the dose-addition method cumulative risk assessment approach. The OIG Analysis addresses the whole mixture approach to the cumulative risk assessment of perchlorate in Appendix A. However, in discussions with ICF International, the lead ICF scientist concurred that the biochemical properties and mechanistic interaction of the four NIS stressors meet all the EPA risk assessment requirements for the use of the dose-addition method (see Section 2.5.1.1).

2.2 Purpose

The OIG is frequently requested to examine the scientific support for a particular EPA decision or program action. Stakeholders often want an entity without a political or scientific bias to independently evaluate the scientific process and procedures used by the Agency. We believe there is a particular need for this type of independent review of regulatory toxicology. Generally, we have been unable to provide this service because we have a limited capacity to do this kind of work. Additionally, cutting edge science is many times a difficult subject to address. For this project, we had on staff a toxicologist with the requisite experience and training to deliver this service.

In conducting this scientific analysis, we sought to use the state of the art techniques and to comply with EPA's risk assessment requirements so that the validity of our evaluation could be defended. We think we have done that. We expected to be challenged and we were. We believe we have responded fairly to all scientific comments and reached a reasonable conclusion. However, we know that other scientific entities will want to contest our findings. In our response to those organizations and people we explain why we think the data we used was valid and we identified the short comings of other data for use in an environmental risk assessment. Stakeholders should carefully consider all points before making a value judgment on the merits of this report.

EPA’s potential regulation of perchlorate in drinking water has been a contentious and divisive environmental issue. The congressional bills H.R. 1747 and S. 150, which would amend the Safe Drinking Water Act to require EPA to regulate perchlorate in drinking water, reflects the interest in addressing this public health issue. We reviewed EPA’s procedures over its regulatory determination process for perchlorate. The completion of a human health risk assessment that derives a perchlorate RfD is a key element in the process of making a regulatory determination. Of particular concern is whether EPA’s perchlorate RfD is protective of human health at all life stages. Since EPA’s final perchlorate RfD is taken directly from the NAS Committee’s recommended perchlorate RfD of 0.0007mg/kg-day, we analzyed whether the NAS Committee’s recommended RfD is protective of human health at all life stages. Further, we analyzed the following scientific issues to assess the effectiveness of EPA’s risk assessment procedures:

- The NAS Committee used an unconventional approach to derive its recommended perchlorate RfD by using a no-effect-level and asserted that this was a conservative, health-protective approach to the perchlorate risk assessment.

- The NAS Committee used a single UF of 10 for intraspecies variation to derive its recommended perchlorate RfD. The NAS Committee stated that its recommended perchlorate RfD needed no additional UFs.

- The NAS Committee stated that the first adverse effect of perchlorate is hypothyroidism. The Committee stated, "Defining the adverse effect is important because it influences how the RfD is derived and ultimately the value of the drinking-water standard."

- EPA's charge directed the NAS Committee to assess the potential adverse effects from perchlorate at various stages of life. The NAS Committee derived its recommended perchlorate RfD based on the TIU in healthy adults observed in the Greer study. However, since the scientific literature reports that the fetal thyroid is more sensitive to a deficiency in TIU, an uncertainty to consider in addressing this public health issue is how well the adult thyroid response to perchlorate measured in the Greer study represents the fetal thyroid response to lack of an adequate TIU.

- The NAS Committee indicates that its recommended RfD is protective of the most sensitive population, "the fetuses of pregnant women who might have . . . iodide deficiency."

- EPA's charge to NAS is written in a single chemical risk assessment approach, which prevents the evaluation of the impact that the other dietary NIS inhibitors (i.e., thiocyanate and nitrate) have on this public health issue.

- EPA's charge to NAS is written in a single chemical risk assessment approach, which prevents the evaluation of the impact that other NIS stressors, such as the lack of iodide, have on this public health issue.

To address these scientific issues, the OIG Analysis applies both mechanistic toxicology and a cumulative risk assessment to this public health issue.

2.3 Calls to Use Cumulative Risk Assessment

We identified that the EPA draft 2002 perchlorate risk assessment, *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization* (NCEA-1-1053), used a single chemical risk assessment approach to a proposed perchlorate RfD of 0.00003 mg/kg-day (corresponding to a DWEL of 1 ppb). Furthermore, we found that the NAS Committee also used a single chemical risk assessment approach to recommend a perchlorate RfD of 0.0007 mg/kg-day (corresponding to a DWEL of 24.5 ppb). Therefore, both EPA and the NAS Committee used a single chemical risk assessment approach to derive their respective perchlorate RfDs. However, since as early as 1992, EPA has known that a single chemical risk assessment is an outdated approach to assessing risk. A single chemical risk assessment characterizes the potential adverse effect and quantifies the risk from only a single chemical pollutant; it does not evaluate the combined effects from multiple chemicals acting through the same mechanism of toxicity.

By contrast, a cumulative risk assessment is defined as "an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors" (EPA 2003, p 6). The application of a cumulative risk assessment has the potential to expand our understanding of the public health impacts of environmental exposures and provides a clearer, more complete picture of a public health issue for making risk management decisions. In 1995, former EPA Administrator Browner stated, "EPA must be responsive and resolve to more openly and fully communicate to the public the complexities and challenges of environmental decision making in the face of scientific uncertainty" (Callahan 2007). In 1997, then-Administrator Browner wrote that a cumulative risk assessment "provides a platform for significant advances in our scientific approach to assessing environmental risks." Furthermore, cumulative risk assessments can reduce risks to the extent that EPA can integrate it into prevention strategies to protect public health.

Experts in the scientific community, various NAS committees, the Science Advisory Board (SAB), the President, Congress, and former EPA Administrator Browner either have identified the need or directed EPA to use a cumulative risk assessment approach to assess the risk to public health from the exposure to environmental contamination:

- The 1992 Expert Panel on the Role of Science at EPA issued the report titled *Safeguarding the Future: Credible Science, Credible Decisions* (EPA/600/9-91/050). The reported pointed out, "A key role of science at EPA is to reduce uncertainties in environmental decision-making." One of the uncertainties identified by the 1992 Expert Panel that needed to be addressed was the " . . . impact of chemical mixtures and other general stressors [on people and the ecosystem] . . . " (EPA 1992, p 14). However, the 1992 Expert Panel noted that EPA has not developed methods to assess the health or environmental effects from multiple stressors (EPA 1992, p 14).

- In 1994, the NAS Committee on Risk Assessment of Hazardous Air Pollutants issued *Science and Judgment in Risk Assessment,* which recommended that EPA move away from single-chemical assessments and to aggregate cancer risks from exposure to multiple compounds (NAS 1994, executive summary 13; EPA 2000, p x (first bullet)).

- Under Section 3-301(b), Presidential Executive Order 12898 (1994) directs federal agencies to identify "multiple and cumulative exposures" in environmental human health analyses, whenever practicable and appropriate.

- In 1996, Congress mandated, under Section 408(b)(2)(C)(i)(III) of the Food Quality Protection Act of 1996, that EPA assess the cumulative risk to children and infants from pesticide chemical residues and other substances having a "common mechanism of toxicity".

- In 1997, former EPA Administrator Browner directed the Agency "to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that integrates multiple sources, effects, pathways, stressors, and populations for cumulative risk analysis in all cases for which relevant data are

available" and to "embrace this cumulative approach" in all major risks assessments (EPA 1997b).

- In 1997, the EPA Science Policy Council stated EPA risk assessments should evolve away from a focus on the potential adverse effect of a single pollutant in one environmental medium.

- In 2007, EPA's Office of the Science Advisor requested SAB to provide recommendations on ways to advance EPA's human health risk assessment practices. On February 28, 2007, SAB recommended to advance cumulative risk assessment methodologies in order to reflect real-world human exposure that includes multiple stressors (SAB 2007).

- In 2008, the NAS Committee on Improving Risk Analysis Approaches Used by the U.S. EPA issued *Science and Decisions: Advancing Risk Assessment,* which recommended that EPA " . . . incorporate interactions between chemical and nonchemical stressors in assessments" (NAS 2008; executive summary, p 9). This NAS Committee stated, "EPA is increasingly asked to address broader public-health and environmental-health questions involving multiple exposures, complex mixtures, and vulnerability of exposed populations issues that stakeholder groups . . . often consider to be inadequately captured by current risk assessments. There is a need for cumulative risk assessments as defined by EPA (EPA 2003)—assessments that include combined risks posed by aggregate exposure to multiple agents or stressors . . . " ((NAS 2008; executive summary, p 9).

- In 2008, the SAB Drinking Water Committee in its draft report on the Third Drinking Water Contaminant Candidate List (CCL3) (December 3, 2008) proposes recommending that "EPA should consider addressing the cumulative effects of chemicals with similar sources and mechanisms (or modes) of action . . . " (SAB 2008).

In contrast to a single chemical risk assessment approach previously used by both EPA and the NAS Committee to characterize the risk from perchlorate, a cumulative risk assessment approach better evaluates the public health risk from exposure to perchlorate and the other NIS stressors. The toxicity pathway identifies that an inadequate supply of iodide (i.e., low TIU) during gestation and possibly during lactation results in increased risk for subtle mental deficits in children. Perchlorate is only one of four NIS stressors (thiocyanate, nitrate, perchlorate, and lack of iodide) that act on the NIS to lower the TIU by the thyroid. The human diet contains all four NIS stressors; diet universally exposes every human to each of the four NIS stressors. Therefore, an individual's TIU level is the combined effect of all four NIS stressors, not just perchlorate exposure. Since a low maternal TIU results in an increased risk for an adverse outcome, EPA should evaluate, through a cumulative risk assessment, the relative contribution that each NIS stressor has on inducing a low TIU. The findings from the cumulative risk assessment produce a broader, more comprehensive understanding and assessment of the factors involved in this public health issue.

2.4 Use of Mechanistic Toxicology

On June 12, 2007, the NAS Toxicity Testing Committee released a report titled *Toxicity Testing in the Twenty-First Century: A Vision and A Strategy* (NAS 2007). The NAS Toxicity Testing Committee recommended a major shift in how the toxicity of chemicals is assessed because of the revolutionary advances in the science (e.g., proteomics, genomics, and biochemistry) in which the increased understanding of the biological mechanisms allows *in vitro* toxicity testing to be applied to environmental risk assessments. The NAS Toxicity Testing Committee's vision is to move away from the traditional descriptive animal toxicity testing to the identification of perturbed biological pathways as the basis for dose-response modeling. Classic descriptive animal toxicity testing determines a dose in which no adverse effects are identified. Descriptive toxicity testing does not develop an understanding or a characterization of the cellular mechanism triggering the toxicity. The NAS Toxicity Testing Committee's vision of toxicity testing is to develop a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by the environmental agent. Subsequent pharmacokinetic modeling would identify a safe human exposure level that prevents the environmental chemical from reaching a toxic tissue concentration. The NAS Toxicity Testing Committee states, "A stronger scientific foundation offers the prospect of improved risk-based regulatory decisions and possibly greater public confidence in and acceptance of the decisions."

The NAS Toxicity Testing Committee's vision can be observed in the evolving perchlorate science. In 2002, EPA released the draft risk assessment *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization*, which proposed a perchlorate RfD of 0.00003 mg/kg-day. EPA's risk assessment relied on observations made in two rat studies conducted by Argus Research Laboratories in 1998 and 2001. These studies are examples of classic descriptive toxicity testing in which various perchlorate doses are evaluated for their potential to produce adverse effects (e.g., brain structural changes) in the most sensitive population – pregnant females, fetuses, and neonates. Although the lack of thyroid hormones during early development can lead to brain damage, these rat studies were not designed to identify or study a specific cellular pathway that is impacted by perchlorate exposure. These studies were also not designed to develop a quantitative, mechanistic, dose-response model of the perturbed cellular pathway that could have led to the observed changes in the linear measurements of several brain regions of the male and female pups (e.g., corpus callosum).

In 2005, the NAS Committee to Assess the Health Implications of Perchlorate Ingestion was charged to review EPA's 2002 draft perchlorate risk assessment. The NAS Committee found that it was impossible to conclude whether perchlorate exposure caused these brain changes because of problems with the method used to make the measurements, and because the observed changes did not follow a dose-effect relationship. The NAS Committee proposed a mechanistic step (i.e., a perturbation of a cellular pathway) that initiates a sequence of subsequent biological events that eventually results in adverse effects in humans. The key cellular event identified was perchlorate inhibiting the NIS, resulting in the decreased uptake of iodide by the thyroid's epithelial cells. The NAS Committee used only the inhibitory effect of perchlorate on this cellular step as the basis to derive a recommended perchlorate RfD. The evaluation of only perchlorate's inhibition does not represent a quantitative, dose-response model of the prominent factors (i.e., iodide, thiocyanate, nitrate, and perchlorate) that impact the uptake

of iodide by the NIS. The NAS Committee relied primarily on the Greer study to develop its recommended perchlorate RfD of 0.0007 mg/kg-day. The Greer study applied descriptive toxicity testing techniques to develop a dose-response curve in order to identify a perchlorate dose in human test subjects that did not inhibit the uptake of iodide by the NIS. The NAS Committee's recommended perchlorate RfD was not based on a quantitative, dose-response model of the prominent factors that impact the uptake of iodide by the NIS. The NAS Committee used a single chemical risk assessment approach despite the fact that the uptake of iodide by the NIS was known to be perturbed by other NIS inhibitors and to be affected by the amount of available iodide in the blood.

OIG's approach to reviewing the perchlorate science is consistent with the NAS Toxicity Testing Committee's vision and strategy for toxicity testing and its implications on risk assessment. In 2004, Tonacchera developed and evaluated an *in vitro* NIS Model of Competitive Inhibition (Tonacchera 2004). This model quantitatively determines the dose-response of the NIS to the presence of all of the following four anions simultaneously: thiocyanate, nitrate, perchlorate, and iodide. The model predicts the thyroidal iodide uptake by correlating the amount of iodide (i.e., iodide nutritional status) for any given total NIS inhibitor load (i.e., total goitrogen load). The Tonacchera Model quantifies the total goitrogen load into a single value by mathematically combining the amount of inhibition contributed from thiocyanate, nitrate, and perchlorate by using potency factors. Tonacchera developed the model from *in vitro* experiments that exposed Chinese hamster ovary (CHO) cells expressing the human NIS protein to various combinations of the four anions and measured the amount of uptake of radioactive iodide by the cells. The use of the Tonacchera Model for human health risk assessment implements the NAS Toxicity Testing Committee's vision of toxicity testing by using a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by perchlorate, and then the subsequent use of pharmacokinetic modeling to identify a human perchlorate exposure level that prevents the perchlorate from reaching a toxic concentration at the NIS. Therefore, the OIG used the Tonacchera Model to conduct a cumulative risk assessment of this public health issue.

2.5 EPA Risk Assessment Procedures

2.5.1 EPA Risk Assessment Guidance

EPA risk assessment guidance provides procedures for combining the risk from multiple chemicals (i.e., a chemical mixture) sharing the same "mode of action." These risk assessment procedures are identified in the following EPA risk assessment guidance documents: the 1986 *Guidelines for the Health Risk Assessment of Chemical Mixtures* (EPA/630/R-98/002) and the 2000 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (EPA/630/R-00/002). EPA assumes the toxicity of individual chemicals in a chemical mixture add together (i.e., dose additivity) when the chemicals have the same mode of action and elicit the same effects. EPA's risk assessment guidance identifies the use of a dose-addition method for combining risk from components of a chemical mixture (e.g., multiple agents or stressors). EPA defines a chemical mixture as the concurrent or sequential exposure of two or more chemicals regardless of source, timing, or location of exposure (i.e., the chemicals do not have to

physically exist as a mixture outside the body or be consumed as a mixture to be a chemical mixture for environmental risk assessment purposes).

EPA's own risk assessment guidance instructs the agency to aggregate the risk from multiple chemicals or stressors sharing the same mode of action. The following sections of EPA's risk assessment guidance provide details on the dose-addition model for combining risk from multiple chemicals having the same mode of action using relative potency factors:

- The 1986 EPA *Guidelines for the Health Risk Assessment of Chemical Mixtures* recommends three approaches for the quantitative health risk assessment of a chemical mixture (e.g., multiple agents or stressors) (EPA 1986; EPA 2000, section 2.2.1). This guidance defines a chemical mixture as the concurrent or sequential exposure of two or more chemicals regardless of source, timing, or location of exposure (i.e., the chemicals do not have to physically exist as a mixture outside the body or be consumed as a mixture to be a chemical mixture for environmental risk assessment purposes) (EPA 1986, p 1). In section 4.1 of this 1986 EPA guidance document, the third recommended approach allows for a risk assessment of a chemical mixture by using a dose-addition model with potency adjustment if the chemicals act as dilutions or concentrations of each other (EPA 1986, p 16).

- In 2000, EPA supplemented this chemical mixture guidance by publishing the *Supplementary Guidance for Conducting Heath Risk Assessment of Chemical Mixtures* (EPA 2000). Section 2.6.1 of this supplementary guidance provides details on using the dose-addition method for combining the risk from multiple of chemicals having the same mode of action (EPA 2000).

2.5.1.1 Applying the Dose Addition Method

Since 1986, the EPA risk assessment procedures have allowed the amount of NIS inhibition from thiocyanate, nitrate, perchlorate, and the lack of iodide to be combined through dose addition by the use of relative potency factors for the purposes of conducting a human risk assessment. The biochemical properties and mechanistic interaction of the four NIS stressors meet the following EPA risk assessment requirements for the use of the dose-addition method:

- Same mechanism of toxicity (all four NIS stressors act on the NIS to limit the amount of iodide uptake by the thyroid)
- Similar dose-response[3] curves (e.g., same slopes, different x-axis intercepts)
- Act independently of one other; act as dilutions of one another
- Known relative potency factors for each NIS stressor
- Behave similarly in terms of the primary physiologic processes (i.e., uptake, metabolism, distribution, and elimination)
- Human exposures of each chemical are known (including at various life stages)

[3] A dose-response is the relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biologically significant changes in incidence and/or in degree of change (response).

Therefore, the OIG Analysis conducted a cumulative risk assessment of this public health issue using the dose-addition method.

By contrast, an alternative approach to conducting a risk assessment is the whole mixture approach, which risk assessors can accomplish by developing a statistical model on human epidemiological data. An example of this approach that has received a lot of attention is the Blount analysis and the subsequent Steinmaus analysis of the National Health and Nutrition Examination Survey (NHANES) 2001-2002 epidemiological study. We address the whole mixture approach for conducting a cumulative risk assessment in Appendix A.

2.5.2 EPA Development of Cumulative Risk Assessments

In 1997, the EPA Administrator directed the Agency "to take into account cumulative risk issues in scoping and planning major risk assessments and to consider a broader scope that integrates multiple sources, effects, pathways, stressors, and populations for cumulative risk analysis in all cases for which relevant data are available" and to "embrace this cumulative approach" in all major risks assessments (EPA 1997b). In May 2003, EPA published *Framework for Cumulative Risk Assessment* (EPA/600/P-02/001F), which establishes a framework to develop guidance on conducting and evaluating cumulative risk assessments and to provide basic concepts and principles to be addressed in a cumulative risk assessment. The document's primary purpose is to offer a simple, flexible structure for conducting and evaluating cumulative risk assessments within EPA. This framework is neither a procedural guide nor a regulatory requirement within EPA. EPA states it expects cumulative risk assessment to evolve with experience. The following section of the *Framework for Cumulative Risk Assessment* identifies that the risk from stressors with a common mode of action are assessed together and that the dose-addition method can be applied:

- In section 3.2.2.1, the EPA *Framework for Cumulative Risk Assessment* identifies that for situations of toxicological similarity, "the stressors are grouped according to the common mode of action . . . during the planning and scoping phase [of the risk assessment]." "For all effects caused by that mode of action, dose addition can be applied to the stressor group."

In May 2007, EPA issued four white papers on how cumulative assessment differs from traditional assessment and how EPA could assess cumulative risk (Callahan 2007; deFur 2007; Sexton 2007; Ryan 2007). The application of cumulative risk assessment has the potential to expand our understanding of the public health impacts of environmental exposures (Fox 2004) and provides a clearer, more complete picture of a public health issue for making risk management decisions (EPA 2003, appendix A). Cumulative risk assessment will reduce risks to the extent that it can be integrated into prevention strategies to track and protect public health (Fox 2004).

On June 27, 2008, EPA issued *Concepts, Methods, and Data Sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures, and Effects: A Resource Document* (EPA/600/R-06/013F), which identifies specific elements of and approaches for implementing cumulative risk assessments. This document demonstrates the feasibility of including

combinations of chemicals, exposures, effects, and their interactions into a cumulative risk assessment, and is meant to assist with the conduct of multichemical, population-focused assessments. However, this resource document specifically identifies in its preface that it is neither a regulatory document nor guidance (EPA 2008, p xvii). Although EPA's Office of Research and Development (ORD) has made progress in developing resource documents to conduct cumulative risk assessments, ORD has not developed Agency-wide guidance or regulations for conducting a cumulative risk assessment.

By contrast, as required by Section 405 of the Food Quality Protection Act of 1996 [P.L. 104-170], EPA's Office of Pesticide Programs (OPP) issued on January 14, 2002, *Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity*. This cumulative risk assessment guidance applies to pesticides and begins by identifying a group of pesticides that induce a common toxic effect by a common mechanism of toxicity. OPP had conducted four cumulative risk assessments on the following classes of pesticides sharing the same mechanism of toxicity: n-methyl carbamate, organophosphate, triazine, and chloroacetanilide.

2.6 Implementing a Cumulative Risk Assessment

Our approach to conducting a cumulative risk assessment was to apply the NAS Toxicity Testing Committee's strategy and vision to this public health issue. The *in vitro* modeling is required because as the complexity of the risk assessment increases (i.e., more factors evaluated), animal testing becomes neither practical nor sensitive enough to observe and define the relationship between an increasing number of stressors. For example, four stressors each administered at three different doses (i.e., high, medium, and low) would require 81 separate animal studies to evaluate the 34 possible combinations. To conduct 81 perchlorate animal studies is impractical. Furthermore, no scientific techniques are available to measure subtle cognitive deficits in rat offspring.

We used TIU as the basis for the cumulative risk assessment because the NAS Committee identified the TIU as the key biochemical event and recommended the use of TIU as the basis for a perchlorate risk assessment. The TIU is the combined effect of all four NIS stressors acting on the thyroid. We identified the Tonacchera Model as a mathematical model that defines the relationship between the four NIS stressors (thiocyanate, nitrate, perchlorate, and lack of iodide) and the TIU by the NIS. Therefore, the Tonacchera Model combines the risk from multiple chemicals into a single variable, the TIU, which measures the cumulative effect on the thyroid to the simultaneous exposure to all four NIS stressors. Thus, the Tonacchera Model utilizes both the NAS Toxicity Testing Committee's strategy and vision and EPA's dose-addition method to conduct a cumulative risk assessment of this public health issue.

The application of an *in vitro* model to assess environmental risk is unprecedented, but is required to address the complexity of multiple stressors affecting this public health issue. Instead of the conventional single chemical risk assessment approach of identifying an external dose that results in an adverse effect, our approach identified the maternal TIU that is associated with subtle mental deficits in a developing child. TIU is not an external dose of a single chemical, but the combined biological effect of all four NIS stressors acting on the thyroid. So

addressing this public health issue requires preventing the excessive exposure to any single NIS stressor to assure the maternal TIU does not reach a level that induces toxicity.

Section 3.1.3 of EPA's *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models* (November 2003) establishes that all environmental models should be corroborated before they are used as the basis of rule-making or regulation. We have corroborated the Tonacchera Model against several available human exposure studies. The OIG Analysis found the calculated TIU to be both a good predictor of an adverse outcome and a good explanation for the observed toxicity of the NIS stressors.

2.6.1 Aspects in Implementing a Cumulative Risk Assessment

Cumulative risk is defined as "the combined risks from aggregate exposure to multiple agents or stressors" (EPA 2003, p 6). The 1992 Expert Panel on the Role of Science at EPA points out that EPA has historically focused on chemical-specific impacts (EPA 1992, p 14). In 1994, NAS issued a recommendation to move away from single-chemical assessments (NAS 1994, executive summary p 13; EPA 2000, p x (first bullet)). In 1997, former EPA Administrator Browner wrote, "For most of our history, EPA has assessed risks and made environmental protection decisions based on individual contaminants . . . . with risk assessments for these chemicals often focused on one source, pathway, or adverse affect" (EPA 1997b). Likewise, in 1997, the U.S. EPA Science Policy Council stated that EPA's risk assessment should evolve away from a focus on the potential adverse effect of a single pollutant in one environmental medium (Callahan 2007). Unfortunately, both EPA's 2002 draft perchlorate risk characterization (EPA 2002a) and the NAS Committee on perchlorate (NAS 2005) focused solely on perchlorate and did not include in their assessments the quantitative impact of the other stressors that are known to affect the uptake of iodide by the thyroid. For this public health issue, perchlorate is not the only stressor that inhibits the uptake of iodide by the NIS. Thiocyanate and nitrate are also NIS inhibitors and human exposure to them is common, from both natural and manmade sources, and is unavoidable in the diet. A cumulative risk assessment of this public health issue must incorporate the inhibition from all three NIS inhibitors to produce a broader, more comprehensive understanding and assessment of the factors involved that lead to the unacceptable adverse effect of permanent, subtle mental deficits in children.

Another critical aspect of a cumulative risk assessment is that a stressor does not have to be the exposure to a chemical, but the "absence of a necessity" (EPA 2003, p 2). This concept is critical to this public health issue; the NIS uptakes iodide from the blood in order to make the iodide-containing thyroid hormones T_3 and T_4. An adequate supply of iodide in the human diet is essential for the production of iodide-containing thyroid hormones and their subsequent supply to the body. If the human diet lacks a sufficient supply of iodide, at specific level of iodide deficiency for a given NIS inhibitor load, the thyroid becomes unable to meet the body's minimum requirement for thyroid hormones. Therefore, the lack of iodide in the diet (i.e., the absence of a necessity) is also a stressor for this public health issue. Subsequent analysis will show the lack of iodide to be the dominant stressor in this public health issue. The iodide nutritional level must be quantitatively factored into the human health risk assessment. Unfortunately, both EPA's 2002 draft perchlorate risk characterization (EPA 2002a) and the NAS Committee on perchlorate (NAS 2005) did not quantitatively factor in iodide nutrition into

their reviews. By contrast, cumulative risk assessment allows for the lack of this necessity to be quantitatively factored into the human health risk assessment in order to fully characterize this public health issue. The relative contribution of the lack of iodide stressor on the uptake of iodide by the NIS is quantitatively defined in the Tonacchera Model (Tonacchera 2004). Furthermore, since the lack of iodide can be shown to be the dominate stressor effecting this public health issue, it is essential that the lack of iodide be quantitatively included in the human risk assessment.

The fundamental task in a cumulative risk assessment is to combine the toxicity of individual chemicals that share the same toxicity pathway. The toxicity of the individual chemicals sharing the same toxicity pathway can be combined additively by using relative potency factors (Callahan 2007; Sexton 2007). Since 1986, EPA guidance has allowed the amount of NIS inhibition from thiocyanate, nitrate, and perchlorate to be combined through dose addition by the use of relative potency factors for the purposes of conducting a human risk assessment. Under section 2.6.1 of the EPA *Supplementary Guidance for Conducting Heath Risk Assessment of Chemical Mixtures*, for chemicals having the same mode of action, similar dose-response curves, if the chemicals act as dilutions of each other and behave similarly in terms of the primary physiologic processes (i.e., uptake, metabolism, distribution, and elimination), and the exposures of each chemical are known, then the procedure suggested for estimating risk uses relative potency factors (EPA 2000, p 28-29). The relative potency factors approach scales the relative toxicity of each chemical (i.e., stressor) to the potency of an index chemical (i.e., typically the best-studied component of the mixture) (EPA 2000, p 29).

Both EPA's 2002 draft perchlorate risk characterization (EPA 2002a) and the NAS Committee on perchlorate (NAS 2005) did not utilize the dose-addition approach to human risk assessment, and did not quantitatively incorporate the other NIS inhibitors into their reviews. In EPA's perchlorate briefing to the NAS Committee on October 27, 2003, EPA stated that the Agency recognizes the "potential cumulative burden of toxicity" of the other NIS inhibitors, but describes comparisons of perchlorate toxicity to the other NIS inhibitors as "nonproductive" (Farland 2003). EPA is referring to the scientific community's comparison of perchlorate toxicity to the other NIS inhibitors: thiocyanate and nitrate. For example, an article in the *European Journal of Endocrinology* indicates that the daily perchlorate equivalent concentration (PEC) intake from the ingestion of thiocyanate and nitrate in the U.S. Department of Agriculture (USDA) recommended diet is 670 times higher than the amount allowed for by the perchlorate RfD (De Groef 2006). This paper also indicates that the concentration of nitrate allowed in drinking water would cause an amount of NIS inhibition that is 12 times greater than the perchlorate RfD. Likewise, Intertox, Inc. also compares the amount of inhibition from thiocyanate and nitrate in the USDA recommended diet against the perchlorate RfD (Bruce 2003, Bruce 2005). The OIG agrees with EPA that comparing perchlorate toxicity to the other NIS inhibitors individually is not a constructive approach to assessing or addressing this public health issue. We have this opinion because the human body is exposed to all three NIS inhibitors concurrently and the resulting effect of each on the uptake of iodide by the thyroid is cumulative and indistinguishable from the others, the perchlorate risk characterization must include exposure to all three NIS inhibitors. The perchlorate risk characterization performed by EPA and NAS is incomplete because the exposure from only a single NIS inhibitor was evaluated. The risk from

the cumulative inhibition effect of all three NIS inhibitors is best assessed through a cumulative risk assessment.

Another critical aspect of a cumulative risk assessment is to incorporate the risk from the background exposure to the stressors because the exclusion of a stressor(s) or the background exposure from a stressor(s) "may seriously distort" the portion of the total estimated risk attributed to the principal stressor of concern (EPA 2003, appendix C). Cumulative risk assessment distinguishes between background exposures and source specific exposures to provide a more complete picture of both total and source-related risks (EPA 2003, appendix C). For a perchlorate cumulative risk assessment, the background exposure levels and sources of each of the four stressors need to be determined and clearly distinguished from a specific source exposure. The Tonacchera Model identifies the lack of iodide in the diet as the dominant stressor. When the contribution of iodide is not quantitatively factored in to the risk assessment (i.e., as in a single chemical assessment done for perchlorate), the result is seriously distorted. Likewise, when the contribution of the other NIS inhibitors is not quantitatively factored in the perchlorate risk assessment, the result is seriously distorted. When concurrent exposure to all three NIS inhibitors is considered, perchlorate's contribution to the body's total inhibitor load is only a small portion of the body's total inhibition load. Knowing the various sources and the amount of contribution of each of the NIS inhibitors to the body's total inhibition load provides a clearer, more complete picture of this public health issue for making risk management decisions.

EPA's *Framework for Cumulative Risk Assessment* stated, "For most exposure situations, hazard and dose response studies of all of the joint effects from the multiple stressors will not be available, so that conclusions will have to be based at least partly on the single stressor information." For perchlorate, a risk characterization cannot be done in humans because adverse health effects have not been clearly demonstrated in any human population exposed to perchlorate. However, the *Framework for Cumulative Risk Assessment* identifies that the risk characterization of a cumulative risk assessment can be based on the adverse effects observed in one of the stressors and subsequently applied to the joint effects from all of the multiple stressors. Therefore, the risk characterization of the four NIS stressors should be done by using a NIS stressor in which adverse effects in children born to mothers with low maternal TIU during pregnancy and nursing have been documented and reported. The excessive maternal exposure to the NIS stressor, the lack of iodide, is the principal NIS stressor in which adverse effects have been documented and reported in children born to mothers with low maternal TIU during pregnancy and nursing. Thus, the OIG Analysis characterized the adverse effects from the lack of iodide stressor and used this information to conduct the initial three steps in a risk characterization (i.e., hazard characterization, dose-response assessment, and exposure assessment) of this public health issue. The exposure to the lack of iodide stressor was related back to the joint exposure level of all four NIS stressors by integrating the joint exposure into a single parameter or "common metric." The single parameter for measuring the NIS stress level is the TIU. The Tonacchera Model approach was to mathematically calculate the resulting TIU level from the concurrent joint exposure to all four NIS stressors.

The cumulative risk assessment "also includes aspects of cumulative risk that may be outside of EPA's current legislative mandates and where expertise outside of the Agency would be needed to address certain questions if they should arise" (EPA 2003, p xviii). EPA authority

extends to the regulation of environmental contaminates. However, according to the Tonacchera Model, the dominant stressor in this public health issue is the iodide nutrition level (i.e., the lack of iodide in the diet). Overseeing the iodide nutrition level is clearly not in EPA's legislative mandate, but addressing the needs of iodide-deficient pregnant women in the U.S. population is necessary to effectively address this public health issue. The NAS Committee was qualitatively aware of the crucial role of an adequate supply of iodide during pregnancy, so much so that the NAS Committee " . . . recommends that consideration be given to adding iodide to all prenatal vitamins" (NAS 2005, p 18). However, under the 1994 Dietary Supplement Health and Education Act, the FDA does not approve dietary supplements or have the legislative mandate to evaluate whether prenatal vitamins should contain iodide (FDA 2007b). Under the Act, the dietary supplement manufacturers are responsible for determining supplement's safety and the selection of ingredients for a dietary supplement (i.e., if iodide is to be added to prenatal vitamins). The medical community, not EPA, is responsible for any practice to identify and treat iodide deficiency in pregnant women with iodide containing prenatal vitamins or other treatment(s). Therefore, EPA's potential regulation of perchlorate exposure from the environment represents only a small portion of both the problem and solution to this public health issue. Furthermore, addressing this public health issue will require a diverse group of agencies, organizations, and disciplines to fully characterize and address the issue.

Another key aspect is that cumulative risk assessments can be qualitative as well as quantitative (EPA 2003, p 7 and section 3.3.4, p 58). This aspect is important because it allows for the incorporation of risk from other chemical exposures in which there is insufficient information to quantify accurately the risk, but scientists know the presence and nature of the risk. With respect to this public health issue, the uptake of iodide is not the only step in the production and use of thyroid hormones in the body that is disrupted by chemical exposures. Numerous inorganic and synthetic chemicals have been documented to interfere with almost every major step in the production, transport, and peripheral tissue metabolism of thyroid hormones (Howdeshell 2002, p 344, table 1). Furthermore, perchlorate, thiocyanate, and nitrate are not the only chemicals that disrupt the uptake of iodide by the thyroid. The following table provides examples of chemicals that interfere with several major thyroid hormone steps:

Thyroid Hormone Step	Examples of Chemicals that Interfere
Iodide uptake by the NIS	2,4-D, Aldrin, Lead, PBBs,
Iodide oxidation by thyroid peroxidase	Lindane, Malathion, Mancozeb
Circulatory transport in blood (e.g., binding to transthyretin)	Dioxtylphthalate, DDT metabolites, Dichloroprop, Difocol, Lindane, Malathion,
Cellular metabolism of T_4 to T_3 by Type I or Type II 5'-deiodinase in peripheral tissues	Cadmium, Lead, PCB, Dixoin
Increase cellular elimination by glucuronidation of T_4/T_3 by the stimulation of the glucuronidase enzyme	Acetochlor, DDT, PBBs, PCBs, Dioxin

Source: Howdeshell 2002 (for full list, see p 344, table 1).

Therefore, any risk assessment and resulting risk management decision(s) addressing this public health issue must also incorporate the nonquantified risk from the exposure to other thyroid hormone disrupting chemicals in order to be fully effective and protective of human health. Section 3.3.4 of EPA's *Framework for Cumulative Risk Assessment* introduces qualitative approaches to risk assessment (EPA 2003). Qualitative approaches may be the only

practical means to overcome the problems of complexity and data deficiencies and provide some insight into the nature and magnitude of cumulative risks (Callahan 2007).

In conclusion, the nature of this public health issue not only meets the requirements to be addressed by a cumulative risk assessment approach, but the complexity of the interaction among three NIS inhibitors and the iodide nutritional level requires the use of a cumulative risk assessment approach to accurately characterize the nature of the problem and to identify effective and cost-efficient solutions to the problem. Presidential Executive Order 12898, the intent of Congress under the Food Quality Protection Act of 1996, the 1997 direction given by former EPA Administrator Browner, and EPA's own risk assessment guidance directs EPA to consider cumulative risk analyses in all cases for which relevant data are available. The documented interaction of the three NIS inhibitors – thiocyanate, nitrate, and perchlorate – and the role of iodide nutrition in the uptake of iodide meet EPA's criteria for using cumulative risk assessment to address this public health issue. The three NIS inhibitors exceed EPA's mode of action requirement by sharing the same mechanism of toxicity (i.e., simple competitive interaction) by inhibiting the uptake of iodide at the NIS. The term "mechanism of activity" is a more detailed understanding and description of the events at the molecular level meant by "mode of action" (EPA 2000, p 10). As also required by EPA guidance for dose addition, the dose-response curves of thiocyanate, nitrate, and perchlorate have the exact same shape (Tonacchera 2004, figure 1). As also required by EPA guidance for dose addition, the three anions act as concentrations or dilutions of each other (Tonacchera 2004). The relative potency factors for each of the three NIS inhibitors are known (Tonacchera 2004). Human exposure data are available on each of the four stressors (i.e., the three NIS inhibitors and iodide nutrition). Since the EPA requirements for using the dose-addition method are met, there are no significant technical reasons not to use cumulative risk assessment to characterize the risk to the public. The only serious technical difficulty in implementing cumulative risk assessment is lack of experience and familiarity with cumulative risk assessment in the risk assessor community.

2.7 Summary of the OIG Approach

In the opinion of the OIG, the complexity of this public health issue requires the use of a cumulative risk assessment approach to better understand and characterize all the factors contributing to this public health issue and to identify an effective and cost-efficient solution to this problem. Based on the review of the EPA risk assessment guidance, the OIG could simply just recommend the Agency conduct a cumulative risk assessment on perchlorate. However, due to the novelty of the cumulative risk assessment approach and the required judgment in applying the science in a cumulative risk assessment, the OIG has undertaken the unusual step of providing an example of how a cumulative risk assessment could be conducted on perchlorate. This example will demonstrate the advantages of using the cumulative risk assessment approach and provide insight into potential cost-effective remedies to this public health issue. The use of an *in vitro* model to evaluate potential remedies of a public health issue is not unfounded; the NAS Toxicity Testing Committee stated, "In some risk contexts, a dose-response model based on *in vitro* results might provide adequate data to support a risk-management decision" (NAS 2007). The application of a cumulative risk assessment approach using the Tonacchera Model will allow the OIG to make recommendations on needed scientific research, comment on the

current regulatory approach to address the issue, and identify potential cost-effective remedies for consideration.

3. Sodium (Na+)/Iodide Symporter (NIS) Inhibitors

3.1 Background

Sufficient exposure to perchlorate can cause human toxicity by competitively inhibiting transport of iodide into the thyroid gland by NIS (NAS 2005, p 6). The NAS Committee identified the inhibition of iodide uptake by the thyroid in humans as the key biochemical event. The NAS Committee recommended using the inhibition of NIS as the point of departure (POD) for human risk assessment. The NAS Committee describes NIS inhibition as a nonadverse effect that precedes all adverse effects from perchlorate exposure. The NAS Committee-recommended RfD of 0.0007 mg/kg-day was established from a perchlorate exposure level that did not cause a decreased uptake of iodide in adults.

Since the NAS Committee recommended using NIS inhibition as the POD for human risk assessment and used the NIS inhibition to establish a perchlorate RfD, a full understanding and a working quantitative model of this key biochemical event is critical to effectively protect human health from the adverse effects from perchlorate exposure. The NIS is a trans-phospholipid membrane protein that actively transports both sodium and iodide ions (i.e., an ion pump) across the cell's plasmid membrane from the blood serum into the thyroid's follicular cells. The ion transport is in the same direction. The concentration of iodide ion from the blood serum into the thyroid's follicular cells is energetically unfavorable. Therefore, the energy from the co-transport of two sodium ions down sodium's electrochemical gradient allows an iodide ion to move against its electrochemical gradient and into the cell (Carrasco 2005).

Although NIS has a high affinity for the iodide ion, NIS is also known to transport a wide variety of monovalent anions with a similar ionic radii to iodide (De Groef 2006; Carrasco 2005). The following monovalent anions are known to be able to concentrate in the thyroid or to inhibit the NIS: pertechnetate (TcO_4^-), perchlorate (ClO_4^-), perrhenate (ReO_4^-), thiocyanate (SCN^-), boron tetrafluoride (BF_4^-), iodide (I^-), nitrate (NO_3^-), bromide (Br^-), chloride (Cl^-), chlorate (ClO_3^-), selenocyanate ($SeCN^-$), periodate (IO_4^-), and bromate (BrO_3^-) (Carrasco 2005; Wolff 1998; Dohan 2007). The relative NIS potencies of some of these anions are as follows (Wolff 1998):

$$TcO_4^- \geq ClO_4^- > Re0_4^- > SCN^- > BF_4^- > I^- > NO_3^- > Br^- > Cl^-$$

Notice that the iodide anion does not have the highest affinity for the NIS. Furthermore, both perchlorate and thiocyanate have a higher affinity for NIS than iodide.

3.2 Relative Potencies of Other NIS Inhibitors

Humans are exposed nutritionally and environmentally to other NIS inhibitors, in addition to perchlorate, which also competitively inhibit the uptake of iodide. The other principal NIS inhibitors of concern of human exposure are thiocyanate (SCN^-) and nitrate ($NO3^-$). As far back as 1953 (Wyngaarden 1953), the relative NIS inhibition potencies of prevalent nutritional and environmental NIS inhibitors (i.e., perchlorate, thiocyanate, and nitrate

perchlorate) have been studied and quantifed repeatedly with excellent agreement in the results. The following table provides a summary of these NIS inhibitor potency studies and their results:

Study	Study Description	Relative Potency* of NIS Inhibitor		
		Perchlorate	Thiocyanate	Nitrate
Tonacchera 2004	*In vivo* CHO** cells expressing the human NIS	1	15	240
Alexander 1966	*In vivo* 50% thyroid weight increase in rats	1	20	550
Alexander 1966	*In vitro* rat thyroid slices	1	20	400
Greer 1966	*In vitro* rat thyroids	1	20	240
Wyngaarden 1953	*In vivo* rat	1	10	300

* Relative Potency is based on molar concentrations of each anion.
** Chinese hamster ovary
Source: De Groef 2005.

3.3 Human Thiocyanate Exposure

Vegetables are a significant and natural source of thiocyanates (Tonacchera 2004). At least 2,500 taxa in the plant kingdom contain cyanogenic glycosides (CGs) (Vetter 2000). Plants use the CGs as a source to generate hydrogen cyanide, which serves as a feeding deterrent to grazing animals or insects. Upon human ingestion, the CG-containing plants release cyanide. The sulfurtransferase enzyme, rhodanese, metabolizes and detoxifies the released cyanide into thiocyanate (WHO 2004; Hasuike 2004). Therefore, the natural act of eating routinely exposes humans to the relatively potent NIS inhibitor thiocyanate. As a result, the human body always has a certain amount of unavoidable exposure to NIS inhibitors and this is referred to as the body's goitrogen load.

Foods particularly rich in thiocyanate include cabbage, broccoli, Brussels sprouts, corn (maize), turnips, rapeseed, and mustard seed. Furthermore, cauliflower, cabbage, radishes, spinach, and tomatoes contain on average 88, 86, 7, 5, and 2 mg/kg (wet weight) of thiocyanate, respectively. Milk contains 2-10 mg/L thiocyanate. In some parts of the world, the consumption of cassava is the major source of thiocyanate. Cassava can contain the equivalent of up to 3,400 mg/kg of thiocyanate (dry weight). Where cassava is not a significant part of the diet, cyanide produced and absorbed by cigarette smoking is the most significant source of thiocyanate.

Thiocyanate serum concentration in nonsmokers is typically in the range of 10-70 µmol/L. By contrast, the thiocyanate serum concentration of smokers is higher, typically in the range of 80-120 µmol/L.

Thiocyanate may have a regulatory function in normal physiology (Middlesworth 1986). Thiocyanate is continuously synthesized in the normal rat. In the absence of all dietary intake of thiocyanate in the rat, the thiocyanate serum concentration actually increased from 500 µg/dl to 800 µg/dl during fasting (Middlesworth 1986, figure 1). These data indicate that serum thiocyanate in rats is maintained in a normal range. These data also imply that humans also synthesize and maintain thiocyanate serum concentrations in a normal range. A "kidney threshold" is reported to exist in humans at a thiocyanate serum threshold of 200-300 µmol/L (Tonnacchera 2004).

By comparison, the EPA perchlorate RfD of 0.0007 mg/kg-day results in a perchlorate serum concentration of 0.014 µmol/L. By contrast, the daily thiocyanate exposure in nonsmokers results in a thiocyanate serum concentration in the range of 10-70 µmol/L. Therefore, thiocyanate exposure is a significant component of the body's goitrogen load.

3.4 Human Nitrate Exposure

Nitrate is also common in food (Tonacchera 2004). Nitrate occurs in green leafy vegetables. The consumption of vegetables is the primary source of nitrate exposure (NAS 1995, p 35). In 1981, the National Research Council (NRC) estimated that 97% of the daily nitrate intake is from diet (99% for vegetarians) (NAS 1995, p 40, table 4-2). However, nitrate is also added to processed meats as a preservative. Furthermore, both surface and ground sources of drinking water commonly contain nitrate due to the agricultural use of inorganic fertilizers, animal manure, and airborne emissions from utilities, factories, and automobiles. In areas of intensive agriculture, fertilizer usually is the predominant source of nitrogen. Animal manure and atmospheric deposition account for smaller amounts of nitrogen contributions nationally than commercial fertilizer, but are significant secondary sources of nitrogen in certain regions.

In vegetables, the highest nitrate concentrations are found in celery, spinach, lettuce, beets, radishes, melon, turnip greens, and rhubarb (over 1000 mg/kg of vegetable), (NAS 1995, p 35). Dairy products contain low concentrations of nitrate and rarely exceed 5 mg/kg in milk (NAS 1995, p 36). The following table provides a list of common foods that contain nitrate:

Common Foods Containing Nitrates		
Milk	Kimchi	Carrots
Bacon	Garlic	Onion
Sausage	Artichoke	Green beans
Pepperoni	Peas	Melon
Beef	Corn	Turnip
Ham	Sweet potatoes	Sweet pepper
Broccoli	Lima beans	Squash
Celery	Cucumber	Cabbage
Lettuce	Tomatoes	Leek
Radish	Parsley	Cauliflower
Spinach	Brussels sprouts	Pumpkin
Beets	White potatoes	Endive
Rhubarb	Eggplant	Kale
Turnip greens		

Source: Bruce 2004.

Human exposure to nitrate is both exogenous and endogenous. Exogenous human nitrate exposure is from diet through the consumption of primarily vegetables. The endogenous human nitrate exposure is from the body's production of nitric oxide, which is subsequently converted into nitrate by various types of cells in the body (NAS 1995, p 37). Therefore, nitrate excretion in urine exceeds the nitrate intake from food and water. Thus, biomonitoring of nitrate in human urine cannot be directly used to assess exogenous nitrate exposure.

The amount of human nitrate exposure has been assessed and estimated several times. In 1981, the NRC estimated nitrate intake from food at 40-100 mg/day for males (NAS 1995, p 38) and that nitrate intake from contaminated water could contribute 22-44 mg/L (NAS 1995, p 38). In 1998, European investigators report an average dietary nitrate intake of 43-131 mg/day (NAS 1995, p 39). In 1989, Van den Brandt estimated the exogenous nitrate intake at 113 mg/day for males and 184 mg/day for females (NAS 1995, p 38). In 1992, Jones estimated exogenous nitrate intake in the United States for omnivores at 76 mg/day and about 260 mg/day for vegetarians (NAS 1995, p 38). Jones estimated the endogenous production of nitrate to be 62 mg/day (NAS 1995, p 38), which contributes about 45% of a human's total nitrate exposure (NAS 1995, p 38). Jones estimated the total exogenous and endogenous nitrate exposure to be 138 mg/day (NAS 1995, p 38).

In studies from Denmark and the United Kingdom, the average daily nitrate intake is estimated to be about 40-50 mg/day for adults (Tonacchera 2004). This nitrate intake value of 40-50 mg/day is on the low end of the nitrate intake estimates reported above. Fortunately, these data also include the corresponding internal nitrate serum concentrations, which allows for the subsequent calculation of both the amount of NIS inhibition acting on the thyroid from nitrate exposure and the relative TIU by the thyroid under various NIS stressor levels. In the Western world, the typical nitrate serum concentration ranges from 10-140 µmol/L with the mean nitrate serum concentration being between 30-50 µmol/L (Tonacchera 2004). The serum half-life of nitrate is 5-8 hours (Tonacchera 2004).

By comparison, the EPA RfD for perchlorate is 0.0007 mg/kg-day, which corresponds to a daily perchlorate exposure limit of 0.049 mg/day for a 70-kg adult. The daily nitrate intake of 40-50 mg is substantially more (i.e., 1000 times greater on a weight basis) than both the daily perchlorate exposure limit calculated from the EPA RfD of 0.049 mg/day for a 70 kg adult and the .0046 mg/day median U.S. exposure to perchlorate estimated by the Centers for Disease Control and Prevention (CDC) biomonitoring data (Blount 2006). Therefore, nitrate exposure is also a significant component of the human body's goitrogen load.

3.5 Human Perchlorate Exposure

The U.S. population is ubiquitously exposed to low levels of perchlorate. In 2006, Blount of the CDC, National Center for Environmental Health (NCEH), assessed the perchlorate exposure in the U.S. population using urinary biomonitoring data (Blount 2006a). As part of the 2001-2002 NHANES, a nationally representative population of 2,820 U.S. residents (ages 6 years and older) provided urine samples for perchlorate testing. All 2,820 urine samples tested found a detectable level of perchlorate (i.e., >0.05µg/L), indicating that perchlorate exposure in the U.S. population is common. The Blount biomonitoring data indicated median adult perchlorate exposure was 4.6 µg/day, and the 95th percentile exposure was calculated to be 16.4 µg/day in an adult. Furthermore, the FDA TDS estimated lower- and upper-bound average perchlorate intakes (i.e., range of dietary perchlorate intake) for 2005-2006 from 5.4 to 6.8 µg/day from food in 25- to 30-year-old women (Murray 2008, table 5). Therefore, both the CDC biomonitoring and the FDA TDS datasets indicate a low-level background exposure to perchlorate across the entire U.S. population.

Potential sources of perchlorate exposure are through the Department of Defense/aerospace sector (e.g., rocket propellant), commercial products (i.e., safety flares and some fireworks (up to 70% content)), agricultural use (i.e., perchlorate is a contaminant in CNF), and natural atmospheric production (Dasgupta 2006). The Department of Defense/aerospace sector use accounts for about 90% of the U.S. annual consumption of perchlorate (Dasgupta 2006). However, the burning of rocket propellants, safety flares, and perchlorate-containing fireworks does not result in a significant perchlorate exposure; 0.05% or less of the original perchlorate is left after use because the fire destroys the perchlorate (Dasgupta 2006). By contrast, the improper disposal of unused rocket propellants, safety flares, and perchlorate-containing fireworks, which allows the unused perchlorate to get into the groundwater and surface water, can result in human perchlorate exposure upon ingestion of contaminated drinking water. Although this type of improper disposal into water sources would generate "hot spots" of perchlorate exposure within the population, the improper disposal into water sources would not generate a uniform background of perchlorate exposure across the entire U.S. population, because EPA data indicate only 3.16% of the 3,858 Public Water Systems tested in the Unregulated Contaminant Monitoring Rule (UCMR) had at least one detection of perchlorate greater than or equal to 5 µg/L (EPA 2007, table 5). Natural production of perchlorate by the oxidation of chloride by lightning or ozone is not a significant source of drinking water contamination because rainfall contains a mean concentration of perchlorate of 0.015 µg/L (max. 0.2 µg/L) (Dasgupta 2006).

A potential route of perchlorate exposure that is consistent with generating a uniform low background exposure level in the entire U.S. population is the contamination of the U.S. food supply with perchlorate. The FDA TDS identifies that vegetables and dairy foods combined account for between 46% and 59% of the total estimated intake of perchlorate by teenagers and adults, respectively (Murray 2008). A potential explanation for the widespread perchlorate contamination of vegetables and dairy foods is the agricultural use of perchlorate-contaminated CNF from 1909 to at least 1993. Over the life of CNF use in the United States, the average perchlorate content of CNF is estimated to be 0.2% (Dasgupta 2006). The agricultural use of CNF is estimated to have resulted in the environmental release of 1.6 million pounds of perchlorate per year into U.S. agricultural fields for at least a 63-year period from 1930 to 1993 (Dasgupta 2006). In short, there are few ways to introduce a chemical into the food chain that are more efficient than using fertilizer as a vector (Dasgupta 2006).

4. Modeling the NIS Stressors' Influence on the Total Iodide Uptake

4.1 Determining the Total NIS Inhibition Load Acting on the Human Body

The total NIS inhibition load (total goitrogen load) acting on the human body is the combined effect of all four common NIS inhibitors (i.e., thiocyanate, nitrate, perchlorate, and iodide). The Tonacchera study investigated the joint effects on the NIS from simultaneous *in vitro* exposure to these four NIS inhibitors (Tonacchera 2004). This study identified the interaction between the NIS inhibitors as being simple competitive interaction and observed no evidence of synergism or antagonism among the NIS inhibitors. These data indicate that these NIS inhibitors interact in a simple additive fashion. In other words, the *in vitro* effect on the NIS for each NIS inhibitor was indistinguishable from that of the others once adjusted for differences in concentration and potencies. Therefore, the total amount of *in vitro* NIS inhibition acting on the NIS is the combined contribution of each anion on a molar potency adjusted basis (i.e., the dose-addition method). Thus, the total inhibition acting on the NIS can be expressed as single PEC representing the same amount of NIS inhibition induced by the joint effect of all four NIS inhibitors.

The total amount of NIS inhibition (total goitrogen load) acting on the NIS *in vitro* is measured as a PEC and is defined in the Tonacchera study by the following equation:

PEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of SCN^- Inhibition) + (Amt of I^- Inhibition)
PEC = $[ClO_4^-] + [SCN^-] / 15 + [NO_3^-] / 240 + [I^-] / 30$

However, the PEC equation needs to be adjusted into two mathematical forms to represent *in vivo* conditions when the amount of total NIS inhibition is being measured in the blood serum (i.e., serum perchlorate equivalent concentration (SPEC)) or measured from the weight of ingested NIS inhibitors consumed in the diet or through contaminated drinking water (i.e., oral perchlorate equivalent concentration (OPEC)).

4.2 Serum Perchlorate Equivalent Concentration (SPEC)

The body's total NIS inhibition is measured by the following SPEC equation (concept adapted* from Tonacchera 2004):

SPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of free SCN^- Inhibition)
SPEC = [Perchlorate] + [Nitrate] /240 + ([total serum SCN^-] x 0.5) /15
SPEC = [Perchlorate] + [Nitrate] /240 + [free SCN^-] /15

Where: [ ] is molarity
Serum inhibition potency of perchlorate = 1
Serum inhibition potency of nitrate relative to perchlorate = 1 / 240
Serum inhibition potency of free thiocyanate relative to perchlorate = 1 / 15
[free SCN^-]* = [total serum SCN^-] x 0.5 [free SCN^- /total serum SCN^-]

* Note: Tonacchera's PEC equation is derived from *in vitro* results and had to be adjusted to reflect *in vivo* conditions for the binding of thiocyanate to albumin in the blood stream (see discussion below).

The SPEC equation relates the total NIS inhibition to the molarity of the each of the NIS inhibitors in the blood serum (i.e., the concentration of each NIS inhibitor in contact with the NIS protein). However, about half the thiocyanate in blood is bound to albumin (Stoa 1957). Therefore, only about half of the thiocyanate in human blood serum is free to interact and to inhibit the NIS protein on the thyroid. The experimental protocol used in Tonacchera's study (from which each of the anion's potency was determined) incubated the CHO cells expressing human NIS in Hank's balanced salt solution, which contains 0.5% bovine serum albumin (Tonacchera 2004). By contrast, human plasma contains 4.8% albumin, by weight (Marieb 1998). This textbook amount of albumin in blood plasma is consistent with the 4.4% and 4.6% found from direct measurements in nonsmoking and smoking controls, respectively (Hasuike 2004, table 1). Therefore, the *in vitro* conditions in Tonacchera's experimental protocol had only about 1/10th the amount of albumin found in blood plasma.

The SPEC equation defined in this document is adapted from Tonacchera's PEC equation by taking into account the *in vivo* conditions of having about 48 g/L of albumin in human blood serum that affect the amount of free thiocyanate. In five human test subjects having a total SCN^- serum concentration ranging from 1.96 mg/L to 2.55 mg/L (i.e., the range is equivalent from 33.7 µmol/L to 43.9 µmol/L, which is in the typical SCN^- range identified by Tonacchera), 47.2 ± 3.4 % of thiocyanate is bound to albumin (Stoa 1957, table 15). Or stated as free thiocyanate, 52.8% of the total thiocyanate in the blood serum is free and unbound to albumin. In rabbit plasma at a higher SCN^- concentration of about 141 & 280 µmol SCN^-/L, 41.3% and 40.7% of thiocyanate is bound to the rabbit albumin, respectively (58.7 % and 59.3 % SCN^- is free) (Pollay 1966, table 1). The amount of free SCN^- increases with increasing SCN^- concentration in the blood. Since SCN^- in blood serum is typically 40 µmol/L, the amount of free SCN^- from Stoa's human data is used. Therefore, the amount of free SCN^- at typical human SCN^- concentrations can be determined by multiplying the total SCN^- concentration by a factor of 0.528. As the amount of SCN^- increases, the amount of free SCN^- also increases, but without having specific data for percent binding of SCN^- to human albumin at different concentrations, the use of 0.5 in the SPEC equation represents a conservative estimate. Therefore, the thiocyanate term is adjusted by multiplying the total serum SCN^- by 0.5 to derive the amount of available free thiocyanate in the blood able to interact and inhibit the NIS.

Our analysis drops the amount of iodide inhibition term (i.e., $[I^-]/30$] in the original Tonacchera PEC equation in order to simply the mathematical expression for use in a cumulative risk assessment. Eliminating a minor term in a polynomial equation is standard mathematical technique used to simplify a problem. Eliminating the $[I^-]/30$ term in the PEC equation would not significantly alter the SPEC or OPEC-calculated values. The amount of NIS inhibition from iodide is a relatively small percentage of the OPEC value (i.e., about 0.1% of the OPEC of a typical adult exposure level). The typical adult consumes about 150 µg iodide/day. The oral inhibition potency of iodide is 1/30. So, a normal adult intake of 150 µg iodide/day represents the same amount of NIS inhibition as 5 µg of perchlorate (i.e., 1/10th the perchlorate RfD of 49 µg/day). Since the perchlorate RfD has been calculated to represent about 1% of the body's total

goitrogen load, the iodide inhibition at 150 µg/day is about 0.1% of the body's total goitrogen load. Furthermore, the lack of iodide is an NIS stressor, which acts in the opposite to its inhibition. As the iodide concentration decreases, resulting in more stress on the NIS, the amount of NIS inhibition from iodide becomes even less significant (i.e., a low intake of iodide at 50 µg/day causes the same amount of NIS inhibition as 1.7 µg of perchlorate (i.e., about 0.03% of the body's total goitrogen load). Conversely, when the iodide concentration increases, the TIU value increases faster than the decrease in the TIU value from the amount of NIS inhibition induced by iodide itself. Therefore, elimination of the iodide term from the Tonacchera PEC equation represents a negligible bias in the calculation of the amount of total NIS inhibition acting on the body.

The SPEC equation can be used when the serum molarities of each NIS inhibitor is measured and reported. However, serum molarity data for NIS inhibitors is not commonly measured or reported in human exposure studies. The oral exposure (i.e., dose) to NIS inhibitors is more commonly determined and measured as the weight consumed through ingestion.

4.3 Oral Perchlorate Equivalent Concentration (OPEC)

The total NIS inhibitory effect measured upon ingestion of the NIS inhibitors is given by the following OPEC equation (concept adapted from Tonacchera 2004; De Groef 2006, p 155):

OPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of SCN^- Inhibition)
OPEC = grams perchlorate + grams nitrate/150 + 0.5 free SCN^- x grams thiocyanate/8.8
OPEC = grams perchlorate + grams nitrate/150 + grams thiocyanate/17.6

Where:
Oral inhibition potency of perchlorate = 1
Oral inhibition potency of nitrate relative to perchlorate = 1/150
Oral inhibition potency of thiocyanate relative to perchlorate = 0.5/8.8 = 1/17.6

The above OPEC equation adjusts the relative NIS inhibitor potencies for the following three factors:

1. The potency is adjusted to reflect the relationship based on the weights of each NIS inhibitor ingested instead of the molarity of each NIS inhibitor in the serum.

2. The potency is also adjusted to take into account the different pharmacokinetic properties of each of the NIS inhibitors upon ingestion (i.e., the different absorption and excretions characteristics of each NIS inhibitor).

3. The amount of SCN^- binding to albumin in human blood serum.

The pharmacokinetic differences are critical in determining the relative NIS inhibition potencies of each anion upon ingestion. The ingested absorption and serum half-lives of each anion have to be taken into account. Only about 50% of thiocyanate is absorbed in the gastrointestinal tract upon ingestion. Meanwhile, 70-90% of ingested perchlorate and 90-100% of ingested nitrate is absorbed in the gastrointestinal tract. Furthermore, the half-lives of

perchlorate, thiocyanate, and nitrate are 8 hours, 6 days, and 5 hours, respectively. Thiocyanate stays in the serum 18-29 times longer than the other anions. For example, only half of the ingested amount of thiocyanate is absorbed upon ingestion, but the amount of thiocyanate absorbed stays in the blood serum much longer, to act on and inhibit the NIS. Furthermore, only about 0.5 of the total SCN- in human blood serum at typical SCN^- concentrations is free to interact with the NIS protein. Therefore, the NIS inhibition potency of ingested thiocyanate is about 6% (i.e., 0.5 ÷ 8.8 x 100%) – the NIS inhibition potency of ingested perchlorate. The essential observation from the pharmacokinetics is that ingested thiocyanate has about one-quarter the potency as ingested perchlorate, but thiocyanate is consumed in mg quantities in the diet while perchlorate is only consumed in µg quantities (i.e., median is 4.6 µg) in the diet. From this preliminary information, thiocyanate appears to contribute the largest amount of NIS inhibition in the body. A more detailed analysis of the relative contribution of each NIS inhibitor is provided in Section 7.2 of this document.

4.4 Tonacchera NIS Model of Competitive Inhibition

The Tonacchera *in vitro* NIS Model of Competitive Inhibition calculates the TIU by the NIS from the concentration of the following four anions: iodide, perchlorate, nitrate, and thiocyanate. The Tonacchera Model is given by the following equation (concept adapted* from Tonacchera 2004):

$$TIU = \text{constant} \times [I^-] / (1.22 + (SPEC))$$

Since the constant is not provided, the TIU can be restated as:

$$TIU \text{ is proportional to } [I^-] / (1.22 + ([\text{Perchlorate}] + [\text{Nitrate}]/240 + [\text{free } SCN^-]/15)$$

where:
The [ ] of each anion is express in µmol/L units

The Tonacchera Model is particularly useful because it models the key biological event in the toxicity of perchlorate – the uptake of iodide by the thyroid by the NIS. The model predicts the uptake of iodide to the simultaneous exposure to all four variables (i.e., iodide, perchlorate, thiocyanate, and nitrate) affecting the uptake of iodide. The ability to predict the amount of uptake of iodide knowing the body's total goitrogen load and the body's iodide nutritional status is a powerful tool for assessing the risk from perchlorate exposure.

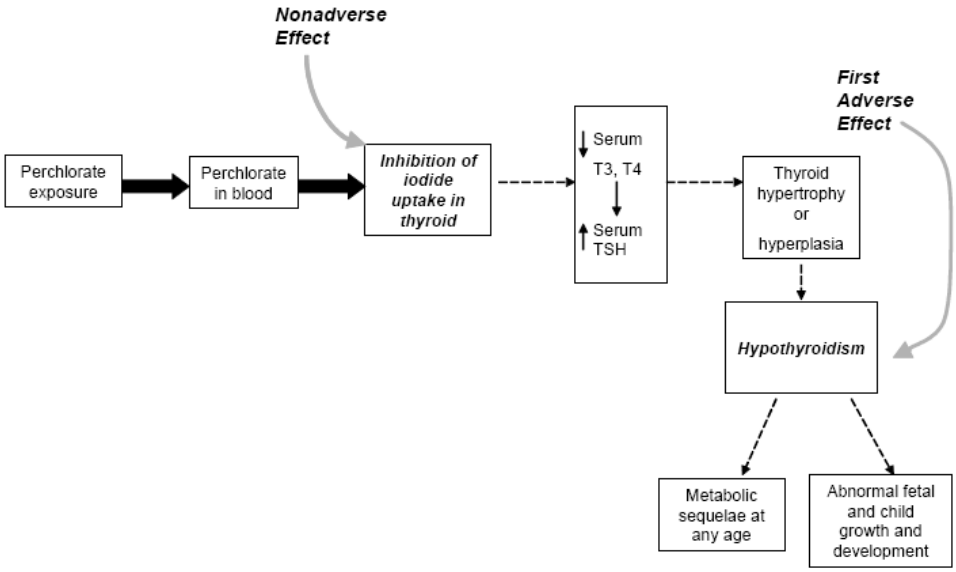
The NAS Committee recommends that the key biochemical event (i.e., the inhibition of iodide uptake) should be used as the basis of the risk assessment (NAS 2005, p 14, 166, 169). Therefore, the TIU is key the biochemical event that is perturbed by the NIS stresssors and should be used as the basis for the risk assessment of this public health issue. The mode of action identifies that a low TIU during pregnancy and nursing increases the risk of subtle mental deficits in the children. However, the cumulative effect of all four NIS stressors on the thyroid (i.e, not just perchlorate exposure) determines the TIU level during the critical time period of pregnancy and nursing. In other words, the cumulative effect of the four NIS stressors argues against perchlorate exposure alone as being directly associated with an adverse outcome. By

contrast, a low TIU during pregnancy and nursing is associated with an adverse outcome. Therefore, the Tonacchera Model allows the TIU to be calculated from the concurrent exposure from all four NIS stressors. Furthermore, the Tonacchera Model allows specific adverse effects to be associated with specific calculated TIU level.

