PSG
PERCHLORATE STUDY GROUP
A coalition of aerospace, defense, chemical and allied industries

October 2, 2008

Michael Baes
PHG Project
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
1515 Clay St. 16th Floor
Oakland, CA 94612

Dear Mr. Baes:

OEHHA recently announced that it will undertake the statutory five-year review of the risk assessment that serves as the basis for the public health goal ("PHG") for perchlorate in drinking water.

To assist in your deliberations, the Perchlorate Study Group ("PSG") hereby submits the attached document, prepared by Intertox: *Perchlorate and Human Health in 2008: The Implications of New Science* ("Intertox Report"). Intertox, led by Dr. Richard C. Pleus, brings longstanding expertise to toxicological issues, notably including perchlorate (for example, Dr. Pleus is a principal author of the Greer Study).

The Intertox Report examines the range of relevant studies, including the landmark National Academy of Sciences 2005 report, *Health Implications of Perchlorate Ingestion.* The result is a comprehensive response to the critical question before OEHHA: Does the risk assessment crafted by OEHHA for the 2004 California PHG remain valid and up-to-date, taking into account subsequent, peer-reviewed studies?

The Intertox Report concludes unequivocally:

> Taken as a whole, including the new science published since 2004, the weight-of-evidence demonstrates that the PHG continues to be a conservative toxicity guideline value and contaminants present in drinking water at or below the PHG would pose no significant health risk to the most sensitive individuals who consume the water on a daily basis over a lifetime. In fact, the latest scientific evidence decreases the uncertainty level noted in the 2004 PHG documentation and demonstrates that values greater than the PHG would provide protection to the most sensitive populations.

We appreciate the important contribution of OEHHA in marshalling and effectively utilizing the best available scientific evidence to achieve comprehensive public health protection. We respectfully submit the Intertox Report to assist you in your mission.

Please do not hesitate to contact Dr. Pleus or me if we can provide additional information or perspective in your deliberations.

Sincerely,

Mr. Michael Girard
Perchlorate Study Group

cc: Joan Denton, Ph.D., Director OEHHA
George Alexeeff, Ph.D., Deputy Director of Scientific Affairs

INTERTOX

PERCHLORATE AND HUMAN HEALTH IN 2008: THE IMPLICATIONS OF NEW SCIENCE

SCIENTIFIC RESPONSE TO THE CALEPA OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT'S (OEHHA) PUBLIC NOTICE OF INITIATION OF RISK ASSESSMENTS FOR CHEMICALS IN DRINKING WATER—JULY 2008

Prepared for:

THE PERCHLORATE STUDY GROUP

October 2, 2008

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EXECUTIVE SUMMARY

The Calderon-Sher Safe Drinking Water Act of 1996 requires the Office of Environmental Health Hazard Assessment (OEHHA) to review and update the risk assessments that form the basis for California's Public Health Goals (PHGs) as appropriate at least every five years. The final PHG for perchlorate was published in March 2004 (OEHHA, 2004).

The scientific process is iterative with new knowledge building upon old knowledge to advance the state of the science. The purpose of this document is to compare and review the basis of the 2004 PHG to peer-reviewed studies that have been published since the release of the PHG. We first detail the inherent conservatism in the risk assessment process (i.e., developing a Reference Dose (RfD) or PHG). We focus on new studies that have been published since 2004 and how they affect the PHG for perchlorate. Since the National Academy of Science (NAS) report *Health Implications of Perchlorate Ingestion* was not available at the time the PHG was created, we examine its impact on the PHG. We also summarize the pertinent studies published since 2004 in the peer-reviewed literature on perchlorate exposure in humans and animals, and on the neurodevelopment of children born to mothers who were hypothyroid during gestation.

Including the new science published since 2004, the weight-of-evidence demonstrates that the PHG continues to be a conservative toxicity guideline value such that contaminants present in drinking water at or below the PHG would pose no significant health risk to the most sensitive individuals who consume the water on a daily basis over a lifetime. In fact, the latest scientific evidence decreases the uncertainty level noted in the 2004 PHG documentation and demonstrates that values greater than the PHG would provide protection to the most sensitive populations.

A. INTRODUCTION/BACKGROUND

This section reinforces the conservative nature of the risk assessment process, particularly to the development of the PHG for perchlorate. We discuss the PHG process and focus on several key aspects of the risk assessment process in which newly published data may affect the PHG. To summarize, the new data demonstrate that the current PHG is conservative and drinking water concentrations at, or even above, 6 ppb are health protective for all populations.

Compared to many environmental pollutants, there exists a strong and wide breadth of scientific knowledge concerning the potential health effects of perchlorate exposure. First, the mechanism of perchlorate action (iodide uptake inhibition) is widely regarded as a temporary and fully reversible biochemical phenomenon, not an adverse effect (OEHHA, 2004; NAS, 2005). Perchlorate effects have a threshold for the point at which there is no inhibition of iodide uptake. This threshold is the no observed effect level (NOEL). This is different than a no observed adverse effect level (NOAEL) in which an effect may occur, but that effect is not adverse. Likewise, a lowest observed adverse effect level (LOAEL) is the lowest dose which can cause an adverse effect to occur. At the NOEL, no effect, adverse or otherwise, occurs.

Perchlorate is non-carcinogenic, it does not accumulate in the body, and its half-life is approximately 8 hours (NRC, 2005). The most sensitive target organ is the thyroid gland and

the most sensitive populations of concern are pregnant women and developing fetuses. Scientific studies regarding potential health effects are predominately based upon human data, with a range of parameters and dose-response data. The literature on human health effects from perchlorate consists mainly of clinical, occupational, or ecological studies. To date, there is only one epidemiological study which measures perchlorate exposures and outcomes in pregnant women and neonates (Tellez et al., 2005). In contrast to ecological studies measuring collective exposures and outcomes, an epidemiological study measures individual exposures and outcomes. There are also some well designed animal studies that have contributed to the health effects database.

The NAS committee concluded "...that using the NOEL for iodide uptake inhibition from Greer et al. (2002) as the point of departure provides a reasonable and transparent approach to the perchlorate risk assessment."

One of the possible early steps in the mechanistic pathway leading to hypothyroidism is a decrease in iodine uptake by the thyroid. As noted by NAS, this effect in itself is not adverse. For it to compromise health, a dose of perchlorate must be sufficient to cause a decrease in iodine uptake leading to changes in the normal amounts of thyroid hormones for an extended period of time (i.e., 6 months or longer). Unless this sustained change in thyroid hormones happens, no adverse effect are expected to occur.

Both the PHG and the RfD are below the dose that causes a decrease in iodine uptake by the thyroid. Thus, the current PHG is conservative and greater doses will still provide protection to the most sensitive individuals.

The United States Environmental Protection Agency (U.S. EPA) has published health-based guidelines or RfDs which are levels of acceptable exposures.[1] The RfD for perchlorate is 0.0007 mg/kg-day which translates to a drinking water concentration of 24.5 ppb[2]. Similar to an RfD, California has developed an "...estimate of the level of [a] contaminant in drinking water that is not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health." (California Health and Safety Code Section 116365 (c)(1)) known as the OEHHA PHG. The U.S. EPA's longstanding definition of the RfD is similar to the PHG except that the PHG is focused on drinking water and the RfD is applied to drinking water and other exposure media.

The PHG for perchlorate is 6 ppb, based on a daily dose of 0.00037 mg/kg-d. This value is based on the assumptions that 60% of the perchlorate exposure is from drinking water, with a body weight to water intake ratio of 25.3 kg-d/L for a pregnant woman and her fetus (OEHHA, 2004). These adjustments result in a PHG water concentration that is more conservative than U.S. EPA's RfD. OEHHA has reported that "...the draft PHG for perchlorate was more extensively reviewed than any of the other 69 PHGs that OEHHA has developed to date."[3] By virtue of its derivation from a reversible biochemical effect (as

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[1] The RfD is "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or BMD, with UFs generally applied to reflect limitations of the data used" (NRC 2005).

[2] Assuming a 70 kg person drinks 2 liters of water per day (http://www.epa.gov/safewater/standard/setting.html).

[3] Drafts of OEHHA's PHG document for perchlorate were submitted to the University of California for two separate rounds of external scientific peer review. OEHHA's normal process only calls for one round of peer review by the University. The U.S. EPA also peer reviewed the document. OEHHA also held two public comment periods and a public workshop on the draft PHG documents. OEHHA began development of the perchlorate PHG in 1998. It is the product of almost six years of assessments and reviews.

opposed to a frank adverse effect), the PHG conservatively derives from conventional risk assessment practice.

Several scientific aspects of both the OEHHA PHG and the U.S. EPA RfD guidelines are worth noting:

- The definitions encompass lifetime exposure.
- The definitions apply to sensitive subgroups within the population. Depending on how small the subgroup is upon which the RfD or PHG is based, it can be a highly precautionary description of the threshold for safe exposure to the larger population. The sensitive population of pregnant women with hypothyroidism is approximately 0.03% of the U.S. population.[4]
- The definitions are an estimate rather than a fixed value, with uncertainty spanning perhaps an order of magnitude or more.
- Exposures are presumed to occur daily over a lifetime rather than episodically. This reflects the common assumption that human health risk rises with lifetime exposure. Perchlorate, however, has a short biological half life, does not bioaccumulate in tissues, and is not metabolized.
- They are both based on a dose that was given in over and above the background dose, which produced no effect, adverse or otherwise (Greer et al., 2002).