5. Deriving a Reference Dose (RfD)

The NAS Committee emphasized that its approach for deriving a perchlorate RfD differs from the traditional single chemical risk assessment approach (NAS 2005, p 15). A traditional single chemical risk assessment derives an RfD from an adverse effect such as a no-observed-adverse-effect-level (NOAEL) or a lowest-observed-adverse-effect-level (LOAEL). By contrast, the NAS Committee used the unconventional approach of using a nonadverse effect to derive its recommended perchlorate RfD. The nonadverse effect was the inhibition of iodide uptake by the NIS, which is labeled a no-observed-effect-level (NOEL). The NAS Committee used the NOEL identified in the Greer study (Greer 2002) and set the perchlorate exposure level of 0.007 mg/kg-day as the POD. The NAS Committee describes its approach of using a NOEL to derive the RfD as conservative and health protective (NAS 2005, p 15).

The NAS Committee also proposed the following mode-of-action model for perchlorate toxicity in humans (see below) (NAS 2005, p 167, figure 5-2). The NAS Committee identified hypothyroidism as the first adverse effect in the continuum of biological effects. A traditional single chemical risk assessment would use the perchlorate dose that just induces hypothyroidism (i.e., a LOAEL), but the NAS Committee states that hypothyroidism should not be used as the basis of a perchlorate risk assessment (NAS 2005, p 166). Overt hypothyroidism is characterized by an elevated TSH level and a below normal T_4 level. Furthermore, subclinical hypothyroidism is characterized by an elevated TSH level and normal T_4 level. Therefore, NAS Committee's mode-of-action model has identified that transient changes of T_4, T_3, and TSH levels within a normal concentration range are nonadverse health effects.



Source: NAS 2005, p 167, figure 5-2.

The final step in deriving an RfD is the application of UFs to the POD. The application of UFs attempts to account for both uncertainty and the variability in the available data (EPA 2002, p 4-38, section 4.4.5). The seven UFs that can be potentially applied to a POD depending in the nature of the dataset are: interspecies UF, intraspecies UF, LOAEL to NOAEL UF,

database UF, subchronic-to-chronic-duration UF, modifying factor, and the Food Quality Protection Act. The Act allows a 10x safety factor to protect infants and children from pesticide residues. The value of each UF depends on the quality of the studies available, the extent of the database, and scientific judgment (EPA 2002, p 4-40, section 4.4.5.1). The NAS Committee wrote, "No absolute rules exist for application of the [uncertainty] factors, and professional judgment is a large component of their use." (NAS 2005, p 29). Each one of these UF can have a value of 1, 3, or 10. If multiple UFs greater than 1 are used, then the UF values are multiplied together (e.g., 10 x 10 x 3 = 300). Since there is overlap in the coverage of uncertainty in the UF, the upper limit of total UF applied to a POD should be no more than 3000 (EPA 2002, p 4-41, section 4.4.5.1).

The NAS Committee used a POD (i.e., labeled as a NOEL) of 0.007 mg/kg-day identified in the Greer study (Greer 2002) and applied a total UF of 10 (i.e., 10x intraspecies UF) to derive the NAS Committee's recommended perchlorate RfD of 0.0007 mg/kg-day as the POD. By contrast, EPA used a POD (i.e., labeled as a LOAEL) of 0.01 mg/kg-day from the Argus rat study (Argus 2001) and applied a total UF of 300 (i.e., 3x intraspecies UF, 10x LOAEL to NOAEL UF, 3x duration UF, and 3x database insufficiency, and a 1x interspecies UF (equivalent of omitting the UF)) to derive the EPA draft perchlorate RfD of 0.00003 mg/kg-day (EPA 2002a, p 7-20–7-23). The following table summaries the differences between how the NAS Committee and EPA derived a perchlorate RfD:

Perchlorate Assessment	Biological Effect Used as the POD	Critical Study and Type of POD	POD Value (mg/kg-day)	Total UF	Proposed RFD* (mg/kg-day)
NAS 2005	Inhibition of iodide uptake by the NIS	Greer 2002 Human Study NOEL (nonadverse effect)	0.007	10	0.0007
EPA 2002	Brain Morphometry, Thyroid Histopathology, Thyroid hormone changes	Argus 2001 Rat Study LOAEL (adverse effect)	0.01	300	0.00003

* Proposed RfD = POD value ÷ Total UF

The scientific disagreement on the manner in which to derive the perchlorate RfD centers around the following issues:

- Should animal data or human data be used to determine the POD (NAS 2005, 14; EPA 2002a)?

- Is the inhibition of iodide uptake an adverse or nonadverse effect (Ginsberg 2005; EPA 2002a, p 7-26, section 7-26)?

- Which UFs should be applied? And if the UF is applied, at what magnitude (i.e., 1, 3, or 10) (Ginsberg 2005)?

Depending on the manner in which each of these scientific concerns is addressed, the total UF applied to the POD will be different. Therefore, in a traditional single chemical risk assessment, the numerical value of the RfD depends significantly on the total UF applied to the

POD. The UFs applied and their size is a matter of scientific judgment in order to account for uncertainty and variability in the data.

5.1 Issues with the Single Chemical Risk Assessment of Perchlorate

A review of every scientific aspect in the perchlorate single chemical risk assessment is beyond the capability, resources, and mission of the OIG. The OIG has identified that due to the interaction of multiple factors, a cumulative risk assessment approach is needed to characterize and address the risk in this public health issue. However, the OIG review specifically comments on the following issues relating to a single chemical risk assessment of perchlorate because the comments provide insight into the science of perchlorate:

- Is the NAS Committee's unconventional approach of using a NOEL to derive its recommended perchlorate RfD conservative and health protective? And, if so, quantify how conservative the NAS recommend perchlorate RfD is as compared to using the traditional single chemical risk assessment approach to deriving an RfD?

- Evaluate whether hypothyroidism is the first adverse effect in the continuum of biological effects from perchlorate exposure.

- Evaluate the application of UFs to deriving a perchlorate RfD in a single chemical risk assessment.

5.1.1 Defining an RfD

To evaluate the NAS Committee's unconventional approach of deriving an RfD, the conventional approach of driving an RfD and a strict regulatory definition of an RfD must be defined. Since an RfD is part of a risk assessment, the term risk assessment is defined:

> **Risk Assessment (in the context of human health):** The evaluation of scientific information on the hazardous properties of environmental agents (hazard characterization), the dose-response relationship (dose-response assessment), and the extent of human exposure to those agents (exposure assessment). The product of the risk assessment is a statement regarding the probability that populations or individuals so exposed will be harmed and to what degree (risk characterization) (EPA 2002; IRIS 2007).

As such, a risk assessment is comprised of four parts: hazard characterization, dose-response assessment, exposure assessment, and risk characterization. The first portion of a risk assessment is to determine the hazardous properties of the environmental agent (i.e., perchlorate) in a hazard characterization, which is defined:

> **Hazard Characterization:** A description of the potential adverse health effects attributable to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the associated dose, route, duration, and timing of exposure (EPA 2002; IRIS 2007).

The hazard characterization identifies the potential adverse health effects in humans resulting from a specific agent. For the health characterization of perchlorate, the NAS Committee states that the first adverse health effect in humans is hypothyroidism, which precedes any potential for mental deficits in offspring.

For a single chemical risk assessment, the hazard characterization of only a specific agent is a critical limitation that can fatally flaw the risk assessment. Multiple agents exerting their toxic effects through the same mechanism are not considered together. This limitation can be without consequence to the single chemical risk assessment if the single chemical is the dominant factor causing the adverse effect. However, if the single chemical being studied is only a minor factor among other stronger factors, this limitation in a single chemical risk assessment can seriously distort the risk characterization. The OIG's use of cumulative risk assessment will demonstrate the latter to be the situation with perchlorate.

The second part of a single chemical risk assessment is the dose-response assessment, which is defined:

> **Dose-Response Assessment:** A determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence or change in a level of response, percent response in groups of subjects (or populations), or the probability of occurrence or change in level of response within a population (EPA 2002).

A dose-response assessment identifies that dose that causes an increased occurrence of a particular biological event in the test subjects. For perchlorate, the NAS Committee identified that a dose of 0.007 mg/kg-day of perchlorate does not statistically inhibit the uptake of iodide by the NIS in adult test subjects as compared to controls subjects.

To conduct the third portion of a risk assessment (i.e., the exposure assessment), the RfD needs to be calculated from the dose-response relationship identified in the dose-response assessment. RfD is defined:

> **Reference dose (RfD):** An estimate of a daily oral exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a benchmark dose lower confidence limit (BMDL), a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used (EPA 2002; IRIS 2007).

Notice that the definition of an RfD is based on avoiding adverse health effects and is derived from a dose associated with an adverse effect (i.e., BMDL, NOAEL, LOAEL, or POD). The definition of BMDL, NOAEL, LOAEL, and POD are defined below and all specifically include the use of adverse effect:

> **BMDL:** A statistical lower confidence limit on the dose or concentration at the benchmark dose (BMD) or benchmark concentration (BMC) respectively (EPA 2002).

> BMD or BMC: A dose or concentration that produces a predetermined change in response of an adverse effect . . . compared to the background (EPA 2002).
>
> **NOAEL:** The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control . . . (EPA 2002; IRIS 2007).
>
> **LOAEL:** The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group (EPA 2002; IRIS 2007).
>
> **POD:** The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower-bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response (EPA 2002; IRIS 2007).

Therefore, an RfD is defined by the adverse effect from which it is derived. The RfD is a dose that is typically one to three orders of magnitude (i.e., 10 to 1000 times) below the NOAEL or LOAEL from with it was derived by the use of uncertainty/variability factors applied to reflect limitations of the data used. Thus, the RfD is set at a dose that is below an exposure that is likely to cause the observed adverse health effects in even the most sensitive group.

The exception to the use of an adverse effect to derive an RfD is that the risk assessment guidance allows the use of the immediate precursor to the adverse effect. This is seen in the definition of a critical effect:

> **Critical Effect:** The first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases (EPA 2002; IRIS 2007).

In regard to perchlorate, since the NAS Committee identified hypothyroidism as the first adverse effect, its immediate precursor in the NAS mode-of-action model (NAS 2005, p 167, figure 5-2) is listed as thyroid hypertrophy or hyperplasia, but hypertrophy and hyperplasia are not typically measured in epidemiological studies. Since overt hypothyroidism is characterized by an elevated TSH level and a below normal T_4 level, and subclinical hypothyroidism is characterized by an elevated TSH level and normal T_4 level, the immediate precursor to hypothyroidism could be considered a perchlorate exposure that induces a statistically significant change in thyroid hormones levels in the exposed group as compared to the control group.

However, the NAS Committee did not derive its RfD from either hypertrophy, hyperplasia, or thyroid hormone changes. The NAS Committee derived its perchlorate RfD from the NOEL observed from the inhibition of iodide uptake by the NIS in the Greer study. A NOEL is defined as:

> **NOEL:** An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control (EPA 2002; IRIS 2007).

In the NAS Committee's mode-of-action mode, the inhibition of iodide uptake by the thyroid is three biological steps before hypothyroidism. Furthermore, the inhibition of iodide uptake in the thyroid is not an immediate precursor to hypothyroidism. When the biology allows for the identification of multiple NOAELs, each associated with different biological response, the highest NOAEL is used for regulatory purposes. For perchlorate, this means a NOAEL identified for changes in thyroid hormones (i.e., the immediate precursor to hypothyroidism). This concept is included in the following NOAEL definition used by EPA's Office of Communications, Education, and Public Affairs (OCEPA):

> **EPA OCEPA NOAEL:** An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, or as precursors to adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the highest one, leading to the common usage of the term NOAEL as the highest exposure without adverse effects.

Therefore, the NAS Committee's unconventional derivation of an RfD from the inhibition of iodide uptake (i.e., NOEL) does not meet the strict definition of an RfD in that it is derived from an adverse effect. Since the NAS Committee's recommended RfD was derived from a biological response several steps before the adverse effect, the POD value used is at a dose below a NOAEL or LOAEL that would be conventionally used for deriving an RfD. Thus, in our opinion, the NAS Committee used a conservative approach of using a NOEL to derived its RfD, but the degree of conservatism (i.e., quantitatively) is not identified because the value of a conventionally derived perchlorate RfD is not known since adverse health effects have not been clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 177).

The NAS Committee also states its recommended RfD is health protective, but evaluating this is difficult because the amount of TIU that causes sufficient thyroid stress in pregnant women to induce mental deficient in their children is not defined in the single chemical risk assessment approach. Furthermore, following the single chemical risk assessment process does not guarantee a health protective RfD. By contrast, the application of a cumulative risk assessment approach expands the understanding of the stressors that affect this public health issue and provide a clearer, more complete picture of this public health issue for making risk-management decisions. Therefore, the cumulative risk assessment results can be used to evaluate whether the NAS Committee-recommended RfD is health protective.

5.1.2 Evaluation of the Application of UFs for Deriving the Perchlorate RfD

The NAS Committee used a nonadverse POD of 0.007 mg/kg-day identified from the human exposure to perchlorate in the Greer study (Greer 2002) to derive its recommended perchlorate RfD. By comparison, the EPA used an adverse POD of 0.01 mg/kg-day identified in the Argus rat study to derive EPA's draft RfD. Note that the respective PODs of 0.007 mg/kg-day and 0.01 mg/kg-day are essentially the same. Therefore, the difference in the NAS and EPA RfDs arise from the selection of which UFs are applied (i.e., interspecies UF, intraspecies UF, LOAEL to NOAEL UF, database UF, subchronic-to-chronic-duration UF, modifying factor) and

the magnitude of each UF applied (i.e., 1, 3, or 10). The NAS Committee used a total UF of 10 while EPA used a total UF of 300.

The EPA risk assessment guidance indicates the preference to use human data over animal data to set a POD. EPA risk assessment guidance states, "Human or animal data can be used to derive an RfD, but when adequate and sufficient human data on the most sensitive effect, the EPA guidance states that the human data should be used" (EPA 2002, p 4-12). Human data is preferred because it minimizes the uncertainty of extrapolating adverse effects observed in animal studies to potential effects in humans. This uncertainty is the source and reason an interspecies UF is often applied when deriving an RfD from animal data (EPA 2002, p 4-42, section 4.4.5.2). Although neither dataset is ideal because the amount of iodide inhibition that induces subtle mental deficits in children during gestation is not identified by these studies, human data are preferred over animal data.

The use of human data is critical for applying a cumulative risk assessment approach to this public health issue. In animal studies, the ability to test the cognitive abilities of the exposed animals is very crude and insensitive to subtle mental deficits. For example, how do you measure a subtle loss of verbal comprehension in a rat? Or, how do you measure a subtle decrease in the attentiveness of a rat? By comparison, measuring subtle mental deficits such as verbal comprehension or ADHD in human children is relatively straightforward. The human exposure to each of the four NIS stressors can be measured or estimated for use in a cumulative risk assessment (i.e., varying the exposure of the four NIS stressors in an animal study is too complicated, expensive, and relatively insensitive to identifying the endpoint of subtle mental deficits). The Tonacchera Model allows the combined effect on the NIS to be determined and identified into a single variable, TIU. In contrast to the limitations of a single chemical risk assessment, a cumulative risk assessment can identify the %TIU in pregnant women that is associated with subtle mental deficits in their children. Therefore, for this public health issue, animal data are not an adequate substitute for human data (i.e., animal data can supplement, but not replace, human data for this public health issue).

The NAS Committee used a nonadverse POD to derive its recommended perchlorate RfD. However, not everyone in the scientific community agrees that the inhibition of iodide uptake is a nonadverse effect (Ginsberg 2005; EPA 2002a, p 7-26, section 7.1.5.1). Toxicologists can have different interpretations of what constitutes an adverse effect (Dorato 2005). An approach to classifying effects as adverse or nonadverse are discussed in Dorato's paper (Dorato 2005, figure 5). If the inhibition of iodide uptake is considered an adverse effect (e.g., a LOAEL), a LOAEL to NOAEL UF would be applied with a magnitude of 3 or 10 (Ginsberg 2005). So, the issue of whether the inhibition of iodide uptake is adverse or nonadverse simply affects the amount of total UFs to be applied to the POD to derive the RfD (see next paragraph – the impact of total UF on this public health issue).

The evaluation of the appropriate selection and magnitude of each UF to be applied to the derivation of a perchlorate RfD is difficult due to the lack of a clear scientific consensus of their application to the perchlorate dataset. The value of each UF depends on the quality of the studies available, the extent of the database, and scientific judgment (EPA 2002, p 4-40, section 4.4.5.1). The NAS Committee wrote, "No absolute rules exist for application of the [uncertainty] factors,

and professional judgment is a large component of their use." (NAS 2005, p 29). However, the total UF used directly affects the calculated RfD value and any resulting regulatory exposure limits set from the RfD. The scientific considerations center around which UF to apply and at what magnitude. However, there is no scientific discussion if the application of UFs to the POD accomplishes the intent of an RfD. An RfD sets an oral exposure limit that is likely to prevent adverse health effects from occurring in the human population over a lifetime.

The perchlorate RfD sets an exposure limit to only one of four known NIS stressors. Even if the perchlorate RfD is never exceeded in a population, the combined effect from various exposure level combinations from the other three uncontrolled NIS stressors (i.e., lack of iodide, thiocyanate, and nitrate) can still generate an unacceptable adverse health effects in humans. All four NIS stressors conspire to limit the amount of iodide uptake in the thyroid, which initiates all subsequent adverse health effects, not just perchlorate exposure. The use of the Tonacchera Model in a cumulative risk assessment approach can identify the $\%TIU_{(LOAEL)}$ and $\%TIU_{(NOAEL)}$ in pregnant women that is associated with subtle mental deficits in their children. The use of UFs should be applied to the $\%TIU_{(LOAEL)}$ or $\%TIU_{(NOAEL)}$ to set a $\%TIU_{(RfD)}$ exposure limit (i.e., the integrated effect of all four NIS stressors) and not the POD for perchlorate. The mode of action identifies that adverse effects are avoided if the amount of TIU in the thyroid is sufficiently high. Limiting exposure of only perchlorate does not guarantee an acceptable TIU level. Only by managing the exposure to all four NIS stressors is the TIU level guaranteed to be a sufficiently high %TIU to avoid adverse effects.

The demonstration of this principle can be seen in the cumulative risk assessment (see Section 9.3). When the total applied UF value is increased from 10 to 500 and is applied only to the perchlorate exposure, the corresponding DWEL shifts from 24.5 ppb and 0.5 ppb, respectively. The calculated increase in %TIU in an adult is estimated to be 0.4% when the perchlorate DWEL is lowered from 24.5 ppp to 0.5 ppb. In a pregnant woman, our analysis estimates a 1.0% increase in the %TIU when the perchlorate DWEL is lowered from 24.5 ppb to 6.1 ppb (see Section 9.5). A change of 1.0%TIU in pregnant women is below the statistical detection in a human radioactive iodide uptake exposure study (e.g, the Greer study). Our cumulative risk assessment estimates that the %TIU would have to fall 50% from a healthy iodide uptake level for pregnant women to potentially start inducing subtle mental deficits in their children. Therefore, the particular total UF (i.e., 10 to 500) applied to the perchlorate POD in a single chemical risk assessment is not particularly significant in maintaining a sufficient iodide uptake during pregnancy. By contrast, our analysis indicates that the application of UF needs to be applied to the $\%TIU_{(NOAEL)}$ level, because a low %TIU (and not perchlorate exposure only) is associated with an adverse outcome. Therefore, the use of a cumulative risk assessment approach is essential to accurately characterize and to understand the risks affecting this public health issue and to formulate an effective response(s) to the problem. The cumulative risk assessment indicates that the dominant stressor in this public health issue is the lack of iodide stressor, and because of iodide deficiency during pregnancy, about 29% of pregnant women are below $\%TIU_{(NOAEL)}$.

5.1.3 First Adverse Effect: Hypothyroidism or Hypothyroxinemia

The NAS Committee identified hypothyroidism as the first adverse effect, but recommended against using hypothyroidism as the basis for a perchlorate risk assessment (NAS 2005, p 166-67). The fetus needs a sufficient supply of T_4 during gestation for proper brain development. The fetus is entirely dependent on the maternal supply of T_4 through mid-gestation (Glinoer 2005, p 1095) and is still sensitive to maternal supply thereafter. Maternal hypothyroidism is toxic to the fetus because it causes a decreased maternal supply of T_4 during gestation. The adverse effects on the fetus from maternal hypothyroidism are reported in a 1999 New England Journal of Medicine study. This study reported on maternal thyroid deficiency and the subsequent neuropsychological development of the children (Haddow 1999). The study identified 47 pregnant women with elevated TSH (i.e., > 6 ul/ml) and another 15 pregnant women with both elevated TSH and low T_4 levels. Of the 48 pregnant women who did not receive treatment for their thyroid deficiency, the full-scale intelligence quotient (IQ) of their children averaged 7 points lower ($p = 0.005$) than the 124 matched control children.

Hypothyroidism is an adverse effect from an extremely low uptake of iodide by the thyroid, but hypothyroidism is not the first adverse effect from perchlorate exposure. Hypothyroxinemia is the first adverse effect from a low uptake of iodide by the thyroid. Iodide deficiency and excess NIS inhibition (i.e., perchlorate exposure) act through the same mechanism (i.e., each result in a low uptake of iodide by the thyroid). The Tonacchera Model indicates that the amount of iodide taken up into the thyroid by the NIS is directly related to the amount of iodide in the blood. If the diet is poor in iodide, the amount of iodide uptake will be low, regardless of how much or how little NIS inhibition is occurring. Therefore, the lack of iodide in the diet has the same mechanism of toxicity and outcome as a high NIS inhibition level; not enough iodide is taken up by the thyroid for the sufficient production of thyroid hormones. Therefore, the adverse effects caused by iodide deficiency are the same adverse effects that would be expected from a high NIS inhibition level when not enough iodide is taken up by the thyroid.

Iodide deficiency is frequently and incorrectly associated with hypothyroidism and increased TSH (Obregon 2005, p 918). The TSH-independent autoregulation is often overlooked, or actually not known to younger Western-trained physicians (Obregon 2005, p 918). Atrophy of the thyroid and overt hypothyroidism occur only when iodide deficiency is accompanied by a high goitrogen load and/or selenium deficiency (Obregon 2005, p 918). Clinical or subclinical hypothyroidism is not observed in uncomplicated iodide deficiency (Obregon 2005, p 918). By contrast, iodide deficiency (i.e., the lack of an adequate uptake of iodide by the thyroid) induces hypothyroxinemia, a thyroid condition characterized by a decreased T_4 serum level and a normal or slightly elevated T_3, without an increase in TSH levels (i.e., TSH levels are normal) (Obregon 2005, p 918). Mild to moderate iodide deficiency is the most widespread cause of maternal hypothyroxinemia (Morreale de Escobar 2004, p U25). The decrease in T_4 in pregnant women with hypothyroxinemia in the first 20 weeks of pregnancy is associated with mental deficits and an increased frequency of ADHD in their children (Vermiglio 1994). Therefore, maternal hypothyroxinemia induces fetal brain damage through the same cause as maternal hypothyroidism – a decreased maternal supply of T_4.

5.1.4 Comparison of the NAS Unconventional RfD with a Conventionally Derived Excess NIS Inhibition RfD

The NAS Committee states that adverse health effects have not been clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 177). This lack of clear demonstration prevents the calculation of a perchlorate RfD using the conventional single chemical risk assessment approach of deriving an RfD from a NOAEL or LOAEL. However, perchlorate is only one of three NIS inhibitors continuously acting on the thyroid to prevent the uptake of iodide. The other two ubiquitously consumed NIS inhibitors are thiocyanate and nitrate. The Tonacchera Model identifies the relative potency factor for each of the three NIS inhibitors relative to perchlorate and identifies the interaction of the chemical mixture as simple competitive interaction (Tonacchera 2004). There is no evidence of synergism or antagonism in chemical mixtures of these three NIS inhibitors. Therefore, the total amount of NIS inhibition acting on the thyroid is the combined contribution of each anion on a molar potency adjusted basis (i.e., a dose-addition method).

The principle of dose addition can be utilized to independently calculate an excess NIS inhibition RfD and compare it to the NAS-recommended perchlorate RfD. Unlike perchlorate in which adverse effects have not been documented in humans, adverse effects have been documented in human populations exposed to excess amounts of the other two NIS inhibitors, thiocyanate and nitrate. Since the NIS inhibition from thiocyanate and nitrate are indistinguishable from the NIS inhibition from perchlorate (Tonacchera 2004), the amount of NIS inhibition in these human populations can be measured and expressed mathematically as a PEC. Then the conventional single chemical risk assessment approach can use the observed NOAELs and LOAELs in these human populations to independently derive an excess NIS inhibition RfD (i.e., a descriptive name for what the perchlorate RfD represents). The excess NIS inhibition RfD (i.e., which was derived through the conventional approach of using a NOAEL or LOAEL) can be compared to the NAS-recommended perchlorate RfD (i.e., which was derived through a unconventional approach of using a NOEL) to quantify how conservative the NAS-recommended perchlorate RfD might be.

The OIG identified the following three epidemiological studies to calculate an excess NIS inhibition RfD in which adverse and nonadverse thyroid effects can be observed in human populations exposed to excess amount of the NIS inhibitors thiocyanate and nitrate:

1. Severe hypothyroxinemia can be observed in the electroplating workers exposed to cyanide resulting in an excess exposure to the NIS inhibitor thiocyanate (Banerjee 1997). (See Section 5.1.4.1).

2. Hypothyroxinemia can be observed in women consuming thiocyanate-preserved cow's milk, which resulted in an excess exposure to the NIS inhibitor thiocyanate (Banerjee 1997b). (See Section 5.1.4.2).

3. Hypertrophy (i.e., an increase in the size of the thyroid) can be observed in children consuming nitrate-contaminated water, which resulted in an excess exposure to the NIS inhibitor nitrate (Tajtáková 2006). (See Section 5.1.4.3).

The NAS Committee states that transient changes in serum thyroid hormones and TSH concentrations are not adverse health effects (NAS 2005, p 166). Furthermore, the NAS Committee states that the serum thyroid hormone concentrations should not be used as the POD for the risk assessment (NAS 2005, p 169). The OIG Analysis of the thyroid function concurs with this assessment. The thyroid is in a continuous dynamic process (i.e., homeostasis) to maintain the serum T_4 and T_3 level within a normal range under ever-changing levels of exposure to the four NIS stressors. Transient changes in serum thyroid hormones and TSH concentrations are a reflection of the thyroid working to maintain equilibrium. In contrast to using transisent changes in serum thyroid hormone and TSH concentrations as the POD, the OIG Analysis identified two thiocyanate exposure studies (i.e., 1. and 2. from above) in which a long-term exposure to excess thiocyanate exceeded the thyroid's ability to adapt and maintain a serum T_4 level within a normal range. This is appropriate because the mode of action for this public health issue identifies that the failure of the thyroid to maintain an adequate serum T_4 level during pregnancy and lactation induces the adverse effects in the offspring. Our analysis identified a study (i.e., 3. from above) in which a long-term NIS stressor exposure to nitrate resulted in hypertrophy in the school-aged children.

5.1.4.1 Calculation of an Excess NIS Inhibition RfD Using the Adverse Thyroid Health Effects Observed in Electroplating Workers Exposed to Cyanide in Adult Men

Background

Sodium cyanide and potassium cyanide are salts used in the electroplating industry. The cyanide containing electroplating baths caused occupational exposure of the electroplating workers to cyanide. Once cyanide is absorbed into the body, about 80% of absorbed cyanide is metabolized to thiocyanate (SCN^-) by the sulfurtransferase enzyme, rhodanese (WHO 2004).

Description of Banerjee's Thiocyanate Study in Adult Males

Of 201 male workers in the plant, 35 male workers had worked in the electroplating process area for at least 5 consecutive years (i.e., the exposed group) (Banerjee 1997). Another 35 male workers who worked outside the manufacturing building were matched for age and dietary habits (i.e., nonexposed group – control). All subjects were selected randomly from among employees who did not use tobacco products and who had no history of thyroid disease.

Five mls of blood were collected from each subject. The blood serum was tested for serum T_4, T_3, TSH, and thiocyanate.

Iodide Nutritional Status

The iodide nutritional status of the test subjects was not evaluated in this study. Specifically, no urinary samples were taken to measure iodide excretion levels in this study.

Banerjee's Thiocyanate Study Results

Both the T_4 and T_3 hormone levels in the exposed workers were all statistically lower (i.e., $p > 0.05$) than the nonexposed workers (i.e., the control group). Both T_4 and T_3 hormone levels in the exposed workers are below the normal range for these thyroid hormones. The exposed workers' TSH value is statistically higher (i.e., $p > 0.05$) than the nonexposed workers. However, the exposed workers' TSH levels were elevated over the controls, but within the normal range of 0.2 to 4.0 µU/ml. The following table summaries the study results:

Study Group	Thiocyanate (µmol/L)	T_4 (µg/dL)	T_3 (µg/dL)	TSH (µU/ml)
35 Nonexposed Workers (all nonsmokers)	90.8 ± 9.02	6.09 ± 0.601	111.0 ± 9.3	1.20 ± 0.301
35 Exposed Workers (all nonsmokers)	316 ± 15.0*	3.81 ± 0.318†	57.2 ± 8.1†	2.91 ± 0.20†
Normal Ranges Cited in the Manufacturers Test Kits		5.5 to 13.5	60 to 200	0.2 to 4.0

* $p < 0.01$
† $p < 0.05$

Source: Banerjee 1997, table 1.

Amount of Excess Thiocyanate

The nonexposed workers (all nonsmokers) had a serum thiocyanate concentration of 90.8 ± 9.02 µmol/L. The exposed workers had a serum thiocyanate concentration of 316 ± 15.0 µmol/L. Therefore the amount of excess thiocyanate measured in the serum was 225.2 µmol of SCN^-/L (i.e., 316 µmol/L - 90.8 µmol/L).

Amount of Excess NIS Inhibition in the Exposed Workers

The amount of excess NIS inhibition from thiocyanate in the exposed workers is converted to the SPEC (concept taken from Tonacchera 2004):

SPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of SCN^- Inhibition)
SPEC = [Perchlorate] + [Nitrate] /240 + ([total serum SCN^-] x 0.5) /15
SPEC = [Perchlorate] + [Nitrate] /240 + [free SCN^-] /15

Where: [ ] is molarity
Serum inhibition potency of perchlorate = 1
Serum inhibition potency of nitrate relative to perchlorate = 1 / 240
Serum inhibition potency of free thiocyanate relative to perchlorate = 1 / 15
[free SCN^-] = [total serum SCN^-] x 0.5 free SCN^- per total serum SCN^-

However, the serum molarity concentration for perchlorate and nitrate are neither measured nor reported in the Banerjee study (i.e., they are potentially confounding factors). Assuming the perchlorate and nitrate exposure in the Banerjee test subjects is constant between the exposed workers and the nonexposed workers (i.e., controls). Then, the SPEC equation can be simplified to express only the excess amount of NIS inhibition from the thiocyanate. Thus:

$$\text{Excess SPEC}_{(\text{Exposed workers})} = ([\text{excess } SCN^-] \times 0.5) \div 15$$
$$= (225.2 \text{ µmoles of } SCN^-/\text{L} \times 0.5) \div 15$$
$$= 7.51 \text{ µmol/L SPEC}$$

Equivalent Amount of Perchlorate Ingestion Corresponding to the Excess SPEC

The Clewell perchlorate physiologically based pharmacokinetic (PBPK) model is used to calculate the external perchlorate exposure (mg/kg-day) from the excess SPEC observed in the exposed workers in the Banerjee test subjects (Clewell 2007). The excess SPEC of 7.51 µmol/L SPEC must be converted into µg/L SPEC unit for use in the Clewell Perchlorate PBPK Model.

$$\text{µg/L SPEC}_{(\text{Exposed workers})} = 7.51 \text{ µmol/L SPEC} \times 99.45 \text{ µg perchlorate/µmol}$$
$$= 747 \text{ µg/L SPEC}_{(\text{Exposed workers})} \text{ or } 0.747 \text{ mg/L SPEC}_{(\text{Exposed workers})}$$

The external perchlorate dose required to generate the amount of excess NIS inhibition observed in the exposed workers is calculated using the Clewell PBPK model as follows:

$$\text{Ext. } ClO4^- \text{ Dose} = 0.747 \text{ mg/L SPEC}_{(\text{Exposed workers})} \times \frac{1.0 \text{ mg/kg-day}}{1.0 \text{ mg/L}}$$

$$= 0.75 \text{ mg/kg-day (rounded)}$$

The exposed workers in the Banerjee study were unable to maintain a normal T_4 or T_3 thyroid hormone level while still maintaining a TSH level in the normal range (refer to table above). Overt hypothyroidism is characterized by an elevated TSH and below normal T_4 while subclinical hypothyroidism is characterized by an elevated TSH and normal T_4. However, hypothyroxinemia is a less severe thyroid condition and is a common condition in pregnant women characterized by low maternal fT_4 levels with normal TSH levels (Kooistra 2006). Therefore, the exposed workers' thyroid hormone levels could be described as severe hypothyroxinemia. Maternal hypothyroxinemia (i.e., during pregnancy) is documented to be associated with mental deficits (e.g., ADHD) in the children of those mothers (Vermiglio 2004). The mechanism of fetal brain damage from maternal hypothyroxinemia is the same as maternal hypothyroidism (i.e., an insufficient maternal supply of T_4 to the fetus during gestation). Therefore, although hypothyroxinemia is not a permanent adverse effect in adult males, maternal hypothyroxinemia is an adverse effect in the most sensitive populations, pregnant women, fetuses, and nursing infants. Thus, the OIG has identified the observed severe hypothyroxinemia in the Banerjee study exposed workers to be an adverse effect (i.e., if severe hypothyroxinemia were to occur in pregnant woman population) from which an excess NIS inhibition RfD can be calculated from.

The excess thiocyanate measured in the exposed workers is converted by the use of the Tonacchera Model and the Clewell perchlorate PBPK model to calculate an external perchlorate dose that corresponds to the LOAEL of NIS inhibition in an adult male human. In other words, an external perchlorate exposure of 0.75 mg/kg-day (or about 55 mg/day for a 70 kg adult male) should generate the same thyroid effects (i.e., severe hypothyroxinemia) observed in the exposed workers in the Banerjee study.

Calculation of an Excess NIS Inhibition RfD from the Excess Thiocyanate Exposure

The severe hypothyroxinemia observed in the Banerjee exposed workers is used to identify the LOAEL for the calculation of the excess NIS inhibition RfD. Therefore, the observed LOAEL in an adult male is:

$$\text{LOAEL}_{(\text{Exposed workers})} = 0.75 \text{ mg/kg-day}$$

The calculation of an NOAEL from a LOAEL is typically accomplished by applying a UF of 10 to the LOAEL (EPA 2002, p 4-44):

$$\text{NOAEL}_{(\text{Exposed workers})} = 0.75 \text{ mg perchlorate equivalent/kg-day} / 10 \text{ UF}$$

$$= 0.075 \text{ mg perchlorate equivalent/kg-day}$$

Since the exposed workers are all men, who are less sensitive than women to adverse thyroid effects, a full UF of 10 is applied to the NOAEL to account for the intraspecies factor to

protect the most sensitive populations. The application of a 10 UF in this calculation is analogous to the NAS Committee applying a total UF of 10 to the NOEL to protect the most sensitive population. The NAS Committee used a NOEL to determine the RfD from the excess perchlorate consumption in the Greer study and a 10 UF was applied to it to derive NAS-recommended perchlorate RfD (NAS 2005, p 16). The OIG is using a UF of 10 for these calculations to allow for the direct comparison of the excess NIS inhibition RfD with the NAS-recommended perchlorate RfD of 0.0007 mg/kg-day. Therefore, the excess NIS inhibition RfD (from excess SCN^- exposure) is calculated as follows:

$$\text{Excess NIS Inhibition } RfD_{(\text{Adult males})} = 0.075 \text{ mg perchlorate equivalent/kg-day} / 10 \text{ UF}$$
$$= 0.0075 \text{ mg/kg-day}$$

The calculated excess NIS inhibition RfD from excess SCN^- exposure in adult males is 0.0075 mg/kg-day. EPA's perchlorate RfD is 0.0007 mg/kg-day obtained from the NAS Committee. The calculated excess NIS inhibition $RfD_{(\text{Adult males})}$ is 10.7 times greater than the NAS-recommended perchlorate RfD.

The advantage of determining the excess NIS inhibition RfD is because it used a conventional risk assessment approach of deriving an RfD by using a LOAEL observed in a human population. The NAS Committee describes its own approach of deriving an RfD from a NOEL as being an unconventional approach, but characterizes it as being a "conservative, health-protective approach to the perchlorate risk assessment" (NAS 2005, p 15). The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's statement that its recommended perchlorate RfD is conservative. The excess NIS inhibition $RfD_{(\text{Adult males})}$ observed in the Banerjee thiocyanate study of electroplating workers indicates that the NAS-recommended perchlorate RfD is conservative by a factor of 10.3.

5.1.4.2 Calculation of an Excess NIS Inhibition RfD Using the Adverse Thyroid Health Effects from the Ingestion of Thiocyanate-Preserved Cow's Milk by Adult Women

Background

In Calcutta, India, local dairies add 30-50 mg/L of thiocyanate to preserve cow's milk in order to activate the lactoperoxidase-thiocyanate-hydrogen peroxide system (LP system). The LP system forms the hypothiocyanite anion ($OSCN^-$), which acts as a bacteriostat to retard the growth of bacteria. The subsequent ingestion of thiocyanate-preserved cow's milk causes a relatively high thiocyanate exposure to the consumer.

Description of Banerjee's Thiocyanate Study in Adult Women

Thirty-five women who had consumed 250 ml/day of thiocyanate-preserved milk for more than 5 consecutive years were designated as the exposed group (Banerjee 1997b). Another 35 women who consumed the same quantity of nonpreserved raw milk were designed the control group (i.e., the nonexposed group). The control group was matched for age and dietary habits. All subjects were selected randomly from a population of similar socioeconomic status and uniform dietary pattern. Furthermore, all test subjects did not use tobacco products and had no history of thyroid disease.

Five mls of blood were collected from each subject. The blood serum was tested for serum T_4, T_3, TSH, and thiocyanate. Iodide levels were measured by urinary iodide concentration (UIC).

Iodide Nutritional Status

The iodide nutritional status of the test subjects was evaluated in this study by UIC. The UIC of the exposed women was found to be 115 μg/L (SE 8.5). The UIC of the control group was found to be 123 μg/L (SE 7.8). The difference in the UICs between the two groups was not statistically significant.

Banerjee's Thiocyanate Study Results

Hypothyroxinemia is a thyroid condition characterized by below normal T_4 and normal T_3 and TSH levels. Hypothyroxinemia was observed in thiocyanate-exposed female in the Banerjee adult female thiocyanate study (Banerjee 1997b). The thiocyanate-exposed women were measured to have below normal T_4, and both normal T_3 levels and statistically elevated TSH levels, but the TSH levels were still in the normal range. This level of hypothyroxinemia is less severe than the hypothyroxinemia observed in the Banerjee adult male thiocyanate study (Banerjee 1997). The exposed females were measured to have a serum thiocyanate exposure of 230 ± 10.0 μmol/L. The control women were measured to have a serum thiocyanate exposure of 90.8 ± 9.0 μmol/L. The observations of the Banerjee study are summarized below:

Study Group	Thiocyanate (µmol/L)	T_4 (nmol/L)	T_3 (nmol/L)	TSH (µU/ml)
35 Nonexposed Females (all nonsmokers)	90.8 ± 9.0	125.4 ± 11.5	1.71 ± 0.16	1.09 ± 0.28
35 Exposed Females (drank SCN^- preserved milk)	230.0 ± 10.0*	87.8 ± 6.6*	2.39 ± 0.32	2.49 ± 0.20*
Normal Ranges Cited in the Manufacturers Test Kits		110 to 279	0.93 to 3.12	0.2 to 4.0

* $p < 0.01$

Source: Banerjee 1997b, table 1.

Amount of Excess Thiocyanate

The control women had a serum thiocyanate concentration of 90.8 µmol/L. The exposed women had a serum thiocyanate concentration of 230 µmol/L. Therefore, the amount of excess thiocyanate measured in the serum was 139.2 µmol of SCN^-/L (i.e., 230 µmol/L - 90.8 µmol/L).

Amount of Excess NIS Inhibition in the Exposed Women

The amount of excess NIS inhibition from thiocyanate in the exposed workers is converted to the SPEC (concept taken from Tonacchera 2004):

SPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of SCN^- Inhibition)
SPEC = [Perchlorate] + [Nitrate] /240 + ([total serum SCN^-] x 0.5) /15
SPEC = [Perchlorate] + [Nitrate] /240 + [free SCN^-] /15

Where: [ ] is molarity
Serum inhibition potency of perchlorate = 1
Serum inhibition potency of nitrate relative to perchlorate = 1 / 240
Serum inhibition potency of free thiocyanate relative to perchlorate = 1 / 15
[free SCN^-] = [total serum SCN^-] x 0.5 free SCN- per total serum SCN^-

However, the serum molarity concentration for perchlorate and nitrate are neither measured nor reported in the Banerjee study (i.e., they are potentially confounding factors). Assuming the perchlorate and nitrate exposure in the Banerjee test subjects is constant between the exposed workers and the nonexposed workers (i.e., controls), then the SPEC equation can be simplified to express only the excess amount of NIS inhibition from the thiocyanate. Thus:

$$\text{Excess SPEC}_{(\text{Exposed workers})} = ([\text{excess } SCN^-] \times 0.5) \div 15$$
$$= (139.2 \text{ µmoles of } SCN^-/\text{L} \times 0.5) \div 15$$
$$= 4.64 \text{ µmol/L SPEC}$$

Equivalent Amount of Perchlorate Ingestion Corresponding to the Excess SPEC

The Clewell Perchlorate PBPK Model is used to calculate the external perchlorate exposure (mg/kg-day) from the excess SPEC observed in the exposed women (Clewell 2007).

The excess SPEC of 4.90 µmol/L SPEC must be converted into µg/L SPEC unit for use in the Clewell Perchlorate PBPK Model.

$$\mu g/L\ SPEC_{(Exposed\ women)} = 4.64\ \mu mol/L\ SPEC \times 99.45\ \mu g\ perchlorate/\mu mol$$
$$= 461\ \mu g/L\ SPEC_{(Exposed\ women)}\ or\ 0.461\ mg/L\ SPEC_{(Exposed\ women)}$$

The external perchlorate dose required to generate the amount of excess NIS inhibition observed in the exposed workers is calculated using the Clewell PBPK model as follows:

$$Ext.\ ClO_4^- Dose = 0.461\ mg/L\ SPEC_{(Exposed\ women)} \times \frac{1.0\ mg/kg\text{-}day}{1.0\ mg/L}$$
$$= 0.461\ mg/kg\text{-}day$$

The exposed women in the Banerjee study were unable to maintain a normal T_4 thyroid hormone level while still maintaining a TSH level in the normal range (refer to table above). The SPEC level at which overt hypothyroidism (i.e., elevated TSH and below normal T_4) or subclinical hypothyroidism (i.e., elevated TSH and normal T_4) is not known by experimental data. However, hypothyroxinemia is a less severe thyroid condition and is a common condition in pregnant women characterized by low maternal fT_4 levels with normal TSH levels (Kooistra 2006). Therefore, the exposed women's thyroid condition could be described as hypothyroxinemia. Maternal hypothyroxinemia (i.e., during pregnancy) is documented to be associated with mental deficits (e.g., ADHD) in the children of those mothers (Vermiglio 2004). The mechanism of fetal brain damage from maternal hypothyroxinemia is the same as maternal hypothyroidism (i.e., an insufficient maternal supply of T_4 to the fetus during gestation). Therefore, although hypothyroxinemia might not have overt adverse symptoms in adult women, maternal hypothyroxinemia is an adverse effect in the most sensitive populations – pregnant women, fetuses, and nursing infants. Thus, the OIG has identified the observed hypothyroxinemia in the Banerjee exposed women to be an adverse effect from which an excess NIS inhibition RfD can be calculated.

The excess thiocyanate measured in the exposed women is converted by the use of the Tonacchera Model and the Clewell perchlorate PBPK model to calculate an external perchlorate dose that corresponds to the LOAEL of NIS inhibition in an adult male human. In other words, an external perchlorate exposure of 0.461 mg/kg-day (or 32 mg/day for a 70 kg adult female) should generate the same thyroid effects (i.e., hypothyroxinemia) observed in the exposed women in the Banerjee study.

Calculation of an Excess NIS Inhibition RfD from the Excess Thiocyanate Exposure

The severe hypothyroxinemia observed in the Banerjee exposed workers is used to identify the LOAEL for the calculation of the excess NIS inhibition RfD. Therefore, the observed LOAEL in an adult male is:

$$LOAEL_{(Adult\ women)} = 0.461\ mg/kg\text{-}day$$

The calculation of an NOAEL from a LOAEL is typically accomplished by applying a UF of 10 to the LOAEL (EPA 2002, p 4-44):

$$NOAEL_{(Adult\ women)} = 0.461 \text{ mg perchlorate equivalent/kg-day} / 10 \text{ UF}$$
$$= 0.0461 \text{ mg perchlorate equivalent/kg-day}$$

Since the exposed women are expected to be more sensitive to NIS inhibition and because the use of hypothyroxinemia is a more subtle adverse thyroid effect than hypothyroidism, a scientific argument could be made to justify a smaller value of 10 for this UF (i.e., LOAEL to NOAEL). However, EPA risk assessment guidance states that a reduction of the intraspecies UF from a default of 10 is to be considered only if the POD is determined from the data obtained from the susceptible subpopulation (EPA 2002, p 4-43). Since nonpregnant women are not the sensitive population for this public health issue, this calculation of the excess NIS inhibition $RfD_{(Women)}$ from the excess exposure to thiocyanate in cow's milk will use a UF of 10. The application of a 10 UF in this calculation is analogous to the NAS Committee applying a total UF of 10 to the NOEL to protect the most sensitive population. From the excess perchlorate consumption in the Greer study, the NAS Committee used a NOEL and applied a UF of 10 to it to derive NAS-recommended perchlorate RfD (NAS 2005, p 16). The use of a UF of 10 in our analysis also allows for the direct comparison of the excess NIS inhibition RfD from thiocyanate-perserved milk in adult women with the NAS-recommended perchlorate RfD of 0.0007 mg/kg-day. Therefore, the excess NIS inhibition $RfD_{(Women)}$ is calculated as follows:

$$\text{Excess NIS Inhibition } RfD_{(Adult\ women)} = 0.0461 \text{ mg perchlorate equivalent/kg-day} / 10 \text{ UF}$$
$$= 0.0046 \text{ mg/kg-day}$$

The calculated excess NIS inhibition RfD from excess SCN^- exposure in adult women is 0.0046 mg/kg-day. EPA's perchlorate RfD is 0.0007 mg/kg-day obtained from the NAS Committee. The calculated excess NIS inhibition $RfD_{(Adult\ women)}$ is 6.6 times greater than the NAS-recommended perchlorate RfD.

The advantage of determining the excess NIS inhibition RfD is it used a conventional risk assessment approach of deriving an RfD by using a LOAEL observed in a human population. The NAS Committee describes its own approach of deriving an RfD from a NOEL as being an unconventional approach, but characterized it as being a "conservative, health-protective approach to the perchlorate risk assessment" (NAS 2005, p 15). The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's statement that its recommended perchlorate RfD is conservative. The excess NIS inhibition $RfD_{(Adult\ women)}$ observed in the Banerjee thiocyanate exposure study in milk indicates that the NAS-recommended perchlorate RfD is conservative by a factor of 6.6.

Discussion

The following findings can be made from the observations in the two Banerjee thiocyanate exposure studies:

- In both Banerjee thiocyanate exposure studies, the first observed adverse effect from excess exposure to NIS inhibition was hypothyroxinemia and not hypothyroidism as stated by the NAS Committee.

- Both Banerjee thiocyanate exposure studies support the concept that the exposure to the other NIS inhibitors, thiocyanate and nitrate, have the same mechanism of toxicity as perchlorate, and an excess exposure on a molar potency adjusted basis is just as toxic as excess exposure to perchlorate. The toxic effects of the three NIS inhibitors are the same and the evaluation of the risk from three NIS inhibitors should not be separated, but should be considered together through a cumulative risk assessment approach.

- Both Banerjee thiocyanate exposure studies support that the mathematical description of the Tonacchera Model for the TIU by the NIS. Hypothyroxinemia is known to be caused by iodide deficiency, which directly results in a low TIU. The Tonacchera Model predicts that a low TIU can also be generated when iodide intake is normal, but the total goitrogen load is excess. In both Banerjee thiocyanate exposure studies, the iodide intake was normal, but due to the excess exposure to NIS inhibitors, the same adverse effect observed with iodide deficiency is generated. This indicates that the lack of iodide acts as an NIS stressor producing the same effect to lower the uptake of iodide in the thyroid as does the exposure to NIS inhibitors.

- The conventionally derived excess NIS inhibition RfD from excess SCN^- exposure in adult males is calculated to be 0.0075 mg/kg-day. The NAS Committee's recommended perchlorate RfD is 0.0007 mg/kg-day. The conventionally derived excess NIS inhibition $RfD_{(Adult\ males)}$ is 10.7 times greater than the NAS-recommended perchlorate RfD. The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's statement that its recommended perchlorate RfD is conservative.

- The conventionally derived excess NIS inhibition RfD from excess SCN^- exposure in adult women is 0.0046 mg/kg-day. The NAS Committee's recommended perchlorate RfD is 0.0007 mg/kg-day. The conventionally derived excess NIS inhibition $RfD_{(Adult\ women)}$ is 6.6 times greater than the NAS recommended perchlorate RfD. The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's claim that its recommended perchlorate RfD is conservative.

- Since both the excess NIS inhibition RfD and the NAS Committee's recommended perchlorate RfD used a single chemical risk assessment approach to derive their respective RfD, both have the same limitations of a single chemical risk assessment.

The pivotal perchlorate study by Greer used by NAS to derive the perchlorate RfD also does not identify or account for the varying amounts of NIS inhibition contributed by the other NIS inhibitors (i.e., nitrate or thiocyanate) among Greer's test subjects. Furthermore, the Greer study does not identify the baseline amount of perchlorate from dietary exposure in the test subjects. Therefore, the perchlorate RfD derived from the Greer study is best described as the amount of excess NIS inhibition from perchlorate (i.e., the additional amount of perchlorate

given to the Greer's test subjects) that is not expected to reduce the amount of iodide uptake by the thyroid in sensitive human populations. Thus, the dataset used here from Banerjee's thiocyanate study is no worse than Greer's study from the perspective of not controlling for the nonmeasured NIS inhibitors. However, the Banerjee thiocyanate study does identify the baseline amount of thiocyanate in the controls. Both studies assume the background NIS inhibitor exposure levels from the nonmeasured NIS inhibitors are the same across the test subjects. Furthermore, neither the Greer study nor the male Banerjee thiocyanate study identified the iodide status of the test subjects, which is known to be a factor in the amount of iodide uptake by the NIS. Therefore, the amount of contributing inhibition from perchlorate and nitrate and the amount of iodide across the test subjects are potential confounding factors in the Greer and Banerjee studies. Obviously, a study in which all four variables (i.e., the amount of perchlorate, thiocyanate, nitrate, and iodide) are measured in the each of the test subjects would be ideal for applying and evaluating the Tonacchera Model, but this dataset is rare in the published literature. Our analysis found only one study (Braverman 2005) that measured all four NIS stressors in a single study population. This study is used to corroborate the calculated %TIU from the Tonacchera Model with the measured %TIU values from actual radioactive iodide uptake measurements reported in this human occupational cohort study (see Section 9.1.5).

5.1.4.3 Calculation of an Excess NIS Inhibition RfD Using the Adverse Effect Hypertrophy Observed in School-aged Children Drinking Nitrate-Contaminated Drinking Water

Description of Tajtáková Nitrate Exposure Study

The Tajtáková nitrate exposure study measured thyroid volume and echogenicity by ultrasound in 342 school-aged children from high-nitrate areas (HNAs) located in the agricultural lowland who consumed drinking water from shallow wells that were highly contaminated with nitrate (51-274 mg/L) in the Kosice Region of Slovakia (Tajtáková 2006). The study compared the HNA children's thyroid volume and echogenicity results against 168 children of the same age from a low-nitrate area (LNA) who consumed low-nitrate drinking water (<2 mg/L). The HNA children's results were also compared to 596 children from the City of Kosice who also consumed low-nitrate drinking water. The ages and genders of the children are as follows:

Study Group	Number of 10-Year-Old Children	Number of 13-Year-Old Children
324 Children from HNA	117 (49 boys / 68 girls)	207 (96 boys / 111 girls)
168 Children from LNA	65 (24 boys / 41 girls)	103 (60 boys / 43 girls)
596 Children from Košice City (also a low-nitrate area)	171 (68 boys / 103 girls)	425 (163 boys / 262 girls)

HNA = high-nitrate area; LNA = low-nitrate area.

Source: Tajtáková 2006.

Blood samples were taking from 315 HNA children and 109 LNA children (no blood samples were taken from the Košice City children). The blood was analyzed for levels of TSH, total thyroxine (tT_4), free triiodothyronine (fT_3), and thyroperoxidase antibodies (anti-TPO) in the serum.

Iodide Nutritional Status

The urinary iodine concentration in the HNA group (n=53) had a mean ± SD of 154 ± 108 µg/L, median 150 µg/L, range 24-320 µg/L, and 79% of the urinary concentrations were > 100 µg/L. The urinary iodine concentration in the LNA group (n=32) had a mean ± SD of 125 ± 48 µg/L, median 126 µg/L, range 60-173 µg/L, and 64% of the urinary concentrations were > 100 µg/L. The recommend urinary iodine concentration for adolescents and children (6-12 yrs) is 100 to 200 µg/L (Delange 2005, p 278). Therefore, the urinary iodine concentration in both HNA and LNA groups indicate satisfactory and healthy iodide intake amounts.

Tajtáková Nitrate Exposure Study Results

In regard to the thyroid volume, both the 10-year-old and 13-year-old HNA children had statistically significant increases in the size of their thyroid (i.e., hypertrophy) as compared to the LNA children and the children from Košice City. The observed thyroid volume in the 10 year olds increased from 4.58 mls in the LNA children to 5.10 mls in the HNA children (i.e., a 11% increase). Furthermore, the observed thyroid volume in the 13 year olds increased from 5.23 mls

in the LNA children to 5.97 mls in the HNA children (i.e., a 14% increase). The thyroid volume results are summarized in the following table:

Study Groups	Thyroid Volume	
	10-year-old Children	13-year-old Children
324 Children from HNA	5.10 ± 0.14 ml	5.97 ± 0.11 ml
168 Children from LNA	4.58 ± 0.17 ml (p <0.02)	5.23 ± 0.15 ml (p < 0.05)
596 Children from Košice City (also a low-nitrate area)	4.77 ± 0.10 ml (p <0.05)	4.87 ± 0.14 ml (p < 0.0001)

HNA = high-nitrate area; LNA = low-nitrate area.

Source: Tajtáková 2006.

In regard to the frequency of hypoechogenicity, both the 10-year-old and 13-year-old HNA children had statistically higher frequencies of hypoechogenicity as compared to the LNA children plus the children from Košice City. The frequency of hypoechogenicity is summarized in the following table:

Study Groups	Frequency of Hypoechogenicity	
	10-year-old Children	13-year-old Children
Children from HNA	16/177 (13.7%) p<0.01	22/207 (10.6%) p<0.03
Children from LNA & Košice City	11/236 (4.7%)	30/528 (5.7%)

Source: Tajtáková 2006.

In regard to TSH levels, of the 315 HNA children tested for TSH, 13 were found to be in the TSH hypothyroid range (i.e., >4.0mU/l). By contrast, of the 109 LNA children tested for TSH, none were found to be in the TSH hypothyroid range.

In regard to anti-TPO values, of the 315 HNA children tested for anti-TPO, 8 were found to be anti-TPO positive. By contrast, of the 109 LNA children tested for anti-TPO, none were found to be anti-TPO positive.

In regard to the blood sample results, the measured tT_4 and fT_3 levels between the HNA and LNA schoolchildren were found to have no statistical difference. The blood sample results for tT_4 and fT_3 are provided in the following table:

Study Groups	Blood Sample Results	
	Total Thyroxine (tT_4)	Free Triiodothyronine (fT_3)
315 Children from HNA (10- & 13-year-old pooled results)	88.9 ± 26.9 nmol/L	5.9 ± 1.0 pmol/L
109 Children from LNA (10- & 13-year-old pooled results)	82.1 ± 22.6 nmol/L	6.0 ± 0.9 pmol/L

Source: Tajtáková 2006

Estimated Excess Nitrate Exposure from Drinking Water in HLA Children

The total number of nitrate-contaminated wells in the study is 45. The total weighted nitrate concentration is 6878 well-mg/L. Therefore, the estimated nitrate concentration in the HLA is 152.8 mg/L (i.e., 6878 well-mg/L divided by 45 wells). Since the LNA children and the Košice children drank water containing up to <2 mg/L nitrate, the amount of excess nitrate

concentration in the HNA children's drinking water ingestion is estimated to be 150.8 mg/L (i.e., 152.8 mg/L – 2.0 mg/L).

Number of Contaminated Wells	Nitrate Concentration Range Reported in the Wells (mg/L)	Mid-Range Nitrate Concentration (mg/L)	Weighted Nitrate Concentration (well-mg/L)
14	214-264	239	3346
16	101-200	150	2400
15	51-100	75.5	1132
Total = 45			**Total = 6878**

Source: OIG Analysis.

The amount of excess nitrate exposure per day in the HNA children is estimated to be 150.8 mg/day (i.e., 150.8 mg of excess nitrate/L x 1 liter/person/day). This is calculated from the amount of excess nitrate concentration in the HNA children's drinking water multiplied by the median drinking water consumption rate of 1 liter/person/day. Since the water consumption rate of children in Slovakia is undocumented, the mean daily average ingestion of community water of 1.0 L/person/day for a consumer in the U.S. population was taken from EPA guidance (EPA 2004, p 4-2). The drinking water consumption rate of Slovakian children may be higher or lower.

Amount of Excess NIS Inhibition from Excess Nitrate in the School-Aged Children

The amount of excess NIS inhibition from excess nitrate in school-aged children is measured by the following ingested-weight basis using the OPEC equation (concept adapted from Tonacchera 2004; De Groef 2006, p 155):

$$\text{OPEC} = (\text{Amt of } ClO_4^- \text{ Inhibition}) + (\text{Amt of } NO_3^- \text{ Inhibition}) + (\text{Amt of } SCN^- \text{ Inhibition})$$
$$= \text{grams of } ClO_4^- + \text{grams of } NO_3^- /150 + 0.5 \text{ free } SCN^- \text{ x grams of } SCN^- /8.8$$

Where:

Oral inhibition potency of perchlorate = 1
Oral inhibition potency of nitrate relative to perchlorate = 1 / 150
Oral inhibition potency of thiocyanate relative to perchlorate = 1 / 8.8

Therefore, the amount of Excess NO_3^- Inhibition is:

$$\text{Excess OPEC}_{(\text{Children})} = \text{grams excess Nitrate} \div 150$$
$$= 150.8 \text{ mg/day} \div 150$$
$$= 1.005 \text{ mg of perchlorate equivalent/day}$$

Calculation of an Excess NIS Inhibition RfD form the Excess Nitrate Exposure

The thyroid hypertrophy observed in the HNA school-aged children is used to identify the NOAEL for the calculation of the excess NIS inhibition RfD. In a study of 143 Nigerian adults, the mean thyroid volume was reported to be 9.55 mls ± 1.82 (Ahidjo 2006). This value is consistent with the thyroid volume range of 8 -12 mls for females and 11 -15 mls for males in

Iceland, Sweden, the Netherlands, and the United States (Langer 1989). Therefore, the thyroid volume in an adult has to approximately double before being considered abnormal. The recommended upper limit for thyroid volume in an adult population is 18 mls (Ivanac 2004). The observed thyroid volume in the 10-year-olds increased from 4.58 mls in the LNA children to 5.10 mls in the HNA children (i.e., an 11% increase). Furthermore, the observed thyroid volume in the 13-year-olds increased from 5.23 mls in the LNA children to 5.97 mls in the HNA children (i.e., a 14% increase). The recommended upper limit for thyroid volume in children under 15 years old is 16 mls (Ivanac 2004). Although a statistically significant increase in thyroid volume was observed between the LNA and HNA children in the Tajtáková study from the increase exposure to nitrate in the drinking water, the increase in thyroid volume was not large enough to be considered a LOAEL.

No statistical difference was found in tT_4 and fT_3 thyroid hormone levels between the HNA and LNA schoolchildren. Since a decreased supply of fT_4 during pregnancy is the proposed mechanism leading to fetal brain damage, the lack of a decrease in the supply of T_4 suggests the observed thyroid volume effect is probably occurring below the NOAEL. Therefore, the excess NIS Inhibition $RfD_{(children)}$ calculated below is probably greater, hence the use of the greater-than symbol (≥).

A NOAEL is expressed on a daily dose-per-kg basis (i.e., mg/kg-day). Therefore, the average weight of the HNA school-aged children is needed, but this data point is not provided in the study. Therefore, the average weight of the HNA school-aged children needs to be estimated. The following table provides weight data on 10- and 13-year-old children to estimate an average weight for the test population:

	10-Year-Old HNA Children			13-Year-Old HNA Children		
Gender	**Number in HNA Group**	**Median Weight (kg)**	**Combined Weight (kg)**	**Number in HNA Group**	**Median Weight (kg)**	**Combined Weight (kg)**
Boys	49	31	1519	96	50	4800
Girls	68	29	1972	111	49	5439
Totals	**117**		**3491**	**207**		**10239**

Source of weight data: EPA 2004, table 7.2.

Therefore, the estimated median weight of the 324 HNA school-aged children is 42.4 kg (i.e., (10239 kg + 3491 kg)/(117 + 207 children)). Therefore, the calculated NOAEL for the median 42.4 kg HNA school-aged child is:

NOAEL ≥ 1.005 mg perchlorate equivalent/day /42.4 kg
≥ 0.0237 mg perchlorate equivalent/kg-day

The NAS Committee applied a total UF of 10 (i.e., an intraspecies factor) to the NOEL to protect the most sensitive population – the fetuses of pregnant women who might have hypothyroidism or iodide deficiency (NAS 2005, p 16). Although 10- and 13-year-old school-aged children are expected to be more sensitive to NIS inhibition than an adult, an intraspecies UF smaller than 10 could be proposed. However, EPA risk assessment guidance states that a reduction of the intraspecies UF from a default of 10 is to be considered only if the POD is

determined from the data obtained from the susceptible subpopulation (EPA 2002, p 4-43). Since children are not the sensitive population for this public health issue, this calculation of the excess NIS inhibition $RfD_{(Children)}$ from the excess exposure to nitrate in school-aged children uses a UF of 10 as follows:

$$\text{Excess NIS Inhibition } RfD_{(Children)} \geq 0.0237 \text{ mg perchlorate eq./kg-day} / 10 \text{ UF}$$
$$\geq 0.0024 \text{ mg perchlorate eq ./kg-day}$$

The calculated excess NIS inhibition RfD from excess nitrate exposure in school-aged children is greater than 0.0024 mg/kg-day. EPA's perchlorate RfD is 0.0007 mg/kg-day, obtained from the NAS Committee. The calculated excess NIS inhibition $RfD_{(children)}$ is at least 3.4 times greater than the NAS-recommended perchlorate RfD.

Determining the excess NIS inhibition RfD is an advantage because it used a conventional risk assessment approach of deriving an RfD by using a NOAEL observed in a human population. The NAS Committee describes its own approach of deriving an RfD from a NOEL as being an unconventional approach, but characterized it as being a "conservative, health-protective approach to the perchlorate risk assessment" (NAS 2005, p 15). The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's statement that its recommended perchlorate RfD is conservative. The excess NIS inhibition $RfD_{(Children)}$ observed in the Tajtáková nitrate exposure study indicates that the NAS-recommended perchlorate RfD is conservative by a factor of at least 3.4.

Discussion

The following findings can be made from the observations in the Tajtáková nitrate exposure study:

- The Tajtáková exposure study supports the concept that the exposure to the other NIS inhibitors, thiocyanate and nitrate, have the same mechanism of toxicity as perchlorate and an excess exposure on a molar potency adjusted basis is just as toxic as excess exposure to perchlorate. The toxic effects of the three NIS inhibitors are the same and the evaluation of the risk from three NIS inhibitors should not be separated, but should be considered together through a cumulative risk assessment approach.

- The conventionally derived excess NIS inhibition RfD from excess nitrate exposure in school-aged children is calculated to be 0.0024 mg/kg-day mg/kg-day. The NAS Committee's recommended perchlorate RfD is 0.0007 mg/kg-day. The conventionally derived excess NIS inhibition $RfD_{(Adult\ males)}$ is > 3.4 times greater than the NAS-recommended perchlorate RfD. The OIG's conventional approach for deriving a perchlorate RfD independently supports the NAS Committee's claim that its recommended perchlorate RfD is conservative.

- Since both the excess NIS inhibition RfD and the NAS Committee's recommended perchlorate RfD used a single chemical risk assessment approach to derive their respective RfD, both have the same limitations of a single chemical risk assessment.

6. Application of the Tonacchera Model to the Chilean Epidemiological Studies

This section discusses the application of the Tonacchera Model to the two epidemiological studies conducted on the perchlorate exposed populations of northern Chile.

6.1 Tellez Study

Dr. Rafael Tellez of the Medicine Service in Puente Alto, Chile, led a longitudinal epidemiological study in northern Chile to evaluate the impact of perchlorate exposure to drinking water of 113.9 µg/L on the maternal thyroid during pregnancy, neonatal thyroid function, the developmental status at birth, breast milk iodide, and perchlorate levels during lactation (Tellez 2005). The Tellez study was conducted in the same three coastal cities in northern Chile (i.e., Antofagasta, Chanaral, and Taltal) as the Crump study that evaluated the impact of perchlorate exposure on school-aged children and newborns (Crump 2000). Since only preliminary results from the Tellez study were available to the NAS Committee, the Tellez study was not used in the formulation of the NAS Committee's conclusions (NAS 2005, p 106).

The Tellez study accounted for both drinking water and dietary exposure to perchlorate. The pregnant mothers from Taltal were found to have perchlorate exposure at about 118.8 µg/day or 2.42 times the EPA RfD. The following table summarizes the perchlorate exposure in the pregnant mothers and neonates in the three northern Chilean cities:

Perchlorate Exposure in Chilean Pregnancy and Neonate Study

Parameter	Perchlorate Exposure Groups		
	Antofagasta	Chanaral	Taltal
Mean Perchlorate in Tap Water	0.5 µg/L < 4.0 µg/L (all samples)	5.82 ± 0.63 µg/L	113.9 ± 13.3 µg/L
Est. Drinking Water Perchlorate Exposure	0.42 µg/day	6.1 µg/day	93.5 µg/day
Est. Dietary Perchlorate Exposure	21.7 µg/day	33.8 µg/day	25.3 µg/day
Est. Median Daily Perchlorate Exposure (i.e., urinary excretion)	22.1 µg/day	40.0 µg/day	118.8 µg/day
Perchlorate Exposure Fraction of EPA RfD of 49 µg/day	0.45 RfD	0.82 RfD	2.42 RfD

Source: Tellez 2005.

The concept of external dose is a crude technique to measure human exposure. As seen in Clewell's perchlorate PBPK model, an external dose of 1 µg/kg-day generates different internal serum concentrations (i.e., internal indices) in each of the following life stages: fetus, neonate, child, adult, pregnant women, and lactating women (Clewell 2007, table 4). The use of an internal indice is critical because the biological impact is directly related to the concentration of perchlorate in direct contact with the NIS (i.e., bathing the symporter). Therefore, internal indices of perchlorate serum concentration are a better measure of exposure than the external perchlorate dose. The same external dose can result in significantly different internal indices. For example, an external dose of 1 µg/kg-day (i.e., 70 µg/day for an adult) would predict an adult's perchlorate serum concentration to be 2 µg/L. However, the same external dose of

1 µg/kg-day in a pregnant mother is predicted to have a 5 µg/L perchlorate serum concentration. Although both the adult and pregnant mother have the same external dose, the pregnant mother has 2.5 times the perchlorate serum concentration in direct contact with the NIS. This means the pregnant mother has 2.5 times the amount of NIS inhibition more than the adult for the same external dose. This is important because the perchlorate RfD is expressed as an external dose, not an internal indice.

The strategy of the 2007 NAS Toxicity Testing Committee was to define the critical internal tissue concentration (i.e., internal indices) that perturbs the biological pathway and then use pharmacokinetic modeling to determine what external dose generates the critical internal dose (NAS 2007, prepub. summary p 5). To apply the NAS Toxicity Testing Committee's approach to the perchlorate RfD, the internal perchlorate serum concentration must be determined at the external RfD dose of 0.7 µg/kg-day. The Clewell Perchlorate PBPK Model predicts a perchlorate serum of 2 µg/L for an adult at an external dose of 70 µg/day (i.e., 1 µg/kg-day or 1.42 times the RfD). If this value is linearly scaled down to the EPA RfD of 49 µg/day in an adult (i.e., 0.7 µg/kg-day), the predicted perchlorate serum concentrations at EPA's RfD in an adult would be 1.4 µg/L. However, the EPA RfD was derived by applying a 10-fold UF to the NOEL of 7 µg/kg-day observed in Greer's subjects (NAS 2005, p 15). Therefore, the perchlorate NOEL corresponds to an external dose of 490 µg/day. The closest point provided in Clewell's paper is that an external dose of 700 µg/day is predicted to generate a perchlorate serum concentration of 10 µg/L in an adult (Clewell 2007, table 4). If this value is linearly scaled down to the perchlorate NOEL of 490 µg/day in an adult (i.e., 7 µg/kg-day), the predicted perchlorate serum concentrations at the NOEL in an adult would be about 7 µg/L. Therefore, any perchlorate serum concentrations above 7 µg/L would be expected to generate observed effects (i.e., changes in the thyroid hormones and TSH levels).

The following table provides a summary of the internal indices exposures in the pregnant mothers and neonates in the Tellez study:

Serum Perchlorate Exposures in Tellez Study

Parameter	Perchlorate Exposure Groups		
	Antofagasta	Chanaral	Taltal
Mothers' Perchlorate Serum at ~16 Weeks of Gestation	< 4 µg/L (n = 3)	< 4 µg/L (n = 7)	10.9 ± 2.1 µg/L (n = 14)
Mothers' Perchlorate Serum at ~32 Weeks of Gestation	< 4 µg/L (n = 5)	< 4 µg/L (n = 5)	13.2 ± 1.7 µg/L (n = 6)
Neonates' Perchlorate Serum at Birth (i.e., newborns)	< 4 µg/L	< 4 µg/L	19.9 ± 5.0 µg/L (n = 14)

Source: Tellez 2005.

The observed perchlorate serum concentration in the pregnant Taltal mothers is 10.9 µg/L at ~ 16 weeks of gestation and 13.2 µg/L at ~32 weeks of gestation. This agrees with the predicted perchlorate concentration estimated by the Clewell PBPK model for pregnant women. An external dose of 70 µg/day is predicted by the model to generate a perchlorate serum concentration of 5 µg/L (Clewell 2007, table 4). A simple linear scaling up of the model's pregnant mothers' perchlorate serum concentration by a factor of 1.7 (i.e., 118.8 µg/day ÷ 70µg/day – because of the daily perchlorate exposure in Taltal of 118.8µg/day) gives a predicted serum concentration for the pregnant Taltal mothers of 8.5 µg/L.

In the Tellez study, the pregnant Taltal mothers had perchlorate serum concentrations of 10.9 µg/L and 13.2 µg/L. The Taltal neonates had an even higher perchlorate serum concentrations of 19.9 µg/L. All of these values are above the perchlorate NOEL observed in Greer's test subjects. Any perchlorate serum concentrations above 7 µg/L would be expected to generate observed effects (i.e., changes in the thyroid hormones and TSH). However, both the pregnant Taltal mothers and the Taltal newborns had normal health function thyroid performance (Tellez 2005, table 3).

As an indicator of thyroid stress, the differences in the TSH levels in the pregnant mothers across the three cities was not statistically significant. However, at each testing interval, the highest perchlorate exposure group (Taltal) always had the lowest TSH level. The T_3, free T_4, and TSH results listed in Table 3 of Tellez's paper for all three testing intervals indicate that the thyroids in the Taltal pregnant mothers are normal, healthy, and not challenged by the relatively large perchlorate exposure (Tellez 2005). The following table summaries the TSH levels in the pregnant mothers:

TSH levels in Pregnant Mothers in the Tellez Study

Parameter	Perchlorate Exposure Groups			Statistical Significance
	Antofagasta	Chanaral	Taltal	
TSH level (µU/ml) at ~16 Weeks of Gestation	2.63	2.81	2.61	0.91
TSH level (µU/ml) at ~32 Weeks of Gestation	3.69	2.55	2.08	0.63
TSH level (µU/ml) Postpartum	8.25	2.34	1.95	0.79

Source: Tellez 2005, table 3.

The differences in the free T_4 and TSH levels in the neonates across the three cities were not statistically significant. The T_3 level in Chanaral was statistically significantly lower than the other two cities. These data indicate that the thyroids in the Taltal neonates are normal, healthy, and not challenged by the relatively large perchlorate exposure. The following table summarizes the thyroid hormones, TSH, and serum perchlorate levels in neonates across the three cities:

Thyroid Hormones and TSH levels in Neonates in the Tellez Study

Parameter	Perchlorate Exposure Groups			Statistical Significance
	Antofagasta	Chanaral	Taltal	
T_3 (ng/dL)	79 ± 13.4	73 ± 17.9	82 ± 20.6	0.03
Free T_4 (ng/dL)	1.07 ± 0.16	1.04 ± 0.13	1.03 ± 0.14	0.73
TSH Level (µU/ml)	6.20 ± 2.96	6.69 ± 4.13	6.31 ± 2.91	0.99
Serum Perchlorate (µg/L)	< 4	< 4	19.9 ± 5.0	0.005

Source: Tellez 2005, table 4.

Given that the perchlorate NOEL determined in the Greer test subjects had an internal indice of 7 µg/L, the pregnant mothers and newborns in Taltal had serum perchlorate levels well above the NOEL. On an internal indices basis, the Taltal pregnant mothers were about 1.6 and 1.9 times the NOEL at ~16 and ~32 weeks of gestation, respectively. Furthermore, the Taltal neonates were about 2.8 times above the NOEL. Application of the Greer NOEL requires the Tellez study to have detected changes in the thyroid hormones and TSH levels in both the Taltal

pregnant mothers and neonates. This is a striking contradiction between the Greer study and the Tellez study that must be explained. It is simply not logical that the Taltal pregnant women and neonates are less sensitive to perchlorate inhibition then the adults in the Greer study. This contradiction between the Greer study and the Tellez study requires evaluating and considering potential confounding variable(s) at work to account for this discrepancy.

The Tonacchera Model identifies and simultaneously quantifies the amount of interaction between the following four variables affecting the uptake of iodide into the thyroid by the NIS: iodide, perchlorate, nitrate, and thiocyanate. The Tonacchera Model evaluates the total NIS inhibition load (i.e., total goitrogen load) acting on the body. The Tellez study does not provide nitrate exposure data in the pregnant mothers, but the study did measure perchlorate serum concentrations at ~16 and ~32 weeks of gestation and thiocyanate serum concentrations at postpartum. The following table summarizes the known NIS inhibitor exposures in the pregnant mothers (note that the NIS inhibitors are also provided in µmol/L to facilitate their use in determining the total goitrogen load acting on the pregnant mothers):

NIS Inhibitor Exposures in Pregnant Mothers in Tellez Study

Parameter	Perchlorate Exposure Groups		
	Antofagasta	Chanaral	Taltal
Perchlorate Serum at ~ 6 Weeks of Gestation	< 4 µg/L (n = 3) < 0.04 µmol/L	< 4 µg/L (n = 7) < 0.04 µmol/L	10.9 ± 2.1 µg/L (n = 14) 0.11 µmol/L
Perchlorate Serum at ~32 Weeks of Gestation	< 4 µg/L (n = 5) < 0.04 µmol/L	< 4 µg/L (n = 5) < 0.04 µmol/L	13.2 ± 1.7 µg/L (n = 6) 0.13 µmol/L
Thiocyanate Serum Postpartum	20.0 ± 10.9 µmol/L (n = 9)	17.4 ± 16.1 µmol/L (n = 21)	12.6 ± 6.4 µmol/L (n = 10)

Source: Tellez 2005.

The total NIS inhibition load (i.e., total goitrogen load) acting on the body is determined by calculating the SPEC as follows (concept taken from Tonacchera 2004):

SPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of free SCN^- Inhibition)
SPEC = $[ClO_4^-]$ + $[NO_3^-]$ /240 + [free SCN^-] /15

Where: [ ] is molarity
Serum inhibition potency of perchlorate = 1
Serum inhibition potency of nitrate relative to perchlorate = 1 / 240
Serum inhibition potency of thiocyanate relative to perchlorate = 1 / 15
[free SCN^-] = [total serum SCN^-] x 0.5 free SCN^- /total serum SCN^-

Using the measured perchlorate serum at ~32 weeks of gestation and thiocyanate serum concentrations at postpartum, the total NIS inhibitor load can be estimated in the pregnant mothers from each of the three cites. However, the Tellez study did not measure the nitrate exposure, and as another NIS inhibitor, nitrate could serve as an uncontrolled confounding variable. However, the amount of NIS inhibition typically contributed from nitrate is only about 11% (i.e., nitrate SPEC of 0.167 µmol/L out of a total SPEC for the adult of 1.501 µmol/L) of

the body's total goitrogen load. In the Western world, the typical nitrate serum concentration ranges from 10-140 µmol/L with the mean nitrate serum concentration being 30-50 µmol/L (Tonacchera 2004). Therefore, an estimated nitrate exposure of 40 µmol/L will be used for all three cities in this calculation of the pregnant mothers' total NIS inhibition load (i.e., since each value is the same, this will not change the relative ranking of total goitrogen loads between the cities). The nitrate exposure in Taltal would have to increase by at least a factor of 2 (i.e., to 80 µmol/L) over nitrate exposure in Antofagasta and Chanaral to cause Taltal to have the largest total goitrogen load. The following are calculations for the total NIS inhibition in the pregnant mothers in each of the three cities from the Tellez study (expressed as SPECs):

Antofagasta SPEC = 0.04 µmol/L + (40.0 µmol/L ÷ 240) + (20.0 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.04 µmol/L + 0.17 µmol/L + 0.67 µmol/L
= 0.88 µmol/L

Chanaral SPEC = 0.04 µmol/L + (40.0 µmol/L ÷ 240) + (17.4 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.04 µmol/L + 0.17 µmol/L + 0.58 µmol/L
= 0.79 µmol/L

Taltal SPEC = 0.13 µmol/L + (40.0 µmol/L ÷ 240) + (12.6 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.13 µmol/L + 0.17 µmol/L + 0.42 µmol/L
= 0.72 µmol/L

The total NIS inhibition load (i.e., total goitrogen load) acting on the neonate can also be determined by calculating the SPEC. The Tellez study did not measure thiocyanate concentrations in neonates. However, the OIG identified four papers that document the transfer of thiocyanate from the mother's blood to the neonate's blood through the placenta (Hauth 1984; Laurberg 2004; Nafstad 1995; Vanderpas 1984). We provided this information above in a table under the section Total NIS Inhibitor Exposure in Fetuses. These four papers clearly report thiocyanate serum concentration ratios of the cord serum to the mother's serum in the range of 68-111%. The observed thiocyanate concentrations in the pregnant mother at the postpartum examination was 20.0 µmol, 17.4 µmol, and 12.6 µmol for Antofagasta, Chanaral, and Taltal, respectively.

The closest match in the data from the four papers is in Hauth's study. The Hauth study reported a thiocyanate serum concentration of 32.2 µmol and 23 µmol in the mother's serum and the neonate's serum, respectively. In the Hauth study, the observed thiocyanate serum concentration ratio of the cord serum to the mother's serum in nonsmokers was 71%. The amount of thiocyanate transfer from the mother to the neonate will be used to estimate the expected thiocyanate concentrations in the neonates in the Tellez study. The amount of nitrate in the neonate's serum is not reported in the Tellez study. Therefore, nitrate is unknown and represents a potential confounding variable. However, the amount of NIS inhibition typically contributed by nitrate in an adult is only about 11% (i.e., nitrate SPEC of 0.167 µmol/L out of a total SPEC for the adult of 1.501 µmol/L) of the body's total goitrogen load. Furthermore, if the nitrate exposure is the same across the three cities, the relative ranking of total NIS inhibition load would not change. The following table summarizes the neonate exposure to NIS inhibitors (note that the NIS inhibitors are also provided in µmol/L to facilitate their use in determining the total goitrogen load acting on the neonates):

NIS Inhibitor Exposures in Neonates in Tellez Study

Parameter	Exposure Groups		
	Antofagasta	Chanaral	Taltal
Perchlorate Serum in Neonates at Birth	< 4 µg/L (n = 4) < 0.04 µmol/L	< 4 µg/L (n = 1) < 0.04 µmol/L	19.9 ± 5.0 µg/L (n = 14) 0.20 µmol/L
Thiocyanate Serum in Pregnant Mother at Postpartum	20.0 ± 10.9 µmol/L (n = 9)	17.4 ± 16.1 µmol/L (n = 21)	12.6 ± 6.4 µmol/L (n = 10)
Estimated Neonate Thiocyanate Serum Using a 70% Equilibrium	14.2 µmol/L	12.4 µmol/L	8.9 µmol/L

Source: Tellez 2005.

The following are calculations for the total NIS inhibition in neonates in each of the three cities in the Tellez study (expressed as SPECs):

Antofagasta SPEC = 0.04 µmol/L + (nitrate ? µmol/L ÷ 240) + (14.2 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.04 µmol/L + unknown nitrate amount + 0.47 µmol/L
= 0.51 µmol/L

Chanaral SPEC = 0.04 µmol/L + (nitrate ? µmol/L ÷ 240) + (12.4 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.04 µmol/L + unknown nitrate amount + 0.41 µmol/L
= 0.45 µmol/L

Taltal SPEC = 0.13 µmol/L + (nitrate ? µmol/L ÷ 240) + (8.9 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.20 µmol/L + unknown nitrate amount + 0.30 µmol/L
= 0.50 µmol/L

The Tellez epidemiological study was designed to compare the thyroid health effects in the high perchlorate exposure city of Taltal against two cities, Antofagasta and Chanaral, which have much lower perchlorate exposure. The authors concluded that " . . . the perchlorate dose in Taltal was not sufficiently high to stress the maternal or neonatal thyroid." However, nitrate and thiocyanate are also known NIS inhibitors and act as potential confounding variables. Since thiocyanate levels were measured in the Tellez study, this analysis shows that the level of thiocyanate exposure in both the pregnant mothers and neonates causes a much greater amount of NIS inhibition than even the amount of NIS inhibition from consuming 118 ppb of perchlorate in the tap water. The relatively low thiocyanate exposure in Taltal causes the Taltal group to actually have the least amount of total NIS inhibition and the least "stressed" or challenged thyroids in the group (i.e., excluding the impact of iodide, to be discussed later). In other words, when considered from a total goitrogen load, the experimental design of this epidemiological study is flipped: Taltal is the control group (i.e., no longer the exposed group), and Antofagasta is the exposed group (i.e., no longer the control group). Actually, since there is only a relatively small difference in the Total NIS Inhibitor Load in the pregnant mothers between Taltal and Antofagasta, the study becomes the equivalent of comparing three control groups together. From the view point of total NIS inhibition load, there is no surprise then that the data show little difference in the thyroid health between the three cities. The following table summarized the relative total NIS inhibition load in the Tellez study:

Relative Total NIS Inhibition Load in the Tellez Study

Parameter	Cities		
	Antofagasta	Chanaral	Taltal
Mean Perchlorate in Tap Water	0.5 µg/L	5.82 µg/L	113.9 µg/L
Estimated Total Perchlorate Exposure	22.1 µg/day	40.0 µg/day	118.8 µg/day
Relative Perchlorate Exposure	Low	medium	high
Estimated Total NIS Inhibition Load in Pregnant Mothers	0.88 µmol/L	0.79 µmol/L	0.72 µmol/L
Est. NIS Inhibition Load in Neonate (excluding nitrate due to a lack of data)	0.51 µmol/L	0.45 µmol/L	0.50 µmol/L
Total NIS Inhibition Load Observed in Pregnant Mothers (i.e., total goitrogen load)	Highest (becomes the experimental group)	In the middle	Lowest (becomes the control group)
Difference in Total NIS Inhibition Load Observed in Neonates (i.e., total goitrogen load)	Not much difference	Not much difference	Not much difference

Source: OIG Analysis; summary of Tellez study results.

The Tellez study can be related back to the conditions in the United States using the Tonacchera Model. In the United States, the typical total NIS inhibition load is 1.500 µmol/L (which, calculated previously in this document, assumes a median serum concentration of 40µmol/L for both nitrate and thiocyanate). In the Tellez study in Chile, the pregnant mothers' total NIS inhibition load was 0.88 µmol/L, 0.79 µmol/L, and 0.72 µmol/L in Antofagasta, Chanaral, and Taltal, respectively. Therefore, the typical U.S. adult has a total NIS inhibition load that is 170%, 190%, and 208% greater than those in Antofagasta, Chanaral, and Taltal, respectively. Furthermore, the median UIC is 140 µg/L in U.S. pregnant women (NAP 2000). In the Tellez study, the entire Chilean study group had a reported median UIC of 269 µg/L which is 92% greater than the United States. The recommended UIC for pregnant and lactating women is reported at 200-300 µg/L (Delange 2005, p 278, table 11E.8). The median UIC indicating optimal iodide nutrition during pregnancy and lactating women could be in the range of 150-230 µg/L (Delange 2004).

The difference in TIU between pregnant women in the United States and Chile can be calculated using the Tonacchera Model TIU is calculated by using the following equation:

TIU = constant x $[I^-]$ / (1.22 + [SPEC])

where: SPEC = $[ClO_4^-]$ + $[NO_3^-]$ /240 + [free SCN^-] /15

Note: The Tonacchera Model uses concentration expressed in units of µmol/L.

Restated:

TIU is proportional to $[I^-]$ / (1.22 + [SPEC])

Estimated TIU in U.S. Pregnant Women

The TIU in the U.S. pregnant women is determined by substituting values into the Tonacchera Model. The $[I^-]$ in the serum is unknown but is directly related to the UIC. The $[I^-]$ is assigned a value of "x". In the United States, the typical total NIS inhibition load is 1.501

μmol/L (which, calculated previously in this document, assumes a median serum concentration of 40μmol/L for both nitrate and thiocyanate).

Therefore, the TIU in the U.S. pregnant women is expressed by:

$TIU_{(U.S.\ pregnant\ women)} \parallel [I^-] / (1.22 + [SPEC])$

where: the symbol || means "proportional to"

Substitution gives:

$TIU_{(U.S.\ pregnant\ women)} \parallel x / (1.22 + (1.501\ \mu mol/L)$

$TIU_{(U.S.\ pregnant\ women)} \parallel x / 2.721$

$TIU_{(U.S.\ pregnant\ women)} \parallel 0.368x$

Estimated TIU in Chilean Pregnant Women

The TIU in the Chilean pregnant women is determined by substituting values into the Tonacchera Model. The $[I^-]$ is unknown but is directly related to the UIC. The median UIC of 269 μg/L is 92% greater than in the U.S. pregnant women. The $[I^-]$ in the Chilean pregnant women is assigned a value of "1.92x". For simplicity of comparison to the United States, the Chilean pregnant mothers' total NIS inhibition load from all three cities is averaged to 0.80 μmol/L (i.e., (0.88 μmol/L + 0.79 μmol/L + 0.72 μmol/L) ÷ 3).

Therefore, the TIU in the Chilean pregnant women is expressed by:

$TIU_{(Chilean\ pregnant\ women)} \parallel [I^-] / (1.22 + [SPEC])$

Substitution gives:

$TIU_{(Chilean\ pregnant\ women)} \parallel 1.92x / (1.22 + (0.80\ \mu mol/L)$

$TIU_{(Chilean\ pregnant\ women)} \parallel 1.92x / 2.02$

$TIU_{(Chilean\ pregnant\ women)} \parallel 0.950x$

Percent Change in TIU of U.S. Pregnant Women as Compared to Chilean Pregnant Women

The percent change in TIU of U.S. pregnant women as compared to Chilean pregnant women from the Tellez study is given by the following equation:

$$\%\ \text{Change in TIU of U.S. Pregnant Women} = \frac{\text{U.S. TIU - Chilean TIU}}{\text{Chilean TIU}} \times 100\%$$

Substitution gives:

% Change in TIU of U.S. Pregnant Women = ((0.368x - 0.950x)/ 0.950x) x 100%

% Change in TIU of U.S. Pregnant Women = - 61%

Therefore, the median U.S. pregnant woman has a 61% less TIU as compared to the Chilean pregnant women in the Tellez study. In other words, the U.S. median pregnant woman has only 39% of the amount of iodide uptake as her Chilean counterpart. In the Tellez study, the entire Chilean cohort had a reported median UIC of 269 μg/L, which is in the middle of the recommended UIC for pregnant and lactating women of 200-300 μg/L (Delange 2005, p 278, table 11E.8). The 61%TIU deficit is driven by the difference in the goitrogen load and iodide nutritional status between the United States and Chile. In other words, U.S. pregnant women

have about twice the goitrogen load as their Chilean counterparts, while having only about half the dietary intake of iodide. Both factors contribute to the decreased uptake of iodide by U.S. pregnant women.

Relative Contribution of the TIU Deficit from Goitrogen Load and from Iodide Nutrition

The Tonacchera Model can be used to assess the amount of TIU deficit attributed to the difference in the goitrogen load and to assess the amount of TIU deficit attributed to the difference in iodide nutrition. This is calculated by analyzing the contribution of each to the TIU separately.

Change in TIU from the change in goitrogen load = ((0.368x - 0.495x)/ 0.495x) x 100%
= - 25.7%

Change in TIU from the change in iodide nutrition = ((x - 1.92x)/ 1.92x) x 100%
= - 47.9%

Note: The 26% and 48% are not added directly together to get the total 61% decrease TIU deficit value. They are combined mathematically in the following way to give 61%:

Total Decrease = 1-((1-0.257)(1-0.479))
= 1-((0.743)(0.521)
= 1-(0.387)
= 0.612 or 61%

To further explain, of the original Chilean TIU, 26% is lost due to the increased U.S. goitrogen load leaving 0.74 TIU. Of the remaining 0.74 TIU, 48% of this amount is lost due to the decreased U.S. iodide nutrition leaving only 0.385 TIU (i.e., 0.74 – (0.74 x 0.48). This leaves 39% of the original TIU, or a 61% decrease from the Chilean TIU (defined as 100% for this comparison).

Therefore, the larger goitrogen load in the United States, by itself, causes the TIU to decrease by 61%. By contrast, the lower iodide nutritional status in the United States, by itself, causes the TIU to decrease by 48%. When the two factors (i.e., increased goitrogen load and decrease availability of iodide) act together, a 61% decrease in the TIU in the United States is predicted by the Tonacchera Model.

6.2 Crump Study

The Crump study compared the thyroid hormone and TSH levels in 9,784 newborns and in 162 school-aged children in three Chilean cities (Crump 2000). The mean urinary iodide, TSH, and T_3 levels were not statistically different in the schoolchildren among the three cities. However, the NAS Committee specifically commented, "Serum free T_4 levels were significantly higher in children in Chanaral and Taltal then Antofagasta . . . the difference was opposite the direction predicted on the basis of competitive perchlorate inhibition of iodide uptake" (NAS 2005, p 100). This contradiction in observed direction of the effect on free T_4 levels should have raised an inquiry into reviewing the mechanism (i.e., because competitive inhibition from perchlorate only does not appear to explain the observed effect). Ideally, this should have triggered the possibility that a confounding variable was at work in the data (i.e., other NIS inhibitors).

The potential confounding variable is the different levels of thiocyanate exposure observed in the Tellez study between Taltal and the other two cities. Based on the trend in amount of total NIS inhibition load observed across the three cities in the Tellez study (identified in the previous section), the trend in the serum free T_4 levels in the Crump schoolchildren is in the direction predicted on the basis of total competitive inhibition of iodide uptake. Using the total goitrogen load better explains the trend in observations seen in the serum free T_4 levels. The following table summarizes the study results in the schoolchildren:

Summary of Crump Study Results of All Schoolchildren by City

Parameter	Antofagasta (n = 60)	Chanaral (n = 60)	Taltal (n = 60)
Perchlorate Concentration in Drinking Water (µg/L)	ND All samples < 4.0 µg/L	5.5 ± 1.6	111.6 ± 6.7
Perchlorate Exposure	very low group	low group	high group
TSH (µU/ml)	3.3 ± 1.8	2.9 ± 1.3	3.0 ± 1.4
Free T_4 (ng/dL)	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.2
T_3 (ng/dL)	208.6 ± 29.7	211.0 ± 20.9	212.0 ± 21.9
Urinary Iodide (µg/L)	756 ± 404	614 ± 357	766 ± 474
Est. Total NIS Inhibition Load in Pregnant Mothers in Tellez Study	1.54 µmol/L	1.37 µmol/L	1.14 µmol/L
Relative Total NIS Inhibition Load Observed in Pregnant Mothers and Neonates in Tellez Study	high	medium	low

Source: Crump 2000, table 2.

For the newborns in the Crump study, the newborn TSH levels were slightly lower in Taltal (highest perchlorate exposure group) as compared with the two low perchlorate exposure cities ($p<0.001$) (Crump 2000). Furthermore, the log serum TSH levels in the Taltal newborns were statistically lower than the other two cities ($p<0.001$); the log TSH levels did not vary significantly between the newborns in Antofagasta and Chanaral (Crump 2000; NAS 2005). Again, the NAS Committee specifically commented, "The lower mean in Taltal is, again, opposite in direction of what would be expected in association with increased exposure to perchlorate" (NAS 2005, p 101). Again, this contradiction in observed direction of the predicted inhibition effect of perchlorate on TSH levels should have raised an inquiry into reviewing the mechanism (i.e., competitive inhibition of perchlorate only does not appear to explain the

observed effect). Ideally, this should have triggered the possibility that a confounding variable is at work in the data (i.e., the other NIS inhibitors).

The potentially confounding variable is the different levels of thiocyanate exposure observed in the Tellez study between Taltal and the other two cities. Based on the trend in amount of total NIS inhibition load of pregnant mothers observed across the same three cities in the Tellez study, the trend in the TSH levels in the Crump neonates is in the direction predicted on the basis of the total competitive inhibition of iodide uptake in the pregnant mothers. Furthermore, by day 7, when the neonate's thyroid is the sole influence on TSH level (i.e, mother's residual influence is gone), the neonate TSH levels are essentially the same across the three cites (see table below). This mirrors the observation that the total goitrogen load estimated in the neonates from the 2005 Tellez study is also essentially the same across the three cities. Using the total goitrogen load better explains the observations seen in the serum TSH levels in the Taltal newborns than the perchlorate exposure level only. The following table summaries the study results in the neonates:

Summary of Crump Study Results of Neonates by City

Parameter	Antofagasta (n = 8888)	Chanaral (n = 468)	Taltal (n = 428)
Perchlorate Concentration in Drinking Water (µg/L)	ND All samples < 4.0 µg/L	5.5 ± 1.6	111.6 ± 6.7
Perchlorate Exposure	Very low group	low group	high group
TSH (µU/ml) Overall Both Genders	3.4 ± 7.8	3.0 ± 2.5	2.4 ± 1.9
Estimated Total NIS Inhibition Load in Pregnant Mothers from the Tellez Study	0.88 µmol/L	0.79 µmol/L	0.72 µmol/L
Relative Total NIS Inhibition Load Observed in Pregnant Mothers and Neonates in Tellez Study	high	medium	low
TSH (uU/ml) Day 7 Both Genders	2.4 ± 2.0	2.1 ± 1.4	2.1 ± 1.7
Est. NIS Inhibition Load in Neonate from the Tellez Study (excluding nitrate - lack of data)	0.51 µmol/L	0.45 µmol/L	0.50 µmol/L

Source: Crump 2000, table 8.

According to the NAS Committee, the major criticisms of the Crump study indicated that the school-aged children had an iodide status different than those in the United States and a higher prevalence of goiter (NAS 2005, p 103). The mean urinary iodide excretion in the children was about 3 times higher than in the United States (NAS 2005, p 103). The NAS Committee finally concluded that the Crump study could be considered in evaluating perchlorate exposure in the United States (NAS 2005, p 105).

The Crump study did not determine the amounts of nitrate or thiocyanate in any of the subjects. The NAS Committee notes the possibility of "uncontrolled confounders" in the Crump study as in any epidemiologic study (NAS 2005, p 103). Since the OIG has shown that the thiocyanate NIS inhibitor was an uncontrolled confounder in the Tellez study, this same confounder probably applies to the Crump study because it involves the same population from the same cites with the same potential difference in dietary habits and explains the observations better.

6.3 Conclusions

The OIG's analysis of the Tellez study shows the advantages of applying cumulative risk assessment to the environmental problem of perchlorate exposure. The biological step perturbed by perchlorate exposure is the uptake of iodide by the NIS. However, the uptake of iodide by the NIS is not just a function of exposure to perchlorate. The amount of iodide uptake by the NIS is the result of the combined effects of simultaneous exposure to all four anions: iodide, perchlorate, nitrate, and thiocyanate. The effects of each anion are defined by the Tonacchera Model, where TIU is proportional to $[I^-] / (1.22 + [SPEC])$. Measuring the internal indices of exposure (i.e., µmol/L concentration in serum) for iodide, perchlorate, nitrate, and thiocyanate is needed to more accurately characterize the amount of stress on the thyroid.

The Chilean pregnant women and neonates have healthy thyroids because their iodide nutritional status is sufficient to tolerate their total goitrogen load. The fact that the relatively high exposure to 118 µg/day of perchlorate from water and food in Taltal represents only a small portion (11%) of their total goitrogen load, and to say that this level of perchlorate exposure did not generate thyroid health problems, is to miss the larger point. The potential impact of perchlorate exposure cannot be assessed without knowing the concurrent exposure to the other NIS inhibitors (nitrate and thiocyanate) and the iodide status of the population/individual. The essential finding from the Tellez study is that the total goitrogen load in Taltal (including the perchlorate) is small compared to the healthy iodide nutritional status.

The critical information needed to characterize this public health problem is determining when the iodide nutritional level is insufficient to tolerate the total goitrogen load. The relationship between the iodide nutritional level and total goitrogen load is dynamic. As the availability of iodide increases, the tolerance for larger total goitrogen loads increases. In other words, iodide-deficient individuals cannot tolerate as much goitrogens as an individual with a healthy iodide intake. Defining when the iodide nutritional level is insufficient for the total goitrogen load and its impact on the regulation of perchlorate is the subject of a later section of this document.

7. Assessment of Total NIS Inhibitor Exposure

7.1 Perchlorate Exposure

To assess the role of perchlorate exposure on this public health issue, the amount of perchlorate exposure must be documented in adults, fetuses, and nursing neonates.

7.1.1 Adults

In 2006, Blount of the CDC, National Center for Environmental Health (NCEH), assessed the perchlorate exposure in the U.S. population using urinary biomonitoring data (Blount 2006a). As part of the 2001-2002 NHANES, a nationally representative population of 2,820 U.S. residents (ages 6 years and older) provided urine samples for perchlorate testing. All 2,820 urine samples tested found detectable levels of perchlorate (i.e., >0.05µg/L), indicating that perchlorate exposure in the U.S. population is common. Blount's biomonitoring results are summarized below:

Population	Blount's U.S. Perchlorate Exposure Assessment from 2001-2002 NHANES Using Urinary Biomonitoring Data	
	Median	95th Percentile
Adults (20 years and older)	0.066 µg/kg-day (4.6 µg/day for a 70 kg adult)	0.234 µg/kg-day (16.4 µg/day for a 70 kg adult)
Reproductive Females (15-44 years old)	0.057 µg/kg-day (4.0 µg/day for a 70 kg adult)	0.214 µg/kg-day (15.0 µg/day for a 70 kg adult)

Source: Blount 2006a.

The perchlorate RfD is 0.7 µg/kg-day (equal to 0.0007 mg/kg-day). For a 70 kg adult, the perchlorate RfD allows the "safe" exposure to 49 µg of perchlorate per day for an adult. Therefore, the median perchlorate exposure is 9.4% and 8.1% of the perchlorate RfD in adults and females of reproductive age, respectively. Furthermore, the perchlorate exposure at the 95th percentile is 33% and 31% of the perchlorate RfD in adults and females of reproductive age, respectively. Therefore, the Blount biomonitoring study indicates that the perchlorate exposure in the majority of adults in the United States is well below the RfD. In fact, Blount's biomonitoring study found that only 11 of the 1,618 adults (i.e., 20 years or older) tested had an estimated perchlorate exposure in excess of the perchlorate RfD of 0.7 µg/kg (i.e., 49 µg per day). This corresponds to about 0.7% of the adult U.S. population that might have perchlorate exposures greater than the RfD.

In May 2007, the FDA's Center for Food Safety and Applied Nutrition estimated the human dietary exposure to perchlorate from the consumption of 27 foods and beverages from samples collected in Fiscal Years 2004 and 2005. The preliminary human perchlorate dietary exposure assessment, "Preliminary Estimation of Perchlorate Dietary Exposure Based on FDA 2004/2005 Exploratory Data," was posted on FDA's Website on May 31, 2007 (FDA 2007a). FDA's preliminary testing represents only 32% of the food and 42% of the beverages in the diet. Since these samples targeted foods grown in areas known to have perchlorate present, the results from this study are not representative of total perchlorate exposures. The remaining 68% of the foods in the diet are expected to contain little to no perchlorate, although this assumption cannot

be verified. Using FDA's stated assumption, FDA's preliminary perchlorate dietary exposure results represent the bulk of the perchlorate exposure expected from the U.S. diet:

Population	FDA's Preliminary Perchlorate Dietary Exposure Estimate	
	Mean	90th Percentile
All Ages (2 and up)	0.053 µg/kg-day (3.7 µg/day for a 70 kg adult)	0.12 µg/kg-day (8.4 µg/day for a 70 kg adult)
Females (15-45 years)	0.037 µg/kg-day (2.6 µg/day for a 70 kg adult)	0.074 µg/kg-day (5.2 µg/day for a 70 kg adult)

Source: FDA 2007a.

On January 2, 2008, FDA published their TDS on perchlorate and iodide (Murray 2008). The TDS is an ongoing market basket survey in which 285 core foods (TDS foods) in the U.S. food supply are collected and analyzed to determine levels of various contaminants and nutrients in those foods. The foods collected in the TDS (referred to as the TDS food list) represent the major components of the diet of the U.S. population. The food list is based on results of national food consumption surveys and is updated from time to time to reflect changes in food consumption patterns. The FDA TDS reported the upper and lower bounds for the average dietary intakes of perchlorate for each of the 14 age/sex groups from FDA's TDS samples collected in Fiscal Years 2005 and 2006. The upper and lower bounds for the average dietary intakes of perchlorate for infants 6 to 11 months and nonpregnant women 25 to 30 years are:

Age/Sex Group Label	FDA's Total Dietary Study: Dietary Intake of Perchlorate	
	Lower and Upper Bound AverageTotal Dietary Intake	90th Percentile Total Dietary Intake
Infants (6 to 11 months)	0.26-0.29 µg/kg-day 2.4 to 2.7 µg/day	Not provided for in the TDS Design*
Women (25 to 30 years)	0.09 to 0.11 µg/kg-day 5.4 to 6.8 µg/day	Not provided for In the TDS Design*

* FDA TDS is not designed to estimate intakes at the extremes (e.g., 10th or 90th percentiles). Murray 2008, p 8.
Source: Murray 2008, table 5.

The FDA TDS study has two significant limitations for characterizing the perchlorate exposure in a risk assessment. Perchlorate exposure during pregnancy and nursing is the critical period in which NIS stressor exposure could induce a low TIU resulting in an increased risk for adverse effects in the developing child. However, the FDA TDS does not provided estimates for dietary intakes for subgroups with specific nutritional needs (e.g., pregnant or lactating women). The second limitation is that the FDA TDS does not provide estimates for the distribution of perchlorate exposure within the age/sex group populations (e.g., 10th and 90th percentiles). Ideally, a risk assessment would benefit from an exposure estimate of the 90th percentile, but the FDA TDS does not provide this type of information.

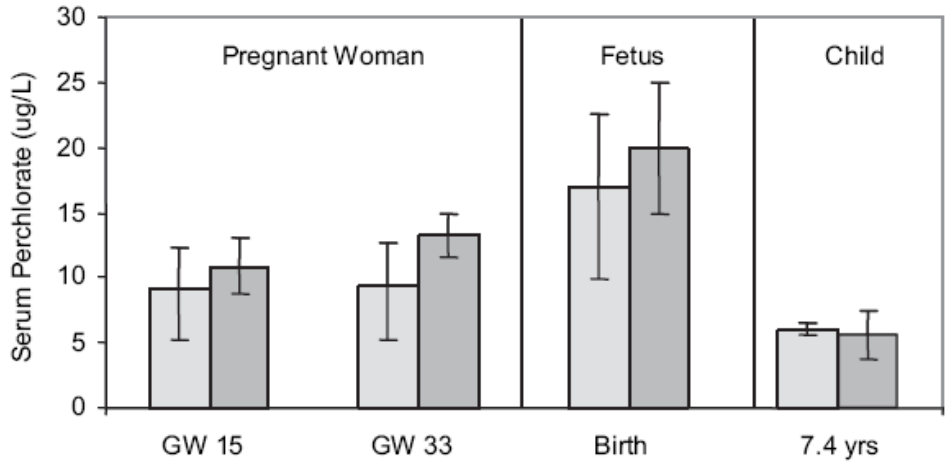
FDA's human perchlorate preliminary dietary exposure assessment and the FDA TDS are in general agreement with the perchlorate exposure measured in Blount's biomonitoring data. Specifically, FDA's preliminary mean perchlorate exposure calculated for an adult is 3.7 µg/day. The FDA TDS estimated a lower and upper bound average total dietary intake for all age/sex groups ≥ 25 years old at 0.08 to 0.12 µg/kg-day (i.e., 5.6 to 8.4 µg/day for 70 kg adult) (Murray 2008, table 5). These values agree with the median perchlorate exposure of 4.6 µg/day estimated from the Blount biomonitoring data. The average perchlorate exposures from the FDA

preliminary data and Blount biomonitoring data are less than 10% the perchlorate RfD of 49 µg/day for an adult. Furthermore, FDA's preliminary dietary exposure assessment at the 90th percentile is calculated at 8.4 µg/day or 17% of the RfD. The estimated perchlorate exposure at the 95th percentile using the Blount biomonitoring data is calculated at 16.4 µg/day, or 33% of the RfD. Both upper percentile values are significantly less than the perchlorate RfD of 49 µg/day for an adult.

7.1.2 Fetuses

Assessing the perchlorate exposure in fetuses is difficult; direct measurements are not possible due to the risk to the child. Therefore, the use of PBPK modeling is required to predict the amount of exposure in the fetus. The Clewell Perchlorate PBPK Model has accurately predicted the serum perchlorate concentrations in sensitive populations including fetuses (Clewell 2007). The Clewell Perchlorate PBPK Model predictions for perchlorate serum concentrations were corroborated against pregnant women at the ~16 and ~32 week of gestation, fetuses at birth observed in the Tellez study, and 7.4-year-old schoolchildren observed in the Crump study (Clewell 2007) (see figure below). This use of the Clewell Perchlorate PBPK Model provides the ability to estimate the fetal perchlorate exposure based on the mother's perchlorate exposure.

Clewell Perchlorate PBPK Model Predicted Perchlorate Serum Concentrations



The PBPK model predicted values (light grey) are the average ± SD (standard deviation). The measured perchlorate serum concentrations (dark grey) are the mean ± SD. The pregnant woman value is from the Tellez Chilean study (Tellez 2005). The 7.4-year-old child value is reported by Gibbs (Gibbs 2004) of the Chilean schoolchildren from the Crump study (Crump 2000).

Source: Clewell 2007, figure 10.

The Clewell Perchlorate PBPK Model predicts that the internal perchlorate serum concentration in a fetus will be 5 times greater then the internal perchlorate serum concentration in an adult from an external perchlorate dose of 0.001 mg/kg-day (Clewell 2007, table 4). Using the Blount biomonitoring data to characterize the perchlorate exposure in U.S. population, if a pregnant woman is exposed to the median adult perchlorate exposure of 4.6 µg/day (i.e., 9.4% of

the RfD), the fetus would be expected have an internal perchlorate serum concentration that corresponds to 47% that of the RfD. Likewise, if a pregnant woman is exposed to the 95th percentile perchlorate exposure for an adult of 16.4 µg/day (i.e., 33% of the RfD), the fetus would be expected have an internal perchlorate serum concentration that corresponds to 1.67 times the perchlorate RfD. Finally, if a pregnant woman is exposed to perchlorate at the RfD, the fetus would be expected have an internal perchlorate serum concentration that corresponds to 5 times the perchlorate RfD.

7.1.3 Nursing Neonates

If exclusively breastfed, nursing infants are dependent on their mothers' milk for all their nutrition, including the essential element, iodide. Both thyroidal and extrathyroidal NIS concentrate iodide from the serum into the thyroid by roughly the same magnitude (i.e., thyroid to serum ration (T/S) is 20- to 40-fold under steady-state conditions) (De La Vieja 2000, p 1093). In cases of iodide deficiency, the TSH stimulates the production of cyclic adenosine monophosphe (cAMP) in the thyroid to enhance the rate of active transport of iodide into the thyroid by the NIS (McMurray 1983, p 170). The T/S ratio may increase up to 300:1. TSH regulates the expression and activity of the NIS in the thyroid gland only (Merrill 2003; Clewell 2003). TSH does not regulate NIS expression in the mammary gland or any other extrathyroidal tissue (Merrill 2003). The NIS expression and regulation appear to be controlled by prolactin and/or oxytocin (Merrill 2003).

Nursing neonates are a sensitive population because an adequate supply of thyroid hormones is required for proper brain development. However, nursing neonates may be at a significant risk due to a relatively large perchlorate exposure (i.e., on a per-body-weight basis) due to the active concentration of perchlorate in breast milk by the NIS. Neonate perchlorate exposure is a function of the weight of the infant, the amount of milk consumption, and the perchlorate concentration of the milk. A 1-month-old infant's median weight is 4.14 kg (EPA 1997a) and these infants consume a mean intake of 673 ml of milk (Dewey 1983). A 6-month-old infant's median weight is 7.53 kg (EPA 1997a), and those infants consume a mean intake of 896 ml of milk (Dewey 1983). Therefore, the mean perchlorate concentration in breast milk that induces a perchlorate RfD exposure of 0.0007 mg/kg-day in a median sized neonate is:

For a 1-month-old infant:

(0.7 µg/kg-day x 4.14 kg body weight) ÷ 0.673 L of milk/day = 4.3 µg/L

For a 6-month-old infant:

(0.7 µg/kg-day x 7.53 kg body weight) ÷ 0.896 L of milk/day = 5.9 µg/L

In fall 2003 and spring 2004, Kirk evaluated the concentrations of perchlorate and iodide in the breast milk of 36 healthy women across 18 States and 23 healthy women across 14 States, respectively (Kirk 2007). The following table summaries the study results for the concentration of perchlorate and iodide found in human breast milk:

Analyte	Perchlorate and Iodide Concentrations in Human Breast Milk			
	Number of Subjects (n)	Range (µg/L)	Mean * (µg/L)	Median (µg/L)
Perchlorate	36	1.4 to 92.2	10.5 ± n/a	3.3
Iodide	23	4.5 to 184.5	63.3 ± n/a	33.5

* No mean statistics provided in the paper. The paper provided an average for each test subject comprising 4 to 6 samples per subject.
Source: Kirk 2005.

Published in February 2007, Kirk evaluated the concentrations of perchlorate, thiocyanate, and iodide in the breast milk of 10 nonsmoking lactating women with five subjects from the Texas Panhandle and one subject from each of the following States: Colorado, Florida, Missouri, New Mexico, and North Carolina (Kirk 2007). Using an average milk consumption of 100 ml/kg-day, the authors found that 3 of 10 subjects averaged breast milk perchlorate concentrations above 7 µg/L, which would cause these 3 infants to exceed the perchlorate RfD of 0.7 µg/kg-day. As the infant grows, the infant's exposure per unit weight decreases. The authors project that an average weight infant consuming an average amount of milk would exceed the perchlorate RfD for the first 2 months of life. Furthermore, the authors found that the median iodide concentration of 55.2 µg/L is substantially below the recommended level of 110-130 µg/day. The following table summarizes the study's results for the concentration of perchlorate and iodide found in human breast milk:

Analyte	Perchlorate and Iodide Concentrations in Human Breast Milk in 10 Nonsmoking Women			
	Number of sample (n)	Range (µg/L)	Mean ± SD (µg/L)	Median (µg/L)
Perchlorate	147	0.5 to 39.5	5.8 ± 6.2	4.0
Iodide	108	3.1 to 334	87.9 ± 80.9	55.2

Source: Kirk 2007.

Between July 2002 and April 2006, breast milk and urine of 57 healthy lactating women in the Boston area were analyzed for perchlorate and iodide concentrations (Pearce 2007). The following table summaries the study's results for the concentration of perchlorate and iodide found in human breast milk:

Analyte	Perchlorate and Iodide Concentrations in Breast Milk and Urine Samples from 57 Healthy Boston Women			
	Number of sample (n)	Range (µg/L)	Mean ± SD (µg/L)	Median (µg/L)
Perchlorate	49	1.3 to 411	33 ± 77	9.1
Urinary Perchlorate	56	0.37 to 127	8.2 ± 19	3.0
Iodide	57	2.7 to 1968	205 ± 271	155
Urinary Iodide	57	25 to 920	155 ± 142	114

Source: Pearce 2007.

Estimate Using the Clewell Perchlorate PBPK Model

The Clewell Perchlorate PBPK Model predicts that the internal perchlorate serum concentration in a 1.5-month-old nursing neonate will be 4 times greater then the internal perchlorate serum concentration in an adult from an external perchlorate dose of 0.001 mg/kg-day (Clewell 2007, table 4). Using the Blount biomonitoring data to characterize the perchlorate exposure in the U.S. population, if a pregnant woman is exposed to the median adult perchlorate exposure of 4.6 µg/day (i.e., 9.4% of the RfD), the nursing neonate would be expected to have an internal perchlorate serum concentration that corresponds to 37% that of the RfD. Likewise, if a pregnant woman is exposed to the 95th percentile perchlorate exposure for an adult of 16.4 µg/day (i.e., 33% of the RfD), the nursing neonate would be expected have an internal perchlorate serum concentration that corresponds to 1.34 times the perchlorate RfD. Finally, if a pregnant woman is exposed to perchlorate at the RfD, the nursing neonate would be expected have an internal perchlorate serum concentration that corresponds to 4 times the perchlorate RfD.

7.2 Total NIS Inhibitor Exposure

Since perchlorate is not the only NIS inhibitor to which these three groups are exposed, the perchlorate exposure must be evaluated in context with the total amount of NIS inhibition on the body (i.e., the goitrogen load). To determine the total goitrogen load, the concurrent amount of exposure to thiocyanate and nitrate must be estimated in each of these groups. Knowing the exposure to each NIS inhibitor in each group allows the total goitrogen load to be determined for each group to assess the contribution of perchlorate to the body's total goitrogen load. Furthermore, the Tonacchera Model also quantifies the impact of the availability of iodide on the thyroid's ability to uptake iodide. Therefore, the iodide nutrition status of each group must be determined and evaluated (see Section 8 of this document).

7.2.1 Total NIS Inhibition Load Acting on Adults

The total NIS inhibition load (i.e., total goitrogen load) acting on the body is determined by knowing the serum concentration of the three environmentally prominent NIS inhibitors: perchlorate, nitrate, and thiocyanate. The serum concentration of each is then used to calculate the SPEC.

The perchlorate serum concentration in an adult was predicted using the Clewell Perchlorate PBPK Model (Clewell 2007). The perchlorate RfD was dervived by applying a 10-fold UF to the NOEL of 7 µg/kg-day measured in Greer's subjects (NAS 2005, p 15). Therefore, the perchlorate NOEL corresponds to an external dose of 490 µg/day. The closest point provided in Clewell's paper is that an external dose of 700 µg/day is predicted to generate a perchlorate serum concentration of 10 µg/L in an adult (Clewell 2007, table 4). If this value is linearly scaled down to the perchlorate NOEL of 490 µg/day in an adult (i.e., 7 µg/kg-day), the predicted perchlorate serum concentrations at the NOEL in an adult would be about 7 µg/L (i.e., 0.070 µmol/L). Likewise, the Clewell Perchlorate PBPK Model predicts a perchlorate serum of 2 µg/L for an adult at an external dose of 70 µg/day (i.e., 1 µg/kg-day or 1.42 times the RfD) (Clewell 2007). If this value is linearly scaled down to the EPA RfD of 49 µg/day in an adult (i.e., 0.7 µg/kg-day), the predicted perchlorate serum concentrations at EPA's RfD in an adult

would be 1.4 µg/L (i.e., 0.014 µmol/L). If this Clewell data is scaled down to the 95th percentile of the U.S. perchlorate exposure level of 16.4 µg/day in an adult measured in the Blount biomonitoring study, the predicted perchlorate serum concentrations at 95th percentile of the U.S. perchlorate exposure level in an adult would be 0.47 µg/L (i.e., 0.0047 µmol/L)(Blount 2006a). If the Clewell data are scaled down to the median U.S. perchlorate exposure level of 4.6 µg/day in an adult measured in the Blount biomonitoring study, the predicted perchlorate serum concentrations at median U.S. perchlorate exposure level in an adult would be 0.13 µg/L (i.e., 0.0013 µmol/L)) (Blount 2006a). The following table summarizes the predicted perchlorate serum concentrations in an adult using the Clewell Perchlorate PBPK Model:

Perchlorate Exposure Level	External Perchlorate Dose in an a 70 kg Adult (µg/day)	Predicted Perchlorate Serum Concentration in an Adult
NOEL (7 µg/kg-day)	490	7.0 µg/L or 0.070 µmol/L
RfD (0.7 µg/kg-day)	49	1.4 µg/L or 0.014 µmol/L
95th Percentile	16.4	0.47 µg/L or 0.0047 µmol/L
50th Percentile	4.6	0.13 µg/L or 0.0013 µmol/L

Source: OIG Analysis.

In the Western world, the typical nitrate serum concentration ranges from 10-140 µmol/L, with the mean nitrate serum concentration being between 30-50 µmol/L (Tonacchera 2004). Thiocyanate serum concentration in nonsmokers is typically in the range of 10-70 µmol/L. By contrast, the thiocyanate serum concentration of smokers is higher and is typically in the range of 80-120 µmol/L (Tonacchera 2004). A thiocyanate serum concentration of 40.5 µmol/L was observed in the controls in Braverman's study of workers at the ammonium perchlorate plant in Cedar City, Utah (Bravermen 2005). Therefore, although variable, the typical nitrate and thiocyanate serum concentrations in the U.S. population are 40 µmol/L and 40 µmol/L, respectively. These values will be used for the calculation of the total NIS inhibition load in a U.S. adult.

The total NIS inhibition load (i.e., total goitrogen load) acting on the body is determined by calculating the SPEC. The SPEC is calculated as follows (concept taken from Tonacchera 2004):

SPEC = (Amt of ClO_4^- Inhibition) + (Amt of NO_3^- Inhibition) + (Amt of free SCN^- Inhibition)
SPEC = $[ClO_4^-]$ + $[NO_3^-]$ /240 + [free SCN^-] /15

Where: [ ] is molarity
Serum inhibition potency of perchlorate = 1
Serum inhibition potency of nitrate relative to perchlorate = 1/240
Serum inhibition potency of thiocyanate relative to perchlorate = 1/15

The following are calculations for the total NIS inhibition in a U.S. adult at the NOEL, RfD, 95th percentile, and median perchlorate exposure levels expressed as SPECs:

SPEC (at NOEL) = 0.070 µmol/L + (40 µmol/L ÷ 240) + (40 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.070 µmol/L + 0.167 µmol/L + 1.333 µmol/L
= 1.567 µmol/L

SPEC (at RfD) = 0.014 µmol/L + (40 µmol/L ÷ 240) + (40 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.014 µmol/L + 0.167 µmol/L + 1.333 µmol/L
= 1.511 µmol/L

SPEC (at 95th CLO_4^-) = 0.0047 µmol/L + (40 µmol/L ÷ 240) + (40 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.0047 µmol/L + 0.167µmol/L + 1.333 µmol/L
= 1.505 µmol/L

SPEC (median ClO_4^-) = 0.0013 µmol/L + (40 µmol/L ÷ 240) + (40 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.0013 µmol/L + 0.167 µmol/L + 1.333 µmol/L
= 1.501 µmol/L

SPEC (w/no ClO_4^-) = 0.0 µmol/L + (40.0 µmol/L ÷ 240) + (40.0 µmol/L x 0.5 free SCN^- ÷ 15)
= 0.0000 µmol/L + 0.1667 µmol/L + 1.333 µmol/L
= 1.500 µmol/L

The following table summarizes the total NIS inhibition in a typical U.S. adult having 40 µmoles/L exposures to both nitrate and thiocyanate at various levels of perchlorate exposure. Furthermore, the table identifies the relative contribution of each NIS inhibitor to the total NIS inhibition:

Relative Contribution of Each NIS Inhibitor to the Total Amount of NIS Inhibition in an Adult

Perchlorate Exposure Level	Total Amount of NIS Inhibition in an Adult (SPEC)	Perchlorate	Nitrate at (40 µmoles/L)	Thiocyanate at (40 µmoles/L)
At the Perchlorate NOEL (7 µg/kg-day)	1.567 µmoles/L	4.5%	10.7%	85.1%
At the Perchlorate RfD of (0.7 µg/kg-day)	1.511 µmoles/L	0.93%	11.1%	88.2%
95th percentile of U.S. population (Blount 2006)	1.505 µmoles/L	0.31%	11.1%	88.6%
50th percentile of U.S. population (Blount 2006)	1.501 µmoles/L	0.09%	11.1%	88.8%
Zero Perchlorate	1.500 µmoles/L	0.00%	11.1%	88.9%

Source: OIG Analysis.

The Tonacchera Model establishes a mathematical equation to calculate the TIU of the NIS from the serum concentrations of the three NIS inhibitors: perchlorate, nitrate, and thiocyanate. The iodide concentration in the serum (i.e., $[I^-]$) is unknown but should be directly related to the UIC. Therefore, $[I^-]$ is assigned a value of "x" in these calculations. The following calculates the TIU at the perchlorate exposure of zero (theoretical) and at the RfD and the amount of change in TIU from the exposure of perchlorate at the RfD and at the NOEL:

The TIU with no perchlorate exposure is expressed by:

$TIU_{(w/\ no\ Perchlorate\ Exposure)} \parallel [I^-] / (1.22 + [SPEC])$

where: the symbol || means "proportional to"

Substitution gives:

$TIU_{(w/\ no\ Perchlorate\ Exposure)} \parallel x / (1.22 + 1.500 \mu mol/L)$

$TIU_{(w/\ no\ Perchlorate\ Exposure)} \parallel x / 2.720$

$TIU_{(w/\ no\ Perchlorate\ Exposure)} \parallel 0.3676x$

The TIU at a perchlorate exposure level of the RfD is expressed by:

$TIU_{(Perchlorate\ Exposure\ at\ RfD)} \parallel [I^-] / (1.22 + [SPEC])$

Substitution gives:

$TIU_{(Perchlorate\ Exposure\ at\ RfD)} \parallel x / (1.22 + 1.511\ \mu mol/L)$

$TIU_{(Perchlorate\ Exposure\ at\ RfD)} \parallel x / 2.731$

$TIU_{(Perchlorate\ Exposure\ at\ RfD)} \parallel 0.3662x$

The change in TIU with perchlorate exposure at RfD = $\frac{TIU\ (RfD) - TIU\ (no\ ClO_4^-)}{TIU\ (no\ ClO_4^-)} \times 100\%$

Substitution gives:

Change in TIU at perchlorate RfD = ((0.3662x - 0.3676x)/ 0.3676x) x 100%

Change in TIU at perchlorate RfD = - 0.38% (i.e., a decrease of 0.4 %)

The TIU at a perchlorate exposure level of the NOEL is expressed by:

$TIU_{(Perchlorate\ Exposure\ at\ NOEL)} \parallel [I^-] / (1.22 + [SPEC])$

Substitution gives:

$TIU_{(Perchlorate\ Exposure\ at\ NOEL)} \parallel x / (1.22 + 1.567\ \mu mol/L)$

$TIU_{(Perchlorate\ Exposure\ at\ NOEL)} \parallel x / 2.787$

$TIU_{(Perchlorate\ Exposure\ at\ NOEL)} \parallel 0.3588x$

Change in TIU with perchlorate exposure at RfD = $\frac{TIU\ (NOEL) - TIU\ (no\ ClO_4^-)}{TIU\ (no\ ClO_4^-)} \times 100\%$

Substitution gives:

Change in TIU at perchlorate RfD = ((0.3588x - 0.3676x)/ 0.3676x) x 100%

Change in TIU at perchlorate RfD = - 2.4% (i.e., a decrease of 2.4 %)

In a typical adult with nitrate and thiocyanate exposures of 40 µmoles/L exposures, the above calculations predict that the exposure to perchlorate at the RfD or at the NOEL will decrease the uptake of iodide by the thyroid by 0.4% and 1.9%, respectively. However, the Tonacchera Model predicts TIU is proportional to $[I^-]$ for all levels of iodide nutrition for any fixed underlying goitrogen load between 0.1 µmol/L to about 12 µmol/L PEC. The TIU vs log PEC relationship becomes nonlinear above 12 µmol/L PEC (Tonacchera 2004, figure 2). Therefore, the TIU could remain unchanged at the perchlorate exposure level of the RfD and at the NOEL in an adult if "x" (iodide concentration in the serum) increases by 0.4% and 2.4%, respectively.

Seasonal and Dietary Variation in NIS Inhibitor Load

By comparison, consumption of common vegetables is a significant and natural source of thiocyanates. In a Norwegian health study of 25,300 people, the mean thiocyanate serum levels (± SD) were 33.9 ± 14.0 µmol/L and 33.4 ± 14.0 µmol/L in nonsmoking males and females, respectively (Foss 1986). The thiocyanate serum levels in 95% of the nonsmokers ranged from10 µmol/L to 60µmol/L. Foods particularly rich in thiocyanate include cabbage, broccoli, Brussels sprouts, corn (maize), turnips, rapeseed, and mustard seed. Foods are known to contain varying quantities of thiocyanate and the content varies considerably from sample to sample. An individual's thiocyanate serum level will vary depending on the frequency and amount of consumption of thiocyanate-containing food types.

For example, a nonsmoking individual consumed 100 grams of raw Brussels sprouts each day for a week to document a rise in thiocyanate serum levels from 31 to 80 µmol/L. Dietary thiocyanate exposure also exhibits a change of about 10 µmol/L, presumably due to seasonal changes in the composition of the diet (Foss 1986). Cabbage is a high thiocyanate content food with consumption higher during autumn than any other time of year. The following table demonstrates the seasonality of thiocyanate serum levels in nonsmokers:

Study Group	Serum Thiocyanate (µmol/L)			
	1st Quarter of the Year	2nd Quarter of the Year	3rd Quarter of the Year	4th Quarter of the Year
Nonsmoking Males (n = 6212)	29.5	30.2	39.4	39.4
Nonsmoking Females (n = 7908)	29.9	30.1	39.9	39.4

Source: Foss 1986.

The purpose of reviewing natural thiocyanate exposure from the diet is to show that a 10 µmol/L change in human thiocyanate serum levels is common. Both the nonsmoking males and females in the Norwegian health study had a ± 14.0 µmol/L SD with regard to the mean (Foss 1986). Furthermore, the seasonal change in thiocyanate serum level in the Norwegian nonsmokers was about 10.0 µmol/L.

In regard to the amount of NIS inhibition, a change of 10.0 µmol/L in human thiocyanate serum levels represents a much greater amount of NIS inhibition than exposure to perchlorate at the RfD in an adult. The amount of NIS inhibition of 10.0 µmol/L in human thiocyanate serum is measured by the Tonacchera Model as SPEC and has value of 0.333 µmol/L (i.e., (10 µmol/L x 0.5 free SCN^-) ÷ 15). By comparison, perchlorate exposure at the RfD has an estimated SPEC of 0.014 µmol/L. Therefore, the fluctuation in the human thiocyanate serum of 10.0 µmol/L causes a change in the amount of NIS inhibition that is about 24 times greater than the amount of NIS inhibition caused by perchlorate exposure at the RfD.

7.2.2 Total NIS Inhibition Load Acting on Fetuses

The total NIS inhibition load (i.e., total goitrogen load) acting on the fetus is determined by estimating the serum concentration in the fetus of the three environmentally prominent NIS inhibitors: perchlorate, nitrate, and thiocyanate. The serum concentration of each is then used to calculate the SPEC in the fetus. Direct measurements of fetal exposures to each of the NIS

inhibitors are not possible due to the unacceptable risk to the unborn child. However, a direct measurement of serum concentration is possible at birth without risk to the fetus by measuring the serum concentration in umbilical cord blood.