Both the PHG and the RfD aim to protect the most sensitive individuals within a population (i.e., pregnant women and their fetuses). Some have argued that the infant is the more sensitive individual in the population; no scientific basis for this has been presented. The weight of evidence shows that the fetal brain is more sensitive to hypothyroidism than the neonate or infant brain (Boelaert and Franklyn, 2005). Neurodevelopmental changes in children are only seen after chronic hypothyroidism (not subclinical hypothyroidism) in the mother (Boelaert and Franklyn, 2005). Breast milk concentrations of perchlorate have been measured at levels greater than the PHG (Kirk et al., 2005; Pearce et al., 2007b). The PHG assumes that if a person consumes 6 ppb of perchlorate not just once, but every day, they will still be consuming an acceptable level of perchlorate. Therefore, if an infant consumes one dose of perchlorate that is greater than the PHG, it is not necessarily harmful. As previously mentioned, the PHG is also inherently conservative in its derivation. And, the PHG assumes that 60% of a person's exposure is due to drinking water and 40% is due to exposures from other media. A solely breast fed infant will not have exposures through other media. These considerations were made by OEHHA in determining the most sensitive population, as summarized in Table 1.

The relative source contribution (RSC) is intended to account for exposures through different media in addition to drinking water. When no other sources are known, the U.S. EPA suggests a value of 80% be allocated to water. In 2004, OEHHA used data that showed low levels of perchlorate were found in food. Hence, the current PHG uses an

[4] Based on 4 million pregnancies per year in the U.S. (http://www.americanpregnancy.org/main/statistics.html) and 2.5% of pregnant women having clinical or subclinical hypothyroidism (Haddow et al., 1991).

Table 1. Comparative Health Protective Doses

Sub-population	BW/WC (kg-d/L)	RSC	UF	Health-protective Concentration (ppb)
Pregnant woman and fetus	25.2	0.6	10	5.6
Lactating woman	26.7	0.6	10	5.9
Infant	5.99	1	3	7.4
Adult	35	0.6	10	7.8

- RSC is 100% for an infant which assumes that all exposure is via breast milk.
- UF is 3 for infant because differences between adults and infants are accounted for in the BW/WC scaling.

Adapted from OEHHA, 2004

RSC of 60%. Murray et al. (2008) published a report that estimated doses of perchlorate through food based on the FDA Total Diet Study (summarized in Section C). Although the dose for the most sensitive population (pregnant women and their fetuses) was not specifically modeled, the estimated dose range for women of child bearing age was 0.09 to 0.11 μg/kg-d. The high end of the range, 0.11 μg/kg-d, is approximately 30% of the dose that the PHG is based on (0.37 μg/kg-d). Based on the data from Murray et al. (2008), an RSC of 70% could be allocated to water to be protective of the most sensitive population.

It is important to note that both the PHG and the RfD are based on the study by Greer et al. (2002). The researchers in this study did not control dietary intake of perchlorate. Therefore, the dose of perchlorate that resulted in a NOEL was in addition to the background level of perchlorate ingested through food and water. If the background dose of perchlorate from all sources was 0.02 to 0.234 μg/kg-d (the 5th and 95th percentile estimated doses from Blount et al. (2007)), then all doses in Greer et al. 2002 underestimate the true dose by this amount.

New studies have emerged since the publication of the PHG in 2004 (summarized in Section C). On a weight-of-evidence basis, they demonstrate no significant health risk to the most sensitive individuals who consume water on a daily basis over a lifetime at environmentally relevant doses. The effects of these new studies on specific aspects of the PHG and RfD risk assessments are summarized in Table 2. On balance, this new scientific evidence supports both the basis of OEHHA's PHG (and U.S. EPA's RfD) and the conclusion that the current 6 ppb value is conservative, and accounts for the most sensitive populations.

A significant degree of attention and concern related to perchlorate exposure has been focused upon the hypothetical iodine-deficient person. However, unless the doses of perchlorate are sufficient to *significantly impair long term (not transient) iodide uptake*, which would be considerably greater than 0.007 mg/kg-d (~240 ppb; based on Greer et al. 2002)) or the OEHHA benchmark dose of 0.0037 mg/kg/day (~120 ppb), perchlorate exposure would not exacerbate iodine deficiency. Thus, the approach used by OEHHA in deriving their PHG remains a conservative and health protective approach that accounts for substantial uncertainty.

Based on the new data reported in U.S. FDA Total Diet Study (Murray et al., 2008), an RSC of 70% could be allocated to water to protect the most sensitive population, pregnant women and their fetuses. The current RSC of 60% continues to provide a conservative margin of safety.

Table 2. Summary of Critical Risk Parameters Used by California OEHHA and U.S. EPA in the Derivation of Toxicity Guidelines and the Impact of New Studies Published Since Their Derivations

Critical Risk Assessment Parameter	OEHHA, 2004 PHG	EPA, 2005 RfD	Any "New Science" (described in this document) Since 2004 to Refute Original Consideration?	Implication of Science Since 2004 PHG
Toxicity Guideline Value	6 ppb	24.5 ppb (assumes 70 kg adult drinking 2 L/d)	All new science demonstrates current PHG is a conservative toxicity guideline value and is health protective. Studies show that higher values would also be equally health protective.	Should provide greater confidence in value already established or provide more certainty (less uncertainty) in the toxicity guideline value.
Sensitive Population of Concern	"(i) pregnant women and their fetuses, especially those who are getting less than a sufficient amount of iodine; (ii) lactating women, especially those who are getting less than a sufficient amount of iodine, (iii) infants; and (iv) individuals with thyroid problems."	The fetuses of pregnant women who might have hypothyroidism or low iodine.	No, this still reflects the appropriate most sensitive population.	No, this still reflects the appropriate most sensitive population.
Assessed from Human Studies?	Yes	Yes	New epidemiological study in the most sensitive population and new ecological, clinical, and occupational studies all support the current PHG.	Human studies are preferred over animal studies. Not only were initial human studies used in establishing toxicity guideline values, the database is now larger with more human studies that demonstrate that the current PHG is a conservative health based value that protects the most sensitive populations.
Point of Departure (POD)	A statistical analysis; the lower limit of a one-sided 95 % confidence interval, called the lower BMD of iodine uptake by the thyroid; the dose is calculated to be 0.0037 mg/kg-day.	"The point of departure is based on a non-statistically significant mean 1.8% (standard error of the mean 8.3%) decline in RAIU in healthy adults following two weeks exposure to a daily perchlorate dose of 0.007 mg/kg-day." (EPA IRIS, 2005)	Braverman et al. (2006); 6 month human volunteer study; doses are 0.5 and 3.0 mg/day potassium perchlorate; n = 13; results are demonstration of iodide uptake inhibition; doses are comparable to low doses in Greer et al. (2002).	OEHHA's BMD is approximately half of the dose of the NOEL reported by Greer et al. (2002). Though a small study population, the Braverman et al. (2006) study supports clinical studies by Greer et al. (2002) and Lawrence et al. (2000; 2001), except this provides oral exposure for 6 months whereas Greer et al., was administered for 14 days.
POD as a dose from which to develop PHG/RFD	Estimated dose equal to 0.0037 mg/kg-day; this estimated dose is approximately half of the NOEL dose of 0.007 mg/kg-day obtained from Greer et al. (2002) used by U.S. EPA.	U.S. EPA used lowest dose in Greer et al. (2002); 0.007 mg/kg-day.	Braverman et al. (2006), as noted above.	No new scientific evidence that refutes initial POD or dose.

Critical Risk Assessment Parameter	OEHHA, 2004 PHG	EPA, 2005 RfD	Any "New Science" (described in this document) Since 2004 to Refute Original Consideration?	Implication of Science Since 2004 PHG
Uncertainty Factor (UF) Rationale	10 (to account for interindividual variability because the subject population in the Greer et al. (2002) study is not the same as the general population).	10 ("an intraspecies uncertainty factor of 10 is applied to protect...the fetuses of pregnant women who might have hypothyroidism or iodide deficiency." (EPA IRIS, 2005)).	There are more studies since 2004 that demonstrate a lack of effect for low environmentally relevant concentrations of perchlorate; see Section C.	More studies where either a weight of evidence or lack of effect would logically provide a greater degree of "certainty. that would support a decrease in UF.
Risk Assessment Assumptions	Use of body weight ratio of 25.2 kg-day/L for the 95th percentile value of the pregnant woman population (see Sensitive Population of Concern, above).	"Iodide uptake inhibition is a key biochemical event that precedes all potential thyroid-mediated effects of perchlorate exposure. Because iodide uptake inhibition is not an adverse effect but a biochemical change, this is a No Observed Effect Level (NOEL). The use of a NOEL differs from the traditional approach to deriving an RfD, which bases the critical effect on an adverse outcome. Using a nonadverse effect that is upstream of the adverse effect is a more conservative and health-protective approach to perchlorate hazard assessment" (EPA IRIS, 2005).	No new studies that refute current assumptions.	More studies where either a weight of evidence or lack of effect would logically provide a greater degree of "certainty" that would support a decrease in UF.
Relative Source Contribution	60%; percent of exposure to perchlorate that is to be allocated to drinking water.	100% (http://www.epa.gov/safewater/standard/setting.html).	No new studies that refute current assumptions.	No new studies that refute current assumptions.

B. CRITICAL COMPONENTS OF THE NATIONAL RESEARCH COUNCIL (NRC)'S 2005 REPORT: *HEALTH IMPLICATIONS OF PERCHLORATE INGESTION*

When the PHG was published in 2004, OEHHA stated that it was aware of the concurrent assessment by the NAS and that "when that evaluation is completed, OEHHA will carefully review the NAS conclusions and will revise the PHG as necessary (Health and Safety code section 116365(e)(1))" (OEHHA, 2004). This section summarizes the NAS report and its effect on the PHG. OEHHA concluded that the NAS report supports their choice of Greer et al. (2002; commonly referred to as the Greer Study) as the critical study determining an appropriate point of departure (POD). The original PHG, which is more conservative than what NAS has recommended, is appropriate for protecting health.

Due to criticism of their 2002 draft risk assessment on perchlorate health effects, the U.S. EPA asked the NAS to independently evaluate the science.[5] The NAS committee recommended basing the RfD on a non-adverse event, inhibition of iodide uptake, as reported by the Greer Study. In the Greer Study, a NOEL for the inhibition of iodide uptake was demonstrated in healthy women (n=6) and one man at 0.007 mg/kg-day of perchlorate. Using a NOEL for a non-adverse event is a conservative method not traditionally used in risk assessment. Inhibition of iodide uptake is not adverse; however, with prolonged inhibition of uptake, there could be a decrease in serum thyroxine (T4) and thyronine (T3) coupled with an increase in serum thyroid stimulating hormone (TSH), which could potentially cause changes in the thyroid and may later result in hypothyroidism. Therefore, by choosing the NOEL, the committee recommended basing an RfD on an effect that is several steps prior to the

The NAS recommended the use of a NOEL determined by Greer et al. (2002) based on a non-adverse effect that occurs several steps prior to the adverse effect.

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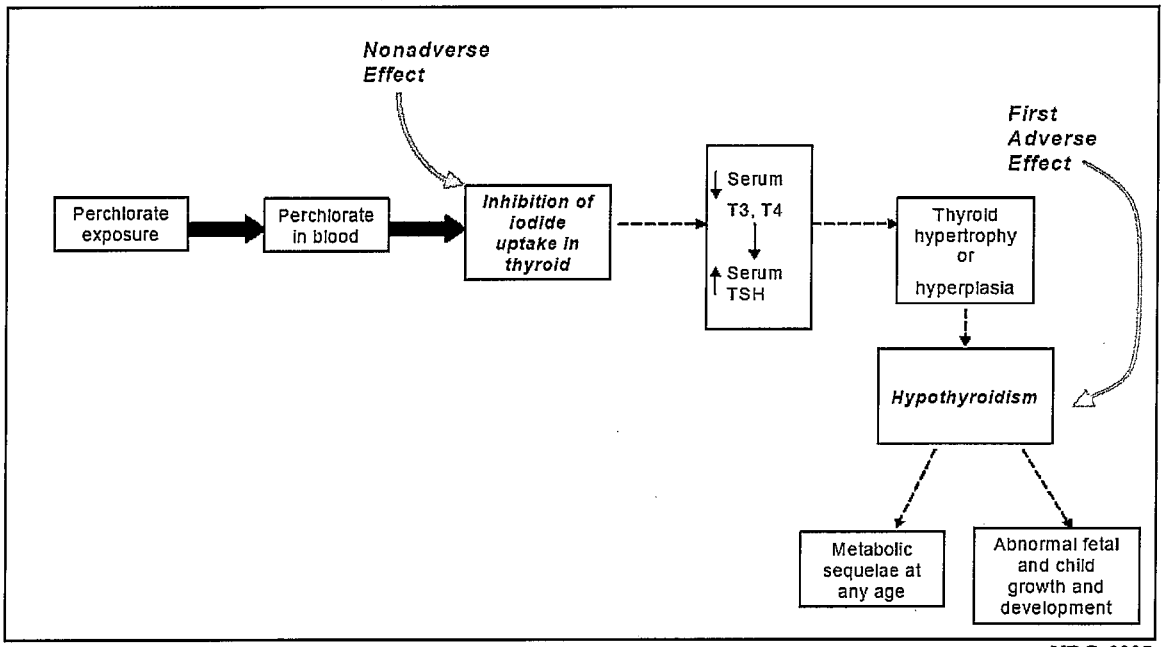
[5] The U.S. EPA, the Department of Defense (DOD), the Department of Energy (DOE), and the National Aeronautics and Space Administration (NASA) asked the NAS, "to assess the potential adverse health effects of perchlorate ingestion from clinical, toxicologic, medical, and public-health perspectives." The result of their thorough analysis was a report titled, *Health Implications of Perchlorate Ingestion* (NRC, 2005). Some of the conclusions of the report were related to NAS assessment of U.S. EPA / National Center for Environmental Assessment (NCEA)'s 2002 draft risk assessment, which was scientifically incomplete in several ways.

For example,

- Regarding U.S. EPA/NCEA's characterization of iodide uptake inhibition as the starting point for an inevitable "cascade" of health effects from perchlorate exposure was incorrect. The committee states, "An important point is that inhibition of thyroid iodide uptake is the only effect that has been consistently documented in humans exposed to perchlorate. Furthermore, the outcomes at the end of the continuum are not inevitable consequences of perchlorate exposure" (p. 13).
- Regarding U.S. EPA/NCEA's incorrect implicit characterization of iodide uptake inhibition as an adverse effect: The Committee "concludes that the first adverse effect in the continuum would be hypothyroidism" (p. 13) and that inhibition of iodide uptake by the thyroid gland is not an adverse human health effect (pp. 14, 166, 169, 174). Specifically, they state, "Inhibition of iodide uptake by the thyroid clearly is not an adverse effect; however, if it does not occur, there is no progression to adverse health effects… " (pp. 166-167, emphasis in original). "[I]t is highly likely that in people with a normal iodide intake the dose of perchlorate would have to reduce thyroid iodide uptake by at least 75% for a sustained period (several months or longer) for iodide uptake and thyroid hormone production to decline enough to cause adverse health effects (equivalent to reducing dietary iodide intake by 75%). In adults, that is likely to require sustained exposure to more than 30 mg of perchlorate per day (0.4 mg/kg per day for a 70-kg person), on the basis of the clinical studies in healthy subjects…" (p. 67). "The committee notes that the NOEL identified by Greer et al. (2002) (0.007 mg/kg per day) [for inhibition of iodide uptake] is lower than all the LOAELs and almost all the NOAELs identified by EPA in studies using rats. The U.S. EPA/NCEA's characterization of transient changes in thyroid hormones or TSH as adverse effects was also wrong: "EPA defined changes in serum thyroid hormone and TSH concentrations as adverse effects. The committee does not agree that transient changes in serum thyroid hormone or TSH concentrations are adverse health effects; they are simply biochemical changes that might precede adverse effects" (p. 13). "The committee does not think that transient changes in serum thyroid hormone or TSH concentrations are necessarily adverse effects" (p. 177).

adverse effect (Figure 1). In addition to this conservative use of a NOEL, the committee suggested use of an uncertainty factor of 10 due to intraspecies variability to account for the most sensitive subpopulation, the fetuses of pregnant women (NRC, 2005). The result is a RfD of 0.0007 mg/kg-day or 24.5 ppb based on a 70 kg person drinking 2 liters of water per day.

Figure 1. Mode-of-Action Model



NRC, 2005

While not available for the 2004 PHG, OEHHA stated they would review the NAS report when available. In an April 2005 memorandum, Dr. Joan Denton stated: "After carefully reviewing the NAS report, OEHHA concludes that the approach we used in developing the perchlorate PHG is valid. The NAS report did not provide "new scientific evidence" indicating that perchlorate "...presents a materially different risk to public health than was previously determined."

In an attachment to the Denton Memorandum, OEHHA states that: "The NAS report made four key findings that strongly support the approach that OEHHA used in developing the PHG."[6]

As to what might be considered an upper level exposure to change thyroid physiology, NAS concluded that people with a normal iodide intake would require a perchlorate dose large enough to lower thyroid iodide uptake by at least 75% for a sustained period of time (several months or longer) to cause thyroid hormone production to decline to the point where hypothyroidism would occur. In adults, that dose is estimated as being no lower than 30 mg/day (0.4 mg/kg per day for a 70-kg person).[7]

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[6] These findings are:

- The health effects of perchlorate should be assessed using data from human studies, rather than animal studies. The NAS specifically recommended the use of the 2002 study by Greer, et al., in which healthy volunteers were administered perchlorate. OEHHA used data from the same study in its perchlorate risk assessment.
- The perchlorate health effect of primary concern is the reduction of the uptake of iodide by the thyroid gland. While not harmful by itself, inadequate iodide uptake may lead to the harmful disruption of proper thyroid function. The NAS report said that the reduction of iodide uptake "is the key event that precedes all thyroid-mediated effects of perchlorate exposure," and that focusing on the reduction of iodide uptake "is the most health protective and scientifically valid approach." OEHHA's assessment similarly focused on the reduction of iodide uptake as the critical health effect.
- The NAS report identified fetuses of pregnant women as the most sensitive subpopulation. OEHHA similarly concluded that pregnant women and fetuses are the most sensitive subpopulation.
- The NAS recommended an uncertainty factor of 10 to ensure adequate protection of pregnant women and fetuses. OEHHA used the same uncertainty factor in calculating the PHG.

"The NAS report calculated a reference dose after first identifying a No Observable Effect Level or NOEL (a generic number identifying a level or perchlorate exposure from any source that would not cause a health effect). OEHHA used a statistical method called the "benchmark dose" to identify a level of perchlorate exposure that would not cause a health effect. A PHG can be calculated from either a NOEL or benchmark dose, and both numbers in this case were obtained using data from the Greer study. The benchmark dose approach is preferred when the number of subjects in a study is relatively small, as in the Greer study, because it involves a statistical analysis and calculation of a 95-percent confidence level using all data points from the study. The NOEL, in contrast, is calculated using only the relatively few data points that appear to identify a no observable effect level. The NAS report said the benchmark dose "can be an improvement" over other approaches, but used the NOEL approach rather than choose between several methods for calculating a benchmark dose.

"While an infant is one of the populations of concern, infants are not considered as sensitive as the pregnant woman and her fetus, based on both biological and effective dose-rate calculations. Although there is no recognized iodine-deficient fraction of the general population, pregnant women are considered to be more likely to be iodide deficient because they have a greater need for iodide and at the same time have a higher urinary iodide excretion rate."

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

"In the NAS report, the committee stated that the choice of a NOEL as the point of departure is unusual, but that given the current scientific knowledge and the need to protect the sensitive subgroups, it is prudent to choose reduction of thyroidal iodide uptake as the endpoint. OEHHA also believes that this endpoint is the prudent choice." (Denton, 2005)

[7] The report stated, "On the basis of the studies of long-term treatment of hyperthyroidism in which patients continued to be given perchlorate after their hyperthyroidism resolved and the clinical studies of healthy adults, the perchlorate dose required to cause hypothyroidism in adults would probably be more than 0.40 mg/kg per day, assuming a 70-kg body weight." (NAS 2005, p. 8) This point of departure has been based on a non adverse effect.