The fetal thiocyanate serum concentration can be estimated by direct measurements of thiocyanate in both maternal and cord serums that have been reported in at least four studies (see table below). The thiocyanate ratio of cord serum to maternal serum observed in nonsmokers is 71%, 88%, and 92% observed in the Hauth, Laurberg, and Vanderpass studies, respectively (Hauth 1984; Laurberg 2004; Vanderpass 1984). For estimating the fetal thiocyanate serum concentration, the thiocyanate ratio of cord serum to maternal serum of 71% observed in the nonsmokers from the Hauth study is used (Hauth 1984). Using the thiocyanate ratio of cord serum to maternal serum of 71% provides the lowest expected value in the fetal thiocyanate serum concentration (i.e., use of either 88% or 92% to estimate the fetal thiocyanate serum concentration would decrease the relative contribution of NIS inhibition from perchlorate). The fetal thiocyanate serum concentration is estimated by multiplying the typical thiocyanate serum concentration found in the Western world of 40 µmol/L by the thiocyanate ratio of cord serum to maternal serum of 71%. Therefore, the typical fetal thiocyanate serum concentration in the Western world is estimated to be at least 28.4 µmol/L. This estimated fetal thiocyanate serum concentration is a conservative value and is based on the direct thiocyanate measures observed in cord blood.

Study (Exposure Type/Description)	Mother's SCN^- Serum Concentration (µmol/L)	Cord SCN^- Serum Concentration (µmol/L)	SCN Ratio Cord Serum/Mother Serum
Hauth 1984			
Smokers	95	72	76%
Passive Smokers	35.9	26	72%
Nonsmokers	32.3	23	71%
Laurberg 2004			
Smokers	84.9 ± 25.4	94.6 ± 31.9	111%
Nonsmokers	54.7 ± 18.2	48.3 ± 15.5	88%
Nafstad 1995			
24 Smokers & 4 Nonsmokers	69	47	68%
Vanderpass 1984			
Central Africa (dietary SCN)	133 ± 7	134 ± 2	101%
Belgian Controls	36 ± 7	33 ± 7	92%

Sources: Hauth 1984; Laurberg 2004; Nafstad 1995; Vanderpass 1984.

The Clewell PBPK model predicts a perchlorate serum concentration (i.e., an internal indice) of 1.4 µg/L (i.e., 0.014 µmol/L) in an adult at an external exposure dose at the RfD. The Clewell PBPK model predicts a pregnant woman's perchlorate serum concentration as being 2.5 times greater than an adult's perchlorate serum concentration at an external exposure dose at the RfD which corresponds to 3.5 µg/L or 0.035 µmol/L. Likewise, the Clewell PBPK model predicts a fetus's perchlorate serum concentration as being 5 times greater than an adult's perchlorate serum concentration at an external exposure dose at the RfD which corresponds to 7 µg/L or 0.070 µmol/L.

Although NIS is present in the placenta, the transport of nitrate from the mother's blood to the fetal blood would be expected (i.e., analogous to the known transport of nitrate from maternal blood into breast milk). However, the OIG could not find a study that reports the amount of nitrate found in cord blood. Although nitrate contributes to fetal NIS inhibition, the amount of NIS inhibition from nitrate is problematic to estimate and is not used in the following calculation of the total NIS inhibition load on the fetal thyroid with a perchlorate exposure at the RfD:

$SPEC_{(RfD)}$ = 0.070 µmol/L + (µmol/L of NO_3^- unknown) + ((40 µmol/L x 0.71 ratio) x 0.5 ÷ 15)
= 0.070 µmol/L + (µmol/L of NO_3^- unknown) + ((28.4 µmol/L) x 0.5 ÷ 15)
= 0.070 µmol/L + (NO_3^- contribution unknown) + 0.947 µmol/L
= 1.017 µmol/L

In a fetus, the perchlorate at the RfD contributes about 6.9% of the total NIS inhibition load acting on the thyroid. (Note: With the contribution from nitrate or the use of a higher SCN^- maternal/cord serum ratio, this estimated value will be smaller.)

The TIU of the fetus with the mother having an external exposure dose at the RfD is expressed by:

$$TIU_{(Fetus)} \parallel [I^-] / (1.22 + [SPEC])$$

Substitution gives:

$$TIU_{(Fetus)} \parallel x / (1.22 + 1.017\ \mu mol/L)$$
$$TIU_{(Fetus)} \parallel x / 2.237$$
$$TIU_{(Fetus)} \parallel 0.4470x$$

The TIU of the fetus with the mother having no perchlorate exposure is expressed by:

$$TIU_{(Fetus\ with\ no\ perchlorate\ exposure)} \parallel [I^-] / (1.22 + [SPEC])$$

Substitution gives:

$$TIU_{(Fetus\ with\ no\ perchlorate\ exposure)} \parallel x / (1.22 + 0.947\ \mu mol/L)$$
$$TIU_{(Fetus\ with\ no\ perchlorate\ exposure)} \parallel x / 2.167$$
$$TIU_{(Fetus\ with\ no\ perchlorate\ exposure)} \parallel 0.4615x$$

The change in $\%TIU_{(Fetus)}$ with mother exposed at RfD versus no perchlorate exposure is given by:

$$= \frac{TIU\ (Fetus\ with\ exposure) - TIU\ (Fetus\ with\ no\ ClO_4^-)}{TIU(fetus\ with\ no\ ClO_4^-)} \times 100\%$$

Substitution gives:

$$\%TIU_{(Fetus)} \text{ w/ mother exposed at RfD} = ((0.4470x - 0.4615x)/ 0.4615x) \times 100\%$$
$$= - 3.1\% \text{ (i.e., a decrease of 3.1 \%)}$$

(Note: With the contribution from nitrate, this estimated value will be < 3.1%.)

7.2.3 Total NIS Inhibition Load Acting on Nursing Infants

The total NIS inhibition load acting on nursing infant's thyroid at a perchlorate RfD exposure to the nursing mother can be estimated by adding the amount of NIS inhibition contributed from perchlorate, nitrate, and thiocyanate together. All three of these NIS inhibitors are found in human breast milk.

In regard to thiocyanate in breast milk, a Norwegian study reported the mean thiocyanate concentration in breast milk as 9.0 mg/L in smokers and 10.5 mg/L in nonsmokers (Dorea 2004). By contrast, the mean thiocyanate concentration in breast milk in the United States is reported to be 4.2 mg/L in smokers and 0.92 mg/L in nonsmokers (Dorea 2004). The reported thiocyanate concentrations in breast milk in nonsmokers, which range from a low of 0.92 mg/L to a high of 10.5 mg/L, are large (i.e., an order of magnitude), which introduces a lot of uncertainty into the estimation. For purposes of estimating (i.e., a conservative estimate) the total NIS inhibition load acting on nursing infant's thyroid at a perchlorate RfD exposure to the nursing mother, the mean thiocyanate concentration in breast milk in the United States of 0.92 mg/L in nonsmokers will be used.

In regard to nitrate concentrations in breast milk, 20 healthy, lactating women in the State of Iowa had their breast milk sampled and tested for nitrate (Dusdieker 1996). The study found 4.4 ± 3.6 mg nitrate/L in human milk (Dusdieker 1996).

The concentration of perchlorate in breast milk when the lactating mother is exposed to perchlorate at the RfD is estimated from the perchlorate PBPK podel in rats. The perchlorate PBPK model in rats predicts that about 50% of the daily maternal perchlorate dose at 0.01 mg/kg-day is transferred to the nursing pup through breast milk (Clewell 2003, 416, (language in the abstract)). If the same relationship holds in humans, lactating women exposed at the perchlorate RfD (i.e., 49 µg/day) would be predicted to transfer 24.5 µg perchlorate/day to the nursing infant. Neonate perchlorate exposure is a function of both the weight of the infant, the amount of milk consumption, and the perchlorate concentration of the milk. A 1-month-old infant's median weight is 4.14 kg (EPA 1997a) and that infant consumes a mean intake of 673 ml of milk (Dewey 1983). A 6-month-old infant's median weight is 7.53 kg (EPA 1997a) and that infant consumes a mean intake of 673 ml of milk (Dewey 1983). Since a 1-month-old infant consumes 0.673 L of milk/day, a breast milk concentration of 36.4 µg perchlorate/L transfers 24.5 µg perchlorate/day to the nursing infant (i.e., the 50% transfer the mother's RfD perchlorate exposure to the nursing infant predicted by the PBPK model). Likewise, a 6-month-old infant consumes 0.896 L of milk/day, a breast milk concentration of 27.3 µg perchlorate/L transfers 24.5 µg perchlorate/day to the nursing infant.

The total NIS inhibition load acting on a nursing infant's thyroid at a perchlorate RfD exposure to the nursing mother estimates perchlorate breast milk concentrations of 36.4 µg/L for a 1-month-old and 27.3 µg/L for a 6-month-old (i.e., perchlorate concentration in breast milk is estimated to be higher because less milk volume is generated to meet the child's needs). These PBPK model estimates of 36.4 µg/L and 27.3 µg/L for perchlorate concentrations in human breast milk are substantially above the measured median perchlorate concentrations in human breast milk of 3.3 µg/L, 4.0 µg/L and 9.1 µg/L (Kirk 2005; Kirk 2007; Pearce 2007). The exposure to each of the NIS inhibitors found in human breast milk is estimated as follows:

For a 1-month-old infant:

36.4 µg $ClO4^-$/L x 0.673 L of milk/day = 24.5 µg $ClO4^-$/day
920 µg SCN^-/L x 0.673 L of milk/day = 619 µg SCN^- /day
4400 µg NO_3^-/L x 0.673 L of milk/day = 2961 µg nitrate/day

For a 6-month-old infant:

27.3 µg $ClO4^-$/L x 0.896 L of milk/day = 24.5 µg $ClO4^-$/day
920 µg SCN^-/L x 0.896 L of milk/day = 824 µg SCN^- /day
4400 µg NO_3^-/L x 0.896 L of milk/day = 3942.4 µg nitrate/day

The total NIS inhibitory effect measured upon ingestion of the NIS inhibitors is given by the following OPEC equation (concept adapted from Tonacchera 2004; De Groef 2006, 155):

OPEC = (Amt of ClO4- Inhibition) + (Amt of NO3- Inhibition) + (Amt of SCN- Inhibition)
OPEC = grams perchlorate + grams nitrate/150 + 0.5 free SCN- x grams thiocyanate/8.8
OPEC = grams perchlorate + grams nitrate/150 + grams thiocyanate/17.6

Where:

Oral inhibition potency of perchlorate = 1
Oral inhibition potency of nitrate relative to perchlorate = 1/150
Oral inhibition potency of thiocyanate relative to perchlorate = 0.5/8.8 = 1/17.6

The total NIS inhibition load acting on nursing infant's thyroid at a perchlorate RfD exposure to the nursing mother is estimated as follows:

For a 1-month-old infant:

$OPEC_{(1\ month)}$ = (Amt $ClO4^-$ Inhibition) + (Amt of $NO3^-$ Inhibition) + (Amt of SCN^- Inhibition)
$OPEC_{(1\ month)}$ = µg perchlorate + µg nitrate/150 + µg thiocyanate/17.6
$OPEC_{(1\ month)}$ = 24.5 µg $ClO4^-$/day + 2961 µg nitrate/day ÷ 150 + 619 µg SCN^- /day ÷ 17.6
$OPEC_{(1\ month)}$ = 24.5 µg ClO_4^-/day + 19.74 µg ClO_4^-equiv./day + 35.2 µg ClO_4^-equiv./day
$OPEC_{(1\ month)}$ = 79.4 µg ClO_4^-equivalent/day

For a 6-month-old infant:

$OPEC_{(6\ month)}$ = (Amt $ClO4^-$ Inhibition) + (Amt of $NO3^-$ Inhibition) + (Amt of SCN^- Inhibition)
$OPEC_{(6\ month)}$ = µg perchlorate + µg nitrate/150 + µg thiocyanate/17.6

$OPEC_{(6\ month)}$ = 24.5 µg $ClO4^-$/day + 3942.4 µg nitrate/day ÷ 150 + 824 µg SCN^- /day ÷ 17.6
$OPEC_{(6\ month)}$ = 24.5 µg ClO_4^-/day + 26.3 µg ClO_4^-equiv./day + 46.8 µg ClO_4^-equiv./day
$OPEC_{(6\ month)}$ = 97.6 µg ClO_4^-equivalent/day

At both 1 month and 6 months, thiocyanate exposure in breast milk contributes the largest portion of the NIS inhibition. These OPEC values were calculated using the lower estimated thiocyanate concentration in breast milk of 0.92 mg/L in nonsmokers. The OPEC values would be much larger if the higher estimated thiocyanate concentration in breast milk of 10.5 mg/L in nonsmokers was used. Using the lowest thiocyanate exposure at 1 month, maternal perchlorate exposure at the RfD is estimated to result in perchlorate contributing about 30% of the nursing infant's total NIS inhibition load. Using the lowest thiocyanate exposure at 6 months, maternal perchlorate exposure at the RfD is estimated to result in perchlorate contributing about 25% of the nursing infant's total NIS inhibition load.

This characterization of a nursing infant's total NIS inhibition load clearly indicates that perchlorate is not acting alone and the use of a single chemical risk assessment of perchlorate to characterize the risk of NIS inhibition acting on a nursing infant's thyroid is incomplete. A cumulative risk assessment approach is needed to characterize and understand the sources and total risk of NIS inhibition acting on a nursing infant's thyroid. Furthermore, a cumulative risk assessment approach is needed to quantify the effect of all four NIS stressor on the TIU by the nursing infant.

7.2.4 Total NIS Inhibition Load Acting on Non-nursing Infants

To calculate or estimate the total NIS inhibition load (i.e., total goitrogen load) acting on the thyroid of non-nursing infants, the non-nursing infant exposure to each of the three environmentally prominent NIS inhibitors (i.e., thiocyanate, nitrate, and perchlorate) must be known. Unfortunately, neither the serum concentrations nor the amount of oral consumption to all three NIS inhibitors are available to calculate or estimate the total NIS inhibition load (i.e., total goitrogen load) acting on the thyroid of non-nursing infants.

The perchlorate exposure in non-nursing infants can be estimated using the results from the 2008 FDA Food Dietary Study. The 2008 FDA Food Dietary Study reports the total perchlorate intake from food for 6- to 11-month-old infants to be 0.26–0.29 µg/kg-day (i.e., not including potential perchlorate exposure from water) (Murray 2008, Table 5). Since the perchlorate RfD is 0.6 µg/kg-day, the perchlorate exposure from food for 6- to 11-month-old infants of 0.26–0.29 µg/kg-day represents 37% to 41% of the perchlorate RfD. This suggests a Relative Source Contribution (RSC) of about 60% for non-nursing infants.

Unfortunately, this estimated perchlorate RSC is derived using a single chemical risk assessment process that is characterized as being outdated. In other words, limiting only perchlorate to protect public health does not insure that the total NIS inhibition load acting on the non-nursing infants is "safe" because the NIS inhibition exposure from thiocyanate and nitrate in the food and water of the non-nursing infant is not considered. The public health risk to non-nursing infants from a potentially excessive amount of NIS inhibitors is not known without knowing the non-nursing infants' exposure to thiocyanate and nitrate. However, the relative

contribution of each NIS inhibitor to the total amount of NIS inhibition load acting on the body is known for adults. In typical nonsmoking U.S. adults, thiocyanate and nitrate from the consumption of food and water contributes the vast majority (i.e., > 99.9%) of the body's total NIS inhibition load (see Section 7.2.1). Since a non-nursing infant's diet contains the same food sources (e.g., vegetables (see Sections 3.3 and 3.4) that contribute thiocyanate and nitrate in an adult's diet, the vast majority of the total NIS inhibition load acting on the thyroid of the non-nursing infant is still expected to be from thiocyanate and nitrate and not from perchlorate. Therefore, from a cumulative risk assessment standpoint, the majority of risk to non-nursing infants from exposure to NIS inhibitors is from thiocyanate and nitrate. Furthermore, to effectively characterize and manage the risk, the exposure to all three NIS inhibitors needs to be determined and the combined exposure needs to be managed to prevent an excessive total NIS inhibition load acting on the non-nursing infant.

Another aspect of a cumulative risk assessment is that the body's tolerance for NIS inhibition is a function of the amount of iodide in the diet. The lack of iodide is also an NIS stressor on the thyroid and also limits the amount of TIU by the thyroid. Therefore, within certain biological limits, the more iodide in the diet, the body can tolerate a larger total NIS inhibition load without adverse effects. The converse of this relationship is also true, within certain biological limits: the less iodide in the diet, the body cannot tolerate as much total NIS inhibition load before adverse effects are observed. In short, there is no single limit to the amount of total NIS inhibition the body can tolerate; the body's toleration of NIS inhibition is on a sliding scale depending on the amount of iodide in the diet.

In regard to the iodide nutrition observed in non-nursing infants, the iodide nutrition level can be estimated using the results from the 2008 FDA Food Dietary Study. The 2008 FDA Food Dietary Study identifies that baby foods and dairy products account for nearly all (90%) of the estimated iodine intake by infants. The 2008 FDA Food Dietary Study reports the range of estimated lower- and upper-bound average iodide intakes for 2003–2004 for 6- to 11-month-old infants to be 144-155 µg/day (Murray 2008, table 7). The 2008 FDA Food Dietary Study indicates that an adequate intake of iodide for 6- to 11-month-old infants is 130 µg/day (Murray 2008, table 7). Therefore, the 2008 FDA Food Dietary Study indicates that the majority of 6- to 11-month-old U.S. infants have an adequate intake of iodide, which allows these infants to tolerate a larger amount of total NIS inhibition load compared to an infant with an iodide-deficient diet. Unfortunately, the 2008 FDA Food Dietary Study was not designed to provide the iodide intake distribution within each population subgroup. This iodide intake distribution within the U.S. population subgroups would help to better characterize the public health risk because the risk to the public from NIS inhibitors increases with the decreasing intake of iodide.

8. Risk Characterization of NIS Stress

In 2003, the EPA *Framework for Cumulative Risk Assessment* identifies, "For most exposure situations, hazard and dose response studies of all of the joint effects from the multiple stressors will not be available, so that conclusions will have to be based at least partly on the single stressor information" (EPA 2003, p 44). For perchlorate, a risk characterization cannot be done in humans because adverse health effects have not been clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 177). However, the EPA *Framework for Cumulative Risk Assessment* allows the risk characterization to be based on the adverse effects observed in one of the stressors, and then be applied to the joint effects from the multiple stressors. Therefore, the risk characterization for NIS stressors must be done by using an NIS stressor in which adverse effects in children born to mothers with low maternal TIU during pregnancy and nursing have been documented and reported. The excessive maternal exposure to the NIS stressor, the lack of iodide, is the principal NIS stressor in which adverse effects have been documented and reported in children born to mothers with low maternal TIU during pregnancy and nursing. Thus, the adverse effects from the lack of iodide stressor can be used to conduct the initial three steps in a risk characterization (i.e., hazard characterization, dose-response assessment, and exposure assessment) to this public health issue. The exposure to the lack of iodide stressor that induces an adverse effect can be related back to the joint exposure level of all four NIS stressors by integrating the joint exposure into a single parameter or "common metric" (EPA 2003, p 55, section 3.3.3.1). The single parameter for measuring the NIS stress level is the TIU. The Tonacchera Model can be used to mathematically calculate the resulting TIU level from the concurrent joint exposure to all four NIS stressors.

The inclusion of the lack of iodide stressor is essential in characterizing and understanding this public health issue. An insufficient uptake of iodide in both the pregnant mother's thyroid and her child's thyroid during gestation and lactation is the initiating biological event that triggers the subsequent potential for permanent brain damage in the developing fetus and nursing infant. An insufficient uptake of iodide can be caused by the exposure to an excessive amount of NIS inhibitors, but an insufficient uptake of iodide can also be caused by the lack of iodide in the diet (i.e., iodide deficiency). The excessive ingestion of NIS inhibitors or an iodide-deficient diet (or a combination of both) can lead to an insufficient uptake of iodide; therefore, excessive NIS inhibitors or the lack of iodide both initiate the same subsequent adverse effects. Thus, critical insight into this public health issue can be learned by studying the adverse effects observed in the children of iodide-deficient pregnant women. The adverse effects observed in the offspring of iodide-deficient pregnant women are the same adverse effects expected to be observed in the offspring of pregnant women with an excessive exposure to NIS inhibitors, because the two groups share the same mechanism of toxicity (i.e., a low TIU).

The 1991 EPA *Guidelines for Developmental Toxicity Risk Assessment* clearly states that human studies are the most appropriate data for conducting developmental toxicity risk assessments (EPA 1991, p 1). Human data avoid the uncertainties of extrapolating from animals studies to humans. Human data also provide the ability to identify subtle mental deficits (e.g., ADHD or verbal comprehension) that are not possible to detect or identify in animal studies. Unfortunately, subtle adverse effects can be observed in the children born to iodide-deficient pregnant women. The type and severity of these adverse effects that occur in the children born

to iodide-deficient pregnant women can be studied and identified. Furthermore, through the use of the Tonacchera Model, the amount of TIU that results in adverse effects to the offspring can be calculated from the iodide exposure levels in the pregnant women. To accomplish this task, the lack of iodide stressor will be evaluated using the traditional risk assessment techniques of a hazard characterization, dose-response assessment, and an exposure assessment.

8.1 Hazard Characterization

EPA's *Framework for Cumulative Risk Assessment* states that a stressor does not have to be the exposure to a chemical, but the "absence of a necessity" (EPA 2003). In terms of the necessity for the thyroid to uptake enough iodide to make a sufficient supply thyroid hormones for proper brain development, the potential absence of iodide in the diet of pregnant women and neonates is the dominant stressor in this public health issue, according to the Tonacchera Model. To characterize the risk from this nonchemical NIS stressor, the same process used for chemical agents is appropriate, except this time the adverse health effects are observed by the lack of exposure. The hazard characterization is the description of the potential adverse health effects attributed to a specific environmental stressor; the mechanisms by which a stressor exerts its toxic effect; and the associated dose, route, duration, and timing of exposure (EPA 2002).

The absence of iodide in the diet (i.e., poor iodide nutrition) acts through the same mechanism of toxicity as perchlorate and the other NIS inhibitors. Although the lack of iodide does not act as an NIS inhibitor, the lack of iodide has the same biological result by preventing an adequate uptake of iodide by the NIS. Therefore, the lack of iodide would have the same adverse health effects as proposed by an excess exposure to perchlorate.

The EPA mode of action for perchlorate proposed a continuum of possible health effects from perchlorate exposure that included the potential for permanent neurodevelopmental deficits in infants (EPA 2002a). The NAS Committee mode of action for perchlorate also identifies a continuum of biological effects that includes the possibility for abnormal fetal and child growth and development (NAS 2005). However, the NAS Committee "emphasizes" that the inhibition of iodide uptake by the thyroid has been the only consistently documented effect of perchlorate exposure in humans (NAS 2005, p 165). The NAS Committee also states that the continuum of possible health effects is only proposed and has not been clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 165). Therefore, the potential perchlorate hazard for neurodevelomental deficits in infants is only theoretical.

By contrast, the neurodevelopmental hazard from this nonchemical NIS stressor (i.e., the lack of iodide) is not theoretical. The neurodevelopmental hazard from this nonchemical NIS stressor is known, characterized, and observed in children born in iodide-deficient populations. Iodide deficiency is fundamental in the cause of endemic cretinism (Delange 2005a). Cretinism is the extreme, irreversible mental retardation and impairment of physical development of children. In severely iodide-deficient populations, the prevalence of cretinism can reach 5% to 15% of the population. However, the combined effect of the four NIS stressors on the uptake of iodide by the thyroid does not reach this level of severity in the United States.

The neurodevelopmental hazard from the lack of iodide is a not threshold effect, but is better characterized by a dose-response relationship. In other words, the severity of mental deficits and the frequency of occurrence of mental deficits increase with the severity of the iodide deficiency. One cross-sectional epidemiological study in children observed a dose-response relationship between UIC and IQ, (i.e, IQ decreases with decreasing UIC) (Santiago-Fernandez 2004). Children born and raised in mild iodide-deficient populations are not reported to have intellectual or cognitive deficits (Delange 2001), but are observed to have slower

reaction times (Lombardi 1995; Vitti 1992). However, children born and raised in moderate iodide-deficient populations have minor, subtle, or overt neurological, psychological, and intellectual deficits (Delange 2005, p 278; Lombardi 1995). These mental deficits are of the same nature but less prominent than the mental deficits found in children born in severe iodide-deficient populations. Brief thyroid failure during fetal development or infancy, when the brain is still developing, is thought to cause these mental deficits.

In 1994, Bleichrodt conducted a meta-analysis of 18 studies comparing the mental development in children between either iodide-sufficient and iodide-deficient regions or iodide prophylaxis (iodide supplement) and a placebo (Bleichrodt 1994). Meta-analysis relies on the fact that statistical significance is highly dependent on sample size. By combining the results from 18 smaller studies, the meta-analysis generated a larger dataset – a total of 2,214 subjects – to identify a statistically significant trend. A meta-analysis generates a Cohen *d*-value where *d* is the difference between the two group means (i.e., control and experimental groups) divided by the standard deviation. A Cohen *d*-value of a $d = 0.2$ is classified as a small effect size, a $d = 0.5$ is classified as a medium effect size, and a $d = 0.8$ is classified as a large effect size. The Bleichrodt meta-analysis of the effects of iodide deficiency on cognitive development found a large effect size with a *d*-value = 0.9. Therefore, the mean IQ scores between iodide-deficient group and the iodide-sufficient group were 0.9 SD or 13.5 IQ points apart. In other words, 82% of the children from the iodide-deficient group score below the average IQ score of a iodide-sufficient child.

The specific adverse mental deficits are documented to be observed in children born to mothers with mild iodide deficiency, moderate iodide deficiency, and hypothyroidism are provided in the following three sections.

Adverse Effects Observed in Mild Iodide-Deficient Populations

Lombardi 1995 Study: The Lombardi epidemiological study evaluating the neuropsychological impairment of mild iodide deficiency during fetal and neonatal life reported delayed reaction times in children aged 6-10 in Tuscany, Italy (Lombardi 1995). The study measured the reaction time between visual stimulus and the response for two child populations: a mildly iodide-deficient area (IDA) and an iodide-sufficient area (ISA). The measurement of simple reaction time is a sensitive test to detect subtle neurological damage from mild iodide-deficiency during fetal and neonatal brain development. The following table summaries the study:

Village	Number of Children	Urinary Iodide Concentration (mean ± SD)	Description of Iodide Level
Borgo a Mozzano	719	80.1 ± 57	Iodide-Deficient Area (IDA)
Marina di Pisa	106	173 ± 95	Iodide-Sufficient Area (ISA)

Source: Lombardi 1995.

Reaction time is known to decrease with age (i.e., explains the observed decrease in reaction time with age in this dataset, but the significance of the study is that reaction time of ISA children is statistically significantly shorter than the reaction time of the IDA children at

each age ($p<0.05$)). The following figure provides the observed reaction times between iodide-deficient and -sufficient children by year (note: the x-axis is age of the child in years):

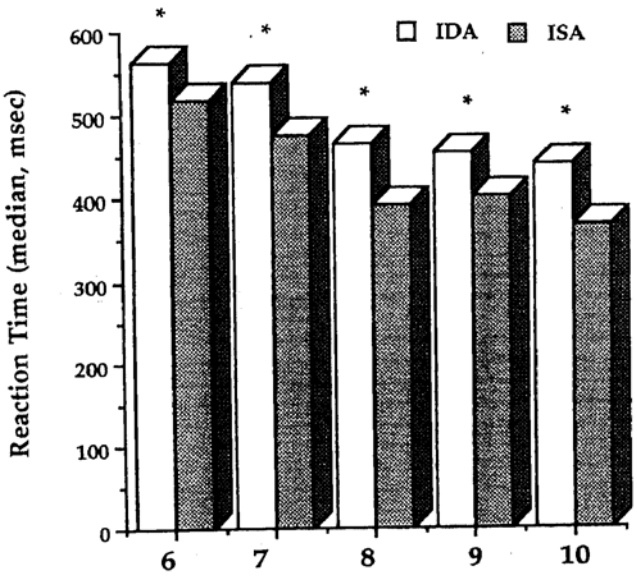


Fig. 1 - *Comparison between RTs (msec) of children born in iodine deficient area (IDA) and in iodine sufficient area (ISA). RTs were expressed as median. *p<0.05.*

Vitti 1992 Study: The Vitti study also evaluated the neuropsychological impairment of mild iodide deficiency during fetal and neonatal life by measuring the reaction times in two groups of schoolchildren in Vagli, Italy (Vitti 1992). Group 1 contained 30 children (mean age 11.5 years) who were born before iodide prophylaxis. Group 2 contained 27 children (mean age 7.3 years) who were born after iodide prophylaxis. This study measured the reaction time between a visual stimulus and the response observed in the two groups and compared them against their age-matched controls living in an ISA. The results are summarized in the following table:

Study Group	Vagli Children Mean RT	Aged-Matched Controls Mean RT*	Statistical Significance
Group 1 (mean age 11.5 yrs) (born before iodide prophylaxis)	355 msec	322 msec	$p < 0.05$
Group 2 (mean age 7.3 yrs) (born after iodide prophylaxis)	473 msec	442 msec	Not statistically significant

*RT – Retention Time
Source: Vitti 1992, table 4.

The Vitti paper discusses that the slower retention times observed in children born in mild IDAs are potentially not related to cognitive impairment, but might be related to slightly altered efficiency of the peripheral nerves to transmit a signal to and from the brain.

Santiago-Fernandez 2004 Study: The Santiago-Fernandez study evaluated the possible relationship between UIC (i.e., a surrogate for iodide intake) and the intellectual capacity observed in 1,221 schoolchildren in the province of Jaen in southeast Spain (Santiago-Fernandez 2004). The mean ages of the children were 10.8 ± 2.9 yrs (range 6-16 years) and were from 1st, 5th, and 8th grades. The IQ was determined by the Cattell's g factor test. IQ is statistically lower in children with UIC <100 µg/L than children with UICs >100 µg/L. This observation is summarized in the following table:

Urinary Iodide Concentration	Intelligence Quotient	Statistical Significance
> 100 µg/L	99.03 ± 15.81	p = 0.01
≤ 100 µg/L	96.40 ± 17.46	

Source: Santiago-Fernandez 2004, table 2.

The IQ level of children is associated with the type of salt consumed. In the United States, iodized salt contains about 100 parts per million (ppm) potassium iodide, which corresponds to 77 µg iodide per gram of salt (Dunn 1998). By contrast, both common salt and sea salt contain negligible amounts of iodide. As a result, iodized-salt-consuming children had the highest level of UIC of about 118 µg/L. The common-salt- and sea-salt-consuming children had a statistically lower level of UIC of about 99 µg/L and 94 µg/L, respectively. The IQ of the iodized-salt-consuming children with the higher iodide intake was about 101, while the IQs of the common-salt- and sea-salt-consuming children with lower iodide intake was statistically lower at about 97 and 96, respectively. These results are summarized in the following table:

Type of Salt Consumed	Iodide Content (µg iodide/g of salt)	Urinary Iodide Conc. (µg/L)	Intelligence Quotient
Iodized [rock salt]	77 µg iodide/g of salt	118.22 ± 77.39[a]	100.63 ± 15.44[c]
Common [noniodized rock salt]	0.7 µg iodide/g of salt*	99.48 ± 68.85[b]	96.64 ± 16.85[d]
Marine [sea salt contains very little iodide]	1.4 µg iodide/g of sea salt**	94.44 ± 70.40[b]	95.50 ± 18.22[d]

Statistical significance between a & b is p = 0.0004
Statistical significance between c & d is p = 0.001

Source: Santiago-Fernandez 2004, table 5; * Aquaron 2000; ** Fisher 1980.

The IQ level of children is associated with the amount of milk consumed. Milk is known to be a significant source of iodide in the diet. As a result, children consuming milk 3 or more times a day have highest levels of UICs of about 119 µg/L, while the children consuming milk 2, 1, or < 1 time(s) per day had a statistically lower level of UIC of about 99, 87, and 81 µg/L, respectively (see table below). The children consuming milk 3 or more times per day or 2 times per day have statistically higher IQs of about 99 and 100, respectively, than children consuming milk 1 or < 1 time per day, who had IQs of 94 and 94, respectively. These results are summarized in the following table:

Frequency of milk intake	Urinary Iodide Conc. (µg/L)	Intelligence Quotient
3 times per day	118.60 ± 79.80[a]	98.01 ± 15.96[c]
2 times per day	98.68 ± 68.70[b]	99.90 ± 16.23[c]
1 time per day	87.10 ± 58.40[b]	93.64 ± 19.41[d]
< 1 time per day	80.82 ± 48.60[b]	93.75 ± 16.93[d]

Statistical significance between a & b is p = 0.0001
Statistical significance between c & d is p = 0.0008
Source: Santiago-Fernandez 2004, table 5.

The UICs in the children of the Santiago-Fernandez study do not reflect the iodide nutritional status of the pregnant mother and do not attempt to measure any potential brain development issues during gestation. Furthermore, the Santiago-Fernandez study is not an interventional study to evaluate whether the observed effects on IQ are reversible if the UIC is increased in the low IQ children.

The major contribution of the Santiago-Fernandez study was to document the sensitivity of the child's thyroid to iodide intake and the resulting cognitive performance of the brain. Another major significant contribution of the Santiago-Fernandez study was to document that iodized salt and milk are significant sources of iodide. Milk is a significant source of iodide in children which is confirmed by the FDA TDS (Murray 2008). Likewise, while iodized salt contains 77 µg iodide/g of salt (Dunn 1998), noniodized salt and sea salt contain only about 0.7 µg iodide/g of noniodized salt (Aquaron 2000) and about 1.4 µg iodide/g of sea salt (Fisher 1980). Therefore, noniodized salt and sea salt do not provide a significant source of iodide in the diet, which results in a corresponding decrease in cognitive performance (Santiago-Fernandez 2004, table 5).

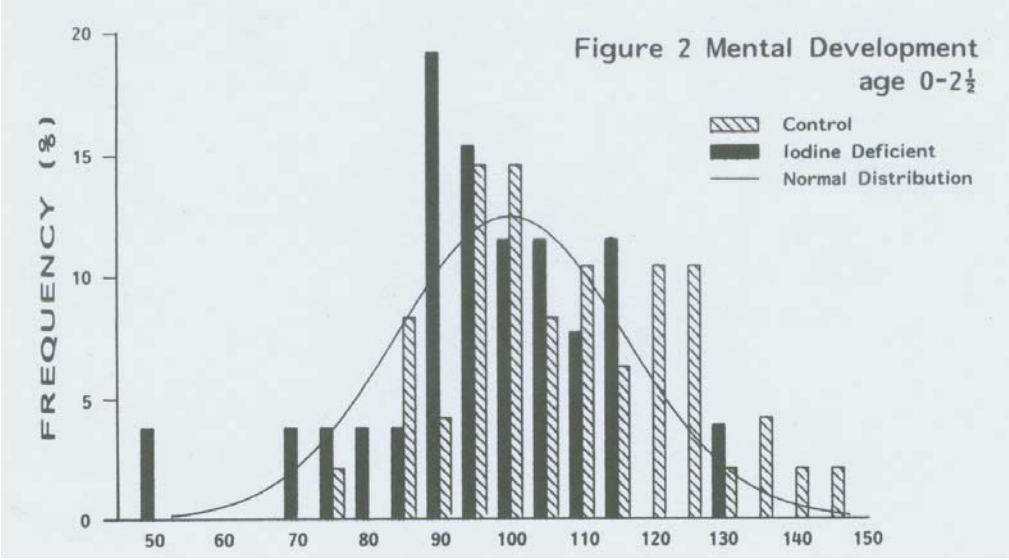
Adverse Effects Observed in Moderate Iodide Deficient Populations

Bleichrodt 1989 Study: A study evaluated the mental and psychomotor development effects on children of moderate iodide deficiency (Bleichrodt 1989). The study evaluated 162 children aged 2 months to 12 years in an IDA in Spain having a mean urinary iodine of 45 ± 41 µg/L. The control group consisted of 193 children aged 2 months to 12 years old in a non-IDA having a mean urinary iodine of 77 ± 46 µg/L. Both T_4 and iodide urinary excretion levels were markedly lower in the iodide-deficient group. The mental development scores are significantly lower ($p \leq 0.01$) across all three age groups (i.e., 0-2.5 years, 2.5-6 years, and 6-12 years). The mental development scores are summarized in the following table:

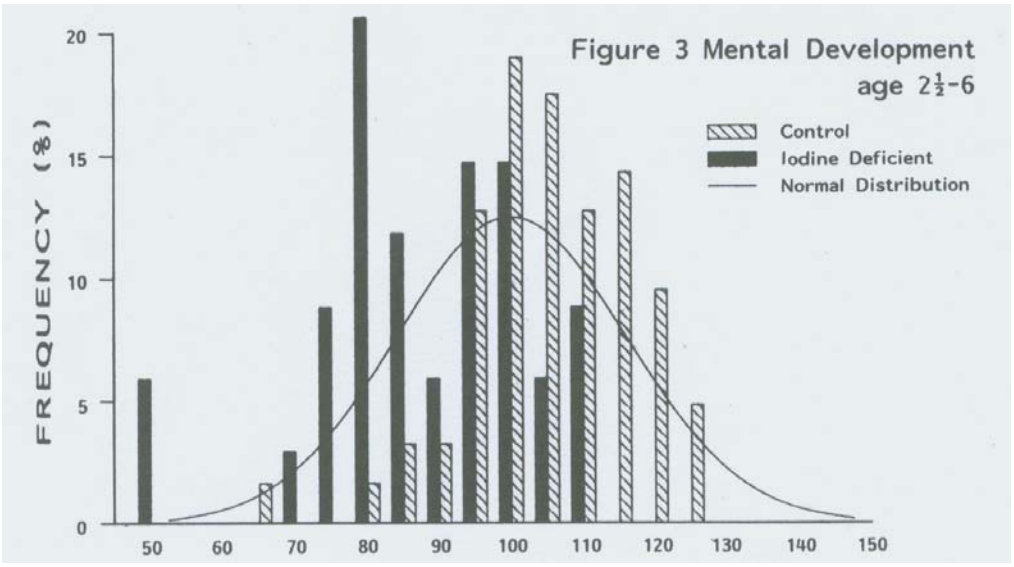
Age Group	Mental Development Scores	
	Control Children	Moderately Iodide Deficient Children
0–2.5 yrs	108.1 ± 16.0	95.9 ± 16.3
2.5–6 yrs	105.1 ± 11.6	88.1 ± 15.0
6–12 yrs	101.6 ± 13.1	88.0 ± 12.7

Source: Bleichrodt 1989, table 3.

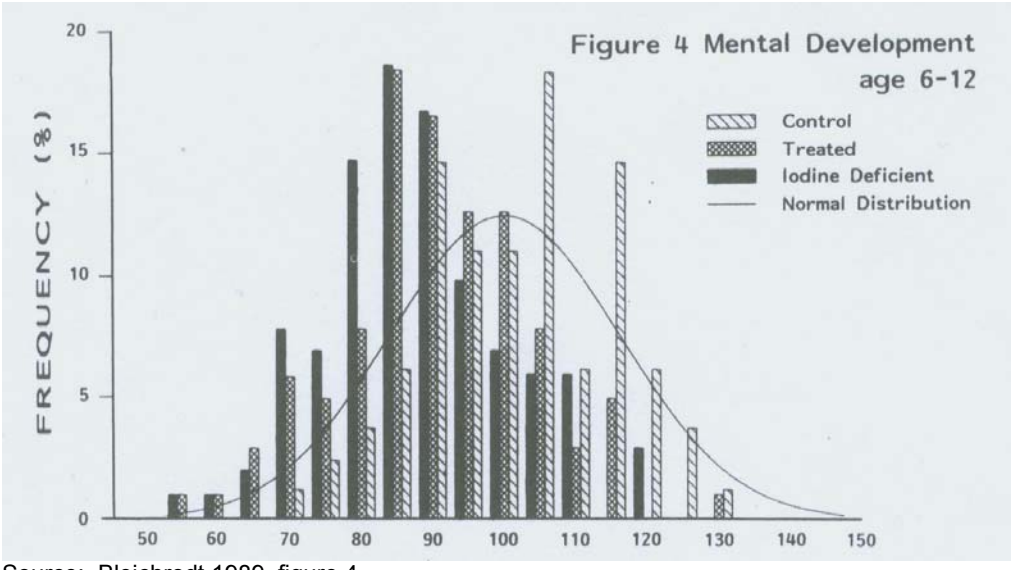
The moderately iodide-deficient group was subsequently treated twice with 2 ml of Lipiodol orally to correct its iodide deficiency and was retested 32 months later. However, the average mental developmental scores and distribution between the moderately iodide-deficient group and the group after having been treated are nearly similar (i.e., mean scores of 90.2 and 88.0 respectively). This observation is consistent with the belief that the mental deficit was irreversible and occurred during fetal and neonatal development and is not a temporary side effect of iodide deficiency. The distribution of mental development scores for the three age groups (0-2.5 years, 2.5-6 years, and 6-12 years) are provided below:



Source: Bleichrodt 1989, figure 2.

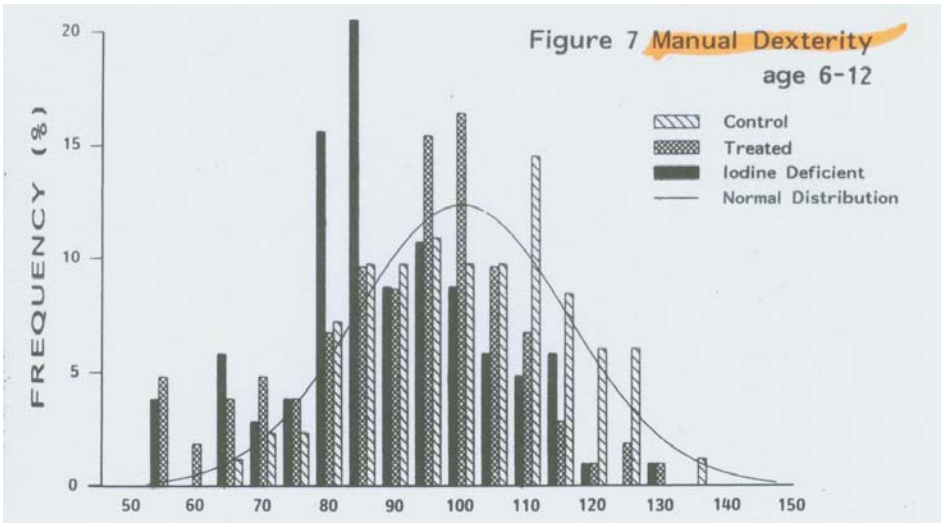


Source: Bleichrodt 1989, figure 3.

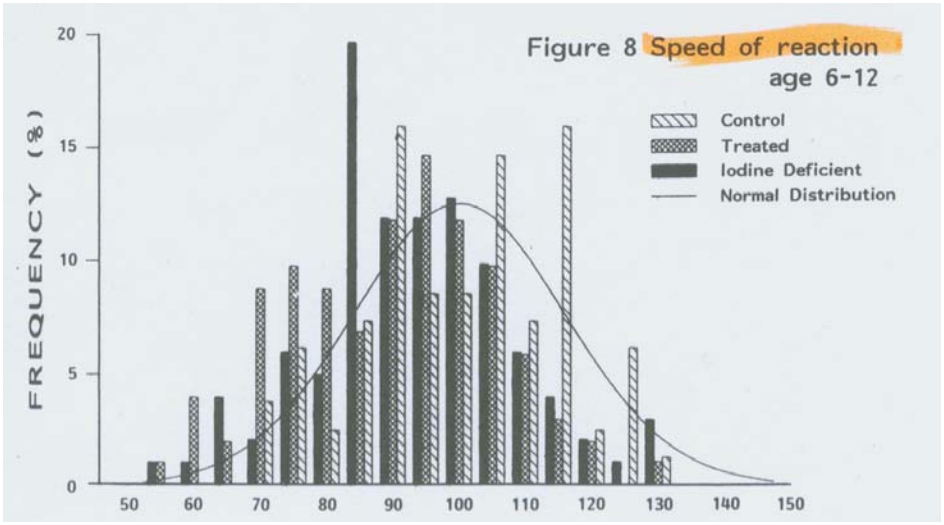


Source: Bleichrodt 1989, figure 4.

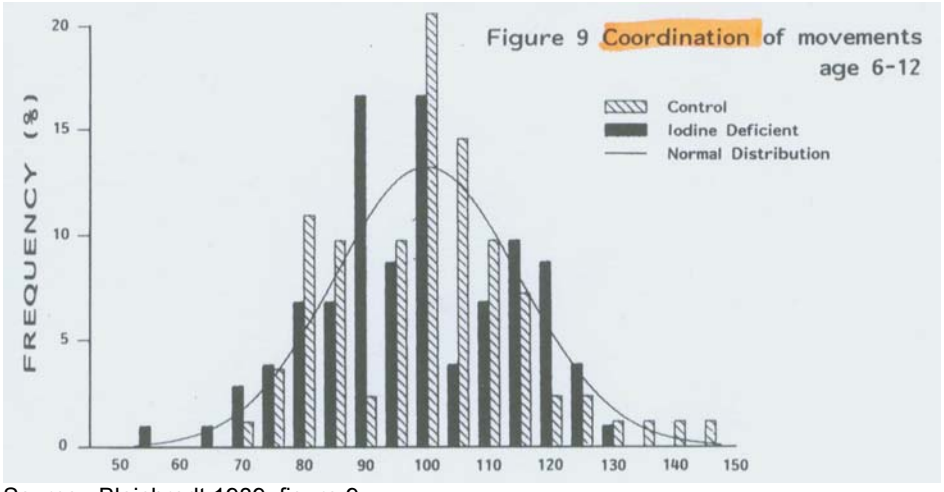
The Bleichrodt study also evaluated the psychomotor development of the 6- to 12-year-olds. The study found the coordination of body movements to be similar between the two groups, but the iodide-deficient children scored significantly lower ($p \leq 0.01$) in manual dexterity and speed of reaction. The moderately iodide-deficient group was subsequently treated twice with 2 ml of Lipiodol orally to correct their iodide deficiency and retested 32 months later. However, the average scores and distribution of scores between the moderately iodide-deficient group and the group after having been treated are nearly identical. This observation is consistent belief that the mental deficit was irreversible and occurred during fetal and neonatal development and is not a temporary side effect of iodide deficiency. The distribution of scores for manual dexterity, speed of reaction, and coordination of movements are provided below:



Source: Bleichrodt 1989, figure 7.



Source: Bleichrodt 1989, figure 8.



Source: Bleichrodt 1989, figure 9.

Vermiglio 2004 Study: The Vermiglio 2004 prospective study evaluated the neuropsychological development of 16 children born to 16 healthy mothers living in a moderately IDA (Area A) and compared them against 11 control children born to 11 age-matched women in a marginally sufficient iodide area (Area B). The urinary iodide excretion level in Area A during pregnancy was 48.1 ± 38.2 µg/day. The urinary iodide excretion level in Area B during pregnancy was 95.2 ± 55.8 µg/day. All children from both Areas A and B were euthyroid at neonatal screening, at the first examination (18-36 months), and at the second examination (8-10 years).

In 2001-2002, at the age of 8-10 years, the children were evaluated for ADHD and their intelligence was tested. In the moderately IDA A, 11 of 16 (68.7%) children had ADHD. Of the 11 ADHD children, 5 had combined-type ADHD subtype, 5 had the predominantly hyperactive-impulsive-type ADHD subtype, and 1 child had predominantly inattentive-type ADHD subtype. None of the control children from Area B had ADHD.

The Wechsler Intelligence Scale for Children (WISC)-III full-scale IQ scores, and subscale scores for ADHD-positive children from Area A, the ADHD-negative children from Area A, and the control children in Area B are summarized below:

TABLE 2. Neuropsychological test scores in ADHD+ve and ADHD−ve children from iodine-deficient area (area A) as compared with iodine-sufficient matched children (area B)

Neuropsychological test	Area A ADHD+ve children (n = 10)[a]	Area A ADHD−ve children (n = 5)	Area B control children (n = 11)	*P* value
Intelligence WISC-III full-scale IQ score (t-IQ)	88.0 ± 6.9	99.0 ± 2.0	110 ± 10	<0.0001[b] <0.05[c] <0.005[d]
WISC-III freedom-from-distractibility score	8.8 ± 2.5	8.9 ± 2.5	10.3 ± 3.1	<0.05[b]
WISC-III verbal IQ score	88.6 ± 11.4	103 ± 2.6	110 ± 12.3	<0.001[b] <0.05[d]
WISC-III verbal section				
General information	9.0 ± 2.6	9.3 ± 3.0	9.6 ± 2.5	NS
General comprehension	7.6 ± 2.4	10.3 ± 2.9	10.9 ± 3.4	<0.05[b]
Arithmetic	8.0 ± 1.5	10.6 ± 3.2	10.7 ± 2.1	<0.005[b] <0.05[d]
Similarities	7.1 ± 4.9	11.3 ± 2.5	12.7 ± 2.9	<0.005[b]
Vocabulary	9.1 ± 2.8	11.0 ± 2.0	14.2 ± 2.5	<0.005[b] <0.05[d]
Digit span	9.7 ± 2.3	8.0 ± 2.6	9.1 ± 2.3	NS
WISC-III performance IQ score	87.8 ± 8.2	94.6 ± 7.1	107.1 ± 8.9	<0.0001[b] <0.05[c]
WISC-III performance section				
Picture completion	5.9 ± 1.2	4.3 ± 2.3	11.3 ± 2.2	<0.00001[b] <0.0005[c]
Picture arrangement	9.0 ± 2.4	11.0 ± 3.6	10.5 ± 1.7	NS
Block design	9.5 ± 3.2	8.7 ± 1.5	11.4 ± 1.9	<0.05[c]
Object assembly	9.5 ± 5.0	14.6 ± 3.5	11.1 ± 2.5	<0.05[c]
Coding	8.7 ± 3.4	8.0 ± 1.0	11.0 ± 4.3	NS
Mazes	8.7 ± 2.6	5.3 ± 3.5	11.0 ± 2.8	<0.05[b] <0.01[c]

NS, Not significant.
[a] Results refer to 10/11 ADHD+ve children due to the fact that in 1/11, extreme inattention and hyperactivity made WISC administration impossible.
[b] ADHD+ve *vs.* controls.
[c] ADHD−ve *vs.* controls.
[d] ADHD+ve *vs.* ADHD−ve.

Source: Vermiglio 2004, table 2.

The intelligence WISC-III full scale IQ scores of 88.0 ± 6.9 for the ADHD-positive children from Area A were statistically lower than the corresponding IQ scores of 110 ± 10 control children from Area B. Of the 15 subscale scores, the following 9 subscale scores of the ADHD-positive children from Area A were found to be statistically lower than the

corresponding scores in the control children from Area B: WISC-III freedom-from-distractibility score, WISC-III verbal IQ score, WISC-III verbal section tests (general comprehension, arithmetic, similarities, vocabulary), WISC-III performance IQ score, and WISC-III performance section tests (picture completion and mazes).

The free T_4 level and TSH level of the pregnant women at weeks 8, 13, and 20 for the three groups (ADHD positive from Area A, ADHD negative from Area A, and controls from Area B) are provided below:

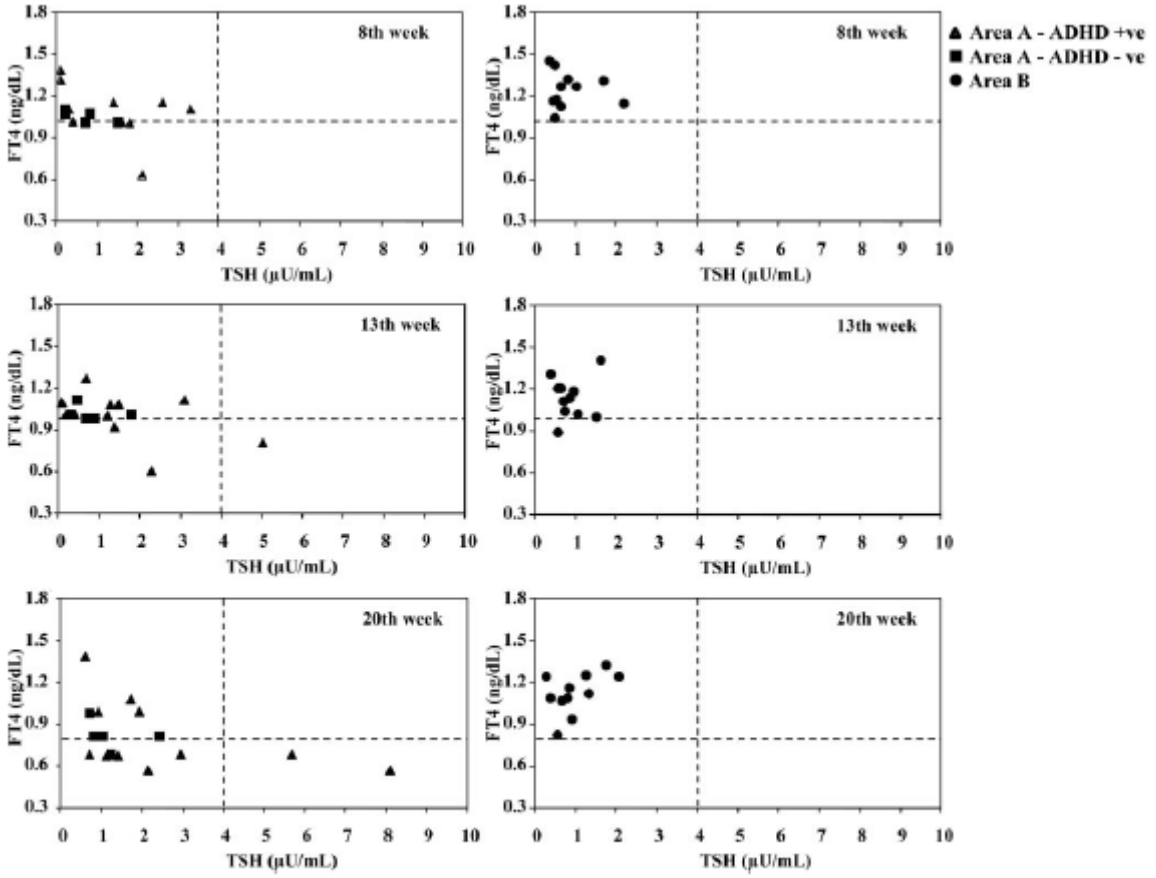


FIG. 2. Serum FT_4 and TSH levels in the 16 area A studied mothers (n = 11 ADHD+ve and n = 5 ADHD−ve subgroups) (*left*) and in the 11 area B pregnant women (*right*) measured at 8, 13, and 20 wk of gestation. The *vertical dotted lines* indicate the highest normal TSH value (4.0 μU/ml). The *horizontal dotted lines* indicate the lowest normal FT_4 values for the gestational week [1.02 ng/dl (13.1 pmol/liter) at 8 wk, 0.98 ng/dl (12.7 pmol/liter) at 13 wk, and 0.85 ng/dl (11.0 pmol/liter) at 20 wk].

Source: Vermiglio 2004, figure 2.

Figure 2 shows that 8 of 16 (50%) pregnant women from the moderately IDA A and 1 of 11 (9.1%) pregnant women from the marginally ISA B (i.e, UIC of 95 µg/L is actually considered mildly iodide deficient) had thyroid failure in early pregnancy in the form of hypothyroxinemia. The term hypothyroxinemia is used to describe pregnant women with normal TSH concentrations (0.4 – 4.0 uU/ml), but have low serum fT_4 values as compared with the range values calculated (mean ± 2 SD) at the same stage of pregnancy (8, 13, and 20 weeks) in a series of 50 healthy women with moderately adequate iodide intake (150–200 µg/day).

Vermiglio stated that ADHD is a developmental disorder involving difficulties with sustained attention, distractibility, poor impulse control, and hyperactivity or inability to regulate activity level to situational demands (Vermiglio 2004). The disorder is believed to arise early in childhood (3-7 years) and is considered organic in pathology. The current view is that dysfunctions of both the frontal and prefrontal lobes, involved in impulsivity and motor activity control, and of cortical and subcortical activity areas, involved in inhibition of irrelevant responses and executive function, are responsible for the disorder.

ADHD is a common behavioral disorder among children. The estimated occurrence of ADHD at 19 years of age is 7.5% (6.5%-8.4% at a 95% confidence interval (CI)) (Barbaresi 2004). A high prevalence ADHD (70%) is reported in children with generalized resistance to thyroid hormone (GRTH). GRTH is a disease caused by mutations in the thyroid receptor-β gene, which reduces the response of tissues to thyroid hormone (i.e., the developing fetal nerve cells do not get enough signal from the thyroid hormone to develop properly). Since the ADHD prevalence in the Vermiglio study was 69% in the moderately iodide-deficient and 87.5% in hypothyroxinemic, it suggests that a decreased signal through the thyroid receptor-β (i.e., by a mutated thyroid receptor-β gene, decreased maternal supply of T_4 to the fetus during the first 20 weeks caused by iodide deficiency, or a combination of both) is associated with ADHD. The Vermiglio study suggests that the rates of ADHD could be significantly reduced by preventing iodide deficiency during pregnancy. Furthermore, the Vermiglio study suggests the possibility that providing a normal thyroid hormone level during gestation may allow a child with GRTH to have normal brain development by not challenging the mutated thyroid receptor-β gene.

The Vermiglio 2004 study is one of four studies reporting that maternal hypothyroxinemia during early pregnancy results in neurodevelopmental deficits in children (Kooistra 2006). Hypothyroxinemia is a common condition in pregnant women characterized by low maternal fT_4 levels with normal TSH levels (Kooistra 2006). Hypothyroxinemia is regarded to be without consequences for the mother and fetus (Kooistra 2006). Hypothyroxinemia reflects a condition in which the pregnant mother has difficulty meeting her own T_4 needs and is unable to meet the fetal demand for T_4 for proper brain development (Kooistra 2006). Fetal hypothyroxinemia is induced by iodide deficiency because the immature fetal thyroid is unable to increase its avidity for iodide despite up-regulation of NIS expression in the fetal thyroid and placenta during iodide deficiency (Delange 2005a). The increased iodide clearance rate will further decrease the iodine stores of the fetal thyroid and decrease fetal T_4 synthesis (Delange 2005a). The transfer of maternal T_3 does not protect the fetal brain. The pregnant woman transfers maternal T_4 until birth, at which time maternal T_4 represents 20% to 50% of the cord serum T_4. Therefore, the fetal thyroid is required to provide the major T_4 during the last half of pregnancy when the fetal thyroid is unable to adapt to a decreased supply of iodide caused by its mother's iodide deficiency. In rats, the fetus can compensate for hypothyroxinemia by increasing the fetal brain deiodinase type 2 activity, which helps protects the fetal brain from T_3 deficiency (Delange 2005a).

Fenzi 1990 Study: A neuropsychological assessment in school-aged children in an area of moderate iodide deficiency found minor impairment of perception, motor, and attentive functions (Fenzi 1990). The epidemiological study evaluated 384 schoolchildren ages 6-14 years in eastern Tuscany having a mean urinary iodine excretion of 39 ± 38 µg/g creatinine. The control group consisted of 352 sex- and age-matched schoolchildren from an ISA with a mean urinary iodine excretion of 88 ± 15 µg/g creatinine. The tT_4, tT_3, and TSH levels were within the normal range for both groups. The study failed to show major differences in the global neuropsychological performance and cognitive performance between the two groups. However, significant differences were observed in subtest for information, verbal, and coding tests that were administered to the third graders. The lower scores in the information and verbal subtests resulted in a lower verbal IQ for the third grader in the moderately IDA. Furthermore, the lower score on the coding test, which is less correlated with global cognitive abilities, suggests minor impairment in perception, motor, and attentive functions.

Vermiglio 1990 Study: In the Vermiglio 1990 study, the visual perceptual integrative motor ability was investigated in 719 6- to 12-year-olds living in two moderately IDAs in Sicily, Italy. A modified Bender-Gestalt test was administered to all 6- to 12-year-old children. The test consists of copying as accurately as possible a number of geometrical figures, explores visual perceptive and neuromotor manual ability, and is affected by specific segments of intellectual function (i.e., memory, spatial concepts, and ability to organize and represent). The children in the moderately deficient areas scored statistically worse on the Bender-Gestalt test than the children in the iodide-sufficient control area. The results are summarized in the following table:

Performance on Bender-Gestalt Test	Moderately Iodide-Deficient Areas A + B (number of children)	Iodide-Sufficient Control Area C (number of children)	Statistical Significance (χ^2 method)
Defective	99 (13.8%)	13 (3.5%)	P < 0.000001
Borderline	124 (17.2%)	14 (3.8%)	P < 0.000001
Nondefective	496 (69.0%)	343 (92.7%)	P < 0.0000005

Source: Vermiglio 1990, table 3.

A Terman-Merrill test was administered to 96 of the 99 defective children and 62 of the 124 borderline children found on the Bender-Gestalt test in the moderately IDAs A + B. The Terman-Merrill test evaluates general intellectual aptitude. Intellectual aptitude deficiencies appeared in the following segments listed in decreasing percentage order of involvement: vocabulary (40%), logical and critical capacities (32%), reading abilities (30%), calculating abilities (28%), handwriting (25%), memory (20%), and comprehension (15%). Of the 96 defective children tested, 91 (i.e., 94.8%) had IQ scores less then 90% on the Terman-Merrill test. Of the 62 borderline children tested, 35 (i.e., 56.4%) had IQ scores less then 90% on the Terman-Merrill test. The results from the Terman-Merrill test are summarized below:

Performance on the Bender-Gastalt Test	Intelligence Quotient Score on the Terman-Merrill Test		
	<90	90-95	96-100
Defective (n = 96)	91 (94.8%)	5	0
Borderline (n = 62)	35 (56.4%)	23	4
Nondefective (n = 12)	0	10	2

Source: Vermiglio 1990, table 4.

Adverse Health Effects Observed in Children of Hypothyroid Pregnant Mothers

A 1999 maternal thyroid deficiency study reported on the subsequent neuropsychological development of the children born to hypothyroid mothers (Haddow 1999). The study identified 47 pregnant women with subclinical hypothyroidism having elevated TSH (i.e., > 6 ul/ml) and normal T_4 levels. The study also identified another 15 pregnant women with overt hypothyroidism having both elevated TSH and low T_4 levels. A survey of pregnant women in the United States identified that 2.5% of pregnant U.S. women have hypothyroidism (Utiger 1999). Likewise, North American and European evaluations identify that up to 0.5% of pregnant women (1 in 200) may have overt hypothyroidism and up to 2.5% of women (1 in 40) have undetected subclinical hypothyroidism before pregnancy (Morreale de Escobar 2004, p U33). Since hypothyroidism is difficult to diagnose, the median time to a hypothyroid diagnosis in women is 5 years (Haddow 1999).

Of the 48 pregnant women that did not receive treatment for their thyroid deficiency, the full-scale IQ of their children averaged 7 points lower ($p = 0.005$) than the 124 matched control children. The children from the 48 untreated pregnant women had statistically poorer performance on the following nine neuropsychological tests:

WISC-III full-scale IQ score	(Intelligence Test)
Number of children with IQ score ≤ 85	(Intelligence Test)
WISC-III freedom-from-distractibility score	(Attention Test)
Continuous Performance Test score > 8%	(Attention Test)
Word Discrimination	(Language Test)
WISC-III verbal IQ score	(Language Test)
PIAT-R* reading-recognition score	(School Performance Test)
WISC-III performance IQ score	(Visual-motor performance)
Pegboard-test score (Nondominant hand)	(Visual-motor performance)

*(PIAT-R) stands for Peabody Individual Achievement Score – Revised

The performance results on all 15 neuropsychological tests were observed to be lower for the untreated children than the control children. However, the statistical significant of this trend was not evaluated.

The children of the 14 treated pregnant women had performance results on 13 of the 15 neuropsychological tests that were comparable to the control children. However, the study did not conduct a statistical comparison between the children of the treated pregnant women with hypothyroidism and the control children (i.e., to determine how effective the treatment was). Even after treatment, the results of the children of the 14 treated pregnant women were worse than both the control and untreated children on the following two tests: Continuous Performance Test (Attention Test) and School difficulties and learning problems (School Performance Test). The performance results for all 15 neuropsychological tests for the children of both the treated and untreated pregnant women are provided below:

TABLE 4. NEUROPSYCHOLOGICAL TEST SCORES AMONG THE CHILDREN OF WOMEN WITH HYPOTHYROIDISM DURING PREGNANCY AS COMPARED WITH THE CHILDREN OF MATCHED CONTROL WOMEN, STRATIFIED ACCORDING TO WHETHER THE HYPOTHYROIDISM WAS BEING TREATED.*

TEST	CHILDREN OF TREATED WOMEN WITH HYPOTHYROIDISM (N=14)	P VALUE†	CHILDREN OF UNTREATED WOMEN WITH HYPOTHYROIDISM‡ (N=48)	P VALUE§	CONTROL CHILDREN (N=124)
Intelligence					
WISC-III full-scale IQ score	111	0.20	100	0.005	107
WISC-III full-scale IQ score ≤85 (%)	0	0.90	19	0.007	5
Attention					
WISC-III freedom-from-distractibility score	103	0.80	97	0.03	102
Continuous Performance Test score >8 (%)¶	50	0.01	33	0.04	19
Language					
Test of Language Development score					
Word articulation	10.5	0.60	10.0	0.6	10.2
Word discrimination	11.4	0.90	10.3	0.02	11.4
WISC-III verbal IQ score	111	0.30	101	0.006	107
School performance					
PIAT-R reading-recognition score	101	0.80	95	0.05	100
PIAT-R reading-comprehension score	105	0.40	96	0.09	101
School difficulties and learning problems (%)¶	29	0.08	21	0.09	11
Repeated a grade (%)¶	7	0.50	8	0.3	4
Visual–motor performance					
Score on Developmental Test of Visual–Motor Integration	102	0.30	94	0.1	97
WISC-III performance IQ score	109	0.30	99	0.01	105
Pegboard-test score					
Dominant hand¶	79	0.40	88	0.06	83
Nondominant hand¶	87	0.70	96	0.04	89

*WISC-III denotes Wechsler Intelligence Scale for Children, third edition, and PIAT-R Peabody Individual Achievement Test, revised.