However, the NAS concluded that the 0.007 mg/kg-day dose NOEL in healthy adults was the best and most scientifically reliable POD. The committee apparently felt uncomfortable extending this conclusion to subpopulations that could be more sensitive. They state,

> "...in pregnant women, infants, children, and people with low iodide intake or pre-existing thyroid dysfunction, the dose required to cause hypothyroidism may be lower." (NAS 2005, p. 8)

Even though the use of a NOEL, rather than a NOAEL, is inherently conservative, the NAS applied an uncertainty factor of 10, which yielded an RfD of 0.0007 mg/kg-d or 24.5 ppb concentration in drinking water.

C. What Has Happened Since the OEHHA 2004 PHG Assessment?

Scientific studies published since OEHHA's PHG assessment for perchlorate in 2004 demonstrate that the current PHG is conservative and health protective and perchlorate concentrations above 6 ppb would also be conservative and health protective for sensitive populations.

Since the publication of the OEHHA perchlorate PHG report in 2004, several key studies have been published that support the conservative nature of the PHG. Overall, these studies demonstrate that perchlorate is an ubiquitous chemical in the environment, food, and the human body. The sources of perchlorate are both natural and anthropogenic. *These studies add to an already robust database, strengthening previous analyses and reducing uncertainty, thereby demonstrating that the current PHG for perchlorate is both conservative and health protective. Perchlorate concentrations equal to or above 6 ppb would also be conservative and health protective for sensitive populations.*

The new studies fall into several categories, which we address below. First, we review commentaries that raise questions about the NAS assessment. This is followed by pertinent human and animal studies that examine the health effects of perchlorate, including the study by Blount et al. (2006) which use the National Health and Nutrition Examination Survey (NHANES) 2001-2002 dataset to determine associations between urinary perchlorate, urinary iodide, and serum thyroid hormones. Finally, we summarize the studies that do not include any antithyroid compound exposure, but do look at the effects of maternal hypothyroidism during pregnancy on children. These key commentaries and studies are summarized below.

1. Addressing Comments Regarding NAS Report:

    a. Ginsberg et al. (2005)

    The authors believe that the rat studies provide important information for use with the human data, and imply that the NAS did not appropriately consider this data. Regarding the Greer Study, they believe that 4 of 7 low (0.007 mg/kg-d) dose subjects showed a perchlorate effect (a decrease in radioactive iodine uptake (RAIU)) and that they believe that the 0.007 mg/kg-d level is a LOAEL.[8] As previously noted, since the background dose of perchlorate was not assessed by the authors of Greer et al. (2002), the dose that was determined to be the NOEL was actually lower than the true dose of perchlorate. *Regardless, the method, interpretation, and results of the Greer Study were reviewed by the NAS committee which determined that the conclusions in the Greer Study, including the statistical analyses, were appropriate. The NAS report provides a*

[8] The article points out that one of the fifteen NAS committee members thought that an additional uncertainty factor of 3 fold should be applied to account for what the person considered a small sample size for the 0.007 mg/kg dose of perchlorate as well as the length of daily for 14 days..."and that "...no chronic exposure studies have been published." Nevertheless, fourteen of the fifteen members disagreed with their colleague. These scientists "acknowledged that the low-dose group in Greer et al. had only seven subjects..." but "the study examined the effects of four doses in a total of 37 subjects." Also, the committee reported four other studies in which healthy adults were given perchlorate with the results of all studies are remarkably similar. There are also "studies of long-term treatment of hyperthyroidism and the studies of occupational and environmental exposure add confidence to the overall database." Finally, they state: "...chronic exposure will have no greater effect than that resulting from short-term exposure, and in fact, it may well have less effect because of the capacity of the pituitary-thyroid system to compensate for iodide deficiency by increasing iodide uptake."

scientifically-based evaluation as well as a science-based rationale for their conclusions.

b. Scinicariello et al. (2005)

The authors focus on genetic mutations at the one documented site of perchlorate action, the sodium iodide symporter (NIS), and genetic mutations in other proteins involved in iodine metabolism but for which there is no documented effect of perchlorate (pendrin and the proteins that make up the organogenesis machinery).

The authors imply that carriers of one copy of mutated NIS will be more sensitive to perchlorate than non-carriers. *They provide no scientific support for this conclusion.* Carriers are non-symptomatic and a rudimentary knowledge of thyroid physiology suggests that they should be able to regulate the production of NIS to compensate for their inactive alleles. The authors fail to acknowledge the differential sensitivity of different sites of perchlorate action. The most sensitive action of perchlorate is inhibition of thyroidal iodide uptake via NIS. At the NOEL for NIS there will be no meaningful effect at any other site of perchlorate action.

The authors discuss Pendred syndrome, an autosomal recessive disorder characterized by sensorineural hearing loss and goiter. Some, but not all, persons with Pendred syndrome have a positive perchlorate discharge test. The role of pendrin in the thyroid is non-essential (as shown by studies in knock-out mice). Pendrin is not directly sensitive to perchlorate. The authors speculate that anyone carrying a mutant pendrin allele will be more sensitive to perchlorate. *The authors provide no scientific support for this statement.*

The authors discuss genetic mutations associated with impaired organogenesis. *There is no evidence to suggest that persons with such a defect will be any more sensitive to perchlorate.*

Finally, genetic variability is accounted for in large-scale epidemiological, ecological, clinical, and occupational studies; therefore, any variability genetics introduces is already part of the inherent intraspecies variability of the subject pool.

2. Significant Peer-reviewed Scientific Studies Since the NAS's 2005 Report:

Compared to many other environmental pollutants, perchlorate has a rich body of human toxicology literature, due to its historical use as a pharmaceutical agent. Since the publication of the PHG, new studies have been published that add to the literature, reducing the PHG's uncertainty.

a. Human Studies:

i. Blount et al. (2006)

Using the NHANES 2001-2002 data set and a cross-sectional study design, this study reports measurements of urinary perchlorate, urinary iodide, and serum TSH, and total T4 levels in men and women over the age of 12. The authors determined that perchlorate levels were not associated with total T4 or TSH levels in men, but were a negative predictor of total T4 and a positive predictor of TSH in women with urinary

iodine less than 100 μg/L. They also found that in women with urinary iodine greater than 100 μg/L, urinary perchlorate was a positive predictor of TSH, but not associated with T4. The significance of evaluating women with urinary iodide less than 100 μg/L is based on World Health Organization (WHO) statement that a median urinary iodine concentration *within a population*, based on spot samples of less than 100 μg/L is indicative of iodine deficiency within the population (WHO 2004).

This study has drawn a great deal of attention as it appears—to the average audience—to demonstrate an effect of perchlorate, albeit at exposures below those which cause any measurable inhibition of iodide uptake. Unfortunately, an association between perchlorate and an effect cannot be concluded for a number of reasons examined below.

First, an important consideration for any study is how the experiment was designed, whether *all pertinent variables were measured*, and if the variables were measured in a logical and temporal manner. We found a number of experimental design issues that caused concern regarding the reliability of information obtained from this study that were used to form conclusions. Our complete assessment is attached as Appendix A. In the appendix, we provide a more thorough discussion regarding the use of only two measures of thyroid function (total T4 and TSH); the use of spot urine samples for iodide status which are neither the preferred, nor most reliable, measure of urinary iodide; and the lack of normalization for the dilution of individual urine samples using urinary creatinine levels.

Further, this type of study cannot determine causation, only *association* between the variables studied. If important variables are missing, then spurious conclusions can be made. Thus, without a full set of variables measured, including a consistent urinary iodide measurement, any association should be examined carefully for reliability. We believe more research should be conducted to better understand the results of this study.

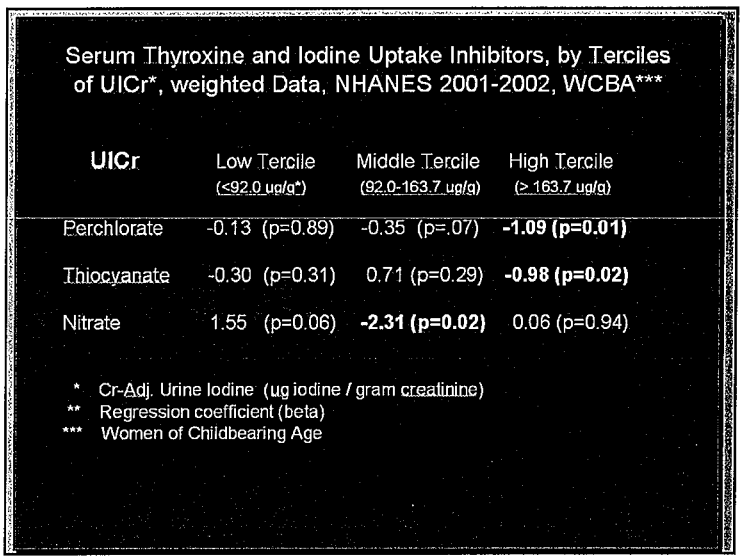
Second, it is important to note among other points made below, that *perchlorate did not actually lower thyroid hormones outside the normal range of values*. Even if it had, the NAS committee does not think that "...transient changes in serum thyroid hormones or TSH concentrations are adverse health effects; they are simply biochemical changes that might precede adverse effects" (NRC, 2005).

Third, not only were the thyroid hormone values within normal clinical ranges, but *urinary perchlorate levels as a surrogate for dose demonstrate that these perchlorate exposures are well below the threshold for zero inhibition of iodide uptake*, a non-adverse effect. Exposures that are below the threshold for iodine uptake inhibition are below the NOEL for perchlorate and are not adverse.

Fourth, the study by Blount et al. does not present *urinary measures normalized to creatinine*. The concentration of dissolved substances in urine, such as iodide, may vary between individuals or between samples from the same individual. Normalization with creatinine is not a perfect method, but is commonly used to account for this variability. At a recent symposium on perchlorate held in conjunction with the Society of Toxicology meeting in Seattle, Lamm et al. (2008) presented their initial reanalysis of the NHANES dataset used by Blount et al. with an

adjustment for urinary creatinine. They considered a subset of women from the Blount et al. study who were of childbearing age (15-44 years old; the Blount et al. study group included all women over the age of 12) as well as the interaction of thiocyanate and nitrate, both in urine. They found there was no significant association between perchlorate and total T4, even for women with urinary iodide less than 100 µg/L (Figure 2). They did find a significant association for women with urinary iodide greater than 163.7 µg/L for both perchlorate and thiocyanate.

Figure 2. Urinary Reanalysis Results of the NHANES Data



Lamm et al., 2008

In a peer-reviewed letter to the editor of *Thyroid*, Gibbs and Landingham (2008) reviewed data from their previously published study (Téllez et al. (2005) which is discussed in Section C), and showed that in a cohort of pregnant women in Chile, *the data do not support the association between environmental perchlorate exposure and changes in thyroid hormones* and are consistent with the recent negative findings by both Pearce et al. (2007b) and Lamm et al. (2007)[9].

Finally, taken from the premise that science is incremental and knowledge builds collectively over time, concerns raised by scientists and related to the Blount et al. (2006) study should be considered an opportunity for further study. According to Dr. Blount, a new study will be conducted. It uses a new data set, NHANES 2007 and includes obtaining data for *eight variables of thyroid function* (total T4, free T4, total T3, free T3, thyroid globulin, anti-thyroid peroxidase, anti-thyroid globulin, and TSH). Furthermore, recognizing that urinary spot iodine or single point iodine measures are not a good indicator of iodine status, *a different approach is being considered.*[10]

[10] Personal Communication between Dr. Richard Pleus and Dr. Benjamin Blount. September 22, 2008.

There are several methodological issues that prevent the reliable interpretation of both Blount et al. (2006) and Steinmaus et al. (2007). These are described in depth in Appendix A.

Briefly,

- Use of spot urine analysis of urinary iodide is not a reliable indicator or iodine status.
- When adjusted for urinary creatinine, which normalizes the dilution of dissolved substances, such as iodide, statistical significance is lost for those women age 15-44 with urinary iodide levels below 100 µg/L.
- Several important confounders were not adjusted for (e.g., diet, medications).

Steinmaus et al. (2007) *used the same dataset as Blount et al. (2006)* to assess the correlation between smoking, thiocyanate and urinary perchlorate, and thyroid hormone levels. Using the same NHANES data set, it is not surprising that many of the same results were noted. The same methodological issues reported for Blount et al. apply to this study. As in Blount et al. (2006), they did not find any interaction between perchlorate and smoking and TSH or total T4 in women with urinary iodine levels greater than or equal to 100 µg/L or in men. They did conclude that in women with urinary iodine less than 100 µg/L, perchlorate increased the risk of lower total T4 and greater TSH, just as was reported in the Blount et al. study. This association was stronger when the woman was also a smoker or had high urinary thiocyanate levels.

ii. Blount et al. (2007)

Blount and colleagues used the NHANES urinary perchlorate data to estimate the total daily dose for adults. These data were adjusted for creatinine. The estimated 95th percentile dose was 0.234 µg/kg-day with a confidence interval of 0.202 – 0.268 µg/kg-d. They also reported that perchlorate was measurable in all of the samples they tested and the urinary levels were higher in children compared to adults. *The estimated doses reported here were lower than the U.S. EPA RfD and the PHG.*

iii. Pearce et al. (2007b)

The objective of the Pearce et al. study was "to determine whether breast milk iodine concentrations in Boston-area women are adequate for infant nutrition, and whether breast milk iodine concentrations may be associated with environmental perchlorate or cigarette smoke exposure." This study addressed one of the areas of uncertainty acknowledged by OEHHA in their PHG. Pearce et al. measured breast milk iodine and perchlorate concentrations as well as iodine, perchlorate, and cotinine in urine. They then compared the levels found in breast milk to 17 commercial infant formulae. *Neither breast milk nor urinary perchlorate levels were significantly correlated with breast milk iodine concentrations.* Although perchlorate was detectable in infant formulae, the levels were lower than that in breast milk. A significant number of women in this study had iodine levels that were insufficient to meet the infant's needs, but the authors did not suggest this was due to perchlorate exposure or that it represents a chronic iodine deficiency. This study provides

scientific information that addresses some of the uncertainties raised by OEHHA regarding the relationship between perchlorate, iodine, and breast milk.

iv. Chang et al. (2003)

Using an ecological study design, Chang et al. used a county-wide measurement of environmentally relevant drinking water perchlorate levels (mean concentration of 10.9 ppb, range excluding the 10% tails was 7 to 15 ppb) and compared them to incidence of Attention Deficit Hyperactivity Disorder (ADHD), autism, and fourth-grade school performance results. *They found that there was no association between the presence of perchlorate in drinking water and any of the endpoints measured.*

This study did not attempt to quantify the amount of perchlorate from food or the ingestion dose of other substances that may cause similar effects on the thyroid. The authors did not directly measure thyroid hormone levels.

v. Braverman et al. (2005)

Although there were two occupational studies published prior to the PHG, there were inadequacies noted by OEHHA, such as short exposure duration, intermittent exposure, small number of subjects, and limited number of endpoints. This study included 29 workers exposed for at least 1.7 years in an ammonium perchlorate plant and 12 unexposed volunteers; variables measured in the study included serum perchlorate, thiocyanate, nitrate, total T4, Free T4, total T3, Tg, and TSH; RAIU; and urinary iodine and perchlorate taken after 3 days off or 3 days working. The authors estimated that "half of the workers experienced average ClO4- doses in excess of 0.33 mg/kg-shift over the year preceding this study." They found that there was a decrease in RAIU, but no change in thyroid hormones compared to unexposed controls. There was a slight increase in T3, T4, and free T4 after 3 days working compared to 3 days off. Again, the NAS committee has stated that, "The committee does not think that transient changes in serum thyroid hormone or TSH concentrations are necessarily adverse effects (NRC, 2005)." *The RAIU dose response curve the authors found was consistent with that of the Greer Study.*

vi. Braverman et al. (2006)

In an effort to study the thyroidal effects of a prolonged (six months) exposure to low perchlorate levels in humans, Braverman et al. exposed 13 healthy volunteers to 0, 0.5, or 3.0 mg/d of potassium perchlorate and measured urinary perchlorate levels, radioactive iodine uptake and serum T3, free T4, TSH and Tg concentrations. The 0.5 and 3.0 mg/d doses are equivalent to 250 and 1500 ppb, assuming a daily water ingestion of 2 liters. The authors concluded that perchlorate at doses of up to 3 mg/day for six months "...had no effect on thyroid function, including inhibition of thyroid iodide uptake as well as serum levels of thyroid hormones, TSH and Tg" and, *"...there was no significant change in the thyroid RAIU during perchlorate administration."*

This study was similar to the Greer Study, but for longer exposures. The doses were 0.5 and 3.0 mg/d and the exposures were for six months. This study also did not restrict or control for dietary intake. Therefore, the doses administered as part of this study were in addition to the background levels of perchlorate ingested—the impact

of dietary iodine intake was not accounted for. The study would have benefited from a larger sample size; however, data from the 14-day Lawrence et al. (2000, 2001) studies, and the Greer Study support the results reported by Braverman et al. These studies all support the NOEL determined by the Greer Study.

vii. Kirk et al. (2005)

With the aim to determine what amount of perchlorate children are exposed to, Kirk et al. measured perchlorate and iodide levels in cow and human breast milk and compared these numbers to corresponding levels of perchlorate in drinking water in the area. Perchlorate was measurable in 81 of the 82 samples measured. The average perchlorate levels in cow milk and human milk were 2 and 10.5 μg/L, respectively. The maximum values of cow and human milk were 11 and 92 μg/L, respectively. *There was no correlation between levels of perchlorate in breast milk and perchlorate in drinking water.* They speculated that there was a correlation between higher levels of perchlorate and lower levels of iodine in breast milk.[11] However, this relationship only existed for the breast milk samples with the highest perchlorate levels (6 subjects out of 82). The authors recognize that this relationship may be coincidental due to the small number of samples with perchlorate levels greater than10 μg/L, stating that "If we take all the available data, there is no meaningful correlation between the perchlorate and iodide levels in breast milk." As with previous studies, due to the design, this study is not able to evaluate a causal relationship. This study provides scientific information and addresses some of the uncertainties raised by OEHHA regarding the relationship between perchlorate, iodine, and breast milk; however, it does not show that perchlorate exposure affects breast milk iodine concentration.

viii. Amitai et al. (2007)

This ecological study aimed to "...assess the effect of gestational perchlorate exposure through drinking water on neonatal thyroxine (T4)" by comparing T4 levels among newborns whose mothers lived in areas where drinking water perchlorate levels were very high (≥340 μg/L), high (42-94 μg/L), or low (<3 μg/L). T4 levels were measured with in 36 to 48 hours after birth, but there was no comment on whether the infants were breast fed or formula fed during the postnatal period. *They found that there were no differences between neonatal T4 levels among the groups.* This study provides evidence that the current PHG and values greater are conservative and health protective to the most sensitive individuals in the population.

ix. Téllez et al. (2005)

This was a longitudinal epidemiologic study of the effects of environmental perchlorate exposure on the thyroidal status of pregnant women and neonate. Perchlorate is found at high levels in Chilean soils and water supplies. The authors measured maternal and neonatal TSH, Tg, and free T4. They also measured neonatal

[11] The Kirk et al study uses an arbitrary and non-scientific method of speculating on this relationship. Kirk et al., write: "...we divide iodide levels [in human breast milk] in two groups, those above 60 μg/L and those below, this being the iodide content for many infant feed formulas"; "we...divide perchlorate content in two groups, high and low, in this case arbitrarily dividing the span of the observed perchlorate range in two equal halves." and "at this point with limited resources, we have been able to analyze only a few samples. Thus, they divide data set using no scientific rationale and then exclude data. The authors state, "If we take all the available data, there is no meaningful correlation between the perchlorate and iodide levels in breast milk."