†The P values are for the comparison of the children of the treated women with the children of the untreated women.

‡One woman received treatment before, but not during, the pregnancy under study.

§The P values are for the comparison of the children of the untreated women with the children of the control women.

¶A higher score or percentage indicates more problems.

Source: Haddow 1999, p 554, table 4.

8.2 Dose-Response Assessment

A dose-response assessment is defined as "a determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence, or change in level of response, percent response in groups of subjects (or population), or the probability of occurrence or change in level of response within a population" (IRIS 2006, EPA 2002).

Ideally, one would like to know the internal dose of free iodide concentration in the blood serum in contact interacting with the NIS to establish the dose. However, determining an internal dose of free iodide in the blood serum is problematic. Healthy levels of inorganic iodide concentrations in serum are very minute and are about 1 pg/dL (Demers 2003, p 77). Due to the difficulty in measuring plasma inorganic iodide (PII) concentration, the measurement of PII is limited to research studies in pregnancy. Since about 90% of ingested iodide is excreted in the urine, the median urinary concentration is the most practical measurement of iodide intake in a population (Dunn 1998). Therefore, in most epidemiological studies, the UIC is used as a surrogate for iodide intake.

The observed biological responses for iodide is inversely proportional to dose (i.e., the more you have in your diet, the less adverse effects that are observed). The dose-response curve for iodide is summarized in the following table:

Observed Biological Response	Iodide Sufficient	Mild Iodide Deficiency	Moderate Iodide Deficiency	Severe Iodide Deficiency
Median UIC (µg/L) in school-aged children and adults (UIC is a surrogate measurement for iodide intake (i.e., dose))	100 to 200	99 to 50	49 to 20	< 20
Prevalence of goiter (%) in school-aged children (Goiter Grades > 0)	< 5.0	5.0–19.9	20.0–29.9	≥ 30
Frequency of thyroid volume (%) in school-aged children (>97th percentile by ultrasound)	< 5.0	5.0–19.9	20.0–29.9	≥ 30
Frequency of elevated serum TSH in newborns (%) (> 5 mU/L whole blood)	≤ 3.0	3.0–19.9	20.0–39.9	≥ 40

Source: Delange 1998.

The frequency of the following biological responses are observed to increase with the the lack of iodide (i.e., decreasing iodide dose): goiter in school-aged children, frequency of thyroid volume >97th in school-aged children, and elevated TSH levels in newborns. This pattern of response to the lack of iodide shows that a segment of the population is more sensitive (i.e., thyroid-sensitive population) than the rest of the population. In ISAs (i.e., little to no thyroid stress), the baseline frequency is that up to 5% of school-aged children have goiter and up to 3% of newborns have elevated TSH. By comparison, in the minimal thyroid stress population induced by mild iodide deficiency, the frequency goiter in school-aged children increases to up to 19.9% and the frequency of elevated TSH in newborns increases to up to 19.9%. In other

words, at least 80% of the newborns are unaffected by mild iodide deficiency, while up to 20% are adversely impacted (i.e., a heterogenous response is observesed in the population). Likewise, in the considerable thyroid stress population induced by moderate iodide deficiency, the frequency goiter in school-aged children increases to up to 29.9% and the frequency of elevated TSH in newborns increases to up to 39.9%. In other words, at least 60% of the newborns are unaffected by moderate iodide deficiency, while up to 40% are adversely impacted (i.e., a heterogenous response is observesed in the population). Furthermore, even in severe IDA, not everyone gets goiter nor does everyone have cretins. For example, in seven severe iodide-deficient cities, the percentage of elevated TSH in newborns did not reach 100% but ranged from 47% in Osh, Kyrgyzstan, to 80% in Lahore, Paskistan, in relation to the severity of the iodide deficiency (Sullivan 1996). In summary, the biological response to iodide deficiency is not uniform across the human population and has to be taken into account in a cumulative risk assessment.

This observation (i.e., not all individuals develop goiters or elevated TSH levels in IDAs) indicates a heterogeneous level of adaptability to iodide deficiency within the human population. A genetic modeling study supports this observation. In euthyroid subjects, quantitative genetic modeling calculated that genetic factors account for 71% (61-78%, CI 95%) of the individual differences in thyroid volume, where as the estimate for unique environmental effects account for only 29% (22-39%, CI 95%) (Hansen 2004). Therefore, the iodide-sensitive thyroid population should be added to the list of sensitive groups for this public health issue. The iodide-sensitive thyroid population is not a trivial concern (e.g., such as an unexplained rare idiosyncratic reaction), because elevated TSH in neonates occurs up to 17% in the mild iodide-deficient population (i.e., 19.9% – 3% background) and up to 37% in the moderately iodide-deficient population (i.e., 39.9% - 3% background). Therefore, neonates have elevated serum TSH more frequently than adults for the same level of iodide deficiency (Delange 1998). Thus, neonates appear hypersensitive to iodide deficiency. Thus, the most sensitive population for cumulative risk assessment purposes is the iodide-sensitive fetus and neonate during gestation and lactation whose mothers are iodide deficient.

Since the early 1970s, newborns are screened for permanent congenital hypothyroidism (CH) (incidence of CH is about 1 in 4,000 births) by measuring the serum TSH (Delange 1998). However, CH screening also detects transient primary hypothyroidism (incidence can be as high as 1 in 10 neonates). Neonatal TSH screening is one of the indicators recommended by the World Health Organization (WHO), the United Nations International Children's Emergency Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) for assessing iodide deficiency disorders and their control (Delange 1998). Neonatal TSH is the single indicator that focuses on potential brain damage, which is the major impact of iodide deficiency (Delange 1998). Neonates with elevated TSH levels are at a high risk of developing subclinical hypothyroidism in infancy and early childhood (Delange 1998).

The Calaciura 2002 study evaluated 56 children with transient neonatal subclinical hypothyroidism at the neonatal TSH screening (Calaciura 2002). All 56 children had a TSH > 20 mU/L at the initial neonatal screening, but during the recall/confirmation examination at 22.2 ± 6.2 days, 33 children (Group I) had a normal fT_4 level and fully normal TSH level (i.e., < 5.0 mU/L) and the remaining 23 children (Group II) had a normal fT_4 level with high normal/slightly

elevated TSH level (i.e., 5-17 mU/L) (Calaciura 2002). Upon follow-up examination in early childhood, 70% of Group I children and 36% of Group II children had mild thyroid dysfunction. Calaciura determined that all infants with elevated TSH at neonatal screening are at risk for developing subclinical hypothyroidism in early childhood (odds ratio 44.6%; 95% CI 38.8-50.4%). Furthermore, for the first time, several different thyroid abnormalities were identified in neonates with elevated TSH levels at birth. Twenty-four of the 56 children had one or more of the following thyroid abnormalities: genetic, immunological, and morphological. This observation documents a partially dysfunctional or underperforming thyroid in a subset of the human population that makes them more sensitive to NIS stressors (e.g., lack of iodide). The adaptability of the thyroid to respond to stress is not uniform in the human population and, therefore, should be thought of as being heterogeneous in the human population. The cumulative risk assessment has to incorporate this difference in the thyroid's adaptability within the human population.

Cognitive deficits in the children of mildly iodide-deficient pregnant women have not been documented (Delange 2001). Unfortunately, these epidemiological studies only compare children from a mild IDA against control children in an ISA. However, the human thyroid's ability to compensate for mild iodide deficiency is not uniform across the population. Up to 17% of humans who may have partially dysfunctional thyroids with limited adaptability have difficulty compensating for mild iodide deficiency. Since neonatal TSH level is the single indicator that focuses on potential brain damage, the partially dysfunctional thyroid subgroup can be tentatively identified by grouping the neonates with an elevated TSH level. The current epidemiological studies evaluating the cognitive performance of children of mildly iodide-deficient pregnant mothers do not segregate the neonates with elevated TSH who are at greater risk of developing mental deficits for evaluation. The potential for lower cognitive performance in these elevated TSH neonates, averaging 8.5% (i.e., 17% ÷ 2) of the population in mild IDAs, is potentially lost in the average cognitive score of the school-aged children (i.e., the cognitive scores from the 91.5% of the normal TSH neonates statistically overwhelms the scores of the 8.5% neonates with elevated TSH). Therefore, in order to confirm that a mild iodide deficiency in pregnant women does not cause any cognitive deficits in partially dysfunction thyroid fetuses/neonates (i.e., a potential sensitive group), the cognitive scores of children who had an elevated TSH level at birth need to be compared to the cognitive scores of appropriate controls (i.e., cognitive scores of children from ISAs or cognitive scores of children from mildly IDAs with normal TSH levels at birth). Therefore, in short, current epidemiological studies have not ruled out the possibility that cognitive deficits could be observed in fetuses/neonates with partially dyfunctional thyroids.

The adverse effect of concern for this public health issue is the potential for mental deficits in fetuses and neonates. The dose-response relationship for mental deficits from the lack of iodide is characterized by the epidemiological studies identified in the hazard characterization assessment. These studies are primarily designed to identify and detect mental deficits. They were not specifically designed to establish a well-documented dose-response curve showing an increased occurrence of mental defects with decreasing dose of iodide. However, these studies show, as required by the definition of a dose-response assessment, a "change in level of response" with decreasing dose of iodide (i.e., a decreasing maternal TIU). The severity of the mental deficits clearly increases as the lack of iodide increases (i.e., as the maternal TIU

becomes lower). The following table summarizes the observed mental deficits in the children of mothers living in IDAs:

Iodide Exposure Levels in Pregnant Women	Observed Mental Deficits in the Children of Mothers in Iodide-Deficient Areas
Iodide sufficient	No increased occurrence of mental or physical effects
Mild iodide deficiency	Delayed reaction time Potential cognitive effects in partially dysfunctional thyroid individuals (see discussion above) Increased frequency of mild thyroid dysfunction in childhood
Moderate iodide deficiency	Subtle, minor, or overt neurological, psychological, and intellectual deficits
Severe iodide deficiency	Cretinism 5–15% prevalence (severe, permanent mental and physical defects)

Source: OIG Analysis.

The NAS Committee concluded the first adverse effect in its mode-of-action model is hypothyroidism and any effects downstream (e.g., neurodevelopmental deficits in infants) of hypothyroidism clearly would be adverse (NAS 2005, p 166). The NAS Committee stated that "developmental deficits" are clinical manifestations of hypothyroidism (NAS 2005, p 29). The NAS Committee identifies hypothyroidism as a deficiency in thyroid hormone production (NAS 2005, p 35). Overt (i.e., primary) hypothyroidism is indicated by high serum TSH concentrations and low T_4 (Ladenson 2005). Mild hypothyroidism is indicated by elevated TSH and normal T_4 (Ladenson 2005). The NAS Committee stated that:

> Iodide can be obtained only by ingestion of food or water that contains it. Therefore, iodide deficiency and reduction in thyroid hormone production can occur if iodide intake is very low. Because the body maintains the serum concentrations of thyroid hormones with narrow limits through feedback control mechanisms, there is remarkable compensation for iodide deficiency. Generally, thyroid production is normal even when iodide intake is quite low. Hypothyroidism occurs only if daily iodide intake is below about 10 to 20 µg (about one-fifth to one-tenth of the average intake in the United States) [10-20 µg of iodide intake equates to severe iodide deficiency]. However, in pregnant women, iodide deficiency of that severity can result in major neurodevelopmental deficits and goiter in their offspring. Lesser degrees of iodide deficiency may also cause important neurodevelopmental deficits in infants and children (NAS 2005, p 6).

The NAS Committee also states:

> Furthermore, among people 15-44 years old, including pregnant women, there are no differences in serum TSH and T_4 concentrations between those with urinary iodide values less than 50 µg/L and those with higher values . . . " (NAS 2005, p 46-47).

These statements represent the NAS Committee's position that neurodevelopmental deficits in infants would not be expected to occur until severe iodide deficiency during pregnancy induces overt hypothyroidism in the mother, resulting in an elevated TSH concentration accompanied by a low T_4.

Unfortunately, the NAS Committee's proposed mode-of-action model for perchlorate is not completely correct. The NAS Committee identifies that adults with moderate iodide deficiency have normal TSH and T_4 levels (NAS 2005, p 48). The NAS adverse effect continuum presumes that pregnant women have to become hypothyroid before neurodevelopmental deficits are observed. However, this statement is not true. For example, due to the added stress of pregnancy, moderately iodide-deficient pregnant women are observed to develop hypothyroxinemia (i.e., normal TSH and low fT_4). The hypothyroxinemia in moderately iodide-deficient pregnant women resulted in mental deficits of their children (Vermiglio 2004, figure 2). Furthermore, under mild iodide deficiency during pregnancy, the maternal thyroid function at delivery is characterized by "relative hypothyroxinemia," which is an increased T_3/T_4 ratio (i.e., indicating a preference for T_3 secretion) and slightly increased TSH levels (i.e., 97% of the pregnant women were still in the normal range) (Glinoer 1992). Likewise, the less-stressed state of mildly iodide-deficient pregnant women are be observed to be euthyroid (Glinoer 1995, figure 4), but they are documented to have children with permanent delayed reaction times (Lombardi 1995; Vitti 1992) and are documented to have up to a 17% increased occurrence of newborns with elevated TSH levels (i.e., subclinical hypothyroidism) over background levels observed in iodide-sufficient populations (Delange 1998). Therefore, pregnant women do not have to develop overt hypothyroidism, as proposed by the NAS Committee, to increase the risk of their offspring developing mental deficits.

Although the maternal hypothalamic-pituitary-thyroid axis has a large ability to adapt to the stress from iodide deficiency without showing obvious changes in TSH and T_4 levels (Soldin 2005, p 184, figures 2 and 3), the fetal thyroid is immature and less able than the mother's thyroid to adapt to the stress induced by mild or moderate iodide deficiency. Fetuses and neonates do not have a thyroidal store of hormones and have a proportionally higher demand for thyroid hormones because they use them for both development and homeostasis, while adults use thyroid hormones primarily for homeostasis only. During the third trimester, the fetal thyroid provides the majority of T_4 (i.e., maternal T_4 represents 20% to 50% of the cord serum T_4) (Delange 2005a). Under iodide-deficient conditions, the immature fetal thyroid is unable to increase its avidity for iodide despite up-regulation of NIS expression in the fetal thyroid and placenta during iodide deficiency (Delange 2005a). The increased iodide clearance rate will further decrease the iodine stores of the fetal thyroid and decrease fetal T_4 synthesis (Delange 2005a). Fetuses and neonates appear to be more sensitive to iodide deficiency than pregnant women (Delange 1998). For mild to moderate iodide deficiency, maternal measurements of TSH, fT_4, and T_4 are not appropriate markers to identify thyroid stress from iodide deficiency (Soldin 2005, p 184). Thyroid function tests are not a measure of iodide sufficiency (Soldin 2005, p 184). Therefore, maternal urinary iodide is the only marker known that can serve as an indirect measure of the amount of fetal thyroid stress induced by maternal mild to moderate iodide deficiency (Soldin 2005, p 184).

During mild and moderate iodide deficiency, the fetus has some ability to adapt to a decreased T_4 hormone supply by increasing the fetal brain type 2 deiodinase (D2) activity, which protects the fetal brain from T_3 deficiency (Delange 2005a, p 737). The compensation mechanism is observed in tracer studies of neonatal rat brains (Bianco 2005, p 123). The increased production of T_3 from T_4 by the increased activity of fetal rat brain type 2 deiodinase and the prolonged residence time of T_4 by the increased activity of fetal rat brain type 3 deiodinase (D3) mitigates the effects from severe iodide deficiency and mild to moderate hypothyroidism (Bianco 2005, p 123). The activation of this fetal rat brain D2-D3 deiodinase compensatory mechanism clearly indicates that the fetal rat brain is not getting an adequate supply of T_4 during severe iodide deficiency and mild to moderate hypothyroidism.

In summary, although the pregnant woman is often euthyroid during iodide deficiency, the fetal thyroid is less able to adapt to this iodide deficiency, resulting in the increased risk for fetal brain damage even though the mother's thyroid hormone level remains normal. Thyroid function tests are not a measure of iodide sufficiency (Soldin 2005, p 184). Therefore, maternal urinary iodide is the only marker known that can serve as an indirect measure of the amount of fetal thyroid stress induced by maternal mild to moderate iodide deficiency (Soldin 2005, p 184). The lowest UIC level in pregnant women in which an adverse effect (e.g., delayed reaction time) is observed in the fetus is about 100 μg/L, which corresponds to a mild iodide deficiency.

8.3 Exposure Assessment

An exposure assessment is defined as "an identification and evaluation of the human population exposed to a toxic agent [i.e., stressor in cumulative risk assessment terms], describing its composition and size, as well as the type, magnitude, frequency, route, and duration of exposure" (EPA 2002).

As the background exposure levels to perchlorate, thiocyanate, and nitrate must be determined to conduct a cumulative risk assessment, the background exposure level to the lack of iodide (i.e., the iodide nutritional status) also needs to be known. As the dose-exposure assessment has identified the iodide levels at which various adverse effects occur, the exposure assessment determines the portion of the population that is exposed at these levels. Since neurological, psychological, and intellectual deficits are known to occur in moderately iodide-deficient populations, the exposure assessment needs to determine the size of the U.S. pregnant women subpopulation that might be moderately iodide deficient. Furthermore, since delayed reaction times and the possibly of cognitive deficits in partially dysfunctional thyroid individuals occur in mildly iodide-deficient populations, the exposure assessment must determine the size of the U.S. pregnant women subpopulation that might be mildly iodide deficient.

U.S. Iodide Nutritional Status

CDC's National Center for Health Statistics (NCHS) surveyed the iodide levels in the U.S. population through NHANES. NHANES is designed to collect data on the health and nutritional status of a statistically representative sample of the U.S. population. According to WHO, iodide-sufficient populations have median UIC greater than 100 µg/L, and no more than 20% of the population should have a UIC below 50 µg/L (Hollowell 1998). Although the median urinary levels in the U.S. population are adequate by the WHO definition, the median UIC has decreased more than 50% from NHANES I (1971-1974) to NHANES III (1988-1994), and remained at this lower value in NHANES 2000 (see table below). The decrease in iodide consumption since 1984 is thought to be due to the reduction in the amount of iodide in milk and the replacement of iodide salts by bromine salts as the dough conditioner in commercial bread production (Hollowell 1998).

Population Group	Urinary Iodide Levels (µg/L)		
	NHANES I (1971-1974)	NHANES III (1988-1991)	NHANES 2000
U.S. Population (6-74 years of age)	320 ± 6 µg/L	145 ± 3 µg/L	161 ± 7 µg/L

Source: CDC/NCHS 2007.

The NHANES 2000 median urinary iodide level of 161 µg/L (95% CI 14.7 – 17.6) agrees with the NHANES III median of 145 µg/L. However, the NHANES 2000 urinary iodide data represent only 1433 samples, which does not allow for detailed analysis of the iodide status of subpopulations (e.g., pregnant or lactating women). Since the NHANES 2000 median agrees with the NHANES III median, the NHANES III distribution data (i.e., the best available dataset) are used to assess the amount of the U.S. population, and the amount of the sensitive pregnant

and lactating population, with inadequate iodide. The following table summarizes the distribution of urinary iodide values determined in the NHANES III survey:

Population Group	Urinary Iodide Concentration Mean (SEM)	Percentiles for Urinary Iodide (µg/L) from NHANES III (1988-1994)			
		5th	10th	25th	50th (Median)
U.S Population (ages 6-71+) (n = 21,298)	275 µg/L (27)	30 µg/L	45 µg/L	81 µg/L	145 µg/L
Pregnant (n = 343)	196 µg/L (11)	43 µg/L*	58 µg/L	92 µg/L	140 µg/L
Lactating (n = 95)	161 µg/L (22)	25 µg/L*	29 µg/L*	81 µg/L*	109 µg/L*

SEM = Standard Error of the Mean
* These values are potentially unreliable in a statistical sense based on an insufficient sample size.

Source: NAP 2000.

The severity of iodide deficiency can be determined by the amount of iodide in the urine. The following table identifies the urinary iodide levels for each of the three categories of iodide deficiency:

Parameter	Classification of Iodine Deficiency		
	Mild	Moderate	Severe
Median Urinary Iodide Levels	99 to 50 µg/L	49 to 20 µg/L	< 20 µg/L

Source: Delange 2005, p 265.

The NHANES III survey documents that 8.1% of males and 15.1% of females are moderately iodide deficient (i.e., UICs < 50 µg/L) (Hollowell 1998). The NHANES III survey identifies that 14.9 % ± 1.2 of the U.S. women of childbearing age are moderately iodide deficient and that 6.9 % ± 1.9 of pregnant U.S. women are moderately iodide deficient (Hollowell 1998). The number of lactating U.S. women with moderate iodide deficiency cannot be precisely determined due to the small sample size in the NHANES III for this category (see * footnote above), but appears to be about 16%. Furthermore, the NHANES III survey indicates that about an additional 22% (i.e., corresponding to about the 29th percentile) of U.S. pregnant women are mildly iodide deficient. The NHANES survey also indicates that about an additional 26% (i.e., corresponds to about the 42nd percentile) of the U.S. lactating women are mildly iodide deficient. The demands of pregnancy on the thyroid cause the UIC to steady decrease from the first to the third trimester (Glinoer 2005). The burdens of lactation continue the trend. Therefore, to have a greater percentage of lactating women than pregnant women both moderately and mildly iodide deficient is consistent with the biology.

The NHANES III survey also documents that the proportion of the U.S. population that was moderately iodide deficient was 4.5 times higher in 1988-1991 than in 1971-1974 (Hollowell 1998). The percentage of women of childbearing age (15-44 years) who were moderately iodide deficient increased 3.8 from the 1971-1974 period to the 1988-1991 period (Hollowell 1998). Furthermore, the percentage of pregnant women who were moderately iodide deficient increased 6.9% from the 1971-1974 period to the 1988-1991 period (Hollowell 1998).

Since the NHANES 2000 data do not provide iodide nutritional information for pregnant women, the OIG checked the current validity of the NHANES III data by evaluating the results

from a 2004 urinary iodide sampling of 100 consecutive healthy, pregnant women in an inner-city obstetric clinic in Boston, Massachusetts (Pearce 2004). The Pearce study shows a similar distribution of mildly and moderately iodide-deficient pregnant women as was observed in the NHANES III survey (Pearce 2004). The Boston sample found 9% of the pregnant women were moderately deficient (i.e., < 50 μg/L UIC). The Boston sample found about 21% of the pregnant women (i.e., the 30th percentile) were mildly iodide deficient (i.e., UIC was greater than 50 μg/L but less than 100 μg/L). Finally, 49% of the Boston sample of pregnant women had UICs below the U.S. recommended dietary allowance (RDA) of 150 μg/L (i.e., corresponds to a daily intake of ~ 220 μg/day for a pregnant women). Therefore, the Boston sampling confirms that the percentage of iodide-deficient pregnant women has remained stable over the last decade since the 1988-1994 NHANES IV.

Criticism of the NHANES III Data to Accurately Assess Iodide Deficiency

The NAS Committee directly commented on the NHANES III survey results that 15% of women of childbearing age and 7% of pregnant women had UIC less than 50 μg/L (NAS 2005, p 46). NHANES measured UIC through a single spot (untimed) analysis. The NAS Committee noted, " . . . distribution of iodide values measured in a spot urine samples is broader than values measured repeatedly in individual subjects (Anderson et al. 2001), this leads to overestimation of the number of subjects with both low and high values" (NAS 2005, p 46). In short, the NAS Committee's opinion is that NHANES overestimates the percentage of U.S. women of childbearing age and pregnant women who are moderately iodide deficient due to the limitations of a single urine spot test.

An Associate Clinical Professor of Medicine and Epidemiology of the Yale School of Medicine provided public comments on the same topic to California's Draft Public Health Goal for Perchlorate in Drinking Water on behalf of the Lockheed Martin Corporation (Borak 2002). The Associate Clinical Professor indicated that the NHANES III survey " . . . exaggerates and perhaps misrepresents the iodine sufficiency of the U.S. population generally and that of pregnant women specifically" (Borak 2002). The Associate Clinical Professor challenged the meaningfulness of the NHANES III survey results that indicate that 15% of women of childbearing age and 7% of pregnant women may be moderately iodide deficient on the following two perspectives:

1. A single spot urine sample reflects very recent dietary intake rather than diet generally. The Associate Clinical Professor also cites the Anderson field study (Anderson 2001), which measured urinary iodide samples in 15 men monthly for 12 months. The variability of urine iodide levels within a month across the individual samples was much greater than the variation of individual means considered across the population (i.e., 6.7% of individual samples were < 25 μg/L but no individual had a yearly average level < 25 μg/L). Therefore, in the Associate Clinical Professor's opinion, the NHANES III survey results " . . . overstates the proportion of subjects with very low (and very high) urinary iodide levels." The Associate Clinical Professor suggested that if multiple urine samples were obtained instead of single spot samples from pregnant women, few (if any) would have levels of < 50 μg/L.

2. In a follow-up study by Hollowell (Hollowell 2002), TSH and total T_4 levels were evaluated in the serum samples collected for the NHANES III survey. This study found that 0.3% had overt hypothyroidism (i.e., elevated TSH and below normal T_4) and 4.3% had subclinical hypothyroidism (i.e., elevated TSH and normal T_4). The Associate Clinical Professor indicated that this study found that moderate iodide deficiency does not predispose an individual to hypothyroidism.

The OIG addresses these criticisms by the NAS Committee and the Associate Clinical Professor regarding the utility of using the NHANES III survey to estimate the frequency of iodide deficiency with in the U.S. population with the following comments:

- The OIG's biggest concern is that both the NAS Committee and the Associate Clinical Professor's comments cite a 12-month "**male"** urine study. The variation in a spot urine sample is expected to vary because of several factors: variation in urine dilution due to the body's level of hydration, diurnal iodide variation, time since last meal, and seasonal iodide intake variation (Demers 2003). However, the essential point is to know how the atypical biology of the most sensitive group (i.e., pregnant women) affects the results of the urinalysis. Pregnancy produces pronounced changes on the body. For the spot urine test, the relevant change is that renal blood flow and glomerular filtration increase, which leads to the increased clearance of iodide from the plasma (Glinoer 2005). This change in the kidneys results in a lower plasma-iodide concentration and the elevated excretion of iodide in the urine. Since pregnant women are both using more iodide (i.e., to provide for the fetus) and are losing more iodide through the kidneys, the recommended iodide for pregnant women is much higher (e.g., RDA of 230 µg iodide intake/day (WHO/UNICEF/ICCIDD RDA of 250-300 µg iodide intake/day) versus 150 µg iodide intake/day for an adult). Since pregnancy causes an increase in the iodide excretion rate, the UIC will appear higher than normal for the given level of iodide intake, thus giving a false reading (i.e., bias high) of the actual iodide nutrition status of the pregnant women (Demers 2003, p 78). Therefore, the increased excretion from the kidneys during pregnancy biases the UIC high, while the spot test can bias the UIC low; thus, the two effects probably cancel each other out. Thus, the NHANES III survey results on the rate of iodide deficiency in pregnant women are probably reasonable, but these results do represent a significant uncertainty in the science concerning this public health issue (i.e., what portion of the U.S. pregnant women population is moderately or mildly iodide deficient?).

- A closely related concern is how to interrupt, for example, a UIC of 50 ug/L in pregnant women. A UIC of 50 ug/L in nonpregnant adults is considered to be moderately iodide deficient. The recommended UIC for healthy, nonpregnant adults is 100 to 200 µg/L (Delange 2005, p 278, table 11E.8). Therefore, the median healthy UIC value is 150 µg/L. Thus, a UIC of 50 ug/L in nonpregnant adults is one-third of normal. However, the recommended healthy UIC in pregnant women is higher. Several credible sources have opined on the recommended UIC for pregnant women. The average recommended UIC for pregnant women from these opinions was 204 µg/L. The following table summarizes the sources and recommended UICs for pregnant women:

Source	Recommended Iodide Intake in Pregnant Women (µg/day)	Recommended Urinary Iodide Concentration (µg/L)
WHO/UNICEF/ICCIDD*	200	200-300
Dr. Francois Delange, MD**	225-300	150-230
WHO Technical Consultant Group***	225-375	150-249
Recommend Dietary Allowances (RDA)†	220†	150††
Average Value =>	258	204

* Source: Delange 2005, p 278, table 11E.8.
** Source: Delange 2004.
*** Source: ATA 2006.
† Source: NAP 2000.
†† Source: Pearce 2004.

Therefore, a UIC of 50 ug/L in pregnant women is a lower fraction of the healthly recommended UIC for pregnant women. A UIC of 50 ug/L in pregnant women is less than one-fourth the average recommended UIC level for pregnant women. A UIC of 50 ug/L in pregnant women could be as much as one-sixth the recommend UIC level if the upper limit of 300 ug/L from the WHO/UNICEF/ICCIDD is used. Therefore, a UIC 50 ug/L in pregnant women represents a greater level of iodide deficiency than the same corresponding UIC of 50 ug/L in nonpregnant adults.

- To assess whether the NHANES III survey data overestimate the percentage of U.S. pregnant women with iodide deficiency, the OIG searched for an independent source of data that could be used to corroborate the NHANES III survey results. Iodide levels in human breast milk (i.e., a different excretion route) are an independent source of data that can be used to independently assess the iodide deficiency status of U.S. lactating women. The observed iodide content of human breast milk can be used to document the frequency of iodide deficiency in U.S. pregnant women. The WHO/UNICEF/ICCIDD uses the following iodide breast milk values to classify the severity of iodide deficiency in goiter endemic areas:

Parameter	Classification of Iodide Deficiency		
	Mild	Moderate	Severe
Iodide Levels In Human Breast Milk	35 to 50 µg/L	20 to 35 µg/L	< 20 µg/L

Source: Delange 1994, p 109.

The urinary iodide results from the NHANES III survey indicate that about 42% of the U.S. lactating women population is iodide deficient. By comparison, three studies measuring the iodide content of breast milk had the following findings:

  - Of the 23 lactating women measured for iodide in the Kirk 2005 study, the iodide content of the breast milk in 14 of 23 lactating women (60%) was measured to be iodide deficient (i.e., < 50 µg/L) (Kirk 2005).

- Of the nine lactating women in the Kirk 2007 study, the iodide content of the breast milk in four of nine lactating women (44%) was found to be iodide deficient (i.e., < 50 µg/L) (Kirk 2007).

- Of the 57 lactating women measured for iodide in the Pearce 2007 study, the median iodide breast milk was reported to be 155 µg/L (range 2.7 - 1968 µg/L) (Pearce 2007). Since only summary statistics were provided (i.e., not individual results for each women), the percentage of iodide-deficient women could not be identified. Pearce points out that differences in analytical methods for measuring total iodide may explain some of the differences between the Pearce 2007 study and the Kirk 2005 and 2007 studies.

Although the sampling size in these three studies is too small to adequately identify the distribution of iodide deficiency in the U.S. lactating population as being mild, moderate, or severe, the percentage of iodide-deficient lactating women in the two Kirk studies (60% and 44%) do independently support the conclusion from the NHANES III survey that about 42% of the U.S. lactating women population is iodide deficient.

- Iodide surveys using the spot test (e.g., NHANES III survey, NHANES 2000, and Boston area study) are appropriate to identify the average UIC of a population. The inherent differences in urine dilution in a spot urine survey are partially compensated for by using a large number (~50) of subjects in each study population (Demers 2003, p 77). Due to these limitations, the spot test urine surveys are not well suited to accurately characterize the size of the high or low UICs in the population. Characterizing the high and low UICs in the population is critical, because the population with high and low UICs is the population susceptible to adverse health effects from iodide.

- Additional iodide survey(s) should be designed to accurately characterize the population with high and low iodide intakes. Iodide intake is best determined by a 24-hour urine sample; however, logistics make this method impractical for epidemiological studies (Demers 2003, p 77). The difference in level of dilution of a spot urine sample can be partially compensated for using results normalized to urine creatinine (i.e., µg iodide excreted/gram creatinine (UI/Cr ratio)), but the diurnal and seasonal cycles of iodine and creatinine urinary excretion are different along with seasonal variations in iodide intake (i.e., higher UIC in winter) (Demers 2003). Furthermore, urinary iodide excretion can vary even in healthy, well-nourished individuals. The Associate Clinical Professor recommends taking more samples to better characterize the UIC. However, this is unlikely to happen because the CDC does not consider iodide nutrition to be a problem or priority (the NHANES 2000 survey only took 1,433 samples to measure iodide instead of the 21,298 samples in the NHANES III survey). Therefore, a practical substitute of the ideal but impractical 24-hour urine sample is the age- and sex-adjusted (UI/Cr) ratio in a fasting morning specimen, which comes close to the true 24-hour iodide excretion if nutrition is generally adequate (Demmers 2003, p 77). Since urinary iodide is diurnal and reaches a median early in the morning or 8-12 hours after the last meal, another practical substitute could be to take spot urine samples at these times (Demers 2003).

- In pregnant women with iodide deficiency, a steady decrease in UIC is seen from the first trimester through the third trimester, hence revealing the underlying tendency toward iodide deficiency associated with the pregnancy (Glinoer 2005). This steady decline in UIC continues through lactation. This trend is seen in the NHANES III survey data with the UIC of lactating women being consistently lower than the UIC of pregnant women for the same percentile. The point is the NHANES III data do not indicate when the spot urine sample was taken during the pregnancy (i.e., which trimester(s)). If the majority of the data points are early on in the pregnancy, the UIC might be biased high and not representative of the last trimester of the pregnancy.

- The Associate Clinical Professor questions the association between iodide deficiency and hypothyroidism. The NAS Committee's mode-of-action model assumes that pregnant women have to become hypothyroid before mental deficits in their children occur. However, the epidemiological studies of iodide deficiency in euthyroid pregnant women show mental deficits in their children (see hazard characterization section above for details). These studies indicate that the mother does not have to be hypothyroid before mental deficits can occur in her children. Although the mother can be euthyroid during iodide deficiency, the fetal thyroid is less able to adapt to the iodide deficiency, resulting in the possibility of permanent lifelong mental deficits. Therefore, the Associate Clinical Professor's issue that the Hollowell's study (Hollowell 2002) does not associate iodide deficiency with hypothyroidism is not critical to assessing the risk. By contrast, iodide deficiency clearly stresses the thyroid of pregnant women; this situation is only partially reversible during late postpartum (Glinoer 1992).

- The Associate Clinical Professor points out that the spot urine samples reflect only recent dietary intake and not yearly averages of UIC. However, due to the speed at which the fetal brain develops, a 2- or 3-week period of insufficient thyroid hormones may be adequate to irreversibly affect the brain's development and result in permanent, lifelong mental deficits in the child. This short time frame is observed in a study of the treatment of congenital hypothyroidism, where neonates who took longer than 2 weeks to have their thyroid hormone levels normalize had significantly lower cognitive, attention, and achievement scores than those infants who had normal thyroid function within 1 or 2 weeks of therapy (Selva 2005).

Assessing Iodide Exposure through the FDA Total Dietary Study

Exposure can also be assessed by estimating the amount of dietary intake. On January 2, 2008, FDA published a TDS measuring the dietary intake of iodide (FDA 2008). The TDS involved the purchase and iodide testing of 285 foods and beverages that represents the "total diet" of the average U.S. population. Iodide has been analyzed for in all TDS foods since 2003. The TDS was designed to provide a reasonable estimate of total dietary exposure to iodide from all foods in the diet. The contribution of iodide from each food group was determined by summing the estimated intakes from all TDS foods within each of the 12 food groups. The percentage of total intake of iodide is calculated for each food group for 14 age/sex groups. The percentage contribution of estimated daily intake of iodide for each food group for three age/sex groups is provided in the following table:

Total Dietary Study (TDS) Food Group	Percentage Contribution (%) of Each TDS Food Group to the Estimated Daily Intake of Iodide for 2003-2004		
	Non-Nursing Infants (6-11 months old)	Children (6 years old)	Women (25-30 years old)
Baby Food	56	0	0
Beverage	1	2	9
Dairy	34	70	49
Egg	2	2	4
Fat/oil	0	0	0
Fruit	2	3	3
Grain	3	14	20
Legume	0	0	0
Mixture	1	5	8
Meat, poultry, fish	0	2	3
Sweets	0	1	2
Vegetable	1	1	2

Source: Murray 2008, table 6.

The FDA TDS identifies that for 6- to 11-month-old non-nursing infants, the baby food group is the dominant source of iodide in the diet, contributing 56% of the total intake of iodide followed by the dairy food group at 34%. Likewise, the FDA TDS identifies that for 6-year-old children, the dairy food group is the dominant source of iodide in the diet, contributing 70% of the total intake of iodide. Furthermore, the FDA TDS also identifies that for 25- to 30-year-old women (i.e., the closest FDA age/sex group corresponding to women of childbearing age), the dairy food group is the dominant source of iodide in the diet, contributing 49% of the total intake of iodide. The FDA TDS clearly identifies that dairy and grain are the most significant sources of iodide in the adult diet (Murray 2008, p 7).

The FDA TDS for iodide also determines the estimated upper and lower average iodide intake for each food group for 14 age/sex groups. The estimated total iodide intake for each age/sex group can then be compared against the estimated average requirement (EAR) and the RDA for each age/sex group. EARs are used to both assess the adequacy of population intakes and as the basis for calculating RDAs for individuals (NAP 2000). RDA is a goal for individual intake. RDAs are set to meet the needs of almost all (i.e., 97% to 98%) individuals in a group (NAP 2000). The estimated upper- and lower-bound average iodide intakes for three age/sex groups are provided in the following table:

Total Dietary Study (TDS) Food Group	Range of Estimated Lower and Upper Bound Average Iodide Intake for 2003-2004 for Each Food Group (µg/person/day)		
	Infants (6-11 months)	Children (6 years old)	Women (25-30 years old)
Baby Food	**82.8 - 88.3**	0.0 - 0.0	0.0 - 0.0
Beverage	0.0 - 1.8	0.1 - 11.3	0.2 - 32.1
Dairy	50.8 - 50.8	**187.9 - 188.0**	**83.2 - 83.2**
Egg	2.5 - 2.5	5.1 - 5.1	6.0 - 6.0
Fat/oil	0.0 - 0.0	0.2 - 0.3	0.6 - 0.7
Fruit	1.6 - 3.1	5.8 - 9.7	4.2 - 7.4
Grain	3.6 - 4.1	37.1 - 39.5	32.3 - 34.8
Legume	0.0 - 0.1	0.2 - 0.5	0.2 - 0.6
Mixture	1.9 - 2.5	11.2 - 13.3	12.6 - 15.8
Meat, poultry, fish	0.5 - 0.6	4.0 - 5.3	4.6 - 6.4
Sweets	0.0 - 0.1	2.7 - 3.4	2.8 - 3.2
Vegetable	0.6 - 1.1	1.2 - 3.9	1.4 - 5.8
Total Iodide Intake	**144 - 155**	**255 - 280**	**148 - 196**
Estimated Average Requirement (EAR)	**130**	**65**	**95** **160 (pregnancy)*** **209 (lactation)***
Recommended Daily Allowance (RDA)*	**130**	**90**	**150** **220 (pregnancy)** **290 (lactation)**

Source: Murray 2008, table 7.
* Source: NAP 2000.

The range of average iodide intakes for the 6- to 11–month-old non-nursing infants subpopulation is above both the EAR and RDA for that subpopulation. Likewise, the range of average iodide intakes for the 6-year-old children subpopulation is also above EAR and RDA for that subpopulation.

Similarly, the range of estimated average iodide intakes for the 25- to 30-year-old women subpopulation is above the EAR for nonpregnant women of this subpopulation. However, the FDA TDS does not estimate intakes for subgroups with specific nutritional needs (e.g., pregnant or lactating women). However, the iodide intake of pregnant and lactating women is the critical information needed to assess the risk from this public health issue. Pregnant and lactating women have increased caloric needs, which would likely result in "somewhat higher" iodide intake values (Murray 2008, p 8). From a population standpoint, and considering the added food intake of pregnant and lactating women, the range of estimated average iodide intakes for the 25- to 30-year-old women subpopulation of 148–196 µg/person/day generally meets the EARs of 95, 160, and 209 µg/person/day for women, pregnant women, and lactating women of this age group. However, even when considering the added food intake of pregnant and lactating women (e.g., estimated at 20% more), the increased estimated average iodide intakes for pregnant and lactating women has difficulty meeting the RDAs of 220 and 290 µg/person/day for pregnant and lactating women. In other words, if the average intake of iodide for a population just meets

the RDA, this means that half of the pregnant and lactating women (i.e., the individuals below the average intake of iodide) are below the RDA for pregnant and lactating women. This finding from the FDA TDS for iodide is fully consistent with the observation that 49% of the Boston sample of pregnant women in the Pearce study had UICs below the U.S. RDA of 150 µg/L pregnant women (i.e., corresponds to a daily intake of ~ 220 µg/day for a pregnant woman) (Pearce 2004). Furthermore, this finding from the TDS for iodide is in agreement with the NHANES III results indicating the 50th percentile of pregnant women (i.e., UIC of 140 µg/L) is below the U.S. RDA of 150 µg/L for pregnant women. Therefore, if the iodide exposure assessment is done by measuring iodide excretion or by measuring iodide intake by food consumption, the iodide exposure assessment comes to the same conclusion that about half (i.e., 50%) of the pregnant women are below the RDA for iodide.

The FDA TDS does not provide the required information needed to evaluate and quantify the risk in the most sensitive group, pregnant women. The perchlorate and iodide intakes in pregnant women are needed assess the risk to pregnant women. The perchlorate intake is essential to quantify the total amount of NIS inhibition occurring in pregnant women. Furthermore, iodide intake is also essential in quantifying the amount of TIU occurring in pregnant women. However, the design of the FDA TDS did not identify pregnant women as one of the 14 age/sex population groups in the study (Murray 2008, p 8).

The perchlorate and iodide intake in pregnant women is needed to characterize the perchlorate and iodide exposure occurring in nursing infants. A nursing mother will pass her perchlorate exposure level (as well as her thiocyanate and nitrate exposoure level) and iodide nutrition level onto her nursing infant. In a cumulative risk assessment, the exposure level to all four NIS stressors in pregnant women must been known and is essential to assessing the risk to nursing infants. However, the FDS TDS does not provide this sought after exposure information in pregnant women. The perchlorate and iodide intakes for pregnant women were not incoporated into the design of the FDS TDS (Murray 2008).

Another critical limitation of the FDA TDS study is that the distribution of iodide intake is not determined in pregnant women. The FDA TDS only determined the statistical range of the average iodide intake. This type of information is useful to evaluate whether the median iodide dietary intake in a population is reasonable. However, adverse health effects from iodide intake in pregnant women occur at the extremes (i.e., the upper and lower 10%). For this public health issue, subtle mental deficits in children occur in either pregnant women who are mildly or moderately iodide deficient. The percentage of pregnant women with low iodide intake (i.e., poor iodide nutrition) is a critical piece of information for the cumulative risk assessment of this public health issue. Unfortunately, the FDA TDS was neither designed for nor provides nutritional information on the proportion of the U.S. pregnant women subpopulation that is either mildly or moderately iodide deficient.

Iodide Exposure Assessment of the Fetus and Nursing Infant is Directly Related to the Mother's Iodide Status

Since the mother is the sole provider of both thyroid hormones and iodide to the developing fetus during pregnancy, the mother's iodide status can directly affect the developing fetus. The fetal thyroid becomes operational only after midgestation (Glinoer 2005, p 1095). During the first and second trimester, the developing fetus is almost exclusively dependent on the mother to provide an adequate supply of thyroid hormones for proper brain development (Glinoer 2005). However, by the third trimester, the supply of thyroid hormones is of essentially fetal origin, but the fetus is still dependent on the mother for an adequate supply of iodide for proper brain development (Glinoer 2005).

During lactation, the mother provides the nursing infant with iodide. The mother's iodide status can directly affect the developing infant. The regulation of iodide into breast milk is only partially known (Laurberg 2002). However, study results observed in Danish mothers indicate that iodide content of breast milk seems to vary in parallel with the iodide intake of the mother (Nohr 1994; Laurberg 2002, figure 1). The results of the iodide study of Danish mothers and infants are summarized below:

Iodide Ratio Evaluated	Ratio (median)	Percentiles (25% - 75%)	Number Tested
Ratio of iodide concentration in breast milk to the mother's urinary iodide concentration	0.96	0.50-1.96	n = 142
Ratio of infant's urinary iodide concentration to mother's iodide breast milk concentration	1.00	0.60-1.64	n = 137
Ratio of infant's urinary iodide concentration to the mother's urinary iodide concentration	1.00	0.65-1.75	n = 141

In other words, this study indicates that, on average in a population, the iodide status of the mother is directly reflected in the iodide concentration of her breast milk. As the breast milk is subsequently consumed by the infant, on average in a population, the infant's iodide status is directly related to the mother's iodide concentration in her breast milk. As such, on average in a population, the mother's iodide status directly corresponds to the iodide status of the nursing infant. Therefore, during lactation, the iodide nutritional status of the lactating mother is a good measure of the iodide nutritional status of the nursing child (Laurberg 2002). For example, if the lactating mother is moderately iodide deficient, then it is reasonable to expect the nursing infant to be also moderately iodide deficient.

The Size of the U.S. Sensitive Population Potentially Affected by the Lack of Iodide

The final task in the iodide exposure assessment is to determine the size of the U.S. sensitive population potentially affected by the lack of iodide. Although about half of pregnant women are iodide sufficient (i.e., RDA for pregnancy of 150 µg/L (Pearce 2004)), a significant portion of the sensitive groups (i.e., pregnant women, fetuses, neonates born with elevated TSH levels, and nursing infants) have either mild or moderate iodide deficiency. The following table provides an estimate of the size of each of the sensitive groups:

Severity of Iodide Deficiency	Sensitive Population	Percentage of 4 Million Annual U.S. Births	Estimated U.S. Population Potentially Affected Per Year
Moderately	Pregnant Women/Fetuses	6.9%	276,000
	Elevated TSH Neonates (Occurrence rate of 17.0% to 36.9% rate above iodide sufficient rate up to 3%)	1.2% to 2.5% (i.e., 0.069 x 0.17; and 0.069 x 0.369)	48,000 to 100,000
	Nursing Infants	3.7% (i.e., 0.069 x 0.53 nursing frequency)	148,000
Mildly	Pregnant Women/Fetuses	22%	880,000
	Elevated TSH Neonates (Occurrence rate of 0% to 16.9% rate above iodide sufficient rate up to 3%)	0% to 3.7% (i.e., 0.22 x 0.0; and 0.22 x 0.169)	0 to 148,000
	Nursing Infants	11.7% (i.e., 0.22 x 0.53 nursing frequency)	468,000

The NHANES III survey identifies that about 6.9% of the U.S. pregnant women are moderately iodide deficient. Since the United States has about 4 million births per year (Martin 2003), up to approximately 276,000 children are born every year to moderately iodide-deficient mothers in the United States (4,000,000 births x 0.069). Therefore, up to 276,000 children are potentially affected every year by the more severe adverse effects associated with low maternal TIU during pregnancy (i.e., maternal UIC less than 50 ug/L).

In addition, the NHANES III survey can be used to identify the percenation of U.S. pregnant women who are mildly iodide deficient. The NHANES III survey identifies approximately the 29th percentile of U.S. pregnant woman population as having a UIC less than 100 µg/L and therefore, as being iodide deficient. The percentage of U.S. pregnant women who are mildly iodide deficient can be estimated by taking the total number of iodide-deficient pregnant women (i.e., 29%) and subtracting the number of moderately iodide deficient pregnant women (i.e., 6.9%). Therefore, approximately 22% (i.e., 29% - 6.9%) of the U.S. pregnant woman population is estimated to be mildly iodide deficient. Since the United States has about 4 million births per year (Martin 2003), up to approximately 880,000 children are born every year to moderately iodide-deficient mothers in the United States (i.e., 4,000,000 births x 0.22). Therefore, up to 880,000 children are potentially affected every year by the minimal adverse effects associated with low maternal TIU during pregnancy (i.e., maternal UIC between 50 and 100 ug/L).

The neonatal TSH level at birth is the principal indicator that identifies fetal thyroid stress and focuses on the potential for neonatal brain damage (Delange 1998). Due to the importance of this indicator, the iodide exposure assessment estimates the number of elevated TSH neonates above the baseline rate observed in iodide-sufficient populations to be 0 to 148,000 and 48,000 to 100,000 neonates per year in mildly and moderately iodide-deficient populations, respectively. This is the portion of the population that may have partially impaired thyroid function, which makes it particularly susceptible to iodide deficiency.

The nursing infant's brain is still developing and still vulnerable to an insufficient uptake of iodide by the thyroid. The nursing infant's iodide nutritional status is directly related (i.e., a ratio of 1.00) to the iodide nutritional status of the mother (Nohr 1994; Laurberg 2002, figure 1). From a population standpoint (i.e., not an individual standpoint), a moderately iodide-deficient nursing mother is likely to have a moderately iodide-deficient nursing infant. Therefore, the number and level of iodide deficiency in nursing infants can be estimated from the number and level of iodide-deficient mothers. Furthermore, the fact that not all mothers breastfeed has to be factored into the estimate. At least 53.6% ± 1.7 of U.S. infants are breastfed at least once, while 22.4% ± 1.2 of U.S. infants are breastfed at 6 months (Li 2002). Therefore, the iodide exposure assessment estimates the number of U.S. nursing infants with mildly and moderately iodide deficiency to be up to 468,000 and 148,000, respectively.

9. TIU Cumulative Risk Assessment

Unlike a conventional single chemical exposure assessment, a cumulative risk assessment accounts for all the known stressors associated with a public health issue. Cumulative risk assessment expands our understanding of the public health impacts of environmental exposures (Fox 2004) and provides a clearer, more complete picture of a public health issue for making risk management decisions (EPA 2003, appendix A). Cumulative risk assessment will reduce risks to the extent that it can be integrated into prevention strategies to track and protect public health (Fox 2004). Therefore, this cumulative risk assessment must account for the following known stressors affecting this public health issue:

1. The amount of NIS inhibition from thiocyanate.
2. The amount of NIS inhibition from nitrate.
3. The amount of NIS inhibition from perchlorate.
4. The lack of iodide in the diet.
5. The heterogeneous thyroid performance within the human population.
6. The interference of the other major steps in the use of thyroid hormones (e.g., production, transport, and peripheral tissue metabolism).

The OIG's approach to conducting a cumulative risk assessment is to apply the NAS Toxicity Testing Committee's vision for toxicity testing to this public health issue. The NAS Toxicity Testing Committee's vision of toxicity testing is to develop a quantitative, mechanistic, dose-response model of the cellular pathway that is perturbed by the stressor(s) (NAS 2007). Subsequent pharmacokinetic modeling would identify a safe human exposure level that prevents the environmental chemical from reaching a toxic tissue concentration. The NAS Toxicity Testing Committee's vision takes advantage of advances in science (e.g., proteomics and genomics) in which the increased understanding of the biological mechanisms allows *in vitro* toxicity testing to be applied to environmental risk assessments. As the complexity of the risk assessment increases (i.e., more factors evaluated), animal testing is neither practical nor sensitive enough to observe and define the relationship between an increasing number of stressors. For example, four stressors each administered at three different doses (i.e., high, medium, and low) would require 81 separate animal studies to evaluate all the possible combinations (i.e., 3^4). To conduct 81 Argus-equivalent perchlorate rat studies is impractical. Furthermore, no scientific techniques are available to measure subtle cognitive deficits in rat offspring. These limitations in conventional animal testing require the implementation of NAS Toxicity Testing Committee's vision of toxicity testing in order to characterize the risk from exposure to the four NIS stressors.

A quantitative, mechanistic, dose-response model of the NIS cellular pathway is available in the published literature. The Tonacchera Model is a multifactorial mathematical equation that characterizes the biological performance of the NIS to uptake iodide to the simultaneous exposure to all four NIS stressors (Tonacchera 2004). The Tonacchera Model defines the relationship between the four NIS stressors (1-4 listed above) and the TIU by the NIS. Therefore, the use of the Tonachera Model results in a single variable, the TIU, that measures the resulting cumulative effect (i.e., integrated effect) of the simultaneous exposure to all four NIS stressors. Since a low TIU result triggers the subsequent adverse effects, knowing the relative

combinations of the four NIS stressors that result in a low TIU is critical to addressing this public health issue. In order to define the combination of the four NIS stressors that result in an unacceptably low TIU requires the development and implementation of a cumulative risk assessment.

The OIG is using of the Tonacchera Model to conduct a cumulative risk assessment that incorporates the four NIS stressors. This is in contrast to the outdated approach of a single chemical risk assessment (EPA 1992; NAS 1994; EPA 1997b; EPA 2000; Callahan 2007). Nevertheless, with any environmental model, any results need to be corroborated with other independent sources of information (e.g., predicted level of stressor exposure is observed to result in adverse effects in humans) (EPA 2003a).

In order to conduct a cumulative risk assessment, the exposure to each of the stressors must be characterized. The human exposure to perchlorate and lack of iodide is relatively well characterized. By contrast, the human exposure to thiocyanate and nitrate is less well characterized. Therefore, this cumulative risk assessment uses only the typical thiocyanate and nitrate exposure levels in the U.S. population to demonstrate the application of cumulative risk assessment to this public health issue. Ideally, the exposure distribution of thiocyanate and nitrate in the U.S. population would be a useful piece of information. The 90th percentile thiocyanate exposure of nonsmokers in the U.S. population would be useful in the subsequent modeling. Likewise, the 90th percentile of nitrate exposure in the U.S. population would also be useful in the subsequent modeling.

9.1 Corroboration of Tonacchera Model with Effects Observed in Humans

The Tonacchera Model must be corroborated with effects observed in humans before being used for risk characterization and before being used to define an RfD based on a %TIU level. Section 3.1.3 of EPA's *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models* (November 2003) establishes the need to corroborate all environmental models before risk assessors use the models as the basis of rule making or regulation (EPA 2003a). The Tonacchera Model represents an environmental model that calculates the TIU by the thyroid based on the environmental exposure to all four NIS stressors. As such, the Tonacchera Model results must be corroborated with effects observed in humans. This corroboration is performed in this section of the OIG Analysis.

"Robustness" is an aspect of model corroboration. Section 3.1.3.2 of EPA's *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models* defines robustness as the capacity of a model to perform equally well across the full range of environmental conditions (EPA 2003a). A model cannot be corroborated against the data or similar data used to generate or calibrate the model. An independent measure of the model's performance is that the model agrees with results from other sources of data. Since the Tonacchera Model is derived from *in vivo* data, all human results represent potential corroboration from a different data source. The OIG Analysis corroborates the Tonacchera Model in Sections 9.1.1 through 9.1.6. The corroboration of the Tonacchera Model is robust because of the multiple points of agreement and the ability to explain the basis of the observed

effect across a variety of human data sources and types. The major points of corroboration are (see the individual sections in 9.1 for a more detailed explanation):

- The calculated %TIU levels for adverse thyroid effects from chronic excess NIS inhibitor exposure in both men and women is consistent with the NAS Committee's statement that a 75% reduction in TIU would be needed to induce adverse effects in adults (which is not the sensitive group) (see Section 9.1.1).

- The Tonacherra Model calculates lower %TIU levels for the more severe thyroid condition, hypothyroidism, than for the less-severe thyroid condition hypothyroxinemia (see Sections 9.1.2 and 9.1.3).

- The calculated %TIU levels for adverse thyroid effects from chronic excess NIS inhibitor exposure show that women are more prone than men to adverse thyroid effects induced by a low TIU, which is consistent with clinical observations (see Sections 9.1.2 and 9.1.3).

- The Tonacchera Models calcluates a decrease in %TIU that agrees with the measured radioactive iodide uptake (RAIU) levels in both the Greer Perchlorate Exposure Study and the Braverman Perchlorate Occupational Exposure Study (see Sections 9.1.4 and 9.1.5).

- The findings from the Tonacchera Model on the role and contribution of each of the NIS stressors to this public health issue are consistent with the role and contribution of iodide and NIS inhibition on the etiology of endemic cretinism, which occurs through the same mechanism of toxicity (see Section 9.1.6).

- The Tonacchera Models provides a quantified, scientific explanation as to why there are no observed health effects from the exposure to 119 ug of perchlorate per day in the Chilean epidemiological studies (see Section 6).

A major uncertainty in the science concerning this public health issue is how much of a reduction in the TIU causes an adverse effect in a pregnant woman, a fetus, or a nursing neonate. In other words, the TIU level associated with adverse effects has not been specifically identified. However, the NAS Committee estimated that a 75% reduction in TIU would be needed to induce adverse effects. Specifically, the NAS Committee states, "To cause declines in thyroid hormone production that would have adverse health effects, iodide uptake would most likely have to be reduced by at least 75% for months or longer" (NAS 2005, p 8). Scientific criticism of the NAS perchlorate assessment states that this NAS statement was not supported by any data, analysis, or references (Ginsberg 2005). Furthermore, a member of the NAS Committee stated to the Massachusetts Department of Environmental Protection's Advisory Committee on Health Effects that the 75% value was not derived by any quantitative analysis but was based on a clinical observation that a loss of a large fraction of the thyroid can be tolerated in adults (MassDEP 2006). The essential point is the 75% reduction in TIU was not determined experimentally from exposure to any of the four stressors and was not determined for the sensitive group: pregnant women and their fetuses (i.e., NAS

characterization of the tolerance of nonpregnant adults is potentially not protective of the sensitive group). To emphasize the limitations of this NAS statement, the concern for the sensitive group can be restated as a question: Can a pregnant woman with three-quarters of her thyroid removed still have enough thyroid hormone production during the added stress occurring during gestation and lactation to have a healthy child without any mental deficits?

The TIU level associated with adverse effects must be identified or estimated. However, determing TIU levels in humans is impractial because of the need to conduct radioactive ^{123}I uptake measurements. By contrast, the Tonacchera Model is able to calculate a TIU level for a given set of exposures to all four NIS stressors in both the human population in general and in the sensitive groups of concern (i.e., pregnant women, fetuses, and nursing neonates). This allows the TIU levels associated with adverse effects to be identified and compared to normal TIU level observed in a healthy human adult. However, the accuracy of the Tonacchera Model to estimate TIU levels must be corroborated with observed human data and information. The corroboration of the Tonacchera Model is performed in remainder of Section 9.1.

9.1.1 NAS Hypothyroidism Statement

The Tonacchera Model indicates that the TIU is directly proportional to the concentration of free iodide in the serum when the total goitrogen load is held constant (see below).

The Tonacchera Model states that

$$TIU \parallel [I^-] / (1.22 + (SPEC))$$

where the symbol || means "proportional to."

If the SPEC is held constant (i.e., the level of NIS inhibitors remains the same), then

$$TIU \parallel [I^-]$$

Thus,

$$TIU_{(Hypothyroidism)} \parallel [I^-]_{(Hypothyroidism)}$$

The $[I^-]$ in Tonacchera Model is the internal concentration of free iodide in the blood serum that is able to directly interact with the NIS. Unfortunately, free iodide concentrations are rarely reported. Healthy levels of inorganic iodide concentrations in serum are very minute and are about 1 pg/dL (Demers, 2003, p 77). Because of the difficulty in measuring PII, the measurement of PII is limited to research studies in pregnancy. Likewise, determining the iodide intake from the iodide content of food is extremely difficult for methodological reasons (Delange 2005, p 267). Therefore, the median urinary concentration is the most practical measurement of iodide intake in a population (Dunn 1998). Iodide balance studies have shown that adults are in balance with their iodide environment (Delange 2005, p 267). About 90% of ingested iodide is excreted in the urine (Dunn 1998), while usually a negligible amount (i.e., 5 µg/day) is excreted in fecal matter. Therefore, in general terms, iodide intake is proportional to urinary elimination. Thus, the UIC is used as a surrogate measure for the iodide concentration in the serum.

The NAS Committee states, "Hypothyroidism occurs only if daily iodide intake is below about 10 to 20 µg (about one-fifth to one-tenth of the average intake in the United States) [an intake of only 10 to20 µg of iodide equates to severe iodide deficiency]" (NAS 2005, p 6). The recommended iodide intake is 150 µg/day for an adult (Delange 2005, p 278, table 11E.8). Therefore, the estimated TIU at which hypothyroidism occurs can be estimated by the ratio of iodide intake associated with hypothyroidism (i.e., range of 10 to 20 µg/day) to a healthy iodide intake of 150 µg/day for an adult. Therefore, the $TIU_{(Hypothyroidism)}$ = (range of 10 to 20 µg/day ÷ 150 µg/day) x 100%) = range of 6.7% to 13.3%. However, the NAS Committee statements that 10 – 20 ug of iodide per day is "about one-fifth to one-tenth of the average intake in the United States." This description of a daily iodide intake of 1/5th to 1/10th corresponds to a $TIU_{(Hypothyroidism)}$ = 10% to 20%.

The recommended UIC for a healthy adult is 100 to 200 µg/L (Delange 2005, p 278, table 11E.8). Therefore, the median healthy UIC value is 150 µg/L. Severe iodide deficiency is characterized by a UIC of < 20 µg/L (Delange 2005, p 265, table 11E.3). Severe iodide deficiency can generate symptoms of hypothyroidism (i.e., low T_4; high TSH) (Delange 2005, p 272, table 11E.6). Therefore, the estimated TIU at which hypothyroidism occurs can be estimated by the ratio of UIC of severe iodide deficiency to median healthy UIC value. Therefore, the $TIU_{(Hypothyroidism)}$ = (20 µg/L ÷ 150 µg/L) x 100%) = 13.3%.

In summary, from the estimates made above from the NAS Committee's hypothyroidism statement, the $TIU_{(Hypothyroidism)}$ is expected to occur in range from 6.7% to 20% with the low teens being most probable (i.e., 13.3%). This is consistent with the NAS Committee statement: "To cause declines in thyroid hormone production that would have adverse health effects, iodide uptake would most likely have to be reduced by at least 75% for months or longer" (NAS 2005, p 8). In other words, the $TIU_{(Hypothyroidism)}$ is expected to occur at 25% or less.

9.1.2 Hypothyroxinemia in Men Exposed to Excess Thiocyanate

The TIU level at which overt hypothyroidism (i.e., elevated TSH and below-normal T_4) or subclinical hypothyroidism (i.e., elevated TSH and normal T_4) is not known by experimental data. However, hypothyroxinemia is a less-severe thyroid condition and is a common condition in pregnant women, characterized by low maternal fT_4 levels with normal TSH levels (Kooistra 2006). Maternal hypothyroxinemia is documented to be associated with mental deficits (e.g., ADHD) in children (Vermiglio 2004). Iodide deficiency induces thyroid changes, characterized by low serum T_4, normal or slightly elevated T_3, and a normal TSH level (i.e., as a consequence of the normal or elevated T_3) (Obregon 2005). Therefore, iodide deficiency induces thyroid changes consistent with hypothyroxinemia. Since both the lack of iodide and NIS inhibitors act through the same mechanism of action, an excess NIS inhibitor exposure will also induce thyroid changes consistent with hypothyroxinemia, observed during iodide deficiency (i.e., a low TIU).

Severe hypothyroxinemia is observed in thiocyanate-exposed electroplating workers in the Banerjee adult male thiocyanate study (Banerjee 1997). The thiocyanate-exposed workers were measured with below normal T_4, just slightly below normal T_3 levels, and statistically elevated TSH levels, but the TSH levels are still in the normal range. The exposed workers were

measured to have a serum thiocyanate exposure of 316 ± 15.0 µmol/L. The observations of the Banerjee study are summarized below:

Banerjee Study Group	Thiocyanate (µmol/L)	T_4 (µg/dL)	T_3 (µg/dL)	TSH (µU/ml)
35 Nonexposed Workers (all nonsmokers)	90.8 ± 9.02	6.09 ± 0.601	111.0 ± 9.3	1.20 ± 0.301
35 Exposed Workers (all nonsmokers)	316 ± 15.0*	3.81 ± 0.318†	57.2 ± 8.1†	2.91 ± 0.20†
Normal Ranges Cited in the Manufacturers Test Kits	–	5.5 to 13.5	60 to 200	0.2 to 4.0

* $p < 0.01$
† $p < 0.05$

Source: Banerjee 1997, table 1.

The Tonacchera Model can be used to calculate the TIU level that corresponds to the hypothyroxinemia observed in the exposed workers in the Banerjee thiocyanate adult male study. The TIU calculations are as follows:

The Tonacchera Model states:

$$TIU \parallel [I^-] / (1.22 + (SPEC))$$

where the symbol || means "proportional to".