birth weight, length, and head circumference. They found that "...perchlorate in drinking water at 114 µg/L did not cause changes in neonatal thyroid function or fetal growth retardation." The levels of iodine in breast milk were not associated with perchlorate exposure. The levels of maternal iodine in urine were intermediate to that found in NHANES I and NHANES III and consistent with WHO recommendations.[12] *This study strengthens the evidence that the RfD and PHG are conservative health based values.*

x. Murray et al. (2008)

The FDA total diet study TDS was "designed to monitor the U.S. food supply for chemical contaminants, nutritional elements, and toxic elements." Based on measurements of perchlorate and iodine in market baskets, the Murray et al. report estimated intakes. The sampling approach is based on "market baskets" that are collected four times per year. Each market basket contains 285 foods, collected simultaneously in three cites within a region. The modeled group estimated to have the greatest dietary exposure to perchlorate was children age 2 (0.35 to 0.39 µg/kg-day). The modeled dose for the most sensitive population, the fetuses of pregnant women, was not specifically modeled, but the estimated dose for women of child bearing age was 0.09 to 0.11 µg/kg-d.[13] This is due to the high intake of dairy products coupled with low body weight. Adult men age 25 to 30 years old were estimated to be exposed to 0.08 to 0.11 µg/kg-day. Of the foods measured in this study, spinach, tomatoes, and cantaloupe had the highest levels of perchlorate and 40, 78, and 24.4 µg perchlorate/kg wet weight of food.

This study can be used to estimate exposures to perchlorate based on actual food concentrations. *Even at the highest estimate, this dose was less than the U.S. EPA RfD of 0.7 µg/kg-day and similar to the PHG of 0.37 µg/kg-day.* The study does not aim to evaluate overall body burden of perchlorate, only dose. Children age 2 have a higher excretion rate than adults or infants do, which would reduce their overall body burden despite the higher intake.

b. Pertinent Animal Studies:

Animal, particularly rodent, studies are an implicit and valuable aspect of toxicology. There are, however, significant differences between human and rodent thyroid physiology. U.S. EPA states, "The fundamental mechanisms involved in the function and regulation of the pituitary-hypothalamus-thyroid system in rats are qualitatively similar to those in humans. However, differences in binding proteins, binding affinities of the proteins for the hormones, turnover rates of hormones, and thyroid stimulation by placental hormones lead to important quantitative differences between the two species. The biochemical and physiologic differences between rats and humans related to the thyroid affect their responses to goitrogens, such as perchlorate. Therefore, although

[12] In the PHG regarding a study by Crump et al. (2000), OEHHA noted that the "...high urinary iodine levels and high goiter prevalence among the schoolchildren in the Chile study make the interpretation of thyroid function data difficult, and together they indicate there may be other confounding factors" (OEHHA, 2004). In Crump et al. (2000), the urinary iodine of schoolchildren was approximately three times higher than children in NHANES III and 75% higher than children in NHANES I. Subsequently, the levels of potassium iodate in iodinized table salt were lowered to 20-60 ppm. In Téllez et al. (2005), the urinary iodine has decreased to levels similar to that found in the U.S.

[13] The estimated doses for women ages 14-16, 25-30, and 40-45 were all 0.09 to 0.11 µg/kg-d.

studies in rats provide useful qualitative information on potential adverse effects of perchlorate exposure, they are limited in their utility for quantitatively assessing human health risk associated with perchlorate exposure" (U.S. EPA, 2005). Since 2004, OEHHA noted two animal studies in their July 2008 Public Notice on the Initiation of Risk Assessments for Chemicals in Drinking Water.[14]

i. Dohán et al. (2007)

The main purpose of the Dohán et al. publication is to demonstrate that perchlorate crosses cell membranes via the NIS; to demonstrate perchlorate's ability to be excreted in breast milk; and to develop a mathematical model to characterize their experimental results. The *in vitro* experiment and system they use is unique, creative, and provides the first evidence of perchlorate crossing a cell membrane using Mardin-Darby canine kidney (MDCK) cells transfected with the human NIS. It provides further evidence regarding questions about breast milk perchlorate levels and whether perchlorate blocks iodide at the symporter or competes against iodide in crossing the NIS. Thus, it decreases uncertainty in the risk assessment process. It is important to remember that this is the first study of its kind, it was conducted *in vitro* (cell culture), and it was conducted in a non-human cell line with artificially introduced human symporters. From a perspective on cell membrane transport, this work provides qualitative information.

The author's discussion of the *in vivo* part of the study's relevance to environmental exposures of perchlorate and human health is, however, far reaching and not supported by the results of this experiment. High acute doses of perchlorate were given to the rats. The rats were given an intraperitoneal injection of nearly 8 mg/kg/d in addition to a drinking water exposure of 13.6 mg/kg/d.[15] The perchlorate doses administered in this study are high enough such that they should effectively block, with complete inhibition, iodide transport by the NIS in these rats, *which provides no information about the effects at environmentally relevant doses. The results cannot be used to draw conclusions about what happens from a human health standpoint or mechanistic function at environmentally relevant concentrations.*

ii. Van Wijk et al. (2008)

This study examined the effects of perinatal and chronic hypothyroidsm on neurological function in rodents using the grip test, balance beam test, open field test, and Morris water maze test. During the last two weeks of pregnancy, dams and their resulting offspring were fed a diet poor in iodide with 750 ppm perchlorate in their drinking water. The pups were fed until sacrifice to simulate chronic hypothyroidism or until weaning to simulate perinatal hypothyroidism. Chronic hypothyroidism had more pronounced effects on development and the authors concluded that "...early effects of hypothyroidism on functional alterations of the developing brain to be partly reversible, and to depend on developmental timing of the deficiency." *The doses were sufficient as to induce frank hypothyroidism, which is not relevant to questions about environmentally relevant doses..*

[14] http://www.oehha.org/water/phg/pdf/PHGinitiation073008.pdf

[15] Assumes a 250 g rat with a daily water intake of 5.5 ml/100 g body weight.

3. Literature Cited by the California OEHHA Reference List and Related to Maternal Thyroidal Effects and Neuropsychological Effects in Offspring:

a. Cooper (2004)

Cooper et al. report the findings of a 2002 conference to develop a consensus among professionals regarding subclinical hypothyroidism. It was determined that subclinical hypothyroidism is defined as serum TSH above 4.5 or 5 mU/L. The consensus was against population based screening, even for pregnant women. Rather, TSH levels may be measured in women who were pregnant or planning on becoming pregnant if there was a personal or family history of hypothyroidism.

b. Haddow et al. (1999)

The study by Haddow et al. measured TSH in serum samples from 25,216 pregnant women in Maine, 62 of which were identified as having been hypothyroid during pregnancy, 47 of which were based on very high TSH levels (at or above the 99.7th percentile of all pregnant women), and 15 of which were based on TSH levels that were moderately high and serum T_4 levels that were moderately low (between the 98th and 99.6th percentile for all pregnant women). Fourteen of the 62 hypothyroid women were treated during their pregnancies for hypothyroidism; the rest were untreated. The study found that the children of the untreated hypothyroid women scored lower on IQ tests in comparison to the children of matched control women who had normal TSH levels during pregnancy but were similar in other respects. However, hypothyroidism in the U.S. is most often caused by chronic autoimmune thyroiditis, not iodine deficiency (Haddow et al., 1999). In this study, 77% of the hypothyroid women had chronic autoimmune thyroiditis. This study does demonstrate neurodevelopmental effects in children born to mothers with TSH levels greater than 98th percentile. Although this study provides meaningful information regarding hypothyroidism and neurodevelopment, it is important to note that these women were likely hypothyroid. Environmentally relevant exposures to perchlorate have not been shown to inhibit iodide uptake and therefore, would not increase the severity of hypothyroidism in already hypothyroid women. The authors do not suggest that a chemical exposure could be responsible for this severity of hypothyroidism. Iodine status was also not mentioned in the article.

c. Pop et al. (1999; 2003)

The studies by Pop et al. (1999; 2003) followed a cohort of children born to mothers in the south-east of the Netherlands with free T4 concentrations at the low end of the range considered normal in that region, as well as levels well-below the normal range. Maternal TSH levels were reported to be somewhere in the range considered normal in that region (this region is reported to have low iodine intake). Thus the mothers can be considered to have been hypothyroxinemic but not hypothyroid. Based on their initial analysis when the children were 10 months of age, the authors concluded that "...low maternal plasma free T4 concentrations during early pregnancy may be an important risk factor for impaired infant development" (Pop et al., 1999). However, based on their subsequent analysis when the children were up to 2 years of age, the authors concluded:

> "Maternal hypothyroxinemia during early gestation is an independent determinant of a delay in infant neurodevelopment. However, when free T4 concentrations increase during pregnancy in women who are

hypothyroxinemic during early gestation, infant development appears not to be adversely affected."

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

NAS also reviewed studies by Haddow et al. and Pop et al. in their 2005 assessment. They found that the studies had significant limitations (e.g., "...test scores were small, and the scores could be confounded by socioeconomic, educational, and other differences between the study groups..."). However, they state: "Nonetheless, if confirmed, they [the studies] emphasize the potential vulnerability of fetuses to decreases in maternal thyroid function." The committee chose to address this in assigning a value for the intraspecies factor. They state that "...the committee recommends use of a full factor of 10 to protect the most sensitive population—the fetuses of pregnant women who might have hypothyroidism or iodide deficiency. The committee views its recommendation as conservative and health-protective, especially given that the point of departure is based on a nonadverse effect that precedes the adverse effect in the continuum of possible effects of perchlorate exposure" (NAS, 2005).

d. Surks et al. (2004)

In this systematic review of subclinical thyroid disease, the authors found that the possible adverse effects due to TSH levels between 4.5 to 10 mU/L were minimal and routine treatment of these patients is unwarranted. They also recommend against population-based screening, but found insufficient evidence to argue for or against routine screening of TSH in pregnant women or women who plan to become pregnant unless there is a family or personal history of hypothyroidism.

4. Other Anticipated Studies:

As presented in a secondary meeting of the Society of Toxicology (SOT)—Seattle 2008:

a. Amitai et al. (2008)

Amitai et al. provide a follow-up to their previously reviewed 2007 publication (reviewed above). In this new study, they locate a subset of the original study population from their previous study and evaluate the children who were exposed *in utero* to "very high" (≥340 µg/L, n=12), "high" (42-94 µg/L, n=43), or "low" (<3 µg/L, n=56) concentrations of perchlorate in the mothers' drinking water for neurodevelopmental deficits (Figure 3). They find that there is no difference between groups of children.

Figure 3. Evaluation Results of Children Exposed to Perchlorate *in utero*

RESULTS OF BAYLEY SCORES

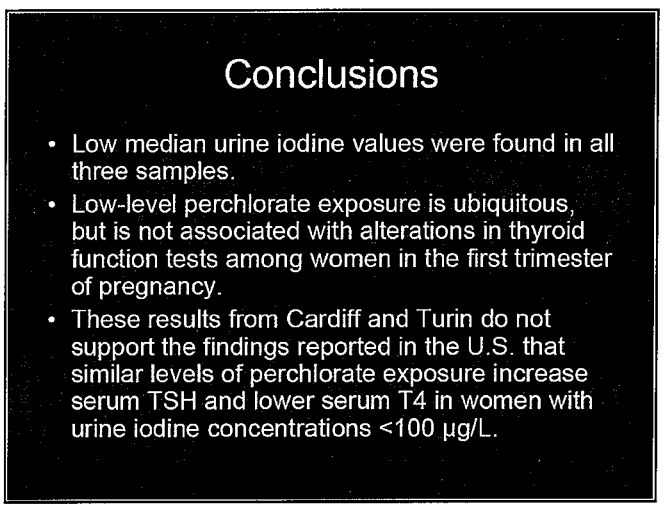
GROUP (N)	T4, UG/DI (±SD)	MENTAL DEVELOPMENTAL INDEX (M DI) (±SD)	PERFORMANCE DEVELOPMENTAL INDEX (PDI) (±SD)
VERY HIGH EXPOSURE (12)	14.42 3.89	110.3 7.7	103.7 6.9
HIGH EXPOSURE (43)	13.67 3.68	105.6 11.0	98.5 10.8
LOW EXPOSURE (56)	14.7 3.27	110.0 9.4	101.8 15.0

Amitai et al., 2008

b. Pearce et al. (2008)

Pearce et al. measured urinary iodine and perchlorate and serum TSH, free T4, TPO antibody, and Tg antibody in first-trimester pregnant women with urinary iodide less than 100 □g/L from Cardiff, Wales; Turin, Italy; and Dublin, Ireland.16 Median urinary perchlorate values were approximately 2 to 3.5 □g/L. They found no association between urinary perchlorate and any of the outcomes measured. The urinary iodine measures from Cardiff, Turin, and Dublin were lower than from women in the U.S., yet urinary perchlorate levels were similar, suggesting similar levels of exposure to perchlorate with lower iodine intake (Figure 4).

Figure 4. Conclusions from Pearce et al. (2008)



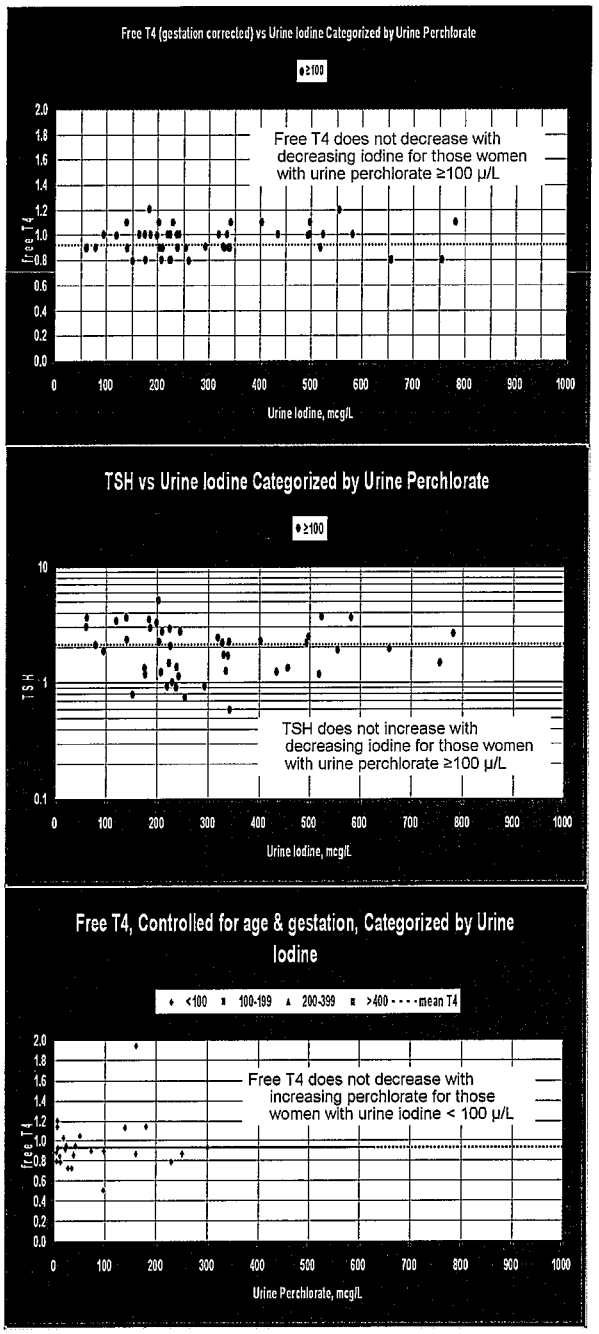
Pearce et al., 2008

[16] Serum measured only in Cardiff and Turin subjects.

c. Gibbs (2008)

Gibbs reported on the further analysis of data used in the Tellez et al. studies. In this presentation, he demonstrated that spot urine iodine levels are not consistent over time, that spot urine iodine levels do not correlate well with iodine nutritional status, and that spot urine iodine levels do not correlate with thyroid function (Figure 5).

Figure 5. Analysis of Data from Gibbs (2008) Presentation



Gibbs, 2008

D. Summary and Conclusion

Since the PHG was published in 2004, more scientific studies have been published, including the NAS report *Health Implications of Perchlorate Ingestion. The results of this assessment demonstrate that the PHG continues to be a conservative toxicity guideline value such that perchlorate concentrations in drinking water at or below the PHG pose no significant health risk to the most sensitive individuals consuming that water on a daily basis over a lifetime. In fact, the latest scientific evidence decreases the uncertainty that was noted in the 2004 PHG and demonstrates that values higher than the PHG would provide the same level of protection to sensitive populations.*

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APPENDIX A

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

A number of scientific issues related to the experimental design of the NHANES dataset and the Blount et al. (2006) analysis make drawing meaningful conclusions from the data extremely difficult. Many of these issues are discussed below, although this list is not exhaustive.

In their paper, Blount et al. (2006) examined the relationship between urinary levels of perchlorate and serum levels of thyroid stimulating hormone (TSH) and total thyroxine (T4) in men and women, aged 12 and older, participating in the NHANES during 2001-2002. They made the following primary observations:

- Perchlorate was not a significant predictor of total T4 or TSH levels in men.
- For women with spot urinary iodine concentrations < 100 µg/L, perchlorate was a significant negative predictor of total T4 ($p < 0.0001$) and a positive predictor of TSH ($p = 0.001$).
- For women with urinary iodine ≥ 100 µg/L, perchlorate was a significant positive predictor of TSH ($p = 0.025$), but not total T4 ($p = 0.550$).

To assess the reliability of these conclusions, we reviewed the Blount et al. (2006) paper and the NHANES 2001-2002 dataset (CDC 2006) used by the authors. We reviewed the same variables reported in the paper and several dozen other variables not reported in the paper but reported in the NHANES 2001-2002 dataset.

The data collected by NHANES is a survey and not a true epidemiological or clinical study. In epidemiological and clinical studies, effort is made to design the study to control for all variables except the variables in question. Surveys are not designed in this manner—their purpose is to collect data from a large number of people over a period of time. NHANES is designed to assess the health and nutritional status of adults and children in the United States (U.S.), combining interviews and physical examinations. It is a major program of the National Center for Health Statistics (NCHS), which is part of the Centers for Disease Control and Prevention (CDC) of the U.S. Public Health Service, and has the responsibility for producing vital health statistics for the nation (CDC, 2006).

NHANES 2001-2002 was designed to continue the collection of information about the health and diet of people in the U.S. that was begun with earlier surveys to fulfill specific goals. These include estimating the number and percent of persons in the general U.S. population and designated subgroups with selected diseases and risk factors; monitoring trends in the prevalence, awareness, treatment, and control of select diseases; monitoring trends in risk behaviors and environmental exposures; studying the relationship between diet, nutrition, and health; and exploring emerging public health issues.

Key components to designing a thyroid study

When conducting a thyroid study, a number of key variables should be considered that are not necessarily included when collecting data for a survey such as NHANES. For instance, thyroid hormone and TSH levels can vary substantially during the day. Free T4 (not total T4, which was measured in NHANES) is the most important thyroid parameter for assessing hypothyroidism. Because hypothyroidism is most commonly caused by one's own immune system, parameters used to assess this effect (including serum antibodies to thyroglobulin and thyroid peroxidase) should also be measured (but were not in the NHANES study). In general, the use of NHANES data to draw conclusions regarding exposures is controversial because of "...the utility of these data to address an existing public health concern, including investigation of etiology, instead of undertaking a study that includes original data collection" (Wartenberg and Buckler, 2001).