If the $[I^-]$ is held constant, a normal $[I^-]$ level is assigned the variable "x." (Note: the variable x corresponds to the normal $[I^-]$ level in the U.S. population of 145 µg/L), then the TIU for normal U.S. exposure (i.e., to be used as the normal TIU reference point) to the four NIS variables is given by the following:

$$TIU_{(Normal)} \parallel \frac{x}{(1.22 + SPEC_{(Normal)})}$$

Since $SPEC_{(normal)}$ was previously calculated in this paper to be 1.501 µmol/L from the median exposures to thiocyanate (SCN^-), nitrate, and perchlorate in the U.S. population, $TIU_{(normal)}$ is:

$$TIU_{(Normal)} \parallel \frac{x}{(1.22 + 1.501)}$$

$$TIU_{(Normal)} \parallel \frac{x}{(2.721)}$$

$$TIU_{(Normal)} \parallel 0.3675x$$

In the Banerjee thiocyanate adult male study, the iodide nutritional level was not determined (which is typical in the epidemiological studies in that they measure only a subset of the four NIS stressors). However, the iodide nutritional level of the

thiocyanate-exposed workers is believed to be sufficient due to the year and location of the study (Gibbs 2006). However, a different Banerjee study did measure the iodide nutritional level in 70 test subjects in India in the same year (Banerjee 1997a). A random sampling of UIC in 50 to 100 people can be used to define the iodide nutritional status of a population (Delange 2005, p 267). In the Banerjee thiocyanate women study, the average UIC of 70 subjects was 119 µg/L (i.e., avg. 115 µg/L and 123 µg/L). Therefore, a UIC of 119 µg/L (i.e., 0.82x) is a reasonable value to use for the $TIU_{(Hypothyroxinemia)}$ calculation in the exposed workers. The $TIU_{(Hypothyroxinemia)}$ calculation will also be been done using the UIC found in the U.S. population of 145 µg/L in order to define an upper limit for the $TIU_{(Hypothyroxinemia)}$ range. Thus, assuming a UIC of 119 µg/L, then the $TIU_{(Hypothyroidism)}$ observed in the workers is given by:

$$TIU_{(Hypothyroxinemia)} \parallel \frac{0.82\ x}{(1.22 + SPEC_{(SCN^-\ Exposed\ Workers)})}$$

Since $SPEC_{(SCN^-\ exposed\ workers)}$ is given by:

$$SPEC_{(SCN^-\ exposed\ workers)} = (ClO_4^-\ Inhibition) + (NO_3^-\ Inhibition) + (free\ SCN^-\ Inhibition)$$

$$SPEC_{(SCN^-\ exposed\ workers)} = [ClO_4^-] + [NO_3^-]\ /240 + ([total\ serum\ SCN^-] \times 0.528)\ /15$$

The amount of perchlorate and nitrate exposure is not measured in the Banerjee thiocyanate study, but the default values observed in the U.S. population for perchlorate and nitrate are used for the calculation:

$$SPEC_{(SCN^-\ exposed\ workers)} = 0.0013\ \mu mol/L + 0.167\ \mu mol/L + (316\ \mu mol/L\ SCN^- \times 0.528)\ /15$$

$$SPEC_{(SCN^-\ exposed\ workers)} = 0.0013\ \mu mol/L + 0.167\ \mu mol/L + 11.12\ \mu mol/L$$

$$SPEC_{(SCN^-\ exposed\ workers)} = 11.29\ \mu mol/L$$

Inserting 11.29 µmol/L for $SPEC_{(SCN^-\ exposed\ workers)}$

$$TIU_{(Hypothyroxinemia)} \parallel \frac{0.82x}{(1.22 + 11.29)}$$

$$TIU_{(Hypothyroxinemia)} \parallel 0.066x$$

The $\%TIU_{(Hypothyroxinemia)}$ in the thiocyanate-exposed adult male workers is given by the following equation:

$$\%TIU_{(Hypothyroxinemia)} = (TIU_{(SCN^-\ exposed\ workers)} \div TIU_{(Normal)}) \times 100\%$$
$$= (0.066x \div 0.3675x) \times 100\%$$
$$= 18.0\ \%$$

Assuming the UIC of the exposed workers was 119 µg/L, the amount of NIS inhibition from the thiocyanate in the exposed workers resulted in a calculated $TIU_{(Hypothyroxinemia)}$ of 18.0% as compared to the normal TIU observed in the U.S. population. Assuming the UIC of the exposed workers was the same as the U.S. population (i.e., 145 µg/L), the amount of NIS inhibition from the thiocyanate in the exposed workers results in a calculated $TIU_{(Hypothyroxinemia)}$ (calculation not shown, but identical to the above expect "0.82x" is replaced with just "x") of 21.8% as compared to the normal TIU observed in the U.S. population.

In summary, as calculated from measurements from the Banerjee adult male thiocyanate study, a $\%TIU_{(Hypothyroxinemia)}$ in the range of 18.0% to 21.8% is shown to induce a T_3 and T_4 thyroid hormone deficiency in adult males consistent with severe hypothyroxinemia. Since hypothyroxinemia is a less-extreme thyroid condition than hypothyroidism, $\%TIU_{(Hypothyroidism)}$ should be less than the $\%TIU_{(Hypothyroxinemia)}$. From the estimates made above from the NAS Committee's hypothyroidism statement, the $\%TIU_{(Hypothyroidism)}$ is estimated to occur in a range from 6.7% to 20%, with the low teens typical (i.e., 13.3%). The $\%TIU_{(Hypothyroxinemia)}$ in males is in the range of 18.0% to 21.8%, which is above the $TIU_{(Hypothyroidism)}$ (i.e., low teens). The calculation of $\%TIU_{(Hypothyroxinemia)}$ for severe hypothyroxinemia in men was performed to corroborate that the results from Tonacchera Model is consistent with information from other sources (e.g., the clinical observations of the NAS experts).

9.1.3 Hypothyroxinemia in Women Exposed to Excess Thiocyanate

Hypothyroxinemia (i.e., below normal T_4 and normal T_3 and TSH levels) is observed in thiocyanate-exposed females in the Banerjee adult female thiocyanate study (Banerjee 1997a). In Calcutta, India, 30-50 mg/L of thiocyanate is added to preserve milk by activating the lactoperoxidase-thiocyanate-hydrogen peroxide system (LP system) found in milk in order to retard the growth of bacteria. The thiocyanate-exposed females are measured to have below normal T_4, and both normal T_3 levels and statistically elevated TSH levels, but the TSH levels are still in the normal range. The exposed females were measured to have a serum thiocyanate exposure of 230 ± 10.0 µmol/L. The observations of the Banerjee study are summarized below:

Study Group	Thiocyanate (µmol/L)	T_4 (nmol/L)	T_3 (nmol/L)	TSH (µU/ml)
35 Nonexposed Females (all nonsmokers)	90.8 ± 9.0	125.4 ± 11.5	1.71 ± 0.16	1.09 ± 0.28
35 Exposed Females (drank SCN^- preserved milk)	230.0 ± 10.0*	87.8 ± 6.6*	2.39 ± 0.32	2.49 ± 0.20*
Normal Ranges Cited in the Manufacturers Test Kits		110 to 279	0.93 to 3.12	0.2 to 4.0

.* $p < 0.01$

Source: Banerjee 1997a, table 1.

The Tonacchera Model can be used to calculate the TIU level that corresponds to the hypothyroxinemia observed in the exposed women in the Banerjee thiocyanate adult female study. The TIU calculations are as follows:

The Tonacchera Model states:

$$TIU \parallel [I^-] / (1.22 + (SPEC))$$

where the symbol || means "proportional to"

If the $[I^-]$ is held constant, a normal $[I^-]$ level is assigned the variable "x," then the TIU for normal U.S. exposure (i.e., to be used as the normal TIU reference point) to the four NIS variables is given by the following:

$$TIU_{(U.S.\ normal)} \parallel \frac{x}{(1.22 + SPEC_{(U.S.\ normal)})}$$

Since $SPEC_{(normal)}$ was previously calculated in this paper to be 1.501 from median exposures to thiocyanate, nitrate, and perchlorate in the U.S. population, $TIU_{(normal)}$ is:

$$TIU_{(U.S.\ normal)} \parallel \frac{x}{(1.22 + 1.501\ \mu mol/L)}$$

$$TIU_{(U.S..normal)} \parallel \frac{x}{(2.721)}$$

$$TIU_{(U.S.\ normal)} \parallel 0.3675x$$

In the Banerjee thiocyanate adult female study, the iodide nutritional level was measured. The average UIC in the expose females was determined to be 115 µg/L (SE 8.5). Since the iodide nutritional level in the U.S. population is 145 µg/L, the $[I^-]$ level in the exposed workers is assigned a value of 0.793x (i.e., 115 µg/L ÷ 145 µg/L). Thus, then the $TIU_{(Hypothyroidism)}$ observed in the workers is given by:

$$TIU_{(Hypothyroxinemia)} \parallel \frac{0.793x}{(1.22 + SPEC_{(SCN^-\ Exposed\ Females)})}$$

Since $SPEC_{(SCN^-\ exposed\ females)}$ is given by:

$$SPEC_{(SCN^-\ exposed\ females)} = (ClO_4^-\ Inhibition) + (NO_3^-\ Inhibition) + (free\ SCN^-\ Inhibition)$$

$$SPEC_{(SCN^-\ exposed\ females)} = [ClO_4^-] + [NO_3^-] /240 + ([total\ serum\ SCN^-] \times 0.528) /15$$

The amount of perchlorate and nitrate exposure is not measured in the Banerjee thiocyanate study, but the default values observed in the U.S. population for perchlorate and nitrate are used for the calculation:

$$SPEC_{(SCN^-\ exposed\ females)} = 0.0013\ \mu mol/L + 0.167\ \mu mol/L + (230\ \mu mol/L\ SCN^- \times 0.528) /15$$

$$SPEC_{(SCN^-\ exposed\ females)} = 0.0013\ \mu mol/L + 0.167\ \mu mol/L + 8.096\ \mu mol/L$$

$$SPEC_{(SCN^- \text{ exposed females})} = 8.26\ \mu mol/L$$

Inserting 8.26 µmol/L for $SPEC_{(SCN^- \text{ Exposed Females})}$

$$TIU_{(Hypothyroxinemia)} \parallel \frac{0.793x}{(1.22 + 8.26)}$$

$$TIU_{(Hypothyroxinemia)} \parallel 0.0836x$$

The %TIU in the thiocyanate exposed females is given by the following equation:

$$\%TIU_{(Hypothyroxinemia)} = TIU_{(SCN^- \text{ exposed females})} \div TIU_{(U.S.\ normal)} \times 100\%$$

$$\%TIU_{(Hypothyroxinemia)} = (0.0836x \div 0.3675x) \times 100\%$$

$$\%TIU_{(Hypothyroxinemia)} = 22.7\%$$

The amount of NIS inhibition from the thiocyanate in the exposed females resulted in a calculated $\%TIU_{(Hypothyroxinemia)}$ of 22.7% as compared to the normal TIU observed in the U.S. population. The Banerjee thiocyanate adult female study, a $\%TIU_{(Hypothyroxinemia)}$ of 22.7% is observed to induce hypothyroxinemia in females. Since hypothyroxinemia is less extreme thyroid condition than hypothyroidism, $\%TIU_{(Hypothyroidism)}$ should be less than the $\%TIU_{(Hypothyroxinemia)}$. From the estimates made above from the NAS Committee's hypothyroidism statement, the $\%TIU_{(Hypothyroidism)}$ is estimated to occur in range from 6.7% to 20%, with the low teens being most probable (i.e., 13.3%). The $\%TIU_{(Hypothyroxinemia)}$ in women is 22.7% and is above the NAS Committee's $TIU_{(Hypothyroidism)}$ of the low teens. The calculation of $\%TIU_{(Hypothyroxinemia)}$ for hypothyroxinemia in women was performed to corroborate the results from Tonacchera Model; the Tonacchera Model results are consistent with information from other sources (e.g., the clinical observations from the NAS experts).

Furthermore, the $\%TIU_{(Hypothyroxinemia)}$ in women is 22.7% and is above the $\%TIU_{(Hypothyroxinemia)}$ found in men of 18.0% to 21.8%. This finding is consistent with the observation that women are more sensitive to adverse thyroid effects than men. In the original Whickman survey, the rate of high serum TSH concentrations was higher in women than men in each decade of age (Vanderpump 2005, p 401, figure 19.2). Furthermore, both the original Whickman survey and the subsequent 20-year follow-up survey found the rate of goiter is higher in women than men (Vanderpump 2005, p 403).

9.1.4 Perchlorate PBPK Model and the Greer Perchlorate Exposure Study

The Tonacchera Model, as with any environmental model, must be corroborated with other independent sources of information (EPA 2003a). The Tonacchera Model can be used to calculate the TIU at various external doses of perchlorate. The Tonacchera Model uses the Clewell Perchlorate PBBK Model to convert external perchlorate doses into internal perchlorate doses in the blood serum. As such, the Tonacchera Model is an example of the NAS Toxicity Testing Committee's (NAS 2007) quantitative, mechanistic, dose-response model of the cellular

pathway that is perturbed by the environmental agent. The Clewell perchlorate PBPK model is the subsequent pharmacokinetic model called on by the NAS Toxicity Testing Committee to convert the internal exposure dose at the cell (i.e., in this case, the perchlorate serum concentration in µg/L) to an external exposure dose (i.e., how much is consumed – mg/kg-day). As seen in the Clewell perchlorate PBPK model, the conversion factor from internal to external perchlorate dose is not constant, but changes due to biological differences across life stages (i.e., adult, fetus, neonate, child, pregnant woman, lactating woman) and changes with external exposure levels (Clewell 2007, p 423, table 4). Therefore, the calculated TIUs will be slightly different for each life stage and external dose level.

The %TIUs calculated by the Tonacchera Model will be compared against the published predicted %TIUs from the Clewell Perchlorate PBBK Model and the %TIUs observed in the Greer study. Since, the Greer study was of adults, this comparison of %TIUs is only for adults and not for the other life stages. The Clewell perchlorate PBPK model predicted the percentage inhibition of TIU across various life stages (Clewell 2007, p 423, table 5). The Greer study calculated a 24-hour TIU from the difference in the amount of RAIU from before and after 14 days of a known external perchlorate exposure at four different levels (Greer 2002, p 931, table 1, 24-hr uptake).

The TIUs calculated by the Tonacchera Model at various external perchlorate doses are provided in the next two tables (see below). Below is an example of the TIU calculations used to populate the values in each of the columns in the next two tables. The resulting internal perchlorate dose (i.e., perchlorate serum concentration) in an adult is calculated for an external perchlorate dose of 0.001 mg/kg-day using the Clewell perchlorate PBPK model (Clewell 2007, p 423, table 4) in the following calculation:

$$\text{Int. } ClO_4^- \text{ Dose}_{(\text{Adult})} = \frac{0.001 \text{ mg}}{\text{kg-day}} \times \frac{0.002 \text{ mg/L Int. } ClO_4^- \text{ Dose}}{0.001 \text{ mg/kg-day Ext. } ClO_4^- \text{ Dose}} = 0.002 \text{ mg/L}$$

The units of the internal perchlorate dose are converted from mg/L to µmol/L using the following calculation:

$$\text{Internal } ClO_4^- \text{ Dose}_{(\text{Adult})} = 0.002 \text{ mg/L} \times \frac{1000 \text{ µg}}{1 \text{ mg}} \times \frac{1 \text{ µmole}}{99.45 \text{ µg}} = 0.020 \text{ µmol/L}$$

The SPEC of an internal perchlorate dose of 0.020 µmol/L is calculated using the following SPEC equation adapted from the Tonacchera Model (typical U.S. exposures for nitrate and thiocyanate are used in for this calculation):

$$\begin{aligned} SPEC_{(\text{Ext. Per. Dose})} &= (ClO_4^- \text{ Inhibition}) + (NO_3^- \text{ Inhibition}) + (\text{free } SCN^- \text{ Inhibition}) \\ &= [ClO_4^-] + [NO_3^-]/240 + ([\text{total serum } SCN^-] \times 0.528)/15 \\ &= 0.020 \text{ µmol/L} + (40 \text{ µmol/L} \div 240) + (40 \text{ µmol/L} \times 0.5 \text{ free } SCN^- \div 15) \\ &= 0.020 \text{ µmol/L} + 0.167 \text{ µmol/L} + 1.33 \text{ µmol/L} \\ &= 1.52 \text{ µmol/L} \end{aligned}$$

The TIU for this external perchlorate dose is calculated using the following Tonacchera Model equation:

$$TIU_{(Ext.\ perchlorate\ dose)} = \frac{x}{(1.22 + SPEC_{(Ext.\ perchlorate\ dose)})}$$
$$= \frac{x}{(1.22 + 1.52\ \mu mol/L)}$$
$$= \frac{x}{2.74}$$
$$= 0.3650x$$

The %TIU for this external perchlorate dose is calculated using the following equation:

$$\%TIU_{(Ext.\ perchlorate\ dose)} = TIU_{(Ext.\ perchlorate\ dose)} \div TIU_{(U.S.\ normal)} \times 100\%$$
$$= (0.3650x \div 0.3675x) \times 100\%$$
$$= 99.3\%$$

The following table provides the TIUs calculated by the Tonacchera Model at various external perchlorate doses (see sample calculations above to see how the values were determined for each column):

External ClO_4^- Dose (mg/kg-day)	Internal ClO_4^- Dose In an Adult (mg/L serum)	Internal ClO_4^- Dose In an Adult (µmol/L)	Serum Perchlorate Equivalent Conc. (SPEC) (µmol/L)	Calculated Total Iodide Uptake (TIU)
0	0.00013 *	0.0013	1.501†	0.3675x
0.000175 (¼ RfD)	0.00035	0.0035	1.505	0.3670x
0.0007 (RfD)	0.0014	0.014	1.511	0.3662x
0.001	0.002	0.020	1.52	0.3650x
0.007 (NOEL)	0.0088**	0.088	1.589	0.3560x
0.01	0.01	0.1	1.60	0.3546x
0.02	0.02	0.2	1.70	0.3325x
0.1	0.1	1.0	2.50	0.2688x
0.5	0.5	5.0	6.50	0.1295x
1.0	1.0	10.0	11.50	0.0786x

* Median perchlorate exposure in U.S. population (Blount 2006a).
† From median NIS inhibitor exposures in the U.S. population (calculated in a previous section).
** Used an estimated conversion factor of 0.009375 mg/L per .0075 mg/kg-day using a linear interpolation from the Clewell perchlorate PBPK model.

The following table provides the %TIUs calculated by the Tonacchera Model at various external perchlorate doses and compares them against the published predicted %TIUs from the Clewell Perchlorate PBBK Model and the %TIUs observed in the Greer study:

External ClO_4^- Dose (mg/kg-day)	Calculated Total Iodide Uptake (TIU)	%TIU Calculated from the Tonacchera Model	%TIU Predicted by the Clewell Perchlorate PBPK Model*	%TIU Measured in the Greer Study†
0	0.3675x	100	-	-
0.000175	0.3670	99.9	-	-
0.0007	0.3662x	99.6	-	ClO_4^- RfD
0.001	0.3650x	99.3	99.4	-
0.007	0.3560x	96.9	-	98.3 ± 8.3 (NOEL)
0.01	0.3546x	96.5	96	-
0.02	0.3325x	93.2	-	83.6 ± 4.1
0.1	0.2688x	73.1	71	55.3 ± 3.9
0.5	0.1295x	35.2	-	32.9 ± 3.8
1.0	0.0786x	21.3	19	-

* Source: Clewell 2007, p 423, table 5.
† Source: Greer 2002, p 931, table 1, 24-hr uptake.

In an adult, the Tonacchera Model calculated a 99.6 %TIU at the perchlorate RfD of 0.0007 mg/kg-day, which corresponds to a 24.5 ppb DWEL. The Tonacchera Model also calculated a 99.9 %TIU at ¼ the perchlorate RfD of 0.0000175 mg/kg-day in an adult which corresponds to a 6.1 ppb DWEL. Therefore, the Tonacchera Model estimated a 0.3% reduction in the TIU when the DWEL is lowered from 24.5 ppb to 6.1 ppb.

The %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.001, 0.01, 0.1, and 1.0 mg/kg-day are in excellent agreement with the %TIU predicted by the Clewell perchlorate PBPK model (see table above). This is expected because the %TIU calculation from the Tonacchera Model uses the Clewell perchlorate PBPK model to convert an external perchlorate dose to an internal perchlorate dose. Although the %TIUs calculated from the Tonacchera Model agree with the %TIUs predicted by the Clewell perchlorate PBPK model, this corroboration of results is not a robust test of the Tonacchera Model because only one stressor (i.e., perchlorate) is varied, while the other three stressors (i.e., lack of iodide, thiocyanate, and nitrate) are held constant.

The %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.007 and 0.5 mg/kg-day are in excellent agreement with the %TIU observed in the Greer study (see table above). However, the %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.02 and 0.1 mg/kg-day are not in particularly good agreement with the %TIU observed in the Greer study (see table above). In short, half of the %TIU values results agree, while the other half of %TIU values do not agree well.

In order to understand the potential sources of differences between the two sets of %TIU values, a reexamination of the Greer study data with consideration to all four NIS stressors is appropriate. The Greer study calculated a 24-hour TIU from the difference in the amount of RAIU from before and after 14 days of a known external perchlorate exposure level. However, the Greer study did not attempt to measure or control for the other three known NIS stressors (i.e., lack of iodide, thiocyanate, and nitrate). Therefore, the other three known NIS stressors can act as confounding variables in the Greer study.

Although the exposures to the other three NIS stressors were not directly measured in the Greer study, the amount of change in an individual's TIU from the other three known NIS stressors can be observed in the 24-hour RAIU results (Greer 2002, Figure 2, provided below). If the contribution from the other three NIS stressors was negligible, the measured 24-hour RAIU should be the same as before exposure (i.e., baseline visit (BV)) and as 15 days post exposure (i.e., P15) when there was no additional external perchlorate dose. At each of the four external perchlorate doses, the average raw 24-hour % RAIUs before exposure (i.e., BV) and 15 days post exposure (i.e., P15) was statistically similar (Greer 2002, table 1, 24-hour uptake column). However, the raw 24-hour RAIUs before exposure (i.e., BV) and 15 days post exposure (i.e., P15) are far from similar for some of the individual test subjects (Greer 2002, figure 2). In figure 2 in the Greer study, if the contribution from the other three NIS stressors was negligible to an individual's TIU, the graphs should be mirror images around exposure day 14 (E14) and the graphs should not have any crossing lines. However, this is not shown in figure 2 of the Greer study.

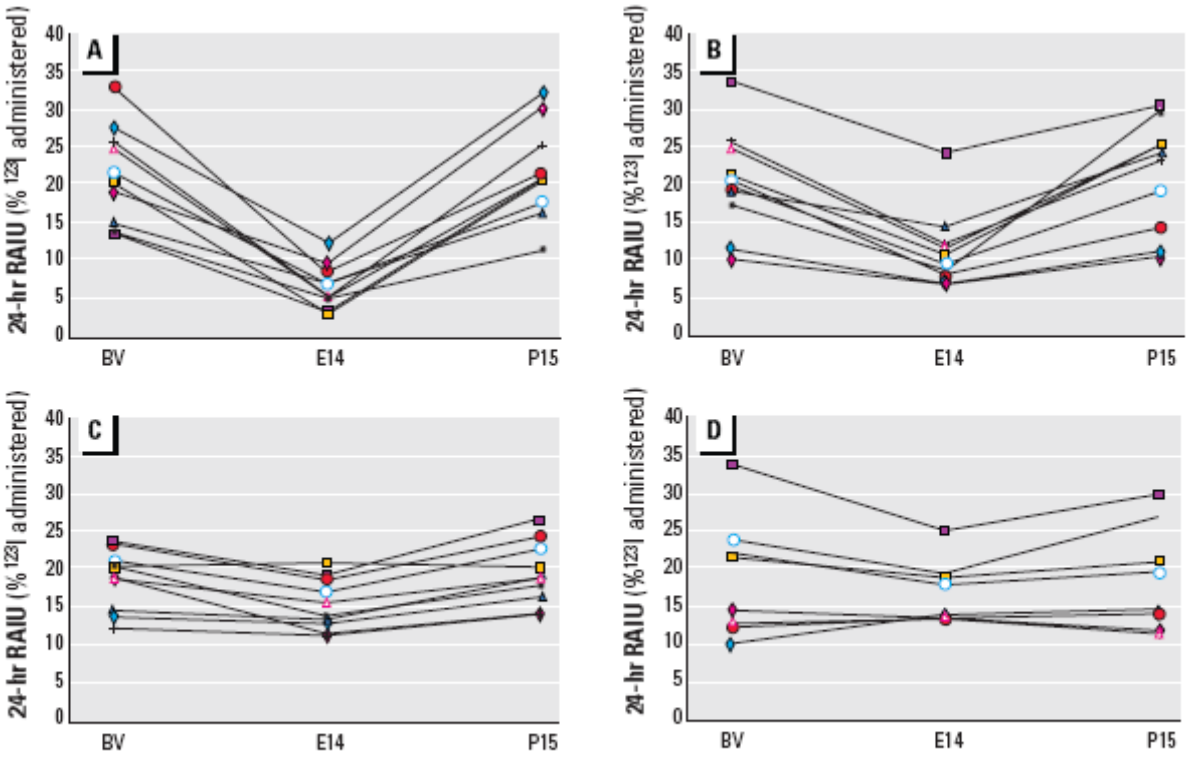


Figure 2. The 24-hr RAIU at the baseline visit and on E14 and P15 for each subject in the (*A*) 0.5-, (*B*) 0.1-, (*C*) 0.02-, and (*D*) 0.007-mg/kg-day dose groups.

Source: Greer 2002, figure 2.

The following table summarizes some of the larger %TIU changes observed in several of the individual test subjects that occurred in the 29 days between the BV and the post 15 days exposure (P15):

External ClO_4^- Dose (mg/kg-day)	Example Test Subject	Estimated Raw RAIU at Baseline Visit*	Estimated Raw RAIU at Post 15 Days*	Individual %TIU change over the 29 days
0.007 (fig. 2 d)	Purple '□'	34	30	12% decrease
0.007 (fig. 2 d)	Blue '◊'	10	14	40% increase
0.02 (fig. 2 c)	Purple '◊'	18	14	22% decrease
0.02 (fig. 2 c)	Blue '◊'	14	19	29% increase
0.1 (fig. 2 b)	Black '*'	17	29	70% increase
0.1 (fig. 2 b)	Red '○'	19	14	26% decrease
0.5 (fig. 2 a)	Red '○'	33	21	36% decrease
0.5 (fig. 2 a)	Purple'◊'	18	30	67% increase

* The raw numerical RAIU data for individuals were not provided in the published Greer study, but were estimated from Greer figure 2 (provide above).

The point of identifying these large changes in %TIU of individual subjects in the Greer study is to highlight that a single chemical risk assessment (i.e., perchlorate only) can seriously distort the perception of risk from perchlorate when the other NIS stressors are not considered. The Greer study is designed to quantify the amount of perchlorate exposure that causes no statistically significant change in the RAIU between before perchlorate exposure and during perchlorate exposure, to identify the NOEL for perchlorate. Then, a UF of 10 was applied by the NAS Committee to derive the perchlorate RfD to ensure perchlorate exposure at the RfD does not cause a significant change (i.e., < 0.2 %TIU) in an individual's TIU to be protective. However, these large changes in %TIU of individual subjects in the Greer study are occurring at each exposure dose without any apparent adverse thyroid effects. A perchlorate-only chemical risk assessment does not attempt to explain how these large changes in %TIU are occurring in the population and does not attempt to identify the source(s) of the effect. If the perchlorate-only chemical risk assessment considers a 1.7 %TIU to be potentially risky, why should the potential risk inherent in double-digit percentage changes in the %TIU observed in the Greer study population be ignored, overlooked, or discounted?

Fortunately, a cumulative risk assessment approach incorporates the risk from the background exposure to all four NIS stressors because the exclusion of one or more stressor(s) or the background exposure from a stressor(s) will seriously distort the portion of the total estimated risk attributed to perchlorate (i.e., the principal stressor of concern) (EPA 2003, appendix C). Furthermore, a cumulative risk assessment distinguishes between background exposures and source-specific exposures to provide a more complete picture of both total and source-related risks (EPA 2003, appendix C). A cumulative risk assessment identifies the amount of risk from the exposure levels observed for each stressor. In regard to this public health issue, the Tonacchera Model identifies the lack of iodide in the diet as the dominant stressor. When the contribution of iodide is not quantitatively factored into the risk assessment (i.e., as in a single chemical assessments done for perchlorate), the result is seriously distorted.

The Greer study measured the RAIU in 37 test subjects at the start of the study and named this RAIU measurement the BV. The observed raw 24-hour RAIU at the BV was 9.8% to 33.7%. Therefore, prior to perchlorate exposure, the 24-hour RAIU varied by a factor of about 3.4 times. The BV results capture the range in %TIUs found in the study's population.

The median 24-hour RAIU at the BV is estimated to be 21.75%. Assigning this as the typical RAIU in the population, the range in %TIUs in the study's population is then estimated to range from 45% to 155% of normal (i.e., 100% is nominal). The Greer BV data show that the TIU is varying ± 55% in the study population prior to perchlorate exposure without adversely effecting thyroid hormone levels. The fT_4, tT_4, tT_3, and TSH levels were measured at BV and were all found to be in the normal range prior to and after the experiment (Greer 2002, table 4).

To manage the risk affecting this public health issue, the 45-155% range in %TIUs of the Greer study's population needs to be understood and explained. The Greer study does not explain this observation. However, the Tonacchera Model provides insight into this observation by quantifying the TIU contribution from each of the stressors. For example, if all the variations in %TIUs in the Greer study population were from variations in the iodide nutritional status, the Greer study population would be expected to have a UIC in the range of 65 µg/L and 225 µg/L. Since the NHANE III survey identified the 10th to the 90th percentile UIC in the U.S. population to be from 45 µg/L to 386 µg/L, the %TIU range observed in the Greer study population can be easily accounted for by the expected variation in iodide nutritional status found in the U.S. population. Furthermore, this range in TIU indicates that variation in an individual's iodide exposure during the experiment represents a large uncontrolled confounding variable in the measurement of the amount of NIS inhibition from perchlorate in the Greer study.

The %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.007 and 0.5 mg/kg-day are in excellent agreement with the %TIU observed in the Greer study (see table above). However, the %TIUs calculated from the Tonacchera Model for an external perchlorate dose for 0.02 and 0.1 mg/kg-day are not in particularly good agreement with the %TIU observed in the Greer study. Agreement in the 0.5 mg/kg-day dose makes sense because the administered perchlorate dose represents a 333% increase in the NIS inhibition load on the body (see table below). Any unbalanced variation in the background exposure levels from the other NIS inhibitions (nitrate and thiocyanate) will have a smaller effect on the observed %TIU. By contrast, in the other three perchlorate dose levels (i.e., 0.007, 0.02, 0.1 mg/kg-day), the administered perchlorate only represents a minor or small fraction of the total NIS inhibition load on the body. Any unbalance variation in the background exposure levels from the other NIS inhibitions, nitrate and thiocyanate, among the test subjects will have a much larger effect on the observed %TIU. And, at all perchlorate exposure levels, the potential unbalanced variation in the iodide intake (i.e., the dominant NIS stressor) among the test subjects has largest potential to confound the observed %TIU.

External ClO_4^- Dose (mg/kg-day)	Internal ClO_4^- Dose in an Adult (µmol/L serum)	Estimated Total SPEC in the Greer Study (µmol/L)*	% of Estimated Total SPEC Contributed by ClO_4^- Dose	Estimated Total SPEC as a % of Normal SPEC
0.007	0.014	1.511	0.9%	101%
0.02	0.2	1.70	11.7%	113%
0.1	1.0	2.50	40%	166%
0.5	5.0	6.50	76%	433% (i.e., a 333% inc. over normal SPEC)

* Background NIS inhibitor exposure in the U.S. population is estimated at 1.501 µmol/L SPEC (see Section 7.2.1).

According to the Tonacchera Model, these potential confounding factors in the Greer study do not invalidate the identification of the NOEL. This is because the Tonacchera Model agrees with the Greer study that a perchlorate dose of 0.007 mg/kg-day produces a minimal change in the %TIU. The Tonacchera Models estimates a 96.9 %TIU at a perchlorate dose of 0.007 mg/kg-day. The Greer study measured a 98.3 ± 8.3 %TIU at a perchlorate dose of 0.007 mg/kg-day. The measured 1.7% reduction in the TIU in the Greer study (Greer 2002, table 1) is not statistically different than a 0.0% reduction in the TIU.

In order to isolate the contribution of perchlorate NIS inhibition on the %TIU, the other NIS stressors must also be measured through the course of the experiment. In this experimental design, any unbalance variation in the background exposure to the other NIS stressor could be mathematically factored out to isolate the only the contribution of perchlorate NIS inhibition on the %TIU. Fortunately, the Braverman perchlorate occupational cohort study took measurements of all four NIS stressors before and during exposure. This will allow the variation in the other NIS stressors to be identified and factored out the confounding variables, if necessary. This will be demonstrated in the next section.

9.1.5 Braverman Perchlorate Occupational Exposure Study

In the Braverman perchlorate occupational exposure study, workers at an ammonium perchlorate production facility in Cedar City, Utah, had the RAIU and all four NIS stressors measured preexposure and during exposure (Braverman 2005). The study also measured the following thyroid parameters: T_4, fT_4, T_3, TSH, and thyroglobulin (Tg). Measurement of all four NIS stressors allows the full application of the Tonacchera Model to the study results.

In the study, the exposed workers had their serum measured for thiocyanate, nitrate, and perchlorate concentrations preexposure and during exposure. During exposure, the exposed workers' mean serum concentrations were 3487.8 µg/L of SCN^-, 7926.8 µg/L of NO_3^-, and 838.4 µg/L of ClO_4^-. Since the Tonacchera Model uses units of µmol/L, the corresponding serum concentrations are 60.0 µmol/L of SCN^-, 127.8 µmol/L of NO_3^-, and 8.43 µmol/L of ClO_4^-. Using these values, the $SPEC_{(During\ exposure)}$ of the exposed workers can be calculated as follows:

$SPEC_{(During\ exposure)} = [ClO_4^-] + [NO_3^-] /240 + [\text{free } SCN^-] /15$
$SPEC_{(During\ exposure)} = 8.43\ \mu mol/L + (127.8\ \mu mol/L \div 240) + (60.0\ \mu mol/L \times 0.5 \text{ free } SCN^- \div 15)$
$SPEC_{(During\ exposure)} = 8.43\ \mu mol/L + 0.53\ \mu mol/L + 2.00\ \mu mol/L$
$SPEC_{(During\ exposure)} = 10.96\ \mu mol/L$

The exposed workers were measured to have a median urinary iodide to creatinine ratio of 174 µg I/g creatinine and an average age of 33.6 years (Braverman 2005, table 3). The NHANES III survey identified the median urinary iodide to creatinine ratio of 30- to 39-year-olds to be 108.3 µg I/g creatinine (i.e., the normal iodide excretion level for this age group) (Hollowell 1998, table 2). Therefore, the perchlorate-exposed workers had a 1.61 times greater urinary excretion than the median urinary excretion of the 30- to 39-year-olds in the NHANES III survey. The following calculation determines the exposed workers $\%TIU_{(During\ exposure)}$ 36% using the $TIU_{(normal)}$ of 0.3675x as the baseline:

Inserting10.96 µmol/L for $SPEC_{(During\ exposure)}$ and 1.61x for the $[I^-]_{(During\ exposure)}$ into the Tonacchera Model gives:

$$TIU_{(During\ exposure)} \parallel \frac{[I^-]_{(Exposed\ workers)}}{(1.22 + SPEC_{(Exposed\ workers)})}$$

$$TIU_{(During\ exposure)} \parallel \frac{1.61x}{(1.22 + 10.96\ \mu mol/L)}$$

$$TIU_{(During\ exposure)} \parallel 0.1322x$$

The $\%TIU_{(During\ exposure)}$ in the exposed is calculated using the following equation:

$$\%TIU_{(During\ exposure)} = (TIU_{(During\ exposure)} \div TIU_{(Normal)}) \times 100\%$$

$$\%TIU_{(During\ exposure)} = (0.1322x \div 0.3675x) \times 100\%$$

$$\%TIU_{(During\ exposure)} = 36.0\ \%$$

The Braverman study reports a mean $RAIU_{(During\ exposure)}$ of 13.5 % ± 7.1% and a mean $RAIU_{(Preexposure)}$ of 21.5 % ± 8.2%. The study determined that the perchlorate exposure in the workers resulted in an average 38% decrease in RAIU (i.e., 38% NIS inhibition) during exposure as compared to the workers' preexposure baseline. The corresponding %TIU for comparison is calculated as: 1 - (13.5% ÷ 21.5%) x 100% = 62.8%. At first glance, the Tonacchera Model calculated $\%TIU_{(During\ exposure)}$ of 36.0 % does not agree with the measured %TIU in the study of 62.8%. However, these two %TIU values cannot be compared directly because of the following two reasons:

- The %TIU values are calculated from different TIU baselines. The Tonacchera Model used a $TIU_{(Normal)}$ of 0.3675x as the baseline, which was calculated from the typical U.S. exposure to the four NIS stressors. The %TIU from the Braverman study used the TIU from the workers' preexposure as the baseline. The $TIU_{(Preexposure)}$ is calculated as 0.3370x, which represents a higher NIS stress level than the typical NIS stress level of 0.3675x. Since all four NIS stressors were measured, the $TIU_{(Preexposure)}$ can be calculated and the raw $RAIU_{(Preexposure)}$ value can be normalized to the NIS stressor condition at the $TIU_{(Normal)}$ for the comparison.

- Since the $TIU_{(During\ exposure)}$ is the integrated effect from combined exposure to all four NIS stressors, to measure only the NIS inhibition from perchlorate requires the exposure from the other three NIS stressors to remain constant. However, the $TIU_{(During\ exposure)}$ is confounded by a substantial change in the median urinary iodide to creatinine ratio between during exposure and during preexposure (i.e., the iodide stressor will be shown to be the dominant stressor in this public health issue). The median urinary iodide to creatinine ratio was 174 µg I/g creatinine during exposure and 133 µg I/g creatinine at preexposure. By contrast, the mean thiocyanate serum concentrations during exposure and preexposure are essentially the same at 7926.8

µg/L and 7638.6 µg/L, respectively (i.e., 2.00 µmol/L SPEC vs 1.90 µmol/L SPEC). Likewise, the mean nitrate serum concentrations during exposure and preexposure are essentially the same at 3487.8 µg/L and 3304.0 µg/L, respectively (i.e., 0.53 µmol/L SPEC vs 0.51 µmol/L SPEC). Since all four NIS stressors were measured, the difference in urinary iodide between preexposure and during exposure can be factored out of the $TIU_{(During\ exposure)}$. Since all four NIS stressors were measured, the $TIU_{(Preexposure)}$ can be calculated and the raw $RAIU_{(Preexposure)}$ value can be normalized to the $TIU_{(Normal)}$ for the comparison.

The $TIU_{(Preexposure)}$ can be calculated, but the $SPEC_{(Preexposure)}$ must be calculated first. During exposure, the exposed workers' mean serum concentrations during the preexposure were measured to be 3304.0 µg/L of SCN^-, 7638.6 µg/L of NO_3^-, and 2.0 µg/L of ClO_4^-. Since the Tonacchera Model uses units of µmol/L, the corresponding serum concentrations are 56.9 µmol/L of SCN^-, 123.2 µmol/L of NO_3^-, and 0.02 µmol/L of ClO_4^-. Using these values, the $SPEC_{(Preexposure)}$ of the exposed workers can be calculated as follows:

$SPEC_{(Preexposure)} = [ClO_4^-] + [NO_3^-] /240 + [\text{free } SCN^-] /15$
$SPEC_{(Preexposure)} = 0.02\ \mu mol/L + (123.2\ \mu mol/L \div 240) + (56.9\ \mu mol/L \times 0.5 \text{ free } SCN^- \div 15)$
$SPEC_{(Preexposure)} = 0.02\ \mu mol/L + 0.51\ \mu mol/L + 1.90\ \mu mol/L$
$SPEC_{(Preexposure)} = 2.43\ \mu mol/L$

The exposed workers at preexposure were measured to have a median urinary iodide to creatinine ratio of 133 µg I/g creatinine and an average age of 33.6 years old (Braverman 2005, table 3). The NHANES III survey identified the median urinary iodide to creatinine ratio of 30- to 39-year-olds to be 108.3 µg I/g creatinine (i.e., the normal iodide excretion level for this age group) (Hollowell 1998, table 2). Therefore, the exposed workers at preexposure had a 1.23 times greater urinary excretion than the median urinary excretion of the 30- to 39-year-olds in the NHANES III survey. The following calculation determines the exposed workers' $\%TIU_{(Preexposure)}$:

Inserting 2.43 µmol/L for $SPEC_{(Preexposure)}$ and 1.23x for the $[I^-]_{(Preexposure)}$ into the Tonacchera Model gives:

$$TIU_{(Preexposure)} \parallel \frac{[I^-]_{(Preexposure)}}{(1.22 + SPEC_{(Preexposure)})}$$

$$TIU_{(Preexposure)} \parallel \frac{1.23x}{(1.22 + 2.43\ \mu mol/L)}$$

$$TIU_{(Preexposure)} \parallel 0.3370x$$

The $TIU_{(Preexposure)}$ of 0.3370x is less than the $TIU_{(Normal)}$ of 0.3675x. Therefore, the $TIU_{(Preexposure)}$ value is only 0.917 of $TIU_{(Normal)}$ (i.e., 0.3370x ÷ 0.3675x). In other words, the four NIS stressors at preexposure is a slightly more stressful NIS condition than the typical exposure to the four NIS stressors found in the U.S. population. The raw $RAIU_{(Preexposure)}$ of 21.5% is multiplied by a factor of 1.09 (i.e., 1/0.917) to estimate what the $RAIU_{(Preexposure)}$ would

have been if preexposure was under the typical NIS stressor exposure conditions found in the U.S. population (i.e., a UIC of 145 µg/L and a $SPEC_{(Normal)}$ of 1.501 µmol/L SPEC). Thus, to normalize the raw $RAIU_{(Preexposure)}$ for a common baseline of comparison to the Tonacchera Model, the normalized $RAIU_{(Preexposure)}$ is calculated as follows:

normalized $RAIU_{(Preexposure)}$ = raw $RAIU_{(Preexposure)}$ x (1/0.917)
normalized $RAIU_{(Preexposure)}$ = 21.5% x (1/0.917)
normalized $RAIU_{(Preexposure)}$ = 23.4 %

The difference in urinary iodide between preexposure and during exposure can be factored out of the raw $RAIU_{(During\ exposure)}$. The exposed workers were measured to have a median urinary iodide to creatinine ratio of 174 µg I/g creatinine. The median urinary iodide to creatinine ratio was 133 µg I/g creatinine at the preexposure. Therefore, the median urinary iodide to creatinine ratio during exposure was 1.30 times greater than at preexposure. The raw $RAIU_{(During\ exposure)}$ must be reduced by a factor of 0.764 to factor out the increased TIU contributed by the higher intake of iodide during exposure. Therefore, the adjusted $RAIU_{(During\ exposure)}$ represents the RAIU caused by only the increase in perchlorate exposure, while the other three NIS stressors are either essentially the same (i.e., thiocyanate and nitrate) or factored out (i.e., as in iodide). The adjusted $RAIU_{(During\ exposure)}$ is calculated as follows:

adjusted $RAIU_{(During\ exposure)}$ = raw $RAIU_{(preexposure)}$ x (1/1.30)
adjusted $RAIU_{(During\ exposure)}$ = 13.5% x 0.764
adjusted $RAIU_{(During\ exposure)}$ = 10.3%

Therefore, the normalized $\%TIU_{(During\ exposure)}$ for comparison to the Tonacchera Model calculated $\%TIU_{(During\ Exposure)}$ of 36.0 % is calculated as follows:

Normalized $\%TIU_{(During\ exposure)}$ = adj. $RAIU_{(During\ exposure)}$ ÷ normalized $RAIU_{(preexposure)}$ x 100%
Normalized $\%TIU_{(During\ exposure)}$ = (10.3% ÷ 23.4%) x 100%
Normalized $\%TIU_{(During\ exposure)}$ = 44% (i.e., a 56% NIS inhibition)

After factoring out the differences in baselines and iodide exposures, the normalized $\%TIU_{(During\ exposure)}$ of 44% compares favorable with the Tonacchera Model calculated $\%TIU_{(During\ exposure)}$ of 36.0 %. The standard deviations in the raw RAIU measurements in the Braverman study were greater than 38% of the measured RAIU value for both during exposure and preexposure. This means the difference in the normalized $\%TIU_{(During\ exposure)}$ and the Tonacchera Model calculated $\%TIU_{(During\ exposure)}$ is well within the statistical variation of the RAIU measurement.

9.1.6 Qualitative Risk Assessment of NIS Stressors

In 2003, EPA's *Framework for Cumulative Risk Assessment* identified that a cumulative risk assessment considers the joint impact of multiple stressors. In Section 3.2.2.1, the *Framework for Cumulative Risk Assessment* states, "Studies on individual stressors can, however, provide informative qualitative information for multistressor assessments, particularly regarding hazard identification" (EPA 2003, p 44). Since adverse health effects have not been

clearly demonstrated in any human population exposed to perchlorate (NAS 2005, p 177), the potential human response to NIS inhibition from perchlorate exposure can be studied by qualitatively evaluating the human response to exposure to the other NIS inhibitors, such as thiocyanate. Therefore, the human biological response to the NIS inhibition from thiocyanate provides insight into the potential human response to other NIS inhibitors, such as perchlorate.

Insight into the Toxicity of NIS Inhibition through the Qualitative Review of Endemic Cretinism

Endemic cretinism is the irreversible severe impairment of intellectual and physical development (Delange 2005a, p 731). In both neurologic cretinism and myxedematous cretinism, cretins are severely mentally retarded. Endemic cretinism occurs in 5-15% of severe goiter populations. Endemic cretinism constitutes the extreme expression of the spectrum of abnormalities in physical and intellectual development from iodide deficiency (i.e., a low maternal %TIU) during gestation. Iodide deficiency is the principal factor in the etiology of endemic cretinism. The degree of iodide deficiency correlates with the frequency of cretinism. Since the level of iodide deficiency is an indirect measure of TIU level, the degree of maternal TIU correlates with the frequency of cretinism. A low TIU during pregnancy is the same mechanism of toxicity as perchlorate. Thus, endemic cretinism occurs through the same mechanism of toxicity as perchlorate and represents a case study with which to qualitatively evaluate the factors influencing the toxicity of NIS inhibition. The main difference between endemic cretinism and the public risk from excess NIS stressor exposure in the United States is that both the severity of the maternal %TIU and the severity of the resulting mental deficits are less in the United States.

Iodide deficiency is the main factor in the etiology of endemic cretinism (Delange 2005a, p 734). Delange makes this conclusion based on the following:

1. The degree of iodide deficiency correlates with the frequency of cretinism
2. The prophylactic action of iodide in preventing endemic cretinism (i.e., correcting iodide deficiency).
3. Cretinism has emerged in previously unaffected populations in the Jimi Valley of New Guinea, due to the recent onset of iodide deficiency when the local, iodide-rich rock salt was replaced with low-iodide industrial salt.

Naturally occurring goitrogens in the diet are also a factor in the etiology of endemic cretinism (Delange 2005a, p 735). Cassava is a staple food in tropical areas. Cassava contains cyanogenic glucosides. Upon ingestion, the cyanogenic glucosides in cassava are hydrolyzed into cyanide, which is subsequently metabolized by the body into thiocyanate. Thiocyanate is a potent NIS inhibitor. However, dietary intake of thiocyanate from properly prepared cassava is not sufficient to induce endemic cretinism alone (i.e., NIS inhibition from thiocyanate is not as strong a factor as iodide deficiency), but thiocyanate NIS inhibition works in conjunction with iodide deficiency to induce endemic cretinism. In other words, the role of thiocyanate on endemic cretinism is entirely due to the aggravation of iodide deficiency (Delange 2005a, p 735). The toxicity of thiocyanate is not fixed, but the critical threshold of inducing endemic cretinism from thiocyanate NIS inhibition is related to the dietary intake of iodide (i.e., as measured by

UIC). Goiter develops when the UIC-to-thiocyanate ratio falls below the critical threshold of about 3 µg of iodide per mg of dietary thiocyanate (Delange 2005, p 267). Therefore, the observed toxicity of thiocyanate NIS inhibition is not fixed, but is related to the dietary intake level of iodide.

The lack of selenium is an additional factor in the etiology of endemic cretinism. However, selenium deficiency works through a different mechanism of toxicity than goitrogens. Since selenium deficiency is believed to be extremely rare in the U.S. population and works through a different mechanism of toxicity, this factor is not directly relevant to the perchlorate public health issue and, therefore, is not discussed here.

The role of the NIS inhibition from thiocyanate on endemic cretinism is consistent with the risk characterization observed in the OIG's cumulative risk assessment of NIS inhibition on this public health issue. Endemic cretinism is the extreme example of abnormalities in physical and intellectual development from extremely low maternal TIU. However, since the mechanism of toxicity of cretinism is the same as that of NIS inhibition from perchlorate, the observed role of the risk factors in endemic cretinism can be qualitatively applied to the perchlorate public health issue. Specifically, the following observations from the OIG Analysis are consistent with the observed role of the risk factors in endemic cretinism:

- The OIG Analysis identifies the lack of iodide stressor (i.e., iodide deficiency) as the dominant NIS stressor in inducing a low maternal TIU resulting in increased risk of subtle mental deficits in the children. This risk characterization is consistent with the dominant role that iodide deficiency has in endemic cretinism.

- The OIG Analysis identifies that the thiocyanate NIS stressor has a smaller impact on lowering TIU than does the lack of iodide stressor. This risk characterization is consistent with the observation that thiocyanate NIS inhibition from cassava consumption is not sufficient by itself to induce endemic cretinism, which makes thiocyanate NIS inhibition only a contributing factor on the etiology of endemic cretinism.

- The OIG Analysis identifies that the amount of NIS inhibition from the typical range of thiocyanate exposure in the United States in iodide-sufficient individuals (i.e., UIC greater or equal to 145 µg/L) is not sufficient to lower maternal TIU enough to increase the risk of subtle mental deficits in her child. However, the amount of NIS inhibition from the typical range of thiocyanate exposure in the United States, in conjunction with iodide deficiency (i.e., UIC less than or equal to 100 µg/L), is sufficient to lower maternal TIU enough to increase the risk of subtle mental deficits in her child. This risk characterization is consistent with and parallels the observation that thiocyanate NIS inhibition from cassava consumption is not sufficient by itself to induce endemic cretinism, but works in conjunction with iodide deficiency to induce endemic cretinism.

- The OIG Analysis identifies that the toxicity of NIS inhibition is not fixed, but the body's ability to tolerate NIS inhibition is directly related to the iodide nutrition level. This risk characterization is consistent with the clinical observations in endemic goiter

regions where the toxicity of thiocyanate NIS inhibition from cassava consumption is not fixed, but has a critical threshold ratio related to the dietary iodide intake level (i.e., 3 µg of dietary iodide per mg of thiocyanate).

- The OIG Analysis identifies that the lack of iodide (i.e., iodide deficiency) during pregnancy (i.e., UIC less than or equal to 100 µg/L) is the major factor increasing the risk of permanent subtle mental deficits in children. The OIG Analysis identified that the most effective and efficient approach to correcting this public health issue of low maternal TIU during pregnancy and nursing is to add iodide to all prenatal vitamins and to ingest them before and during pregnancy and nursing. This risk characterization is consistent with the clinical observations in endemic goiter populations where endemic cretinism is prevented by correcting iodide deficiency through the injection of iodized oil (Delange 2005a, p 741-42), (i.e., correcting low maternal TIU).

Therefore, the qualitative evaluation of the roles of iodide deficiency and NIS inhibition in the etiology of endemic cretinism are consistent with the cumulative risk characterization of this public health issue posed by the potential for inducing low maternal TIU from excessive NIS inhibitor exposure.

9.2 Assessing the Contribution of Each NIS Stressor on the TIU

9.2.1 Evaluation of the Amount of NIS Inhibition Contributed by Each NIS Inhibitor

A critical aspect of a cumulative risk assessment is to incorporate the risk from the background exposure to the stressors because the exclusion of a stressor(s) or the background exposure from a stressor(s) "may seriously distort" the portion of the total estimated risk attributed to the principal stressor of concern (EPA 2003, appendix C). Cumulative risk assessment distinguishes between background exposures and source-specific exposures to provide a more complete picture of both total and source-related risks (EPA 2003, appendix C). As shown by the Tonacchera experiment used to derive the Tonacchera Model, the three NIS inhibitors, thiocyanate, nitrate, and perchlorate, act together to inhibit the uptake of iodide by the NIS (Tonacchera 2004). The inhibition of the NIS by thiocyanate, nitrate, and perchlorate is "indistinguishable" from a concentration or dilution of each other (Tonacchera 2004). Therefore, the NIS inhibition contribution by perchlorate exposure has to be evaluated in context to the total body's burden of NIS inhibition.

To address this aspect of a cumulative risk assessment, the typical background exposure to thiocyanate and nitrate was estimated in the U.S. population to evaluate the amount of the inhibition of the NIS of each NIS inhibitor at various perchlorate exposures. As calculated previously in this document, the following table summarizes the results:

Relative Contribution of Each NIS Inhibitor To the Total Amount of NIS Inhibition in an Adult

Perchlorate Exposure Level	Total Amount of NIS Inhibition in an Adult (SPEC)	Perchlorate	Nitrate at (40 µmoles/L)	Thiocyanate at (40 µmoles/L)
At the perchlorate NOEL (0.007 mg/kg-day)	1.567 µmoles/L	4.5 %	10.7%	85.1%
At the perchlorate RfD of (0.0007 mg/kg-day)	1.511 µmoles/L	0.93%	11.1%	88.2%
95th percentile of U.S. population (Blount 2006)	1.505 µmoles/L	0.31%	11.1%	88.6%
50th percentile of U.S. population (Blount 2006)	1.501 µmoles/L	0.09%	11.1%	88.8%
Zero perchlorate	1.500 µmoles/L	0.00%	11.1%	88.9%

This table clearly shows that when the amount of NIS inhibition from perchlorate exposure is evaluated in context to the typical amount of NIS inhibition contributed from the routine exposure to thiocyanate and nitrate, the amount of NIS inhibition from perchlorate exposure is relatively small. At the perchlorate RfD of 0.007 mg/kg-day in an adult, the amount of NIS inhibition from perchlorate exposure is less than 1%. Therefore, as identified in EPA guidance, the exclusion of the background exposure to thiocyanate and nitrate in single chemical risk assessment "seriously distorts" the portion of the total estimated risk attributed to the principal stressor of concern, perchlorate (EPA 2003, appendix C).

As indicated by the Tonacchera Model, perchlorate is only one of four stressors that contribute to the amount of iodide taken up by the NIS. A thorough cumulative risk assessment identifies the exposure levels of the stressors that result in adverse effects. For this public health issue, this means that this cumulative risk assessment must identify the level of TIU in pregnant women that leads to fetal brain damage. Once the critical level of TIU is identified, then each of the four NIS stressors must be managed to prevent the critical TIU level from being exceeded.

9.2.2 Evaluation of %TIU as a Function of Total NIS Inhibition Load

The amount of iodide taken up by the thyroid is the integrated effect from the exposure of all four NIS stressors. However, the three NIS stressors (thiocyanate, nitrate, and perchlorate) are "indistinguishable" from a concentration or dilution of each other (Tonacchera 2004). Therefore, their combined NIS inhibitor exposure can be expressed as a single variable reflecting the total NIS inhibition load on the body (i.e., SPEC), which is measured in µmol/L SPEC. Thus, the Tonacchera Model is simplified into a two-variable equation. This simplification allows the TIU to be expressed as a function of the body's total NIS inhibition load (i.e., SPEC) at a given level of iodide nutrition (e.g., normal iodide intake level). Therefore, to examine this impact of SPEC on TIU, the iodide is held constant, but the SPEC is allowed to change. The following is an example calculation:

The Tonacchera Model stated as a two-variable equation:

$$TIU \parallel [I^-] / (1.22 + (SPEC))$$

If the $[I^-]$ is held constant (i.e., at a stable iodide nutritional level – "x"), the total NIS inhibition exposure level (i.e., SPEC) can be calculated for any given TIU level by using the following equation:

$$TIU_{(Normal)} \times \text{fraction of TIU} \parallel \frac{x}{(1.22 + SPEC_{(\%TIU)})}$$

Given $TIU_{(Normal)}$ in the U.S. population for an adult is 0.3675x, then solving for $SPEC_{(\%TIU)}$ becomes

$$SPEC_{(\% TIU)} \parallel \frac{x}{0.3675x \times (\text{fraction of TIU})} - 1.22$$

For a TIU level of 25% of normal, the $SPEC_{(25\% TIU)}$ becomes

$$SPEC_{(25\% TIU)} \parallel \frac{x}{0.3675x \times (0.25)} - 1.22$$

$$SPEC_{(25\% TIU)} \parallel \frac{x}{0.091875x} - 1.22$$

$$SPEC_{(25\% TIU)} \parallel 10.88 - 1.22$$

$SPEC_{(25\% TIU)} \parallel 9.66$

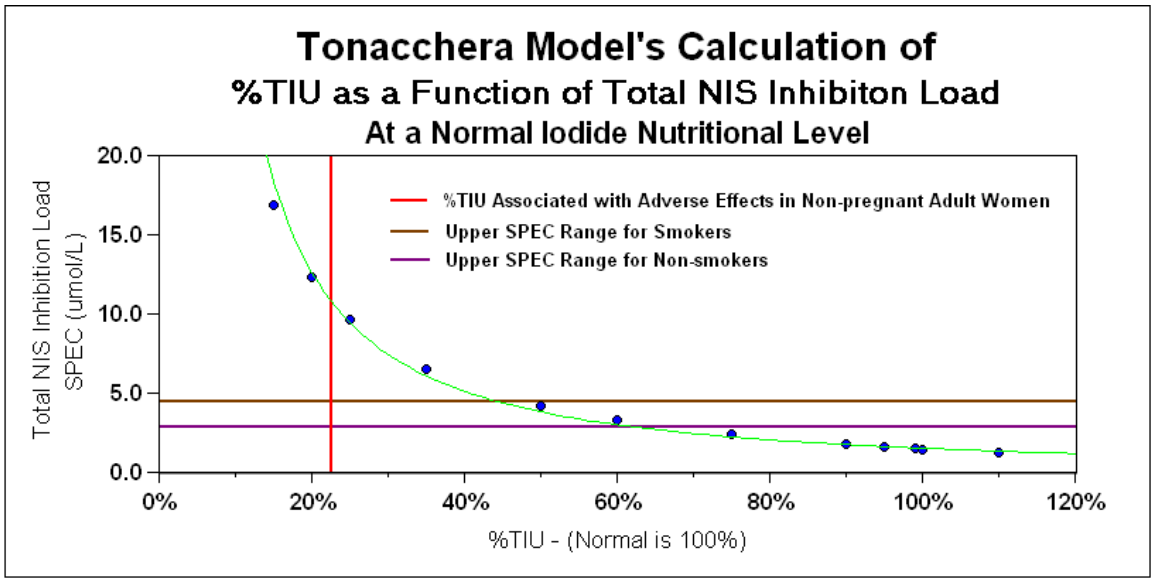
This calculated value estimates that a total goitrogen load of 9.66 µmol/L SPEC at a typical U.S. iodide nutritional level will induce the TIU to be lowered to 25% of the normal TIU. Since the $SPEC_{(Median)}$ is 1.501 µmol/L, an increased NIS inhibitor exposure amount of 8.16 µmol/L SPEC, or 544% increase of normal TIU, is needed to lower normal TIU to 25%. Using the Tonacchera Model to calculate the values, the following table provides the $SPEC_{(\%TIU)}$ for an adult at several %TIU levels:

%TIU Level of Normal	Iodide Nutrition (%) of Normal	$SPEC_{(\%TIU)}$ (µmol/L)	Additional SPEC Exposure Above U.S. Normal* to Achieve TIU Level (µmol/L)	Percent Increase in Additional NIS Inhibitor Load Needed to Generate Specified TIU Level (%)
15	100	16.92	15.41	1027
20	100	12.38	10.88	725
25	100	9.66	8.16	544
35	100	6.55	5.05	337
50	100	4.22	2.72	181
60	100	3.32	1.81	121
75	100	2.42	0.92	62
90	100	1.80	0.30	20
95	100	1.64	0.14	9.3
99	100	1.528	0.027	1.8
100 (normal TIU)	100	1.501	0	0
110	100	1.25	-0.25	-16.7

* U.S. normal SPEC is 1.501 µmol/L (see calculation in Section 7.2.1).
Source: OIG Analysis; Tonacchera Model calculations.

This modeling of %TIU as a function of the body's total NIS inhibition load at normal iodide intake shows that the thyroid can tolerate a lot of exposure to NIS inhibitors before changes in thyroid hormone production. As observed in the adult males and females in the Banerjee thiocyanate studies, hypothyroxinemia is not expected to be observed until %TIU falls at or below 22.7% (Banerjee 1997; Banerjee 1997b). Therefore, in an adult with a normal iodide intake, the thyroid can tolerate up to an additional 8.16 µmol/L SPEC of NIS inhibition above the background NIS inhibition load of 1.501 µmol/L SPEC before adult thyroid is unable to maintain T_4 production. This represents a 544% increase in the body's normal total goitrogen load. Only under rare exposure conditions to NIS inhibitors does the total goitrogen load increase to this point in the Western world (e.g., from occupational exposures or from medicines). By comparison, the amount of NIS inhibition contributed by perchlorate at the RfD in an adult is only 0.014 µmol/L SPEC (i.e., a 0.9% increase of the normal goitrogen load on the body).

The modeling of %TIU as a function of Total NIS Inhibition Load at a normal iodide intake is graphically presented below:



Source: OIG Analysis – graphic representation of the table immediately above.

Since the range of thiocyanate and nitrate exposure in the Western world is known, the upper range for the total NIS inhibition load in the Western world can be calculated for both smokers and nonsmokers using the Tonacchera Model. The total NIS inhibition load is separated into smokers and nonsmokers because the act of smoking is a significant source of cyanide, which is subsequently metabolized into the NIS inhibitor thiocyanate. For nonsmokers, the thiocyanate serum concentration is typically in the range of 10-70 µmol/L. By contrast, for smokers, the thiocyanate serum concentration is higher and is typically in the range of 80-120 µmol/L. In the Western world, the typical nitrate serum concentration ranges from 10-140 µmol/L (Tonacchera 2004). Therefore, the upper range of the total NIS inhibitor load in a nonsmoker is then calculated using140 µmol/L for serum nitrate, the70 µmol/L for serum thiocyanate, and 0.0047 µmol/L for perchlorate (i.e., the 95th percentile observed in the Blount Biomonitoring Study (Blount 2006a (see Section 7.2.1)). Therefore, the $SPEC_{(Upper\ range\ nonsmoker)}$ is given by the following calculation:

$SPEC_{(Upper\ range\ nonsmoker)} = [ClO_4^-] + [NO_3^-] /240 + [free\ SCN^-] /15$
$SPEC_{(Upper\ range\ nonsmoker)} = 0.0047\ µmol/L + (140\ µmol/L \div 240) + (70\ µmol/L \times 0.5\ free\ SCN^- \div 15)$
$SPEC_{(Upper\ range\ nonsmoker)} = 0.0047\ µmol/L + 0.583\ µmol/L + 2.333\ µmol/L$
$SPEC_{(Upper\ range\ nonsmoker)} = 2.92\ µmol/L$

A nonsmoker with a normal iodide intake exposed to NIS inhibitors at an $SPEC_{(Upper\ range\ nonsmoker)}$ of 2.92 µmol/L (purple line in the graph above) has a calculated %TIU of 66%. This is well above the %TIU associated with hypothyroxinemia or hypothyroidism. Therefore, at a normal iodide nutritional level, the upper observed range for total NIS inhibition load in the nonsmoking population does not sufficiently decrease the %TIU level to prevent the thyroid from adapting and maintaining thyroid hormone production in the normal range.

Likewise, the upper range of the total NIS inhibitor load in a smoker is then calculated using 140 µmol/L for serum nitrate, 120 µmol/L for serum thiocyanate, and 0.0047 µmol/L for

perchlorate (i.e., the 95th percentile observed in the Blount biomonitoring study (Blount 2006a (see Section 7.2.1)). Therefore, the $SPEC_{(Upper\ range\ smoker)}$ is given by the following calculation:

$SPEC_{(Upper\ range\ smoker)} = [ClO_4^-] + [NO_3^-] /240 + [free\ SCN^-] /15$
$SPEC_{(Upper\ range\ smoker)} = 0.0047\ \mu mol/L + (140\ \mu mol/L \div 240) + (120\ \mu mol/L \times 0.5\ free\ SCN^- \div 15)$
$SPEC_{(Upper\ range\ smoker)} = 0.0047\ \mu mol/L + 0.583\ \mu mol/L + 4.000\ \mu mol/L$
$SPEC_{(Upper\ range\ smoker)} = 4.59\ \mu mol/L$

A smoker with a normal iodide intake exposed to NIS inhibitors at an $SPEC_{(Upper\ range\ smoker)}$ of 4.58 µmol/L (brown line in the graph above) has a calculated %TIU of 47%. This is well above the %TIU associated with hypothyroxinemia or hypothyroidism. Therefore, at a normal iodide nutritional level, the upper observed range for total NIS inhibition load in the smoking population does not sufficiently decrease the %TIU level to prevent the thyroid from adapting and maintaining thyroid hormone production in the normal range.

To summarize, the graph of %TIU as a function of total NIS inhibition load at a normal iodide nutrition level identifies that the cumulative effect of all three NIS inhibitors (even at the upper range of exposure to each NIS inhibitor found in the U.S. population) does not exceed the thyroid's ability to compensate for the stress. In other words, the largest total NIS inhibition loads observed in the U.S. population are insufficient to lower the %TIU below the critical level of 22.7% to result in hypothyroxemia. Therefore, this analysis identifies that the essential role and contribution of the lack of iodide NIS stressor (i.e., the fourth NIS stressor) is to induce a sufficiently low %TIU (i.e., 22.7% in nonpregnant women) to decrease serum T_4 levels below the normal range. The added stress from the lack of iodide NIS stressor is the critical factor affecting this public health issue. An iodide-deficient diet is required to induce a low enough %TIU level to result in T_3 or T_4 serum level below the normal range.

Perchlorate Occupational Cohort Study

In the Braverman perchlorate occupational cohort study, the ammonium perchlorate workers (i.e., all were males) at Cedar City, Utah, facility had their serum measured for thiocyanate, nitrate, and perchlorate (Braverman 2005). The mean serum concentrations during exposure were 3487.8 µg/L of SCN^-, 7926.8 µg/L of NO_3^-, and 838.4 µg/L of ClO_4^-. Since the Tonacchera Model uses units of µmol/L, the corresponding serum concentrations are 60.0 µmol/L of SCN^-, 127.8 µmol/L of NO_3^-, and 8.43 µmol/L of ClO_4^-. Using these values, the SPEC of the exposed workers can be calculated as follows:

$SPEC_{(Exposed\ workers)} = [ClO_4^-] + [NO_3^-] /240 + [free\ SCN^-] /15$
$SPEC_{(Exposed\ workers)} = 8.43\ \mu mol/L + (127.8\ \mu mol/L \div 240) + (60.0\ \mu mol/L \times 0.5\ free\ SCN^- \div 15)$
$SPEC_{(Exposed\ workers)} = 8.43\ \mu mol/L + 0.53\ \mu mol/L + 2.00\ \mu mol/L$
$SPEC_{(Exposed\ workers)} = 10.96\ \mu mol/L$

In reviewing the table above, an adult with a SPEC of 10.96 and normal iodide intake would be expected to have %TIU of about 22.3%. This %TIU is barely above the $\%TIU_{(Hypothyroxinemia)}$ range of 18.0% to 21.8% in the Banerjee adult male thiocyanate study that induced a T_3 and T_4 thyroid hormone deficiency in adult males consistent with severe hypothyroxinemia. The calcuated %TIU of 22.3% from a total NIS inhibition load of 10.96

µmol/L at a normal iodide nutrition level is in the range in which hypothyroxinemia would be expected, but the thyroid hormones in the perchlorate-exposed workers are well within the normal range. This is because the TIU integrates the exposure to all four NIS stressors (i.e., not just the NIS inhibitors). The iodide stressor is not included in the SPEC value (i.e., the calculation held iodide nutritional level at a normal level of 100%).

If the exposed workers had a normal or below-normal iodide intake, their %TIU would be low enough to cause adverse thyroid hormone effects (e.g., hypothyroxinemia). However, the exposed workers in the Braverman study were measured to have an median urinary iodide to creatinine ratio of 174 µg I/g creatinine and an average age of 33.6 years (Braverman 2005, table 3). The NHANES III survey identified the median urinary iodide to creatinine ratio of 30- to 39-year-olds to be 108.3 µg I/g creatinine (i.e., the normal iodide excretion level for this age group) (Hollowell 1998, table 2). Therefore, the perchlorate-exposed workers had a 1.61 times greater urinary excretion than the median urinary excretion of the 30- to 39-year-olds in the NHANES III survey. The following calculation shows that the above-median intake of iodide by the exposed workers increased their %TIU from a potentially adverse TIU level of 22.3% to an asymptomatic TIU level of 36% (i.e., the increased iodide intake in the exposed workers protected them from adverse thyroid effects):

Inserting 10.96 µmol/L for $SPEC_{(Exposed\ workers)}$ and 1.61x for the $[I^-]_{(Exposed\ workers)}$ into the Tonacchera Model gives:

$$TIU_{(Exposed\ workers)} \parallel \frac{[I^-]_{(exposed\ workers)}}{(1.22 + SPEC_{(Exposed\ Workers)})}$$

$$TIU_{(Exposed\ workers)} \parallel \frac{1.61x}{(1.22 + 10.96\ \mu mol/L)}$$

$$TIU_{(Exposed\ workers)} \parallel 0.1322x$$

The $\%TIU_{(Exposed\ workers)}$ in the exposed is calculated using the following equation:

$$\%TIU_{(Exposed\ workers)} = (TIU_{(Exposed\ Workers)} \div TIU_{(Normal)}) \times 100\%$$

$$\%TIU_{(Exposed\ workers)} = (0.1322x \div 0.3675x) \times 100\%$$

$$\%TIU_{(Exposed\ workers)} = 36.0\ \%$$

An exposed worker with an exposure to NIS inhibitors of $SPEC_{(Exposed\ worker)}$ of 10.96 µmol/L and a iodide intake 1.61 times greater than normal has a calculated %TIU of 36%. This is well above the %TIU associated with hypothyroxinemia or hypothyroidism. Therefore, the exposed workers in the Braverman study would be expected to have thyroid hormones in the normal range, and they were found to have thyroid hormones in the normal range. In other words, the exposed workers' increased iodide intake during exposure protected them from adverse thyroid effects.

9.2.3 Evaluation of the Role of Iodide Nutrition on the Ability to Tolerate NIS Inhibition

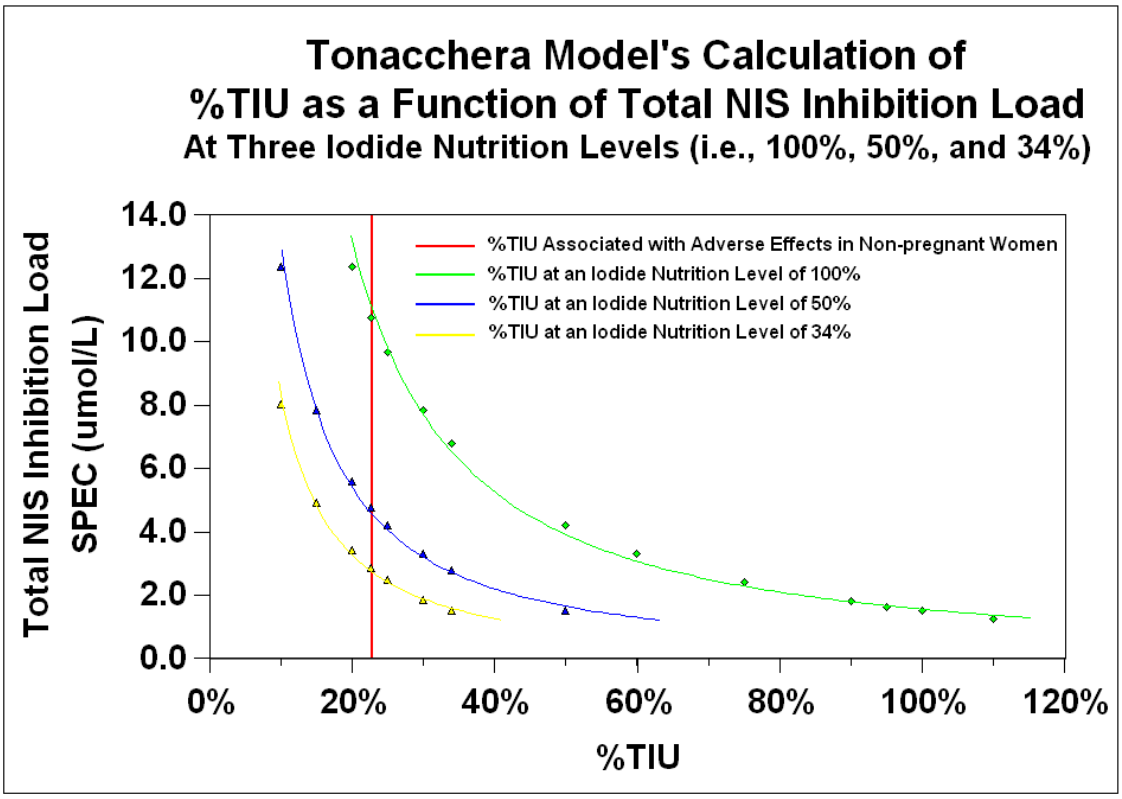
The Tonacchera Model can also be used to evaluate the impact of iodide nutrition on the amount of iodide taken up by the NIS. The three iodide nutrition levels modeled are the fractions 0.34, 0.50, and 1.00 of normal which correspond to a UIC of 50 µg/L, 72.5 µg/L, and 145 µg/L at a $SPEC_{(normal)}$ of 1.501 µmol/L SPEC. The UICs of 50 µg/L, 72.5 µg/L, and 145 µg/L correspond to borderline moderate iodide deficiency, mild iodide deficiency, and sufficient iodide levels. The following table calculates the $SPEC_{(\%TIU)}$ for each of the three iodide nutrition levels for a given %TIU level (sample calculation not shown, but similar to previous calculations):

%TIU Level of Normal	Iodide Nutrition Fraction of Normal	$SPEC_{(\%TIU)}$ (µmol/L)	Additional SPEC Exposure above U.S. Normal†† to Achieve TIU Level (µmol/L SPEC)	Increase in Additional NIS Inhibitor Load Needed to Generate Specified TIU Level (%)
10	0.34*	8.03	6.53	435
15	0.34*	4.94	3.45	230
20	0.34*	3.41	1.90	127
22.7	0.34*	2.85	1.35	90
25	0.34*	2.48	0.98	65
30	0.34*	1.86	0.36	24
34	0.34*	1.501	0.0	0.0
10	0.50†	12.38	10.88	725
15	0.50†	7.85	6.35	423
20	0.50†	5.58	4.08	272
22.7	0.50†	4.77	3.27	218
25	0.50†	4.22	2.72	181
30	0.50†	3.32	1.81	121
34	0.50†	2.78	1.28	85
50	0.50†	1.501	0	0.0
15	1.00	16.92	15.41	1027
20	1.00	12.38	10.88	725
22.7	1.00	10.77	9.27	618
25	1.00	9.66	8.16	544
34	1.00	6.78	5.28	352
50	1.00	4.22	2.72	181
100 (normal TIU)	1.00	1.501	0	0.0

* A 0.34 fraction of iodide nutrition corresponds to borderline moderate iodide deficiency (i.e., UIC of 50 µg/L ÷ normal UIC of 145 µg/L in an adult).
† A 0.50 fraction of iodide nutrition corresponds to mild iodide deficiency (i.e., UIC of 72.5 µg/L ÷ normal UIC of 145 µg/L in an adult).
†† U.S. normal SPEC is 1.501 µmol/L (see calculation in previous section).

The table shows that as iodide nutrition decreases, the thyroid is less able to tolerate a large NIS inhibition load. For example, to maintain a %TIU above the asymptomatic %TIU of 22.7% in an non-pregnant adult, at an iodide nutrition fraction of 1.0, 0.5, and 0.34, the body can tolerate up to a total NIS inhibition load on the body of 6.78, 2.78, and 1.501 µmol/L SPEC, respectively. An iodide nutrition fraction of 0.34 corresponds to borderline moderate iodide deficiency. To summarize, the body's ability to cope with NIS inhibitors decreases rapidly as the iodide intake decreases.

The modeling of %TIU as a function of total NIS inhibition load at three iodide intake levels of 100%, 50%, and 34% is graphically presented below:



Source: OIG Analysis – graphic representation of the table immediately above.

This graph demonstrates that the lack of iodide NIS stressor dramatically lowers the %TIU level for any given total NIS inhibition load (i.e., shifts the curve to the left). For example, decreasing the normal iodide nutrition level in half (i.e., from 100% to 50%) at a constant typical total NIS inhibition load proportionally decreases the %TIU in half (i.e., from 100% to 50%). By contrast, doubling the typical total NIS inhibition load from 1.501 µmol/L SPEC to 3.0 µmol/L SPEC at a constant normal iodide nutrition level results in a %TIU of 64.5% (i.e., a decrease of 35.5%). Therefore, for the NIS stressor exposure conditions in this example, the lack of iodide NIS stressor has about 1.4 times the effect as the same amount of change in the total NIS inhibition load. This clearly demonstrates that the lack of iodide NIS stressor has a larger effect on the %TIU than all the three other NIS stressors combined. Therefore, the lack of iodide NIS stressor is the dominant stressor affecting the public health issue.

As seen in the Banerjee thiocyanate study in male electroplating workers (Banerjee 1997), a %TIU of about 20% is symptomatic and presents as severe hypothyroxinemia. In this study, the low %TIU was brought on by a large total goitrogen load on the thyroid. However, since the %TIU is a function of all four NIS stressors, a low %TIU causing the symptoms of severe hypothyroxinemia can also be brought on by a borderline moderate iodide deficiency with a total goitrogen load found in the upper range of the U.S. population. For example, adult males with a 20 %TIU, at iodide nutrition levels of 1.0, 0.5, and 0.34, can tolerate total NIS inhibition loads of up to 12.38, 5.58, and 3.41 µmol/L SPEC, respectively. Since the upper range of the total NIS inhibition load found in the U.S. population is 4.59 µmol/L SPEC for smokers and 2.92 µmol/L SPEC for nonsmokers, a portion of only the male smoker subpopulation with borderline moderate iodide deficiency (i.e., iodide nutrition fraction of 0.34) with the highest observed levels of the total NIS inhibition load would exceed the tolerable limit of 3.41 µmol/L SPEC. Therefore, a portion of the borderline moderate iodide-deficient male smokers would be expected to show symptoms of hypothyroxinemia. By comparison, borderline moderate iodide-deficient male nonsmokers would not be expected to show symptoms of hypothyroxinemia. Furthermore, all the male nonsmokers and the smokers at iodide nutrition factions of 1.0 and 0.5 would be below the maximum tolerable SPEC values for their iodide nutritional status (i.e., their %TIU > 20%).

As seen in the Banerjee thiocyanate exposure study on females (Banerjee 1997b), a %TIU of 22.7% induced hypothyroxinemia in nonpregnant females. In this study, the low %TIU was brought on by a large total goitrogen load on the body. However, since the %TIU is a function of all four NIS stressors, a low %TIU causing the symptoms of hypothyroxinemia can also be brought on by a borderline moderate iodide deficiency with a total NIS inhibition load found in the upper range of the U.S. female population. For example, adult females with a 22.7 %TIU, at iodide nutrition levels of 1.0, 0.5, and 0.34, can tolerate total NIS inhibition loads of up to 10.77, 4.77, and 2.85 µmol/L SPEC, respectively. Since the upper range of the total NIS inhibition load found in the U.S. population is 4.59 µmol/L SPEC for smokers and 2.92 µmol/L SPEC for nonsmokers, both female smokers and nonsmokers with borderline moderate iodide deficiency (i.e., iodide nutrition fraction of 0.34) with the highest observed levels of the total NIS inhibition load would exceed the tolerable limit of 2.85 µmol/L SPEC. Therefore, a portion of the borderline moderate iodide-deficient female smoker and nonsmoker subpopulations would be expected to show symptoms of hypothyroxinemia. By comparison, all the female nonsmokers and the smokers at iodide nutrition factions of 1.0 and 0.5 would be below the maximum tolerable SPEC values for their iodide nutritional status (i.e., their %TIU > 22.7%).

The clinical observations in areas of endemic cretinism show that the consumption of cassava is not the cause of this health problem, but serves to aggravate the underlying problem of severe iodide deficiency in these areas (Delange 2005a, p 735). The consumption of cassava causes a large NIS inhibition load on the body, but is not large enough to cause health problems on its own. Only when the consumption of cassava occurs in conjunction with a low iodide intake do health problems occur. The above table and graph express the same finding as that observed in the clinical observation in areas of endemic cretinism. In other words, the table and graph state that with a healthy iodide intake, the consumption of NIS inhibitors is not large enough to induce health problems. However, if iodide intake is low enough, the consumption of

moderate amounts of NIS inhibitors can induce health problems; but once again, the root problem of the health issue is iodide deficiency, not the moderate consumption of NIS inhibitors. The next section also identifies iodide intake as the dominant stressor and quantifies the impact on this public health issue.

In conclusion, the NIS inhibitor exposure levels found in the U.S. population are not individually sufficient to induce adverse health effects. However, combined with the added NIS stress caused by iodide defiency, the cumulative effect of all four NIS stressors on the TIU by the thyroid is sufficient to induce adverse effects in the U.S. population. Therefore, NIS inhibitors are not the source of the problem, but act to modulate the toxicity of iodide deficiency.

9.2.4 Evaluation of the Relative Influence of Each NIS Stressor

A critical aspect of a cumulative risk assessment is that a stressor does not have to be the exposure to a chemical, but the "absence of a necessity" (EPA 2003, p 2). The role of iodide nutrition is critical to this public health issue. The Tonacchera Model can be simplified into a two-variable equation that establishes the relationship between the amount of iodide and the body's total NIS inhibition load, which is measured in µmol/L SPEC. The following table shows the relationship between iodide nutrition and the body's total NIS inhibition load (a sample calculation is not shown, but is similar to previous examples):

%TIU Level of Normal	Iodide Nutrition (%) of Normal	SPEC (µmol/L)	Reduction in SPEC Exposure From U.S. Normal* to Achieve TIU Level (µmol/L)	Percent Change in Total NIS Inhibitor Load (%)
99	100	1.528	0.027	1.8
99	99	1.501	0.0	0.0
100 (normal TIU)	100	1.501	0.0	0.0
100	99.4	1.486	0.015	- 1.0
100	99	1.474	0.027	- 1.8
101	100	1.474	0.027	- 1.8

* U.S. normal SPEC is 1.501 µmol/L (see calculation in previous section).

This table shows that the lack of iodide stressor is the dominant NIS stressor of the four NIS stressors. A 1% decrease in the iodide nutrition level has the same effect as lowering the body's total NIS inhibition load by 1.8%. In short, the lack of iodide stressor has about twice the impact on the %TIU as does the impact on the %TIU of all three NIS stressors combined at their typical exposure level. Therefore, by not being able to include the nonchemical stressor, lack of iodide, the conventional single chemical risk assessments for perchlorate "seriously distort" the portion of the total estimated risk attributed to the principal stressor of concern (i.e., perchlorate). By implementing a cumulative risk assessment approach, the contribution of the lack of iodide stressor is quantitatively factored into the total risk, and the portion of total risk attributed to perchlorate and each of the other three NIS stressors can be measured.

The Tonacchera Model can also be used to quantify the impact on the TIU from each of the four NIS stressors. The following table calculates the percent reduction in TIU as a result of

a 25% change in the typical concentration of each NIS stressor (a sample calculation is not shown, but is similar to previous examples):

Modeled Condition	SPEC (µmol/L)	Raw TIU Value	%TIU of Baseline at $SPEC_{(RfD)}$ & Normal [I^-]	Reduction in TIU as a Result of Modeled Condition
$SPEC_{(RfD)}$ + normal [I^-]	1.511†	0.36617x	100 (baseline)	-
$SPEC_{(RfD)}$ + 25% ↓ [I^-]	1.511	0.27463x	75.0	25%
$SPEC_{(RfD)}$ (25%↑ [ClO_4^-])*	1.5175	0.36530x	99.8	0.2%
$SPEC_{(RfD)}$ (25%↑ [NO_3^-])*	1.5558	0.36026x	98.4	1.6%
$SPEC_{(RfD)}$ (25%↑ [SCN^-])*	1.8473	0.32602x	89.0	11.0%

↑ means "increase in"
↓ means "decrease in"
* Modeled serum condition is at normal [I^-]
† Estimated typical total NIS inhibition load at the perchlorate RfD
Note: The table uses a 25% change in each of the NIS stressors so that the perchlorate effect would be on scale (i.e., a fraction of a % change in TIU).

The table shows the change in %TIU as the result of a 25% change in serum concentration of each of the NIS stressors. At the estimated total NIS inhibition load on the body at the perchlorate RfD of 1.511 µmol/L SPEC, an isolated 1% change in the serum concentration of each of the stressors (i.e., lack of iodide, thiocyanate, nitrate, and perchlorate) induces changes in %TIU of 1%, 0.44%, 0.06%, and 0.008% respectively. Therefore, the relative impact of the stressors on this public health issue can be described by the following mathematical expression:

Lack of Iodide > SCN^- >> NO_3^- >> ClO_4^-

Where: " > " means "greater than"
" >> " means "much greater than"

The relative impact of each of the NIS stressors can be described in the following words: the lack of iodide (i.e., iodide nutrition) is the dominant stressor (i.e., strongest NIS stressor), thiocyanate exposure is a medium-strength stressor, nitrate exposure is a weak NIS stressor, and perchlorate exposure is a very weak NIS stressor. The relative impact of each of the NIS stressors is useful information when evaluating the course of action to take to effectively address this public health issue.

9.2.5 Assessing the Effectiveness of Lowering the Total NIS Inhibition Load to Compensate for Poor Iodide Nutrition

The Tonacchera Model can be used to assess the effectiveness of potentially reducing the body's total NIS inhibition load to compensate for poor iodide nutrition to maintain a normal TIU level. The following table summaries the modeled results (a sample calculation is not shown, but is similar to previous examples):

TIU Level (%) of Normal	Iodide Nutritional Level Fraction of Normal	Calculated SPEC Exposure Required to Maintain Normal TIU level (µmol/L)	Reduction in SPEC Exposure Needed to Maintain Normal TIU Level (µmol/L)	Percentage Reduction in SPEC Exposure (%)
100	1.00	1.501*	0	0
100	0.99	1.474	0.027	1.8
100	0.98	1.447	0.054	3.6
100	0.95	1.365	0.136	9.1
100	0.935	1.324	0.176	11.8
100	0.90	1.229	0.272	18.1
100	0.75	0.821	0.680	45.3
100	0.69	0.657	0.843	56.2
100	0.50	0.140	1.361	90.7

* 1.501 µmol/L SPEC is the baseline amount of total NIS inhibition acting on the body.

The table shows that reducing the total NIS inhibition load acting on the body is neither practical nor biologically achievable approach for compensating for poor iodide nutrition. A 1% decrease in the iodide nutritional level (i.e., corresponds to a mean UIC of 143.6 µg/L) can be offset by decreasing the body's total NIS inhibition load by 0.027 µmol/L SPEC. By comparison, the amount of NIS inhibition in an adult contributed by perchlorate at the RfD to the body's total NIS inhibition load is only 0.014 µmol/L SPEC.

Likewise, the table shows that a 7.5% decrease in the iodide nutritional level (i.e., corresponds to a mean UIC of 136 µg/L) can be offset by decreasing the body's total NIS inhibition load by 0.176 µmol/L SPEC. By comparison, the amount of NIS inhibition contributed by both perchlorate exposure at the RfD and the typical nitrate exposure in an adult is only 0.181 µmol/L SPEC. So, all exposure to perchlorate at the RfD and nitrate would have to be eliminated to offset a 7.5% decrease in the body's intake of iodide.

Furthermore, the table shows that a 31% decrease in the iodide nutritional level to borderline mild iodide deficiency (i.e., corresponds to a mean UIC of 100 µg/L) can be offset by decreasing the body's total NIS inhibition load by 0.843 µmol/L SPEC. To achieve this, all of the exposure to perchlorate at the RfD (i.e., 0.014 µmol/L SPEC), all of the typical nitrate exposure (i.e., 0.167 µmol/L SPEC), and half of the typical thiocyanate exposure (i.e., 0.667 µmol/L SPEC) would have to be eliminated to offset the decrease in %TIU from borderline mild iodide deficiency. Such a reduction in the total NIS inhibition load is not practical. All nitrate-containing foods would have to be eliminated from the diet, to include bacon, beef, sausage, potatoes, and carrots, and most of the foods rich in thiocyanate would have to be eliminated from

the diet, to include cabbage, broccoli, Brussels sprouts, corn (maize), cauliflower, cabbage, radishes, spinach, tomatoes, and milk.

Furthermore, thiocyanate may have a regulatory function in normal physiology (Middlesworth 1986). Thiocyanate is continually synthesized in the normal rat. In the absence of all dietary intake of thiocyanate in the rat, the thiocyanate serum concentration actually increased, from 500 µg/dl to 800 µg/dl, during the fasting (Middlesworth 1986, figure 1). These data indicate that serum thiocyanate in rats is maintained in a normal range. This suggests that significantly reducing serum thiocyanate in humans from a normal range is not biologically achievable.

In conclusion, reducing the average exposure to NIS inhibitors is not an effective solution to compensate for iodide deficiency. The only effective manner to address the adverse health effects of a low %TIU resulting from iodide deficiency is to increase the intake of iodide in the diet.

9.3 Application of UFs in a Multifactorial Cumulative Risk Assessment

The conventional approach of deriving an RfD in a single contaminant characterization is to apply one or more UFs to a LOAEL, NOAEL, or BMD, to address uncertainties in the data to ensure the RfD is below an exposure that might induce an adverse effect in a sensitive population. The NAS Committee applied a UF of 10 to the NOEL to derive the recommend perchlorate RfD of 0.0007 mg/kg-day. The number, type, and magnitude of UFs to apply in the derivation of an RfD is a matter of scientific judgment. This is a major source of contention in the derivation of a perchlorate RfD. The following table calculates the estimated total goitrogen load in an adult for various applications to UFs to the perchlorate NOEL of 0.007 mg/kg-day, which correspond to several DWELs (sample calculation not shown, but similar to previous calculations):

Description of Dose Level	Drinking Water Equivalent Level (DWEL)	External Dose (mg/kg-day)	Corresponding Uncertainty Factor (UF)	Calculated Total Goitrogen Load (µmol/L SPEC)
Greer NOEL	245 ppb	0.007	0	1.567
ClO_4^- RfD	24.5 ppb	0.0007	10	1.511
California Drinking Water Limit	6.0 ppb	0.00017†	40.8†	1.5034
Massachusetts Drinking Water Limit	2.0 ppb	0.000057†	122.5†	1.5012
EPA draft RfD	1.0 ppb	0.00003	233.3*	1.5006
Provided to assess the impact of a large UF on the TIU	0.5 ppb	0.000014	500	1.5003

* EPA's draft perchlorate RfD was 0.00003 mg/kg-day external dose, but was derived from a different POD using a UF of 300.

† For this evaluation of the impact of applying different UFs to the setting of a perchlorate RfD on the TIU, the external perchlorate dose and UF listed were back calculated from the Greer NOEL to arrive at the State's Drinking Water Limits (this not the manner in which the States set their limits).

The following table summaries the calculated TIU, %TIU, and percentage increase in TIU in an adult at various applications to UFs to the perchlorate NOEL of 0.007 mg/kg-day, which corresponds to several DWELs (sample calculation not shown, but similar to previous calculations):

Description of Dose Level	Drinking Water Equivalent Level (DWEL)	Calculated Total Iodide Uptake in an Adult (TIU)	Percentage TIU of Baseline (%)	Change in %TIU from Baseline
Greer NOEL	245 ppb	0.35881x	100 baseline	0
ClO_4^- RfD	24.5 ppb	0.36617x	102.05	2.05
California Drinking Water Limit	6.0 ppb	0.36718x	102.33	2.33
Massachusetts Drinking Water Limit	2.0 ppb	0.36748x	102.42	2.42
EPA draft RfD	1.0 ppb	0.36756x	102.44	2.44
Provided to assess the impact of a large UF on the TIU	0.5 ppb	0.36761x	102.45	2.45

Applying a UF of 10 to the NOEL reduces the perchlorate DWEL from 245 ppb to 24.5 ppb, which would increase an adult's TIU by 2.05%. Applying a UF of 40.8 to the NOEL reduces the perchlorate DWEL to 6.0 ppb; this further reduction in the perchlorate DWEL from 24.5 ppb to 6.0 ppb increases an adult's TIU by only 0.28%. Applying a UF of 122.5 to the NOEL reduces the perchlorate DWEL to 2.0 ppb; this further reduction in the perchlorate DWEL from 6.0 ppb to 2.0 ppb increases an adult's TIU by only 0.09%. Applying a UF of 233.3 to the NOEL reduces the perchlorate DWEL to 1.0 ppb; this further reduction in the perchlorate DWEL from 2.0 ppb to 1.0 ppb increases an adult's TIU by only 0.02%. Applying a UF of 500 to the NOEL reduces the perchlorate DWEL to 0.5 ppb; this further reduction in the perchlorate DWEL from 1.0 ppb to 0.5 ppb increases an adult's TIU by only 0.01%.

In short, this calculation shows that the use of an increasing UF value has the surprising result of having only a minimal impact on the TIU. This is contrary to the intent of the use of UFs in deriving an RfD. The explanation of this result is that a single chemical risk assessment is based on the assumption that the chemical is the principal cause (i.e., dominant stressor) of the adverse effect. So, if the exposure to the single chemical is kept sufficiently below the POD, the adverse effect will be avoided in even the most sensitive human subpopulation. However, perchlorate is only one of four stressors that affect the TIU of the NIS. Perchlorate also contributes the least amount of stress of the four NIS stressors and explains why the application of a UF to perchlorate exposure has a minimal effect on the TIU. Since the mechanism of toxicity results from the lack of a sufficient uptake of iodide to make an adequate supply of thyroid hormones for proper brain development during gestation and lactation, the concept of UFs should be applied to the TIU, which addresses the integrated result from the simultaneous exposure to all four stressors. The magnitude of the TIU is the single variable that determines a favorable or unfavorable outcome in this public health issue. In other words, since a low TIU leads to the adverse effect of brain damage, the minimum TIUs observed in the human population need to be kept above the TIU level that results in adverse effects. To apply UFs to

the TIU, the equivalent LOAEL or NOAEL TIU value must be determined; this will be discussed in the next section.

9.4 Determining %TIU Levels of Concern

9.4.1 Identifying a $\%TIU_{(LOAEL)}$ in Pregnant Women to Prevent Subtle Mental Deficits in Children

The Tonacchera Model can be used to estimate the LOAEL %TIU in pregnant women when fetal brain damage occurs. The Tonacchera Model states:

$$TIU \parallel [I^-] / (1.22 + (SPEC))$$

where: the symbol || means "proportional to"

If the SPEC is held constant (i.e., the level of NIS inhibitors remains the same), then,

$$TIU \parallel [I^-]$$

Therefore, when the $SPEC_{(Normal)}$, then $\%TIU_{(LOAEL)}$ is given by:

$$\%TIU_{(LOAEL)} = ([I^-]_{(Fetal\ brain\ damage)} \div [I^-]_{(Normal\ pregnant\ women)}) \times 100\%$$

Since subtle fetal brain damage is known to occur in moderately iodide-deficient pregnant women (Bleichrodt 1989; Vermiglio 2004; Vermiglio 1990), as before, using UIC as a surrogate, the $[I^-]_{(Fetal\ brain\ damage)}$ is 50 µg/L. The other information need is the recommended healthy UIC in pregnant women (i.e., $[I^-]_{(Normal\ pregnant\ women)}$). Several credible sources have opined on the recommended UIC for pregnant women. Instead of relying on a single source, the average recommended UIC for pregnant women was determined and found to be 204 µg/L. The following table summarizes the sources and recommended UICs for pregnant women:

Source	Recommended Iodide Intake in Pregnant Women (µg/day)	Recommended Urinary Iodide Concentration (µg/L)
WHO/UNICEF/ICCIDD*	200	200-300
Dr. Francois Delange, MD**	225-300	150-230
WHO Technical Consultant Group***	225-375	150-249
Recommend Dietary Allowances (RDA)†	220†	150††
Average Value =>	258	204

* Source: Delange 2005, p 278, table 11E.8.
** Source: Delange 2004.
*** Source: ATA 2006.
† Source: NAP 2000.
†† Source: Pearce 2004.

Therefore, the $\%TIU_{(LOAEL)}$ in pregnant women is calculated as follows:

$$\%TIU_{(LOAEL)} = ([I^-]_{(Fetal\ brain\ damage)} \div [I^-]_{(Normal\ pregnant\ women)}) \times 100\%$$
$$\%TIU_{(LOAEL)} = (50\ \mu g/L \div 204\ \mu g/L) \times 100\%$$
$$\%TIU_{(LOAEL)} = 24.5\%$$

From the observed subtle brain damage in the offspring of moderately iodide-deficient pregnant women (Bleichrodt 1989; Vermiglio 2004; Vermiglio 1990), the LOAEL in pregnant women is estimated to be 24.5 %TIU. This agrees with 22.7%$TIU_{(Hypothyoxinemia)}$ observed to generate hypothyroxinemia in women exposed to excess amounts of thiocyanate (Banerjee 1997b). Furthermore, inducing hypothyroxinemia in pregnant women is consistent with the proposed mechanism that an insufficient supply of maternal fT_4 during gestation starves the developing fetal brain of an adequate supply of thyroid hormones, resulting in fetal brain damage. Also of note, 50% of the moderately iodide-deficient pregnant women in the Vermiglio 2004 study were observed to have hypothyroxinemia. Eleven of the 16 children born to moderately iodide-deficient pregnant women in the Vermiglio 2004 study had ADHD.

The 24.5 %$TIU_{(LOAEL)}$ integrates the combined effects of all four NIS stressors into a single variable. In a cumulative risk assessment, this is the numerical value that the concept of UFs shall be applied to. In a single chemical risk assessment, UFs are applied to the LOAEL or NOAEL to derive an RfD to address the uncertainties inherent in the data. If this concept is applied to the %$TIU_{(LOAEL)}$, typically a UF of 10 is applied to a LOAEL to estimate a NOAEL. And as in the case of the NAS Committee, an intraspecies UF of 10 was applied to derive the perchlorate RfD. Using this simplified template for the estimation of UFs to apply to the %$TIU_{(LOAEL)}$, a total UF of 100 would be used. The application of a UF of 100 to 24.5 %$TIU_{(LOAEL)}$ (i.e., to back off the adverse effect level by two orders of magnitude) would derive a %$TIU_{(RfD\ equivalent)}$ = 2450%. This value corresponds to a UIC of about 5000 µg/L. This is biologically unrealistic. Obviously, the conventional rules of applying UFs during a single chemical risk assessment do not apply to this cumulative risk assessment.

9.4.2 Identifying a %$TIU_{(NOAEL)}$ in Pregnant Women to Prevent Subtle Mental Deficits in Children

The cumulative risk assessment of this public health issue recognizes that the dose-response curve for iodide exposure to the thyroid is a u-shape curve (Delange 2005, p 281). When the UIC of pregnant women is below 50 µg/L, adverse effects are observed in their children, but when the UIC of pregnant women goes above 500 µg/L (ATA 2006), other adverse clinical effects can occur. By analogy, since %TIU represents the same mechanism (i.e., the amount of iodide available to the thyroid), the %TIU dose-response curve will also be u-shaped. A UIC of 50 µg/L and 500 µg/L result in a %TIU in a pregnant woman of 24.5 %$TIU_{(LOAEL)}$ and 245 %$TIU_{(Excess\ limit)}$, respectively.

EPA's Integrated Risk Information System defines a NOAEL as:

> *The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects* (IRIS 2007).

The traditional use of a NOAEL in a single chemical exposure is as the lowest chemical exposure level without adverse effects. The typical use of a NOAEL assumes that increasing chemical exposure produces an increasing the severity or frequency of adverse effects.

However, the %TIU dose-response curve is u-shaped. An adverse effect occurs when the %TIU is either too high or too low. Therefore, the application of the NOAEL concept to a u-shape dose-response curve generates the need for two NOEALs to prevent insufficient uptake of iodide and to prevent an excessive uptake of iodide. For the purposes of identifying each NOAEL, the following labels will be used: $\%TIU_{(NOAEL)}$ and $\%TIU_{(NOAEL\ for\ excess)}$. A UIC above 500 µg/L in pregnant women (ATA 2006) is excessive and results in a $\%TIU_{(NOAEL\ for\ excess)}$ of 245%.

The key is to define a $\%TIU_{(NOAEL)}$ that is " . . . [the lowest %TIU] exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control." A $\%TIU_{(Insufficient\ NOAEL)}$ can be derived from the following adverse effects occurring in children of mildly iodide-deficient pregnant women:

- Mildly iodide-deficient pregnant women have children with statistically significant delayed reaction times to visual stimuli (Lombardi 1995; Vitti 1992). Although not a particularly severe adverse effect, delayed reaction times to visual stimuli is a sensitive test for a subtle change that is reproducible between studies and is undesirable.

- The heterogeneous thyroid performance within the human population (i.e., the 5th item to be addressed by this cumulative risk assessment) can be observed in the children of mildly iodide-deficient pregnant women. Newborns of mildly iodide-deficient women have an increased frequency of 0 to 16.9% of elevated TSH above the < 3% background levels observed in iodide-sufficient areas. In other words, at least 83% of the newborns are unaffected by mild iodide deficiency, while up to 16.9% are potentially adversely affected. Although a mildly iodide-deficient pregnant woman is able to adapt to mild iodide deficiency and will probably remain euthyroid throughout pregnancy, the fetus's thyroid is less able to adapt to iodide deficiency. Fetal hypothyroxinemia is induced by iodide deficiency because the immature fetal thyroid is unable to increase its avidity for iodide despite up-regulation of NIS expression in the fetal thyroid and placenta during iodide deficiency (Delange 2005a). The increased iodide clearance rate will further decrease the iodine stores of the fetal thyroid and decrease fetal T_4 synthesis (Delange 2005a). Tracer studies in mild hypothyroid neonatal rats (i.e., elevated TSH without below normal fT_4) have observed increased D2 deiodinase activity of the central nervous system (CNS) in order to compensate for a decreased supply of T_3 in the CNS. This implies that newborns with elevated TSH (analogous to mild hypothyroidism in the neonatal rats) may have had to activate their D2 deiodinase compensatory mechanism in the CNS (Bianco 2005, p 123). This indicates that the fetal thyroid may not be making enough T_4 during mild iodide deficiency and increases the possibility that subtle brain damage may have occurred. However, epidemiology studies have neither identified nor evaluated this subgroup (i.e., elevated TSH newborns) for the possibility of subtle mental deficits. Neonatal TSH is the single indicator that focuses on potential brain damage, which is the major impact of iodide deficiency (Delange 1998). An additional adverse effect in neonates with elevated TSH is observed in a study by Calacirua, in which infants with elevated TSH at neonatal screening are at risk for developing subclinical hypothyroidism in early childhood (odds ratio 44.6%; 95% CI 38.8-50.4%) (Calaciura

2002). Delange also points out that neonates with elevated TSH levels are at a high risk of developing subclinical hypothyroidism in infancy and early childhood (Delange 1998).

Clearly, the observed delayed reaction time, the increased occurrence of elevated TSH in newborns, and the increased risk of developing subclinical hypothyroidism meet the NOAEL definitional requirement that the NOAEL exposure level must be above the level of "increases in the frequency or severity of adverse effect(s)." Since mild iodide deficiency in pregnant women is characterized by a UIC between 100 µg/L to 50 µg/L, using a UIC of 100 µg/L prevents a %TIU exposure level that induces an increased frequency of elevated TSH levels in the iodide-sensitive subpopulation. Therefore, the $\%TIU_{(NOAEL)}$ in pregnant women is calculated as follows:

$$\%TIU_{(NOAEL)} = ([I^-]_{(\text{inc. freq. of elevated TSH in newborns})} \div [I^-]_{(\text{Normal pregnant women})}) \times 100\%$$
$$\%TIU_{(NOAEL)} = (100\ \mu g/L \div 204\ \mu g/L) \times 100\%$$
$$\%TIU_{(NOAEL)} = 49\%$$

9.4.3 Identifying a $\%TIU_{(RfD)}$ in Pregnant Women to Prevent Subtle Mental Deficits in Children

EPA's *Framework for Cumulative Risk Assessment* (EPA 2003) defines an (RfD) as, "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime (EPA 2003)."

EPA's risk assessment guidance states that a reduction of the intraspecies UF from a default of 10 is to be considered only if the POD is determined from the data obtained from the susceptible subpopulation (EPA 2002, p 4-43). Since the POD (i.e., $\%TIU_{(NOAEL)} = 49\%$) was determined in the susceptible subpopulation, an intraspecies UF of 1 should be used. However, the $\%TIU_{(NOAEL)}$ should not be used as the $\%TIU_{(RfD)}$, because the uptake of iodide is not the only step in the production and use of thyroid hormones in the body that is disrupted by chemical exposures. Numerous inorganic and synthetic chemicals have been documented to interfere with almost every major step in the production, transport, and peripheral tissue metabolism of thyroid hormones (Howdeshell 2002, p 344, table 1). The following table provides examples of chemicals that interfere with several major thyroid hormone steps:

Thyroid Hormone Step	Examples of Chemicals that Interfere
Iodide uptake by the NIS	2,4-D, Aldrin, Lead, PBBs,
Iodide oxidation by thyroid peroxidase	Lindane, Malathion, Mancozeb
Circulatory transport in blood (e.g., binding to transthyretin)	Dioxtylphthalate, DDT metabolites, Dichloroprop, Difocol, Lindane, Malathion,
Cellular metabolism of T_4 to T_3 by Type I or Type II 5'-deiodinase in peripheral tissues	Cadmium, Lead, PCB, Dixoin
Increase cellular elimination by glucuronidation of T_4/T_3 by the stimulation of the glucuronidase enzyme	Acetochlor, DDT, PBBs, PCBs, Dioxin

Source: Howdeshell 2002 (for full list - see p 344, table 1).

As stated in the EPA's *Framework for Cumulative Risk Assessment*, an aspect of a cumulative risk assessment is that it can be qualitative as well as quantitative (EPA 2003). This

aspect is necessary to incorporate risk from other chemical exposures in which there is insufficient information to accurately quantify the amount of risk, although the presence and nature of the risk is known. The rational for using a nonquantitative approach for setting the $\%TIU_{(RfD)}$ is also based on the understanding of the mechanism of action for thyroid hormones (Yen 2005). Proper fetal/neonate brain development occurs when a sufficient supply of intracellular T_3 binds to the thyroid hormone receptor TRβ-1. The T_3/TRβ-1 is a ligand-regluated transcription factor that along with other transcriptional cofactors causes the expression of genes containing thyroid hormone response elements in the promoter region (Yen 2005).

In short, thyroid hormones represent a "chemical signal" in the body that regulates the transcription of genes during CNS development. Although the $\%TIU_{(NOAEL)}$ provides enough uptake of iodide by the thyroid to make a sufficient supply of T_4, subsequent loses of T_4 in subsequent thyroid hormone steps due to the potential interference by other industrial chemicals (e.g., higher than normal cadmium exposure during gestation) could result in a insufficient suppy of T_4 at the target brain tissue. In other words, the "chemical signal" started out strong enough at the thyroid, but was weakened by interference from other industrial chemicals along the way that resulted in an insufficient "chemical signal" in the developing brain cell. The amount of interference by these other industrial chemicals is qualitatively known to occur, but is not easily quantified for use in a conventional risk assessment. However, a cumulative risk assessment allows this interference effect to be qualitatively factored into the assessment. The concept is that if the original "chemical signal" is made strong enough, it may tolerate some interference along the way and still be strong enough at the end to be correctly interrupted and allow for the appropriate level of gene transcription to occur for proper brain development.

The qualitative factor proposed is the equivalent of using a 1.5 UF. Therefore, the $\%TIU_{(RfD)}$ in pregnant women is calculated as follows:

$\%TIU_{(RfD)} = \%TIU_{(NOAEL)} \times 1.5\ UF$
$\%TIU_{(RfD)} = 49\% \times 1.5\ UF$
$\%TIU_{(RfD)} = 74\%$

At a typical 1.501 µmol/L SPEC level, a %TIU of 74% corresponds to a UIC in pregnant women of 151 µg/L. Since the median UIC in pregnant women in the NHANES III survey was 140 µg/L, this means about half of U.S. pregnancies each year occur below the $\%TIU_{(RfD)}$. This indicates a clear need to increase the %TIU levels in this half of the U.S. pregnant women population with these low %TIUs.

9.5 Calculating the %TIU in Pregnant Women at Various Perchlorate Exposures

The Clewell perchlorate PBPK model identifies that the internal perchlorate exposure (i.e., internal indices) is not the same for each life stage for the same external perchlorate dose (Clewell 2007, p 423, table 4). Specifically, the conversion factor from an external to internal perchlorate dose is 2.5 times higher at 0.001 mg/kg-day for a pregnant woman than for an adult. In other words, a pregnant woman experiences 2.5 times more NIS inhibition from higher serum perchlorate level than an adult for the same external perchlorate dose of 0.001 mg/kg-day. Therefore, the calculated TIUs in pregnant women will be slightly lower then the calculated TIUs in adults (see previous table in Section 9.1.4) at the same external perchlorate dose. The following table summaries the calculated TIUs for pregnant women with various external perchlorate exposures levels (sample calculation not shown, but similar to previous calculations):

External ClO_4^- Dose (mg/kg-day)	Internal ClO_4^- Dose in Pregnant Women (µmol/L)	Serum Perchlorate Equivalent Conc. (SPEC) in Pregnant Women (µmol/L)	Iodide Nutritional Level in Pregnant Women (Fraction of Normal)	Calculated Total Iodide Uptake (TIU)	%TIU Calculated from the Tonacchera Model
0.000066* (in nonpregnant adults)	0.0013*	1.501†	1.00	0.3675x	100 (nonpregnant adult %TIU reference level)
0.000066*	0.00325	1.503	1.00	0.3672x	99.9
0.000175 (¼ RfD)	0.0088	1.510	1.00	0.3663x	99.7
0.0007 (RfD)	0.035	1.536	1.00	0.3628x	98.7
0.007 (NOEL)	0.399**	1.900	1.00	0.3205x	87.2
0.000066*	0.00325	1.503	0.49***	0.1799x	49.0
0.000175 (¼ RfD)	0.0088	1.510	0.49***	0.1795x	48.8
0.0007 (RfD)	0.035	1.536	0.49***	0.1778x	48.4
0.007 (NOEL)	0.399**	1.900	0.49***	0.1570x	42.7

* Median perchlorate exposure in U.S. population (Blount 2006a).
† From median NIS inhibitor exposures in the U.S. population (calculated in Section 7.2.1).
** Used an estimated conversion factor of 0.0425 mg/L per .0075 mg/kg-day using a linear interpolation from the Clewell perchlorate PBPK model.
*** Average recommended urinary iodide concentration in pregnant women = 204 µg/L (see Section 9.4.1). Therefore, an iodide nutritional level of 0.49 in pregnant women corresponds to 100 µg/L, which is the urinary iodide limit for mild iodide deficiency.

In a pregnant woman at the average recommended iodide nutrition level, the Tonacchera Model calculates a 98.7 %TIU at the perchlorate RfD of 0.0007 mg/kg-day, which corresponds to a 24.5 ppb DWEL. By comparison, the calculated %TIU in adults at the perchlorate RfD is 99.6% (see table in Section 9.1.4). Therefore, the Tonacchera Model estimates that a pregnant woman's %TIU is affected more by the same external perchlorate exposure than is an adult's %TIU (i.e., a decrease of 0.9 %TIU). In a pregnant woman, the Tonacchera Model also calculates a 99.7 %TIU at a perchlorate exposure level of 0.0000175 mg/kg-day (i.e., a quarter of

the perchlorate RfD level), which corresponds to a 6.1 ppb DWEL. Therefore, in a pregnant woman, the Tonacchera Model estimates a 1.0% increase in the %TIU when the perchlorate drinking water exposure is lowered from 24.5 ppb to 6.1 ppb.

By contrast, in a pregnant woman with mild iodide deficiency (i.e., with a UIC of 100 µg/L) , the Tonacchera Model calculates a 48.4 %TIU at the perchlorate RfD of 0.0007 mg/kg-day, which corresponds to a 24.5 ppb DWEL. This is a decrease of 50.3%TIU as compared to a pregnant woman at the average recommended iodide nutrition level having a same perchlorate exposure at the perchlorate RfD (i.e., 98.7% minus 48.4%). In other words, for a pregnant woman with perchlorate exposure at the RfD, decreasing her iodide intake from the average recommended iodide nutrition level (i.e., a UIC of 204 µg/L) to a mildly iodide-deficient level (i.e., a UIC of 100 µg/l) lowers her TIU level by 50.3%TIU. By comparison, a pregnant woman with mild iodide deficiency and a perchlorate exposure level of 0.0000175 mg/kg-day (i.e., a quarter of the perchlorate RfD), which corresponds to a 6.1 ppb in drinking water, would have a calculated %TIU of 48.8%. Therefore, in a pregnant woman with mild iodide deficiency, the Tonacchera Model estimates only a 0.4 % increase in the %TIU when the perchlorate drinking water exposure is lowered from 24.5 ppb to 6.1 ppb. Clearly, an increase of 0.4% in %TIU in mildly iodide-deficient pregnant women estimated from the potential act of lowering the perchlorate drinking water exposure from 24.5 ppb to 6.1 ppb is not sufficient to offset the 50.3% decrease in %TIU from the loss of iodide in the diet during mild iodide deficiency during pregnancy. This example in mildly iodide-deficient pregnant women shows that the lack of iodide is the dominant stressor affecting this public health issue and the risk to the public from a low %TIU during pregnancy and lactation cannot be managed without addressing iodide deficiency within this sensitive subpopulation.

Evaluating these results from another perspective, the Greer study observed a %TIU of 98.2% ± 8.3% at the perchlorate NOEL of 0.007 mg/kg-day. Therefore, the predicted 1.0% reduction in the TIU in pregnant women when the DWEL is lowered from 24.5 ppb to 6.1 ppb is not statistically detectable in a human experiment. From the Greer data, a 1.0% change in the %TIU is only 1/12th the random variation observed in the %TIU at the perchlorate NOEL. By contrast, the Greer study measures a %TIU range of 45% to 155% in the population before the exposure to perchlorate (i.e., in the 37 test subjects at the BV). Therefore, %TIU varied ± 55% in Greer's study population without measuring any thyroid hormones outside the normal ranges for TSH, fT_4, tT_4, or tT_3. Potentially regulating perchlorate at a DWEL of 6.0 ppb instead of 24.5 ppb prevents about a 1% change in TIU in pregnant women; a 1%TIU change is only a small fraction of the ± 55% normal variation observed in the %TIU at baseline in the Greer study population. Therefore, decreasing the perchlorate drinking water concentration from 24.5 ppb to 6.0 ppb will not have a significant effect on the %TIU observed in people. A single chemical risk assessment of perchlorate only characterizes a small portion of this public health issue and does not explain the factors that cause the TIU to vary ± 55% in the Greer study population. Furthermore, managing only the exposure to perchlorate and not managing the other NIS stressors will have little effect of ensuring the %TIU is above the NOAEL for pregnant women. This cumulative risk assessment estimates the $\%TIU_{(NOAEL)}$ = 49% for pregnant women. Only by managing the exposure to all four NIS stressors can the %TIU be held above the $\%TIU_{(NOAEL)}$ for pregnant women.

Using a cumulative risk assessment approach to characterize this public health issue, the large variation in the %TIU observed in the Greer study can be easily explained. The NIS stressor that can account for such a large variation in the %TIU observed in the Greer study population is the dominant NIS stressor – iodide. If all the variation in %TIU was from the variation in iodide nutrition alone, the Greer study population would be expected to have a UIC in the range of 65 µg/L and 225 µg/L. This agrees with iodide variation observed in the NHANE III survey, which identified the 10th to the 90th percentile UIC in the U.S. population to be 45 µg/L to 386 µg/L.

A 1.0% reduction in %TIU could be offset by a 1.0% increase in the UIC in pregnant women. At a borderline iodide-deficient UIC of 100 µg/L in pregnant women, a 1.0% increase in %TIU is caused by the additional intake of about 1.1 µg of iodide. The American Thyroid Association (ATA) recommends that pregnant and lactating women take a daily prenatal vitamin/mineral supplement containing 150 µg of iodide (ATA 2006). Taking an iodide-containing prenatal vitamin/mineral supplement would easily offset a 1% reduction in %TIU. Furthermore, since iodized salt in the United States contains about 100 ppm potassium iodide or 77 µg iodide/gram of salt (Dunn 1998), the increased consumption of 14.3 mg of iodized salt would provide the 1.1 µg of iodide required to offset the 1% reduction in %TIU. Since a pinch of table salt weighs about 460 mg, 14.3 mg of salt is equivalent to about 1/32nd of a pinch of salt. Since a smidgen of table salt weighs about 230 mg, 14.3 mg of salt is equivalent to about 1/16th a smidgen of salt.

10. Approaches to Address this Public Health Issue

10.1 %TIU Exposure Levels of Concern

Any effective remedy must ensure that pregnant and nursing women have a sufficient TIU to prevent subtle mental deficits in their children. The cumulative risk assessment determined that a $\%TIU_{(insufficient\ RfD)}$ of 74% in pregnant and nursing women is the exposure level at which the risk begins for this public health issue. As the %TIU decreases below 74%, the level of risk increases. The risk levels of concern are characterized as follows:

- If the combination of exposures to the four NIS stressors induces a %TIU less than 74% (i.e., the $\%TIU_{(Insufficient\ RfD)}$ level), the possibility for mental deficits exists. At a typical total NIS inhibition load, a %TIU corresponds to a UIC of 151 µg/L in pregnant women.

- If the combination of exposures to the four NIS stressors induces a %TIU less than 49% (i.e., the $\%TIU_{(NOAEL)}$ level), the fetus and nursing infant are under thyroid stress (and the thyroid-sensitive subpopulations are at greater risk) and the potential for mental deficits increases. At a typical total NIS inhibition load, a %TIU corresponds to a UIC of 100 µg/L in pregnant women.

- If the combination of exposures to the four NIS stressors induces a %TIU less than 24.5% (i.e., the $\%TIU_{(LOAEL)}$ level), mental deficits occur in a percentage of the offspring population. The lower the %TIU, the frequency and severity of the mental deficits increases. At a typical total NIS inhibition load, a %TIU corresponds to a UIC of 50 µg/L in pregnant women.

10.2 Remedy Approach

The public health issue of potential subtle mental deficits in children is initiated by an insufficient amount of iodide being taken up by the thyroid in pregnant women, fetuses, and nursing infants. An excess exposure to any one (or more) of the four NIS stressors (i.e., lack of iodide, thiocyanate, nitrate, and perchlorate) can result in a TIU less than 74%TIU in pregnant women. Unfortunately, the current federal and State remedy being considered or implemented to address this public health issue is the exposure to only one of the four NIS stressors. The federal and State governments are considering or have implemented a maximum contaminant limit (MCL) for perchlorate, which will limit the perchlorate exposure from drinking water.

Using a cumulative risk assessment approach, everyone in the U.S. population has a continual exposure to all four NIS stressors. An excess exposure to any one (or more) of the four NIS stressors can result in a TIU less than 74 %TIU in pregnant women. To address the risk, the exposure levels to all four NIS stressor have to be considered and potentially managed. To manage the risk, the proposed goal should be to ensure that the 90th percentile of pregnant women have a %TIU greater than $\%TIU_{(RfD)}$ of 74%. Currently, the exposure to NIS stressors results in about half of all pregnant women having a %TIU less then the $\%TIU_{(RfD)}$. Furthermore, the proposed goal should be to ensure that >99th percentile of pregnant women have a %TIU greater than the $\%TIU_{(NOAEL)}$ of 49%. Currently, the exposure to NIS stressors

results in about 29% of all U.S. pregnant women having a %TIU less then the $\%TIU_{(NOAEL)}$ value. Most troubling, the current exposure to NIS stressors results in up to 7% of all U.S. pregnant women having a %TIU less then the $\%TIU_{(LOAEL)}$ of 24.5%.

10.2.1 Exposure Management of Each NIS Stressor

An adequate amount of iodide uptake in pregnant women, fetuses, and nursing infants is addressed by preventing an excess exposure to any one (or more) of the four NIS stressors. The cumulative risk assessment identified that the relative influence of each of the NIS stressors on this public health issue can be described as follows:

Lack of iodide > SCN^- >> NO_3^- >> ClO_4^-

Where: " > " means "greater than"
" >> " means "much greater than"

Therefore, the management of the lack of iodide stressor has the largest effect on the %TIUs value observed in pregnant women, followed by the management of thiocyanate exposure. By contrast, the management of nitrate and perchlorate will have the least effect on the %TIUs observed in pregnant women. However, the exposure management to all four NIS stressors is required to ensure pregnant women have a healthy %TIU level, which is greater than 74%.

10.2.1.1 Lack of Iodide Stressor

The cumulative risk assessment has identified that the lack of iodide NIS stressor is the dominant stressor in this public health issue. Unfortunately, the iodide exposure assessment in the sensitive group indicates that 6.9% of pregnant women are moderately iodide deficient and an additional 22% are mildly iodide deficient. The iodide exposure assessment indicates that up to 29% of the U.S. population of pregnant women is at a higher risk for mental deficits in their children. Obviously, the amount of iodide in the diet of a significant portion of the U.S. pregnant women population is dangerously low. Therefore, correcting poor iodide nutrition among pregnant and lactating women is needed to ensure the healthy brain development of the fetus and nursing infant.

This is where using a cumulative risk assessment approach to this public health issue is critical. A cumulative risk assessment allows stressors that are outside EPA's legislative mandates, authority, or expertise to be identified and addressed in cooperation with other federal agencies (EPA 2003, xviii), health organizations, and technical disciplines. EPA authority extends to the regulation of environmental contaminates. However, according to the Tonacchera Model, the dominant stressor in this public health issue is the iodide nutrition level (i.e., the lack of iodide in the diet) in pregnant and lactating women. Overseeing and correcting the iodide nutrition level is clearly not in EPA's legislative mandate; however, addressing the iodide deficiency in U.S. pregnant women is essential to effectively addressing this public health issue.

The NAS Committee was fundamentally aware of the crucial role of iodide nutrition during pregnancy. The NAS Committee wrote in the summary of their report that "[The NAS Committee] . . . recommends that consideration be given to adding iodide to all prenatal vitamins" (NAS 2005, p 18). The NAS Committee also wrote that same statement as the last line in its report (NAS 2005, p 196). The issue of whether prenatal vitamins should contain 150 µg of iodide falls into the legislative authority the National Institutes of Health, the FDA (see further discussion below), and the charter of the Institute of Medicine (IOM). The NAS Committee's recommendation for adding iodide to all prenatal vitamins should or could be addressed through the Federal Government's Perchlorate Interagency WorkGroup. However, this NAS recommendation has not been acted upon within that environmental arena.

10.2.1.1.1 Iodide Supplementation During Pregnancy and Lactation

The NAS Committee, ATA, and leading thyroid experts have recommended iodide supplementation during pregnancy and lactation. The NAS Committee recommends that consideration be given to adding iodide to all prenatal vitamins (NAS 2005, p 18, 196). ATA recommends that pregnant and lactating women take a daily prenatal vitamin/mineral supplement containing 150 µg of iodide (ATA 2006). Zimmerman and Delange have encouraged both the European Thyroid Association and the European Office of ICCIDD to recommend that supplement manufacturers include 150 µg/day iodide in prenatal supplements (Zimmermann 2004). Mild to moderate iodide deficiency during pregnancy adversely affects thyroid function of the mother and newborn and mental development of the offspring; these adverse effects can be prevented or minimized by iodide supplementation (Zimmermann 2004).

Unfortunately, not all prenatal vitamin supplements contain the recommended 150 µg iodide, and the use of iodide-containing prenatal vitamins is unacceptably low. The IOM currently does not recommend including iodide in prenatal vitamins (Sullivan 2007). Therefore, many prenatal vitamins do not contain iodide (Sullivan 2007). Only 1 of the 19 vitamins (i.e., 5%) listed for prenatal use in the *2006 Physician's Desk Reference* contained 150 µg of iodide, while the other 18 vitamins listed contained no iodide (ATA 2006). Pearce identifies an abstract that reports 44 of 69 (i.e., 64%) prenatal vitamins marketed in the United States contain some level of iodide (Pearce 2007a). In the Boston study of 57 lactating women, only 41 (72%) took a multivitamin, but only 3 lactating women (7%) took multivitamins containing iodide (Pearce 2007). The Boston study concludes that "47% of women sampled may have been providing breast milk with insufficient iodine to meet infants' requirements" (Pearce 2007). Furthermore, younger women of lower education and of certain ethnic groups tend not to use supplements on a regular basis (Sullivan 2007).

The 234 pregnant women taking no iodide supplement in the NHANES III survey had a median UIC of 141 µg/L (ATA 2006). By contrast, the 100 pregnant women taking a daily 150 µg iodide supplement in the NHANES III survey had a median urinary concentration of 169 µg/L (ATA 2006). The fraction of women excreting excessive amounts of iodide (i.e., > 500 µg/L) was not appreciably higher than those not taking a daily iodide supplement. ATA concludes that taking a 150 µg iodide supplement per day during pregnancy "appears to be safe" (ATA 2006). Benefits of correcting iodide deficiency outweigh the risks (Delange 2005, p 283).

The risk from iodide intake is U-shaped, meaning that there is a risk from too little or too much iodide. Our analysis identified that an iodide supplement of about 150 µg of iodide per day will reduce the risk from a low TIU during pregnancy and nursing. This is consistent with Glinoer's recommendation for the use of a prenatal vitamin pill containing 100-200 µg of iodide per day (Glinoer 2005, p 1092). However, the responsibility of assessing the need for and implementing prenatal vitamin use belongs to the medical community.

10.2.1.1.2 Participation of the Medical Community

A cumulative risk assessment allows stressors that are outside EPA's legislative mandates, authority, or expertise to still be addressed by involving the expertise of other federal agencies (EPA 2003, page xviii), health organizations, and technical disciplines. The medical community has the authority to determine whether iodide-containing prenatal vitamins should be recommended to address this public health issue. Our analysis identifies that taking iodide-containing prenatal vitamin will reduce the risk from a low TIU during pregnancy and nursing. Furthermore, our analysis identifies that iodide-containing prenatal vitamins should be taken at least 3 months before pregnancy, during pregnancy, and during nursing.

In regard to the concern that pregnant women with a UIC above 150 µg/L taking a prenatal vitamin containing iodide might develop health problems from excess iodide, the 234 pregnant women taking no iodide supplement in the NHANES III survey had a median UIC of 141 µg/L (ATA 2006). By contrast, the 100 pregnant women taking a daily 150 µg iodide supplement in the NHANES III survey had a median UIC of 169 µg/L (ATA 2006). The fraction of women excreting excessive amounts of iodide (i.e., > 500 µg/L) was not appreciably higher than those not taking a daily iodide supplement. ATA concludes that taking a 150 µg iodide supplement per day during pregnancy "appears to be safe" (ATA 2006). In general, the benefits of correcting iodide deficiency outweigh the risks (Delange 2005, p 283).

An approach to avoiding this potential for excess iodide exposure during pregnancy is to simply measure the UIC during prenatal care. If the UIC is below 150 µg/L, the use of an iodide-containing prenatal vitamin is warranted. If the UIC is above 150 µg/L, the use of an iodide supplement may not be necessary, and the potential risk of inducing excess iodide intake is eliminated. On a related concern, if the pregnant woman's UIC is below 50 µg/L (i.e., indicating moderate iodide deficiency), the medical community may need to consider more aggressive intervention, such as iodide supplementation and levothyroxine (Glinoer 1995), to avoid or minimize the severity of subtle mental deficits in her child.

The most appropriate preventative and therapeutic approach to avoiding excessive stress on the pregnant mother's thyroid from the inadequate uptake of iodide is to systematically increase the iodide supply as early as possible during gestation and continuing through lactation if the mother intends to breast feed (Glinoer 2005, p 1092). This can be easily achieved by the use of a multivitamin pill containing 100-200 µg of iodide per day (Glinoer 2005, p 1092). However, if an individual has been iodide deficient (i.e., mild or moderate) for a long time before pregnancy, a lag period of about one trimester (i.e., 3 months) is inevitable before the thyroid function normalizes following the onset of iodide supplementation (Glinoer 2005). This is the

reason for recommending taking iodide containing prenatal vitamins 3 months prior to pregnancy, if possible.

The effectiveness of iodide supplementation during pregnancy can be observed in the six randomized, controlled trials of iodide supplementation involving a total of 450 pregnant women with mild to moderate iodide deficiency (Zimmermann 2004, Table 2). In all six trials, iodide supplementation resulted in a significant increase in maternal UIC. The six trials found no increase in maternal thyroid autoimmunity or in the prevalence or severity of postpartum thyroid dysfunction. Iodide supplementation is efficacious for both the mother and newborn. Iodide supplementation is associated with significantly reduced maternal thyroid volume and generally lowers maternal TSH levels. Iodide supplementation prevents or minimizes the enlargement of newborn thyroid volume and increased Tg levels. However, there are no clinical studies on the effect iodide supplementation has on long-term outcomes, such as a child's mental development.

Results of Treatment of Mild Iodide Deficiency during Pregnancy with Iodide Supplementation

The benefits of iodide supplementation can be observed in a 1995 prospective double-blind, randomized trail study of 180 euthyroid women with excess thyroid stimulation who were divided into three equal groups (Glinoer 1995). Excess thyroid stimulation was determined by meeting the following criteria: serum Tg > 20 μg/L and (fT_4 index < 1.23 or T_3/T_4 ratio > 25 $x10^{-3}$). The screening of 2,000 pregnant women resulted in 180 pregnant women (i.e., 9% of the screened population) meeting the excess thyroid stimulation criteria. Only pregnant women at less than 16 weeks of gestation were enrolled in the study. Treatment began from the day of enrollment to delivery. (Note: iodide supplementation in this study did not start 3 months before pregnancy or at conception). Sixty women received a daily placebo (no treatment); another 60 women received a daily 100 μg iodide supplement, and 60 women received a daily dose of 131 μg iodide supplement and a daily dose of 100 μg levothyroxine.

The results from the1995 prospective double-blind, randomized trail study show the benefits of supplementing pregnant women with iodide:

- Mean serum Tg in newborns of mothers with iodide supplementation was significantly lower versus the controls (65 ± 6 μg/L vs 113 ± 9 μg/L (p = 0.0001), respectively).

- The mean UIC in newborns of mothers with iodide supplementation was significantly higher versus controls (77 ± 8 μg/L vs 43 ± 4 μg/L (p = 0.0001), respectively). Even with the improvement of 100 μg iodide/day supplementation, the mean neonate UIC of 77 μg/L falls still short of the WHO-recommended mean neonate UIC of 150 μg/L (Delange 2005). Furthermore, optimal neonate UIC is reported be as high as 180-225 μg/L (Delange 2004). Since the study used only a 100 μg dose of iodide/day supplementation, the NAS Committee's and ATA's recommendation for prenatal vitamins to contain 150 μg iodide is supported by these data.

- The mean thyroid volume in newborns of mothers with iodide supplementation was significantly less versus controls (0.76 ± 0.05μg/L vs 1.05 ± 0.05 μg/L (p = 0.0001),

respectively). In other words, the mean thyroid volume in newborns without maternal iodide supplementation was 40% larger than newborns with maternal iodide supplementation. Furthermore, glandular hyperplasia was already present at birth in 10% of the neonates without maternal iodide supplementation (Glinoer 2005, p 1092). By contrast, no instance of neonatal goiter is observed in neonates born to mothers with iodide supplementation (Glinoer 2005, p 1092).

- Mothers with iodide supplementation have significantly higher iodide levels in their breast milk versus controls (61 ± 10 μg/L vs 29 ± 2 μg/L ($p = 0.001$), respectively). Breast milk less than 50 μg/L is considered by WHO/UNICEF/ICCIDD to indicate iodide deficiency in the population (Delange 1994 p,109). Clearly, iodide supplementation dramatically improves the iodide levels in breast milk, which directly improves the iodide nutrition of nursing infants in which critical brain development is occurring.

These observations indicate that iodide supplementation during pregnancy reduces the stress on the fetal thyroid so that the risk for developing subtle mental deficits is minimized.

10.2.1.2 Thiocyanate Stressor

The cumulative risk assessment has identified that the thiocyanate NIS stressor at typical exposure concentrations is a medium-impact stressor in this public health issue. Common, healthy vegetables in the diet are a significant and natural source of thiocyanates (Tonacchera 2004). Foods particularly rich in thiocyanate include cabbage, cauliflower, broccoli, Brussels sprouts, corn (maize), mustard seed, and milk. For example, a nonsmoking individual consumed 100 grams of raw Brussels sprouts each day for a week to document a rise in thiocyanate serum levels from 31 to 80 μmol/L. Furthermore, dietary thiocyanate exposure exhibits a seasonal change of about 10 μmol/L presumably due to seasonal changes in the composition of the diet (Foss 1986). Therefore, the human body is continuously and unavoidable exposed to thiocyanate.

Thiocyanate may have a regulatory function in normal physiology (Middlesworth 1986). Thiocyanate is continually synthesized in the normal rat. In the absence of all dietary intake of thiocyanate in the rat, the thiocyanate serum concentration actually increased from 500 μg/dl to 800 μg/dl during the fasting (Middlesworth 1986, Figure 1). These data indicate that serum thiocyanate in rats is maintained in a normal range. A "kidney threshold" is reported to exist in humans at a thiocyanate serum threshold of 200-300 μmol/L (Tonnacchera 2004). These data imply that humans also synthesize and maintain thiocyanate serum concentrations in a normal range. Therefore, any remedy to significantly lower thiocyanate exposures – to decrease serum thiocyanate concentration, which will increase the %TIU in adult and in particular pregnant women – is not practical and will not be effective, due to the maintenance of thiocyanate serum concentrations by the body.

The human nonsmoker thiocyanate exposure is typically in the range of 10-70 μmol/L in the serum, and most occurs naturally through the consumption of healthy vegetables. Except for the potential for occupational exposures to cyanide (e.g., electroplaters), the amount of

uncontrolled environmental cyanide exposure in the U.S. population is negligible (i.e., cyanide is metabolized by the body into thiocyanate). By contrast, the major source of excess thiocyanate exposure in humans occurs through smoking. The act of smoking increases the mean thiocyanate serum concentration by a factor of 2.5x (i.e., from 40 to 100 µmol/L). The thiocyanate exposure in smokers is typically in the range of 80-120 µmol/L. The increased risk of fetal brain damage from smoking while pregnant is yet another reason to encourage pregnant women not to smoke. Encouraging individuals not to smoke is clearly an activity outside EPA's legislative mandate and authority.

In reviewing the potential for cyanide exposure in the U.S. population, the U.S. drinking water limit (i.e., MCL) for cyanide is 0.2 mg/L (De Groef 2006). The consumption of 2 liters water at the cyanide MCL per day would result in an exposure of 0.4 mg cyanide/day. This amount of cyanide would be metabolized into 894 µg thiocyanate/day (i.e., assuming 100% conversion). Using the OPEC equation to convert exposure to 894 µg thiocyanate/day into perchlorate equivalent amount results in 51 µg OPEC (i.e., the perchlorate RfD = 49 µg/day). Therefore, consumption of 2 liters of drinking water contaminated at the MCL for cyanide induces the same amount of NIS inhibition as perchlorate exposure at the RfD.

10.2.1.3 Nitrate Stressor

The cumulative risk assessment has identified that the nitrate NIS stressor at typical exposure concentrations is a weak impact stressor in this public health issue. Human exposure to nitrate is both exogenous and endogenous. Exogenous human nitrate exposure is from diet through the consumption of primarily vegetables. Nitrate is common in food (Tonacchera, 2004) and occurs in green leafy vegetables. Nitrate is also added to processed meats as a preservative. Nitrate commonly contaminates both surface water and groundwater sources of drinking water. Furthermore, the endogenous human nitrate exposure is from the body's production of nitric oxide, which is subsequently converted into nitrate by various types of cells in the body (NAS 1995, page 37). Therefore, humans are continuously and unavoidably exposed to nitrate.

Most nitrogenous materials in natural waters tend to be converted to nitrate, so all sources of combined nitrogen, particularly organic nitrogen and ammonia, should be considered as potential nitrate sources (EPA 2008). Primary sources of organic nitrates include human sewage and livestock manure, especially from feedlots. The primary inorganic nitrates which may contaminate drinking water are potassium nitrate and ammonium nitrate, both of which are widely used as fertilizers (EPA 2008). According to the Toxics Release Inventory, releases to water and land totaled over 112 million pounds from 1991 through 1993. The largest releases of inorganic nitrates occurred in Georgia and California at 12.0 and 21.8 million pounds (EPA 2008). EPA's 1999 study on the occurrence of contaminants in Public Water Systems (PWSs) regulated under the Safe Drinking Water Act identified that of the 8,988 groundwater PWSs tested for nitrate, 85.9% had at least one analytical result above the minimum reporting limit, 11.4% had at least one analytical result above ½ MCL, and 2.3% had at least one analytical result above the MCL (EPA 1999, Appendix A).

In studies from Denmark and the United Kingdom, the average nitrate intake is estimated to be about 40-50 mg/day for adults (Tonacchera 2004). This value is on the low end

of the nitrate intake estimates reported by the National Academy of Sciences (see Section 3.4 above; NAS 1995, p 35-43) and represents a bias low estimate of the average daily nitrate intake. In the Western world, the typical nitrate serum concentration ranges from 10-140 µmol/L with the mean nitrate serum concentration being between 30-50 µmol/L (Tonacchera 2004). The Tonacchera Model indicates that at typical nitrate exposure level of 40 µmol/L, nitrate contributes only about 11% of the body's typical total NIS inhibition load of 1.501 µmol/L. Potentially reducing the nitrate exposure will only marginally improve the %TIU values in humans and in particular pregnant women.

The U.S. drinking water MCL for nitrate is 10 mg of nitrate nitrogen per liter[4] (EPA 1991b), which is equivalent to 44 mg of nitrate per liter (NAS 1989, p 5, 11, 45). The conversion factor between these two analytical units is 1 mg of nitrate nitrogen per liter, equal to 4.429 mg of nitrate per liter. Therefore, the consumption of 2 liters water at the EPA nitrate MCL per day would result in an exposure of 88 mg nitrate/day (i.e., this is about twice the average daily intake of nitrate). Using the OPEC equation to convert exposure to 88 mg nitrate/day into PEC results in a 587 µg/day OPEC (i.e., the perchlorate RfD = 49 µg/day). Therefore, consumption of 2 liters of drinking water contaminated at the nitrate MCL induces 12 times the amount of NIS inhibition as perchlorate exposure at the RfD (De Groef 2006).

In 1999, EPA identified that 2.3% of the groundwater PWSs tested for nitrate had at least one analytical result above MCL (EPA 1999, appendix A). Furthermore, in 1999, EPA identified that 11.4% of groundwater PWSs tested for nitrate had at least one analytical result above ½ MCL (EPA 1999, appendix A). The consumption of 2 liters of drinking water contaminated at the ½ nitrate MCL induces 6 times the amount of NIS inhibition as perchlorate exposure at the RfD. In regard to private groundwater wells, 9% of domestic groundwater wells (irrespective of depth to groundwater) sampled by the U.S. Geological Survey's National Water-Quality Assessment Program during 1993-2000 had nitrate levels above the EPA nitrate MCL of 44 mg/L for drinking water (Nolan 2002). This nitrate contamination of private groundwater wells is a potential health issue for the estimated 42 million Americans that drink unregulated groundwater from their own private wells. By comparison, the consumption of drinking water contaminated with 3.7 mg/L of nitrate (i.e., 1/12th the nitrate MCL) has the equivalent NIS inhibition as the consumption of drinking water contaminated at 24.5 ppb perchlorate. Considering the nitrate contamination of both PWSs and private wells, about 10% of U.S. population is consuming nitrate-contaminated water at levels that induce at least 6 times more NIS inhibition than perchlorate exposure at the RfD. Therefore, lowering the public's nitrate exposure provides a more meaningful opportunity to lower the public's NIS inhibition load (i.e., a more meaningful opportunity to lower public risk) than to lower the public's perchlorate exposure below the perchlorate RfD.

[4] The current EPA MCL for nitrate is drinking water is expressed as 10 mg nitrate nitrogen per liter (i.e., which is the mass of nitrogen present in the nitrate form per liter), which is equivalent to 44 mg of nitrate per liter (NAS 1989, 5, 11, 45). The conversion factor between these two analytical units is 1 mg of nitrate nitrogen per liter = 4.429 mg of nitrate per liter (NAS 1989, 10).

10.2.1.4 Perchlorate Stressor

The cumulative risk assessment has identified that the thiocyanate NIS stressor at the RfD exposure concentrations is a very weak impact stressor in this public health issue. The FDA's perchlorate TDS identified that perchlorate is present at very low levels in food, primarily through the consumption of dairy and vegetables (Murray 2008, p 5). Furthermore, both surface and ground sources of drinking water can be contaminated with perchlorate. Therefore, the human body is continuously and unavoidably exposed to low levels of perchlorate.

The FDA TDS estimates a perchlorate intake of 5.4 to 6.8 µg/day from food in 25- to 30-year-old women (Murray 2008, table 5). The FDA TDS estimate of perchlorate intake agrees with the CDC biomonitoring data that estimates the median perchlorate exposure in the U.S. population is 4.6 µg/day (Blount 2006). The Tonacchera Model indicates that a median perchlorate exposure level of 4.6 µg/day, observed in the CDC biomonitoring data, contributes only about 0.09% of an adult body's typical total NIS inhibition load of 1.501 µmol/L. Furthermore, the Tonacchera Model indicates that a perchlorate exposure at the RfD contributes only 0.9% (less than 1%) of an adult body's typical total NIS inhibition load of 1.501 µmol/L. Finally, the Tonacchera Model also estimated a 0.3% increase in %TIU in adults and 1.0% increase in the %TIU of pregnant women if the actual perchlorate drinking water exposure is reduced from 24.5 ppb to 6.1 ppb. Therefore, potentially reducing the maximum perchlorate exposure in drinking water from 24.5 ppb to 6.0 ppb or lower will not significantly improve the %TIU values in adults and, in particular, pregnant women.

The remedy of potentially decreasing the perchlorate DWEL from 24.5 ppb to 6.1 ppb will help only a very small portion of the at-risk population. Conceptually, a theorectial 1.0% increase in %TIU applied to the entire at-risk poplution would be expected to increase at most 2% of the pregnant woman population above of critical %TIU level of 49% (i.e., $\%TIU_{(NOAEL)}$), which would leave about 27% of the pregnant woman population below the critical %TIU level. However, the potential remedy of lowering the perchlorate DWEL from 24.5 ppb to 6.0 ppb will increase the %TIU in only a small subset of this population. The EPA's Unregulated Contaminant Monitoring Rule (UCMR 1) data indicate only 2.13% of the PWSs are contaminated with perchlorate greater than 7 µg/L, which effects a population size of approximately 2.2 million people (EPA 2007, table 5). Therefore, about 0.7% of the U.S. population (i.e., 2.2 million ÷ 300 million x 100%) is potentially exposed to drinking water containing > 7 ug/L and potentially able to benefit from the lowering of perchlorate exposure in drinking water. Assuming pregnant women are evenly distributed throughout the U.S. population, the act of lowering the perchlorate DWEL from 24.5 ppb to 6.0 ppb would be expected to increase at most 0.014% of the annual pregnant woman population (i.e., 2% x 0.007) above of critical %TIU level of 49%. This potential remedy leaves 28.99% of the estimated 29% of U.S. pregnant woman population with a %TIU < 49% still at risk for subtle mental deficits in their children. In other words, the act of lowering perchlorate drinking water exposure below 24.5 ppp provides a neglible decrease in the size of the at-risk population. By contrast, implementing prenatal vitamins containing iodide has the potential to pull all 29% of the iodide-deficient pregnant women in the United States successfully above the critical $\%TIU_{(NOAEL)}$ value, and at a relatively low implementation cost.

A 1.0% reduction in %TIU could be offset by a 1.0% increase in the UIC in pregnant women. At a borderline iodide-deficient UIC of 100 µg/L in pregnant women, a 1.0% increase in %TIU is caused by the additional intake of about 1.1 µg of iodide. ATA recommends that pregnant and lactating women take a daily prenatal vitamin/mineral supplement containing 150 µg of iodide (ATA 2006). Taking an iodide-containing prenatal vitamin/mineral supplement would easily offset a 1% reduction in %TIU. Furthermore, iodized salt in the United States contains about 100 ppm potassium iodide or 77 µg iodide/gram of salt (Dunn 1998). The increased consumption of 14.3 mg of iodized salt would provide the 1.1 µg of iodide required to offset the 1% reduction in %TIU. Since a pinch of table salt weights about 460 mg, 14.3 mg of salt is equivalent to about 1/32nd of a pinch of salt. Since a smidgen of table salt weights about 230 mg, 14.3 mg of salt is equivalent to about 1/16th a smidgen of salt.

In reviewing the exposure to NIS inhibitors in drinking water, about 11.4% of groundwater PWSs tested for nitrate had at least one analytical result above ½ MCL, which corresponds to an amount of NIS inhibition 6 times greater than the amount of NIS inhibition from perchlorate exposure at the DWEL (i.e., at the RfD). Furthermore, about 2.3% of groundwater PWSs tested for nitrate had at least one analytical result above MCL, which corresponds to an amount of NIS inhibition 12 times greater than the amount of NIS inhibition from perchlorate exposure at the RfD. Furthermore, these data indicate that a significant portion of the U.S. population has a much higher risk from NIS inhibition from nitrate from drinking water than the risk from NIS inhibition from perchlorate exposure from drinking water at the RfD.

From a risk management standpoint, the Federal Government and the States should weigh the effectiveness of potential remedies to this public health issue and the cost of implementing each remedy against the health benefits to be derived by each remedy. The following are three potential remedies that can potentially address this public health issue; each has varying degrees of effectiveness:

- Add iodide to prenatal vitamin supplements and implement their use before and during pregnancy and lactation.

- Reassess the nitrate drinking water MCL and consider lowering the MCL to decrease the public's NIS inhibition exposure.

- Lower the perchlorate drinking water exposure level from 24.5 ppb to 6 ppb or lower.

11. Conclusion

The OIG Analysis of the scientific literature identified that the risk from perchlorate exposure is only part of the larger public health issue that is defined by the subtle mental deficits occurring in children born to mothers with low maternal TIU during pregnancy and nursing. The TIU results from the combined biological effect of four NIS stressors acting on the thyroid: thiocyanate, nitrate, perchlorate, and lack of iodide. Diet constantly exposes everyone to each of the four NIS stressors, and an individual's TIU level is the result of the combined effect of all four NIS stressors, not just perchlorate exposure. The OIG Analysis concludes that a single chemical risk assessment of perchlorate is not sufficient to assess and characterize the combined human health risk from all four NIS stressors. However, both EPA's draft perchlorate RfD from the Argus rat study and the NAS Committee's recommended perchlorate RfD from the Greer study used a single chemical risk assessment approach. By contrast, our cumulative risk assessment better characterizes the nature and sources of risk affecting this public health issue.

All four NIS stressors meet EPA's risk assessment guidance requirements for conducting a cumulative risk assessment using the dose-addition method. In the OIG Analysis, we conducted a cumulative risk assessment and determined that the risk from each of the four NIS stressors is not equal. The OIG Analysis also independently confirmed that EPA's perchlorate RfD is conservative and protects human health, but limiting perchlorate exposure does not effectively address this public health issue. Potentially lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public's risk. By contrast, addressing moderate and mild iodide deficiency that occurs in about 29% of the U.S. pregnant and nursing population appears to be a more effective approach of increasing TIU to healthy levels during pregnancy and nursing, thereby reducing the frequency and severity of permanent mental deficits in children.

The NAS Committee recommended that ". . . consideration be given to adding iodide to all prenatal vitamins" (NAS 2005, p 18). Our independent cumulative risk assessment came to a similar conclusion. The most effective and efficient approach for reducing the health risk of permanent mental deficits in children from low maternal TIU during pregnancy and nursing is to require that iodide be added to all prenatal vitamins and to encourage women to take them before and during pregnancy and while nursing.

Appendix A

"Whole Mixture" Approach to the Cumulative Risk Assessment of Perchlorate

An alternative approach to conducting a cumulative risk assessment is the "whole mixture" approach. Risk assessors can accomplish a whole mixture approach by developing a statistical model on human epidemiological data. An example of this whole mixture approach that has received a lot of attention is the Blount analysis (Blount 2006, Table 6) and the subsequent Steinmaus analysis of the NHANES 2001-2002 epidemiological study. The Blount analysis and the Steinmaus analysis represent statistical models fitted to the NHANES 2001-2002 epidemiological data. The Blount analysis predicted a decrease of 1.64 µg/dL in tT_4 serum from a 13 µg/day perchlorate exposure in women ≥ 12 yrs of age with a UIC ≤ 100 µg/L due to the NIS inhibition of perchlorate. These women are moderate to mildly iodide deficient. The subsequent Steinmaus analysis (Steinmaus 2007) on the same NHANES 2001-2002 epidemiological data concluded that increasing thiocyanate exposure potentiates the rate of decrease in tT_4 with increasing perchlorate exposure.

Using EPA criteria, our review indicates that the whole mixture approach to risk assessment of perchlorate (i.e., Blount analysis and the Steinmaus analysis) is not sufficiently developed and corroborated to be either the basis for developing a perchlorate RfD or the basis for establishing a potential perchlorate drinking water limit for the following reasons:

1. Section 3.1.3 of EPA's *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models* (November 2003) establishes the need to corroborate all environmental models before risk assessors use the models as the basis for rulemaking or regulation (EPA 2003a). The Blount and Steinmaus analyses represent a statistical model of the toxicity of perchlorate; their findings must be corroborated with other epidemiological data and other human exposure studies of perchlorate before they should be used as the basis for rulemaking. No human epidemiological study has corroborated the results of the Blount and Steinmaus analyses. To the contrary, the available epidemiological data of perchlorate-exposed pregnant women with UIC ≤ 100 µg/L contradict the findings of the Blount and Steinmaus analyses. These epidemiological studies included the following:

    a. A cohort of 789 pregnant women (i.e., 396 from Cardiff, Wales; 311 from Turin, Italy; and 82 from Dublin, Ireland) with perchlorate exposure levels shows no association between urinary perchlorate levels and free T_4 levels in the pregnant women as a group or in the subgroup of pregnant women with UIC ≤ 100 µg/L (Pearce 2007b).

    b. A cohort of 230 pregnant women (i.e., 128 from Los Angeles, California; 102 from Córdoba, Argentina) with low perchlorate exposure levels predicted by the Blount analysis to induce a reduction of tT_4 shows no association between urinary

perchlorate levels and free T_4 levels in the pregnant women as a group or in the subgroup of pregnant women with UIC ≤ 100 µg/L (Pearce 2008).

c. From the 2005 Tellez cohort study (Tellez 2005) in three Chilean cities, a reanalysis of the data identified 16 of the pregnant women had UIC ≤ 100 µg/L (Gibbs 2008). The reanalysis shows no association between urinary perchlorate levels and free T_4 levels in this subset of pregnant women with UIC ≤ 100 µg/L.

2. Pregnant women with a tT_4 decrease of 3.2 µg/dL from a normal tT_4 level have hypothyroidism. Researchers have observed that the children of these women have lower cognitive, attention, and motor performance scores (Haddow 1999, tables 2 and 4). However, we have identified adverse effects in children of pregnant women under less thyroid stress than hypothyroidism (i.e., hypothyroxinemia and low TIU). Therefore, the Blount analysis, which predicted a decrease of 1.64 µg/dL in tT_4 from a 13 µg/day perchlorate exposure in women with a UIC ≤ 100 µg/L, is anticipated by our technical review by ICF International to be sufficient to induce adverse effect in the children. Thus, from the Blount analysis, 13 µg of perchlorate exposure per day could be considered the LOAEL. However, the NAS Committee derived its recommended perchlorate RfD from a NOEL of 0.007 µg/kg-day. Assuming a 70-kg person and a LOAEL of no less than 10 times greater than the NOEL, the estimated LOAEL equivalent from the Greer study would be at least roughly 4,900 µg/day. The Blount analysis suggests that the perchlorate toxicity is significantly more toxic (i.e., more than 300 times more toxic) than what the NAS Committee and all previous perchlorate studies have indicated. Typical human exposures to the other common NIS inhibitors – thiocyanate and nitrate – induce substantially more NIS inhibition than 13 µg/day of perchlorate, but are not observed to induce thyroid hormone changes in the Blount analysis. Therefore, the increased toxicity of perchlorate reported in the Blount analysis would not be accounted for by the known mechanism of perchlorate toxicity: the inhibition of NIS. Furthermore, the Blount analysis does not propose a new mechanism of perchlorate toxicity that accounts for their reported increased toxicity of perchlorate. Until researchers can explain the increased toxicity of perchlorate by a verified biological mechanism, regulators should not use the Blount analysis as a basis for developing a perchlorate RfD, nor should it be used as the basis for establishing a drinking water limit.

3. The NHANES 2001-2002 epidemiological urinary data are from a single, untimed spot urinalysis and can vary due to dilution or concentration of the urine (i.e., due to an individual's hydration status). A common technique to eliminate this variation in the spot urinalysis is to normalize the iodide urine concentration against creatinine, resulting in the analysis expressing iodide excretion as µg of iodide/g of creatinine (i.e., µg/g creatinine) instead of µg of iodide/L of urine. Lamm has successfully repeated Blount's statistical analysis of the NHANES 2001-2002 epidemiological data only when his analysis measures UIC as µg/L (Lamm 2007). However, when Lamm repeats the statistical analysis using only women of childbearing age (ages 15-44) and UIC measured as µg/g creatinine, the statistical significance of the relationship between decreasing tT_4 with increasing perchlorate exposure in women with low UIC is lost. The statistical significance of this relationship should be present regardless of whether the analysis

expresses UIC in µg/L or µg/g of creatinine. This inconsistency suggests that the relationship between tT_4 and perchlorate reported in the Blount analysis might be an artifact in the NHANES dataset.

4. Section 3.2 of EPA's *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* establishes a major requirement for the use of a whole mixture risk assessment approach – the composition of the mixture must be stable over time; the relative proportions of chemicals in the mixture are roughly constant so that the mixture can be treated as though it were a single chemical (EPA 2000). Examples of EPA whole mixture risk assessments include Araclor 1016, Araclor 1254, and coke oven emissions. However, human exposure to each of the four NIS stressors varies independently based on the dietary intake preferences of the individual. Therefore, the mixture of the four NIS stressors is not a fixed, stable composition. Thus, a whole mixture approach is not well suited to assessing the toxicity for this public health issue since exposure to each of the four NIS stressors varies.

5. The OIG Analysis indicates that the toxicity of perchlorate is not fixed and cannot be described by a single RfD; rather, the toxicity of perchlorate changes in response to the exposure level of the other three NIS stressors, especially the lack of iodide stressor. From clinical observations of populations with endemic cretinism and from the OIG Analysis, at higher iodide nutrition levels, the body can tolerate a higher total goitrogen load. The converse is also true: at lower iodide nutrition levels, the body's tolerance of total NIS inhibition is much less. The Blount analysis also observes this trend in the toxicity of perchlorate. The Blount analysis reports that 13 µg/day of perchlorate exposure induces toxicity (i.e., a decrease in tT_4) in women with UICs $\leq$ 100 µg/L, but the same 13 µg/day of perchlorate exposure is nontoxic in women with UICs $\geq$ 100 µg/L. From both clinical observations (i.e., in Africa and India) and our cumulative risk assessment, reducing NIS inhibition exposure cannot correct a low TIU caused by iodide deficiency. Only the addition of iodide to the diet can correct a low TIU caused by iodide deficiency.

6. Section 3.1.3.2 of EPA's *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models* defines robustness as the capacity of a model to perform equally well across the full range of environmental conditions (EPA 2003a). This guidance further elaborates that if the data used to calibrate the model are identical or statistically similar to the dataset used to corroborate a model, the study did not provide an independent measure of the model's performance. Therefore, the Steinmaus analysis does not corroborate the Blount analysis, because both studies use the same NHANES 2001-2002 dataset. Furthermore, CDC plans to use the next NHANES dataset to continue to examine this interface of environmental perchlorate exposure, iodine levels, and tobacco smoke exposure (thiocyanate). However, repeating the analysis in the next NHANES dataset would not represent an independent evaluation of the robustness or the performance of the model because it is still a statistically similar dataset.

7. The December 13, 2006, ATA Public Health Statement commented on the Blount analysis. ATA identified that the NHANES 2001-2002 measured serum thyroxine as tT_4 rather than as free T_4. Free T_4 is the most frequently used clinical measurement and the physiologically available form of thyroxine.

8. The scientific literature reports that several dietary NIS stressors biologically affect the uptake of iodide, not to mention other chemical exposures affecting other subsequent biological steps in the production of T_4 that could contribute to the decrease in serum tT_4 observed in the Blount analysis. The Blount analysis reports an R^2 value of 0.240. R^2 is a statistical value called the "proportion of variation explained," and is the proportion of scatter in a dataset that the statistical model explains. The R^2 value of 0.240 reported in the Blount analysis shows that perchlorate accounts for only 3% of the variation seen in the serum tT_4 (Charnley 2008). If only 3% of the tT_4 change predicted by the Blount analysis was from perchlorate, the toxicity level of perchlorate reported in the Blount analysis would be more consistent with the other perchlorate studies and with the NAS-recommended perchlorate RfD.

Appendix B

References

Ahidjo A, Tahir A, Tukur MA. 2006. Ultrasound determination of thyroid gland volume among adult Nigerians. The Internet Journal of Radiology 2 (4). http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijra/vol4n2/thyroid.xml (accessed February 13, 2008).

Alexander WD, Wolff J. 1966. Thyroidal iodide transport VIII, relation between transport goitrogenic and antigoitrogenic properties of certain anions. Endocrinology 78:581-90.

Amitai Y, Winston G, Sack J, Lewis M, Blount BC, Valentin-Blasini L, Fisher N, Isreali A, Leventhal A. 2007. Gestational exposure to high perchlorate concentrations in drinking water and neonatal hhyroxine levels. Thyroid 17(9):843-50.

Anderson S, Pedersen KM, Pedersen IB, Laurberg P. 2001. Variations in urinary iodine excretion and thyroid function. A 1-year study in healthy men. Eur J Endocrinol 144(5):461-65.

Aquaron R. 2000. Iodine content of non-iodized salts and iodized salts obtained from the retail markets worldwide. 8th World Salt Symposium 2:935-40.

Argus. 2001. Hormone, thyroid and neurohistological effects of oral (drinking water) exposure to ammonium perchlorate in pregnant and lactating rats and in fetuses and nursing pups exposed to ammonium perchlorate during gestation or via maternal milk. Argus 1416-003. Argus Research Laboratories, Inc., Horsham, PA.

American Thyroid Association (ATA). 2006. Iodine supplementation for pregnancy and lactation – United States and Canada: Recommendations of the American Thyroid Association. First draft initiated by Dr. John T. Dunn before his untimely death in 2004. Draft was completed by members of the Public Health Committee of ATA. Thyroid 16(10):949-51.

Banerjee KK, Dishayee A, Marimuthu P. 1997. Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. Journal Occupational Environmental Medicine 39(3):258-60.

Banerjee KK, Marimuthu P, Bhattacharyya P, Chatterjee M. 1997a. Effect of thiocyanate ingestion through milk on thyroid hormone homeostasis in women. British Journal of Nutrition 78:679-81.

Barbaresi W, Katusic S, Colligan R, Weaver A, Pankratz V, Mrazek D, Jacobsen S. 2004. How common is attention-deficit/hyperactivity disorder? Towards resolution of the controversy: Results from a population-based study. Acta Paediatr Suppl 93(445):55-59.

Barnes DG, Dourson M. 1988. Reference dose (RfD): Description and use in health risk assessments. Regulatory Toxicology and Pharmacology 8:471-86.

Bianco AC, Larsen PR. 2005. Chapter 7: Intracellular pathways of iodothyronine metabolism. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins (ISBN 0-7817-5047-4).

Bleichrodt N, Born MP. 1994. Chapter 19: A metaanalysis of research on iodine and its relationship to cognitive development. 279-285. In *The Damaged Brain of Iodine Deficiency: Cognitive, Behavioral, Neuromotor, Educative Aspects*. JB Stanbury, Ed. New York: Cognizant Communication Corp.

Bleichrodt N, Del Rey FE, Morreale de Escobar G, Garcia I, Rubio C. 1989. Iodine deficiency, implications for mental and psychomotor development in children. 269-287. In *Iodine and the Brain*. Delong GR, Robblins J, Condliffe PG, Eds. New York: Plenum Press.

Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL. 2006a. Perchlorate exposure of the U.S. population, 2001-2002. Journal of Exposure Science and Environmental Epidemiology, 1-8.

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

Borak JB. 2002. Comment on: [California's] draft public health goal for perchlorate in drinking water (peer review draft March 2002). Prepared on behalf of Lockheed Martin Corporation. Jonathan Borak, MD, DABT, Associate Clinical Professor of Medicine and Epidemiology, Yale School of Medicine, New Haven, CT.

Braverman LE, XueMei H, Pino S, Cross M, Magnani B, Lamm SH, Kruse MB, Engel A, Crump KS, Gibbs JP. 2005. The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. Journal of Clinical Endocrinology & Metabolism 90(2):700-706.

Bruce GM, Peterson MK, and Pleus RC. 2004. Comparative contribution of perchlorate and anti-thyroid agents in American diets to iodide uptake inhibition. Paper presented at the 32nd JANNAF Propellant Development & Characterization and 21st Safety & Environmental Protection Joint Meeting. Seattle, WA, July 29, 2004.

Bruce G. 2005. Letter regarding perchlorate/nitrate/thiocyanate comparison written to David Huber, U.S. EPA/Office Ground Water Drinking Water/Risk Management Division, July 13, 2005.

Calaciura F, Motta RM, Miscio G, Fichera G, Leonardi D, Carta A, Trischitta V, Tassi V, Sava L, Vigneri R. 2002. Subclinical hypothyroidism in early childhood: A frequent outcome of

transient neonatal hyperthyrotropinemia. Journal of Clinical Endocrinology & Metabolism 87(7): 3209-14.

Callahan MA, Sexton K. 2007. If cumulative risk assessment is the answer, what is the question? Environmental Health Perspectives, 115(5):799-806.

Carrasco N. 2005. Chapter 4: Thyroid hormone synthesis. In *The Thyroid: A Fundamental Clinical Text*. 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Charnley G. 2008. Perchlorate: Overview of risks and regulation. Food and Chemical Toxicology. (Epub ahead of print - March 10, 2008). doi:10.1016/j.fct.2008.03.006

Clewell RA, Merrill EA, Gearheart JM, Robinson PJ, Sterner TR, Mattie DR, Clewell III HJ. 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 stages. Journal of Toxicology and Environmental Health, Part A, 70:408-28.

Clewell RA, Merrill EA, Yu KO, Mahle DA, Sterner TR, Fisher JW, Gearhart JM. 2003. Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiological-based pharmacokinetic modeling. Toxicology Sciences 74:416-36.

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

CDCP/NCHS. 2007. Centers for Disease Control and Prevention/National Center for Health Statistics (CDCP/NCHS). http://www.cdc.gov/nchs/products/pubs/pubd/hestats/iodine.htm (accessed July 25, 2007).

Dasgupta KP, Dyke JV, Kirk AB, Jackson WA. 2006. Perchlorate in the United States analysis of relative source contributions to the food chain. Environ. Sci. Technol. 40:6608-14.

DeFur PL, Evans GW, Hubal EAC, Kyle AD, Morello-Frosch RA, Williams DR. 2007. Vulnerability as a function of individual and group resources in cumulative risk assessment. Environmental Health Perspectives 115(5):817-24.

De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R. 2006. Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid-related health effects. Eur J Endocrinol 155(1):17-25.

Delange FM, Dunn JT. 2005. Chapter 11E: Iodine deficiency. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Delange FM. 2005a. Chapter 49: Endemic cretinism. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Delange F. 2004. Optimal iodine nutrition during pregnancy, lactation and the neonatal period. Int J Endocrinol Metab 2:1-12.

Delange F. 2001. Iodine deficiency as a cause of brain damage. Postgraduate Medical Journal 77:217-20.

Delange F. 1998. Screening for congential hypothyroidism used as an indicator of the degree of iodine deficiency and of its control. Thyroid 8(12):1185-92.

Delange F. 1994. The disorders induced by iodine deficiency. Thyroid 4(1):107-28.

De La Vieja A, Dohan O, Levy O, Carrasco N. 2000. Molecular analysis of the sodium/iodide symporter: Impact on thyroid and extrathyroid pathophysiology. Physiol Rev 80:1083-1105.

Demers LM, Spencer CA. Eds. 2003. Laboratory support for the diagnosis and monitoring of thyroid disease. Laboratory Medicine Practice Guidelines prepared by the National Academy of Clinical Biochemistry. Franklin, TN: Durik Adverstising, Inc. Available from the American Association of Clinical Chemistry at www.AACC.org. Monograph originally published in Thyroid, 13(1):1-126.

Dewey KG, Lönnerdal B. 1983. Milk and nutrient intake of breast-fed infants from 1-6 months: Relation to growth and fatness. J Pediatr Gastroenterol Nutr 2(3):497-506.

Dohan O, Portulano C, Basquin C, Reyna-Neyra A, Amzel LM, Carrasco N. 2007. The Na+/I-symporter (NIS) mediates electroneutral active transport of the environmental pollutant perchlorate. Proceedings of the National Academy Science 104(51):20250-55.

Dorato MA, Engelhardt JA. 2005. The no-observed-adverse-effect-level in drug safety evaluations: Use, issues, and definition(s). Regulatory Toxicology and Pharmacology 42:265-74.

Dorea JG. 2004. Maternal thiocyanate and thyroid status during breast-feeding. Journal of American College of Nutrition 23:97-101.

Dorne JLCM, Renwick AG. 2005. Forum: The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicological Sciences 86(1):20-26.

Dunn JT. 1998. Editorial: What's happening to our iodine? Journal of Clinical Endocrinology and Metabolism 83(10):3398-3400.

Dusdieker LB, Stumbo PJ, Kross BC, Dungy CI. 1996. Does increased nitrate ingestion elevate nitrate levels in human milk? Arch Pediatr Adolesc Med 150:311-14.

EPA 2010. ORD Assistant Administrator Paul Anastas memorandum titled: ORD: The Path Forward (Dated March 4, 2010). Washington, DC: U.S. Environmental Protection Agency,

Office of Research and Development.
http://v26265ncay001.aa.ad.epa.gov/opencms/export/ord@work/organization/labscentersoffices/ioaa/The_Path_Forward.html

EPA. 2009. *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals.* EPA/100/K-09/001. Washington, DC: U.S. Environmental Protection Agency, Office of the Science Advisor, Science Policy Council.

EPA. 2009a. EPA memorandum from OSWER Assitant Administrator to Regional Administrators. Subject: Revised Assessment Guidance for Perchlorate, January 8, 2009. http://www.epa.gov/swerffrr/documents/perchlorate_memo_01-08-09.pdf.

EPA. 2009b. *A Citizen's Guide to Radon: The Guide to Protecting Yourself and Your Family From Radon.* EPA 402/K-09/001. Washington, DC: U.S. Environmental Protection Agency, Indoor Air Quality, January 2009. http://www.epa.gov/radon.

EPA. 2008. *Consumer Factsheet on Nitrates/Nitrites.* U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water. http://www.epa.gov/OGWDW/contaminants/dw_contamfs/nitrates.html (accessed March 4, 2008).

EPA. 2008a. *Interim Drinking Water Health Advisory for Perchlorate*. EPA 822-R-08-025. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Health and Ecological Criteria Division, December 2008. http://www.epa.gov/safewater/contaminants/unregulated/perchlorate.html.

EPA. 2007. *Drinking Water: Regulatory Determinations Regarding Contaminants on the Second Drinking Water Contaminant Candidate List— Preliminary Determinations.* Published in the Federal Register on May 1, 2007 (72 FR 24015).

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

EPA. 2006. *Peer Review Handbook.* 3rd Ed. EPA/100/B-06/002. Washington, DC: U.S. Environmental Protection Agency, Science Policy Council.

EPA. 2004. *Estimated Per Capita Water Ingestion and Body Weight in the United States – An Update.* EPA-822-R-00-001. Washington, DC: U.S. Environmental Protection Agency, Office of Water/Office of Science and Technology, October 2004.

EPA. 2003. *Framework for Cumulative Risk Assessment*. EPA/600/P-02/001F. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment.

EPA. 2003a. *Draft Guidance on the Development, Evaluation, and Application of Regulatory Environmental Models*. Prepared by the Council for Regulatory Environmental Modeling. Washington, DC: U.S. Environmental Protection Agency, Office of Science Policy, Office of Research and Development, November 2003.

EPA. 2002. *A Review of the Reference Dose and Reference Concentration Processes*. Risk Assessment Forum. EPA/630/P-02/002F. Washington, DC: U.S. Environmental Protection Agency, December 2002.

EPA. 2002a. *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization* (External Review Draft). NCEA-1-0503. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, January 16, 2002.

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

EPA. 2000. *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures*. Risk Assessment Forum. EPA/630/R-00/002. Washington, DC: U.S. Environmental Protection Agency.

EPA. 1999. *A Review of Contaminant Occurrence in Public Water Systems.* EPA 816-R-99-006. Washington, DC: U.S. Environmental Protection Agency, Office of Water, November 1999.

EPA. 1997a. Chapter 7: Body Weight Studies, Table 7-1. In *Exposure Factors Handbook, Volume 1 - General Factors*. U.S. Environmental Protection Agency, Office of Research and Development/National Center for Environmental Assessment.
http://www.epa.gov/ncea/efh/pdfs/efh-chapter07.pdf (accessed August 5, 2007).

EPA. 1997b. EPA Administrator's memorandum to the Agency implementing "*Cumulative Risk Assessment Guidance – Phase I Planning and Scoping,*" July 3, 1997.

EPA. 1997c. *Guidance on Cumulative Risk Assessment. Part 1. Planning and Scoping.* Washington, DC: U.S. Environmental Protection Agency, Science Policy Council, July 3, 1997.

EPA. 1993. *Reference Dose (RfD): Description and Use in Health Risk Assessments – Background Document 1A, March 15, 1993*. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment.
http://www.epa.gov/iris/rfd.htm (accessed December 10, 2009).

EPA. 1992. *Safeguarding the Future: Credible Science, Credible Decisions*. A report of the Expert Panel on the Role of Science at EPA. EPA/600/9-91/050. Washington, DC: U.S. Environmental Protection Agency.

EPA. 1991. *Guidelines for Developmental Toxicity Risk Assessment.* Risk Assessment Forum. EPA/600/FR-91/001. Washington, DC: U.S. Environmental Protection Agency. Published in the Federal Register on December 5, 1991 (56 FR 63798).

EPA. 1991b. National Primary Drinking Water Regulations; Final Rule (56 FR 3526-3597). Also listed in 40 CFR § 141.15.

EPA. 1986. *Guidelines for Health Risk Assessment of Chemical Mixtures*. Federal Register 51(185):34014-25. Also published by EPA as EPA/630/R-98/002.

EPA SAB. 2004. *Science and Research Budgets for the U.S. Environmental Protection Agency for Fiscal Year 2007 – An Advisory Report by the Science Advisory Board.* EPA-SAB-ADV-06-003. Washington, DC: U.S. Environmental Protection Agency, Office of the Administrator, Science Advisory Board, March 30, 2006. http://www.epa.gov/science1/pdf/sab-adv-06-003.pdf

Farland WH, Jarabek AM. 2003. Perchlorate risk characterization: US EPA technical perspectives. Presentation made to the NAS Committee to Assess the Health Implications of Perchlorate Ingestion. Washington, DC, October 27, 2003.

FDA. 2007a. Preliminary estimation of perchlorate dietary exposure based on FDA 2004/2005 exploratory data. http://www.cfsan.fda.gov/~dms/clo4ee.html (accessed June 6, 2007).

FDA. 2007b. *Overview of Dietary Supplements*. http://www.cfsan.fda.gov/~dms/ds-oview.html#regulate (accessed December 11, 2007).

Fenzi GF, Giusti LF, Aghini-Lombardi F, Bartalena L, Marcocci C, Santini F, Bargagna S, Brizzolara D, Ferretti G, Falciglia G, Monteleone M, Marcheschi M, Pinchera A. 1990. Neuropsychological assessment in schoolchildren from an area of moderate iodine deficiency. J. Endocrinol. Invest. 13:427-31.

Fisher PW, L'Abbe M. 1980. Iodine in iodized table salt and sea salt. Can. Inst. Food Sci. Technolo. J. 13(2):103-4.

Fox MA, Tran NL, Groopman JD, Burke TA. 2004. Toxicological resources for cumulative risk: An example with hazardous air pollutants. Regulatory Toxicology and Pharmacology 40: 305-11.

GAO. 2007. *Government Auditing Standards (July 2007 Revision).* GAO-07-713G. Washington, DC: U.S. Government Accountability Office.

Gibbs G, van Landingham C. 2008. Urinary perchlorate excretion does not predict thyroid function among pregnant women. Thyroid (Letter to the editor – in peer review; manuscript ID: THY-2007-0377.R1).

Gibbs JP. 2006. A comparative toxicological assessment of perchlorate and thiocyanate based on competitive inhibition of iodide uptake as common mode of action. Human and Ecological Risk Assessment 12(1):157-73.

Ginsberg G, Rice D. 2005. The NAS perchlorate review: Questions remain about the perchlorate RfD. Environ Health Perspectives 113(9):1117-19.

Glinoer D. 2005. Chapter 80: Thyroid disease during pregnancy. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Glinoer D, De Nayer P, Delange F, Lemone M, Toppet V, Spehl M, Grun J, Kinthaert J, Lejeune B. 1995. A randomized trial for the treatment of mild iodine deficiency during pregnancy: Maternal and neonatal effects. Journal of Clinical Endocrinology and Metabolism 80(1):258-69.

Glinoer D, Lemone M, Bourdoux P, De Nayer P, Delange F, Kinthaert J, Lejeune B. 1992. Partial reversibility during late postpartum of thyroid abnormalities associated with pregnancy. Journal of Clinical Endocrinology & Metabolism 74(2):453-57.

Glinoer D, Delange F, Laboureur I, De Nayer P, Lejeune B, Kinthaert J, Bourdoux P. 1992. Maternal and neonatal thyroid function at birth in an area of marginally low iodine intake. Journal of Clinical Endocrinology & Metabolism 75(3): 800-805.

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

Greer MA, Stoot AK, Milne KA. 1966. Effect of thiocyanate, perchlorate and other anions on thyroidal iodine metabolism. Endocrinology 79:237-47.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon JG, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. The New England Journal of Medicine 341(8):549-55.

Hansen PS, Brix TH, Bennedbaek FN, Bonnema SJ, Kyvik KO, Hegedus L. 2004. Genetic and environmental causes of individual differences in thyroid size: A study of healthy danish twins. The Journal of Clinical Endocrinology & Metabolism 89(5): 2071-77.

Hasuike Y, Nakanishi T, Moriguchi R, Yoshinaga O, Nanami M, Hama Y, Naka M, Miyagawa K, Izumi M, Takamistsu Y. 2004. Accumulation of cyanide and thiocyanate in haemodialysis patients. Nephrology Dialysis Transplant 19(6):1474-79.

Hauth JC, Hauth J, Drawbaugh RB, Gilstrap LC, Pierson WP. 1984. Passive smoking and thiocyanate concentrations in pregnant women and newborns. Obstet Gynecol 63(4):519-22.

Hollowell JG, Staehling NW, Flanders WD, et al. 2002. Serum TSH, T4, and thyroid antibodies in the United States population (1988-1994): National Health and Nutrition Examination Survey (NHANES III). Journal Clinical Endocinology & Metabolism 87:489-99.

Hollowell JG, Staehling NW, Hannon WH, Flanders DW, Gunter EW, Maberly GF, Braverman LE, Pino S, Miller DT, Garbe PL, DeLozier DM, Jackson RJ. 1998. Iodine nutrition in the United States. Trends and public health implications: Iodine excretion data from National Health and Nutrition Examination Surveys I and III (1971-1974 and 1988-1994). J Clin Endocrinol Metab 83(10):3401-8.

House 2007. Perchlorate: Health and Environmental Impacts of Unregulated Exposure. Hearing before the Subcommittee on Environmental and Hazardous Materials of the Committee on Energy and Commerce. House of Representative, 110th Congress, First Session. April 25, 2007. Serial No. 110-35. 38-495 PDF. Washington, DC: U.S. Government Printing Office.

Howdeshell KL. 2002. A model of the development of the brain as a construct of the thyroid system. Environmental Health Perspectives 110(3):337-48.

IRIS. 2007. *Glossary of IRIS Terms. EPA's Integrated Risk Information System (IRIS).* http://www.epa.gov/iriswebp/iris/gloss8.htm (accessed December 27, 2007).

Ivanac G, Rozman B, Skreb F, Brkljacic B, Pavic L. 2004. Ultrasonographic measurement of the thyroid volume. Coll. Antropol. 28(1):287-91.

Kirk AB, Dyke JV, Martin CF, Dasgupta PK. 2007. Temporal patterns in perchlorate, thiocyanate, iodide excretion in human milk. Environmental Health Perspectives 115(2):182-86.

Kirk AB, Martinelango PK, Tian K, Dutta A, Smith EE, Dasgupta PK. 2005. Perchlorate and iodide in dairy and breast milk. Environmental Science and Technology 39(7):2011-17.

Kooistra L, Crawford S, van Baar AL, Brouwers EP, Pop VJ. 2006. Neonatal effects of maternal hypothyroxinemia during early pregnancy. Pediatrics 117(1):161-67.

Ladenson PW. 2005. Chapter 66: Diagnosis of hypothyroidism. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Lamm, S.H., Hollowell, J.G., Engel, A., Chen, R. 2007. Perchlorate, thyroxine, and low urine iodine association not seen with low creatinine-adjusted urine iodine among women of childbearing age. Thyroid 17 (s1), S-51.

Langer P. 1989. Normal thyroid size versus goiter - postmortem thyroid weight and ultrasonographic volumetry versus physical examination. Endocrinology Exp 23(2):67-76.

Lombardi FA, Pinchera A, Antonangeli L, Rago T, Chiovato L, Bargagna S, Bertucelli B, Ferretti G, Sbrana B, Marcheschi, Vitti P. 1995. Mild iodine deficiency during fetal/neonatal life and neuropsychological impairment in Tuscany. J. Endocrinol. Invest. 18:57-62.

Laurberg P, Nohr SB, Pedersen KM, Fμglsang E. 2004. Iodine nutrition in breast-fed infants is impaired by maternal smoking. The Journal of Clinical Endocrinology & Metabolism 89(1):181-87.

Laurberg P, Andersen S, Knudsen N, Ovesen L, Nohr SB, Bulow Pedersen I. 2002. Thiocyanate in food and iodine in milk: From domestic animal feeding to improved understanding of cretinism. Thyroid 12(10):897-902.

Li R, Ogden C, Ballew C, Gillespie C, Grummer-Strawn L. 2002. Prevalence of exclusive breastfeeding among U.S. infants: The Third National Health and Nutrition Examination Survey (Phase II, 1991-1994). Research and Practice 92(7):1107-10.

Marieb E. 1998. Chapter 18: Blood. In *Human Anatomy & Physiology*. 4th ed. Menlo Park, CA: Benjamin/Cummings Science Publishing. ISBN: 0-8053-4360-1.

Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. 2003. Births: Final data for 2002. National Vital Statistics Reports 52(10):1-114. Published by CDC.

Massachusetts Department of Environmental Protection (MassDEP). 2006. *Final Perchlorate Regulations, Response to Comment # 21 from the New England Water Works Association*, June 19, 2006.

McLanahan ED, Anderson ME, Campbell Jr. JL, Fisher JW. 2009. Competitive inhibition of thyroid uptake of dietary iodide by perchlorate does not describe perturbations in rat serum total T4 and TSH. Environmental Health Perspective 117(5):731-38.

McMurray WC. 1982. *A Synopsis of Human Biochemistry: With Medical Applications*. Philadelphia: Harper & Row Publishers, Inc., ISBN 0-06-141642-8.

Merrill EA, Clewell RA, Gearhart JM, Robinson PJ, Sterner TR, Yu KO, Mattie DR, Fisher JW. 2003. PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat. Toxicological Sciences 73:256-69.

Middlesworth, LV. 1986. Potential metabolic significance of blood thiocyanate. Endocrinologia Experimentals 20:17-22.

Morreale de Escobar G, Obregon MJ, Del Rey E. 2004. Role of thyroid hormone during early brain development. European Journal of Endocrinology 151:U25-U37. ISSN 0804-4643.

Murray CW, Egan SK, Kim H, Beru N, Bolger PM. 2008. U.S. Food and Drug Administration's total diet study: Dietary intake of perchlorate and iodine. Journal of Exposure Science and Environmental Epidemiology 2008:1-10.

Nafstad P, Magnus P, Stray-Pedersen B. 1995. Opposing placental gradients for thiocyanate and cotinine at birth. Early Hum Dev 42(1):73-79.

National Academies Press (NAP). 2000. Appendix G: Biochemical indicators for iron, vitamin A, and iodine from the third National Health and Nutrition Examination Survey. In *Dietary Reference Intake for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc,* 690-691. Washington, DC: National Academies Press. ISBN-13: 978-0-309-07290-8.

National Academy of Science (NAS). 2008. *Science and Decisions: Advancing Risk Assessment.* NAS Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. Washington, DC: National Academies Press. ISBN-13: 978-0-309-12046-3.

NAS. 2008a. *Phthalates and Cumulative Risk Assessment: The Task Ahead.* NAS Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. Washington, DC, National Academies Press. ISBN-13: 978-0-309-12841-4.

NAS. 2007. *Toxicity Testing in the Twenty-First Century: A Vision and a Strategy.* Committee on Toxicity Testing and Assessment of Environmental Agents. Washington, DC, National Academies Press. ISBN-10: 0-309-10988-4.

NAS. 2005. *Health Implications of Perchlorate Ingestion.* Washington, DC: National Academies Press. ISBN 0-309-09568-9.

NAS. 2001. *Food Safety Policy, Science, and Risk Assessment: Strengthening the Connection: Workshop Proceedings.* Food Forum, Food and Nutrition Board. Washington, DC: National Academies Press. ISBN 0-309-56512-X.

NAS. 1995. *Nitrate and Nitrite in Drinking Water.* National Research Council, Commission on Life Sciences, Subcommittee on Nitrate and Nitrite in Drinking Water. Washington, DC: National Academy Press. ISBN-10: 0-309-08370-2.

NAS. 1994. *Science and Judgment in Risk Assessment*. National Research Council, Commission on Life Sciences, Committee on Risk Assessment of Hazardous Air Pollutants. Washington, DC: National Academy Press.

NASDA 2009. NASDA October 8, 2009 letter to EPA Office of Water's August 19, 2009 request for supplemental perchlorate comments (Docket ID No. EPA-HQ-OW-2009-0297). The National Association of State Departments of Agriculture (NASDA), 1156 15th Street NW, Suite 1020, Washington, D.C. 2005. http://www.nasda.org/cms/7196/20728/20741/24460.aspx

Nohr SB, Laurberg P, Borlum KG, Pedersen KM, Johannesen PL, Damm P, Fuglsang E, Johansen A. 1994. Iodine status in neonates in Denmark: Regional variations and dependency on material iodine supplementation. Acta Paediatr 83(6):578-82.

Nolan BT, Hitt KJ, Ruddy BC. 2002. Probability of nitrate contamination of recently recharged groundwaters in the conterminous United States. Environmental Science and Technology, 36(10): 2138-45.

Obregon MJ, Escobar del Rey F, Morreale de Escobar G. 2005. The effects of iodine deficiency on thyroid hormone deiodination. Thyroid 15(8):917-29.

Pearce EN, Spencer CA, Mestman J, Lee R, Bergoglio LM, Mereshian P, He X, Leung AM, Braverman LE. 2008. "Thyroid Function is Not Affected by Environmental Perchlorate Exposure in First Trimester Pregnant Women from California and Argentina." Presentation at Annual Meeting of the Endocrine Society, San Francisco, CA, June 15-18, 2008.

Pearce EN, Leung AM, Blount BC, Bazrafshan HR, He X, Pino S, Valentin-Blasini L, Braverman LE. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. Journal of Clinical Endocrinology & Metabolism 92(5):1673-77.

Pearce EN. 2007a. National trends in iodine nutrition: Is everyone getting enough? Thyroid 17(9):823-27.

Pearce EN, Lazarus JH, Smyth PPA, He X, Dall'Amico D, Parkes AB, Burns R, Smith DF, Maina A, Leung AM, Braverman LE. 2007b. Thyroid function is not affected by environmental perchlorate exposure in first trimester pregnant women. Thyroid 17 (s1), S-133 [abstract only].

Pearce EN, Bazrafshan HR, He X, Pino S, Braverman LE. 2004. Dietary iodine in pregnant women from the Boston, Massachusetts area. Thyroid 14(4): 327-28.

Pollay M, Stevens A, Davis Jr. C. 1966. Determination of plasma-thiocyanate binding and the Donnan ratio under simulated physiological conditions. Analytical Biochemistry 17:192-200.

Ryan PB, Burke TA, Hubal EAC, Cura JJ, McKone TE. 2007. Using biomarkers to inform cumulative risk assessment. Environmental Health Perspectives, 115(5): 833-40.

Santiago-Fernandez P, Torres-Barahona R, Mulela-Marinez JA, Rojo-Martinez G, Garcia-Fuentes G, Garriga J, Leon AG, Soriguer F. 2004. Intelligence quotient and iodine intake: A cross-sectional study in children. J. Clin. Endocrinol. Metab. 89:3851-57.

Science Advisory Board (SAB). 2008. *SAB Advisory on EPA's Draft Third Drinking Water Contaminant Candidate List (CCL 3)(Draft 3, 2008)* (EPA-SAB-07-00_). Committee Response to Charge Question 4. SAB Drinking Water Committee on the Third Drinking Water Contaminant Candidate List (CCL3). http://www.epa.gov/sab (accessed December 18, 2008).

SAB. 2007. SAB's February 28, 2007 memo to EPA Administrator Stephen L. Johnson regarding *Consultation on Enhancing Risk Assessment Practices and Updating EPA's Exposure Guidelines*. EPA-SAB-07-003.

Selva KA, Harper A, Downs A, Blasco PA, LaFranchi SH. 2005. Neurodevelopmental outcomes in congenital hypothyroidis: Comparison of initial T4 dose and time to reach target T4 and TSH. Journal of Pediatrics 147:775-80.

Sexton K, Hattis D. 2007. Assessing cumulative health risks from exposure to environmental mixtures – three fundamental questions. Environmental Health Perspectives, 115(5): 825-32.

Soldin OP, Tractenberg RE, Pezzullo JC. 2005. Do thyroxine and thyroid-stimulating hormone levels reflect urinary iodine concentrations? Ther Drug Monit 27:178-85.

Steinmaus C, Miller MD, Howd R. 2007. Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 National Health and Nutrition Examination Survey. Environmental Health Perspectives 115(9):1333-38.

Stoa KF. 1957. Studies on thiocyanate in serum with some supplementary investigations in saliva, urine, and cerbrospinal fluid. 17. Bergen, Norway: University Bergen Medical Yearbook.

Sullivan KM. 2007. Iodine supplementation for pregnancy and lactation: United States and Canada: Recommendations of the American Thyroid Association. Thyroid 17(5):483-84.

Sullivan KM, May W, Nordenberg D, Houston R, Maberly GF. 1997. Use of thyroid stimulating hormone testing in newborns to identify iodine deficiency. Journal of Nutrition 127:55-58.

Tajtakova M, Semanova Z, Tomkova Z, Szokeova E, Majoros J, Radikova Z, Sebokova E, Klimes I, Langer P. 2006. Increased thyroid volume and frequency of thyroid disorders signs in school children from nitrate polluted area. Chemosphere 62:559-64.

Tellez RT, Chacon PM, Abarca CR, Blount BC, Van Landingham CB, Crump KS, Gibbs JP. 2005. Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. Thyroid 15(9):963-87.

Ting D, Howd RA, Fan AM, Alexeeff GV. 2006. Development of a health-protective drinking water level for perchlorate. Environmental Health Perspectives 114(6):881-86.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J. 2004. *Relative* Potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid 14:1012-19.

Utiger RD. 1999. Editorial: Maternal hypothyroidism and fetal development. New England Journal Medicine 341(8):601-2.

Vanderpas J, Bourdoux P, Lagasse R, Rivera M, Dramaix M, Lody D, Nelson G, Delange F, Ermans AM, Thilly CH. 1984. Endemic infantile hypothyroidism in severe endemic goiter area in Central Africa. Clinical Endocrinology 20(3):327-40.

Vanderpump MPJ. 2005. Chapter 19: The Epidemiology of Thyroid Diseases. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Vetter J. 2000. Plant cyanogenic glycosides. Toxicon 38:11-36.

Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, Castagna MG, Mattina F, Violi MA, Crisa A, Artemisia A, Trimarchi F. 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. Journal of Clinical Endocrinology & Metabolism 89(12):6054-60.

Vermiglio F, Sidoti M, Finocchiaro MD, Battiato S, Presti VPL, Benvenga S, Trimarchi. 1990. Defective neuromotor and cognitive ability in iodine-deficient schoolchildren of an endemic goiter region in Sicily. Journal of Clinical Endocrinology and Metabolism 70(2):379-84.

Vitti P, Lombardi FA, Antonangeli L, Rago T, Chiovato L, Pinchera A, Marcheschi M, Bargagna S, Bertuccelli B, Ferretti G, Sbrana B. 1992. Mild iodine deficiency in fetal/neonatal life and neuropsychlological performances. Acta Med Austriaca 19:57-59.

Wenzel KW, Lente JR. 1984. Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves' disease: Evidence against and immunosuppressive action of thionamide drugs. Journal of Clinical Endocrinology Metabolism 58(1):62-69.

Wolff J. 1998. Perchlorate and the thyroid gland. Pharmacological Reviews 50(1):89-105.

World Health Organization (WHO). 2004. Hydrogen cyanide and cyanides: Human health aspects. 61. Concise International Chemical Assessment Document.

Wyngaarden JB, Stanbury JB, Rapp B. 1953. The effect of iodide, perchlorate, thiocyanate, and nitrate administration upon the iodide concentrating mechanism of the rat thyroid. Endocrinology 52:568-74.

Yen PM. 2005. Chapter 8: Genomic and nongenomic actions of thyroid hormones. In *The Thyroid: A Fundamental Clinical Text.* 9th ed. Philadelphia: Lippincott Williams & Wilkins. ISBN 0-7817-5047-4.

Zimmermann M, Delange F. 2004. Iodine supplementation of pregnant women in Europe: A review and recommendations. European Journal of Clinical Nutrition 58:979-84.

Appendix C

List of Acronyms and Abbreviations

ADHD	attention-deficit/hyperactivity disorder
ATA	American Thyroid Association
BMC	benchmark concentration
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BV	baseline visit
CDC	Centers for Disease Control and Prevention
CG	cyanogenic glycosides
CH	congenital hypothyroidism
CHO	Chinese hamster ovary
CI	confidence interval
ClO_4^-	perchlorate
CNF	Chilean nitrate fertilizer
CNS	central nervous system
d	Cohen's *d*-value
DWEL	drinking water equivalent level
EAR	estimated average requirement
EPA	U.S. Environmental Protection Agency
FDA	Food and Drµg Administration
fT_3	free triiodothyronine
fT_4	free thyroxine
GAO	Government Accountability Office
HA	health advisory
HRL	health reference level
HNA	high-nitrate area
I^-	iodide
I_2	iodine
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
IDA	iodide-deficient area
IOM	Institute of Medicine
IQ	intelligence quotient
IRIS	Integrated Risk Information System
ISA	iodide-sufficient area
LNA	low-nitrate area
LOAEL	lowest-observed-adverse-effect-level
MCL	maximum contaminant limit
mg	milligram
mg/day	milligram per day
mg/kg-day	milligram per kilogram per day
mg/L	milligram per liter
mU/l	milli-international-units per liter
NAP	National Academies Press

NAS	National Academy of Sciences
NCEH	National Center for Environmental Health
NHANES	National Health and Nutrition Examination Survey
NIS	sodium iodide symporter
nmol/L	nanomole per liter
NO_3^-	nitrate
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NPDWR	National Primary Drinking Water Regulation
NRC	National Research Council
OCEPA	Office of Communications, Education, and Public Affairs
OIG	Office of Inspector General
OPEC	oral perchlorate equivalent concentration
p	probability
PBPK	physiologically based pharmacokinetic
PEC	perchlorate equivalent concentration
pg/dL	picogram per deciliter
PII	plasma inorganic iodide
POD	point of departure
ppb	part per billion
ppm	part per million
RAIU	radioactive iodide uptake
RDA	recommended dietary allowance
RfD	reference dose
RPF	relative potency factor
RSC	relative cource contribution
SAB	Science Advisory Board
SCN^-	thiocyanate
SD	standard deviation
SE	standard error of the mean
SPEC	serum perchlorate equivalent concentration
T_3	triiodothyronine
T_4	thyroxine
TDS	Total Dietary Study
Tg	thyroglobulin
TIU	total iodide uptake
%TIU	percent total iodide uptake
TSH	thyroid-stimulating hormone (a.k.a. thyrotropin)
tT_3	total triiodothyronine
tT_4	total thyroxine
UCMR	Unregulated Contaminant Monitoring Rule
UF	uncertainty factor
UIC	urinary iodide concentration
UNICEF	United Nations International Children's Emergency Fund
µg	microgram
µg/day	mircrogram per day

µg/dL	microgram per deciliter
µg/kg-day	microgram per kilogram per day
µg/L	microgram per liter
µmol/L	micromole per liter
µU/ml	micro-international-units per milliliter
USDA	U. S. Department of Agriculture
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children

Appendix D

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

For this appendix, go to the following:

www.epa.gov/oig/reports/2010/20100419-10-P-0101_appD.pdf

Appendix E

OIG Response to Comments on OIG Scientific Analysis of Perchlorate (External Review Draft)

For this appendix, go to the following:

www.epa.gov/oig/reports/2010/20100419-10-P-0101_appE.pdf

Appendix F

Distribution

Office of the Administrator
Assistant Administrator for Research and Development
Assistant Administrator for Water
Director, Office of Children's Health Protection and Environmental Education
Agency Follow-up Official (the CFO)
Agency Follow-up Coordinator
General Counsel
Associate Administrator for Congressional and Intragovernmental Relations
Associate Administrator for Public Affairs
Audit Follow-up Coordinator, Office of the Administrator
Audit Follow-up Coordinator, Office of Research and Development
Audit Follow-up Coordinator, Office of Water
Acting Inspector General