Study variables of concern

We were able to confirm the dataset Blount et al. (2006) used in the paper and generally replicate the statistical results obtained by Blount et al. This allows us to examine the experimental design of the study with more confidence. Based on our review, we observed several key variables that caused us to question the conclusions presented in the Blount et al. paper. These variables are summarized below.[17]

A. Relying upon the urinary iodide measurements for classification of study subjects into "low" or "high" iodine groups is not scientifically appropriate.

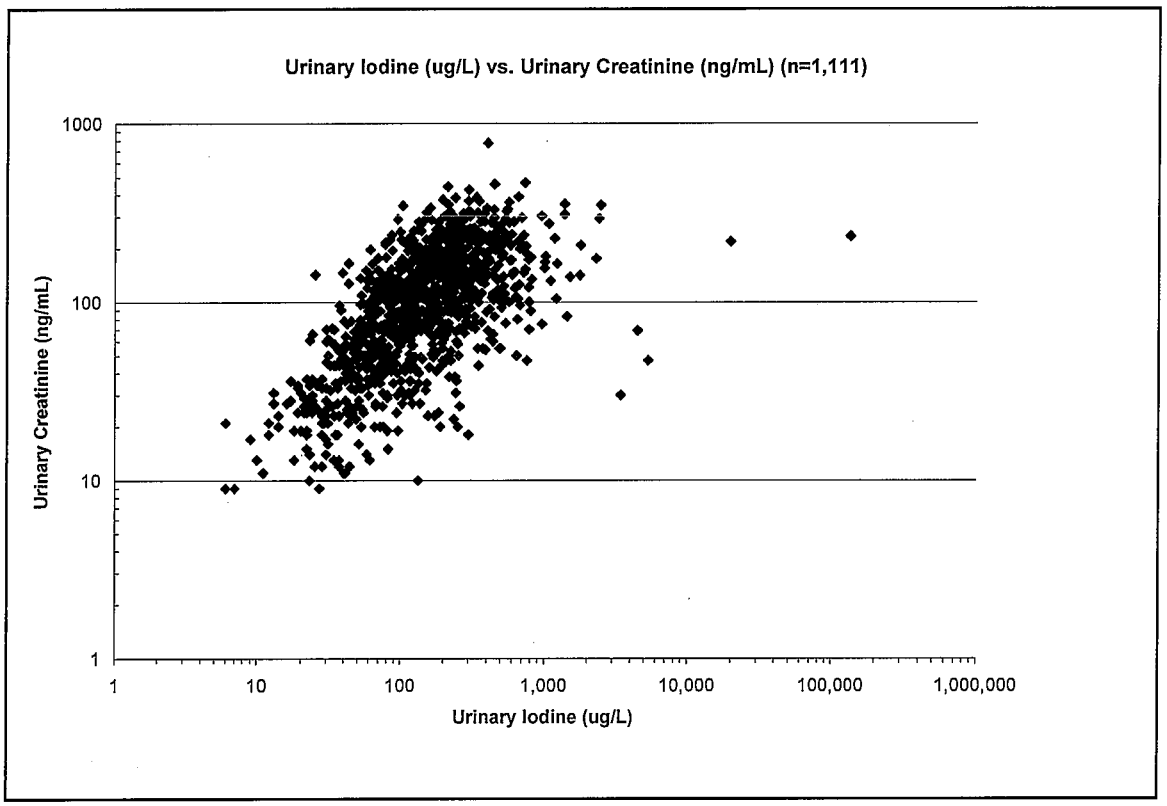
- Urinary iodine concentrations in the study group ranged from 6 to 136,161 µg/L. The geometric mean was 127 µg/L (SE = 5.4) and the 5th and 95th percentiles were 22 and 568 µg/L, respectively. Our findings for this group are consistent with the summary of urinary iodine measurements in NHANES 2001-2002 published by the CDC (Caldwell et al., 2005). If taken at face value as representative of chronic iodine nutrition, the upper limits of these measurements likely exceed safe levels and should be a source of some alarm for the CDC. For example, elevated TSH levels and hypothyroidism have been associated with daily iodine intakes in excess of 750 µg/day by adults (NAS 2001). However, these measurements were taken in spot samples and are thus not necessarily indicative of an individual's iodine status.
- Urinary creatinine concentrations were highly variable from subject to subject (range 9 to 774 ng/mL), indicating significant differences in sample dilution and, likely, urinary output.
- The WHO has stated that a median urinary iodine concentration *within a population*, based on spot samples of <100 µg/L is indicative of iodine deficiency within the population (WHO, 2004). The median urinary iodine concentration for the study population evaluated by Blount et al. (n =1,111) is 133 µg/L. Thus, assuming that iodine deficiency exists within this population is inappropriate—

[17] This evaluation is primarily based on the sample of 1,111 women aged 12 and older (i.e., the "study group"), described by Blount et al., from whom urine samples for perchlorate and serum thyroid hormone/TSH concentrations were measured, and excluding those who had a reported history of thyroid disease, were taking thyroid medications or a small subset of other thyroid-active drugs, or had extreme (high) levels of TSH or extreme (low) levels of T4. It should also be noted that total T4 measurements were performed at two separate laboratories which may have introduced some bias.

the WHO criteria are not meant to diagnose iodine deficiency in individuals (Borak, 2005). Conversely, the best way to establish *individual* urinary excretion of iodine (or any urinary analyte) is to collect 24-hour samples (Bourdoux, 1998). However, without a reliable measure or indicator or iodine status, comparison s make suing this measure could be spurious.

- Figure 1A illustrates the variability in urinary iodine concentrations in the Blount et al. dataset.

Figure 1A. Variability of Urinary Iodine from Blount et al. (2006)



B. Characteristics of individual members of the study group vary widely. Because many of these variables could impact overall subject health and, potentially, thyroid hormone or TSH levels, it is impossible to establish causality based on this dataset. The following is a sample of some of these variables—many others, both reported in and not reported in the dataset, could potentially impact thyroid measurements.

- NHANES is a *survey* designed to assess the health and nutritional status of adults and children in the U.S. It is not a controlled epidemiological study. Up to 3,400+ different variables are reported in the online NHANES 2001-2002 dataset in association with specific study subjects. The potential for spurious associations between variables is significant.
- 48% of those who responded were active smokers (42% reported smoking "everyday" and 6% "some days"; only 301 subjects out of 1,111 study subjects had a reported response to this question). 21% of smokers reported smoking 15 or more cigarettes per day. Smoking status of study subjects under 18 years of age (29% of all study subjects) was not reported due to privacy concerns. In

addition, 21% of study subjects reported living in a home with a smoker. *Smoking is a known source of thiocyanate and other goitrogens. In smokers, cyanide from cigarette smoking is likely the most important source of SCN^- in the body* (Tonacchera et al., 2004). Ingbar (2000) states that *thiocyanate as well as the pyridine components of cigarette smoke are likely a cause of lower T4 and T3 levels in serum of heavy smokers.* Smoking and thiocyanate were found to be significant in a later analysis of the same data (Steinmaus et al., 2007).

- 105 reported being pregnant at the time of the examination. *Pregnancy is known to have an effect on thyroid economy with significant changes in iodine metabolism and serum thyroid binding proteins* (Lazarus, 2005a; 2005b).
- Although Blount et al. excluded subjects who were taking thyroid medications (e.g., levothyroxine) or certain other thyroid active agents (e.g., propylthiouracil or methimazole), subjects taking any other kind of drug were included. 43% of study subjects reported taking at least one prescription drug within the month preceding their examination interview. This includes subjects taking medications known to directly affect the thyroid including lithium, amiodarone, and carbamazepine. *Many drugs affect tests of thyroid function through alterations in the synthesis, transport, and metabolism of thyroid hormones, as well as via influences on thyrotrophin (TSH) synthesis and secretion. Despite effects on circulating thyroid hormone and TSH levels, few drugs result in important changes in clinical thyroid state, but difficulty in interpretation of thyroid function tests often results. Commonly prescribed drugs including anti-convulsants, non-steroidal anti-inflammatory drugs, steroid hormones and heparin may result in abnormal thyroid function tests in the absence of clinical features of thyroid dysfunction. In contrast, lithium and iodine containing drugs, including radiographic contrast agents and amiodarone, may result rarely in overt thyroid disease* (Davies and Franklyn 1991; Gittoes and Franklyn 1995; Wenzel 1996; Daminet and Ferguson 2003).
- Many reported having one of a number of specific potentially severe medical conditions; however, only a limited list of conditions was reported. For example, 14 reported having had heart failure, 3 had a current liver condition, 71 had current or past cancer, 13 had been told at some time they have weak or failing kidneys, and an unreported number (due to privacy concerns, presumably) had hepatitis C. *Kidney failure is known to causes alterations in thyroid hormone metabolism known as nonthyroidal illness syndrome* (Rosolowska-Huszcz et al., 2005). *Hepatitis C patients on interferon therapy were found to be more susceptible to autoimmune thyroid diseases* (Muratori et al., 2005).
- 4% of adult study subjects are underweight (per CDC guidelines, BMI <18.5), 20% are obese (BMI ≥30 and <40), and 4% are morbidly obese (BMI ≥ 40). *Associations between body weight and thyroid hormone and TSH levels have been reported in obese and morbidly obese individuals* (Sari et al., 2003; Michalaki et al., 2006).
- Many report other types of exposures. 16% of study subjects reported using pesticides within their home during the previous month. A small number of women (16) reported using cocaine or other street drugs within the previous year, with one reporting using a drug on a total of 200 days during the previous year—

because of the nature of this information, it could be assumed that this statistic is underreported. Illicit drug use status for subjects under age 18 was not reported. *Associations between pesticide exposure and changes in thyroid hormone/TSH levels have been reported in some animal studies* (Beard and Rawlings 1999; Jeong et al., 2006). *T4 levels have been shown to decrease in rats following amphetamine administration* (Budziszewska et al., 1996).

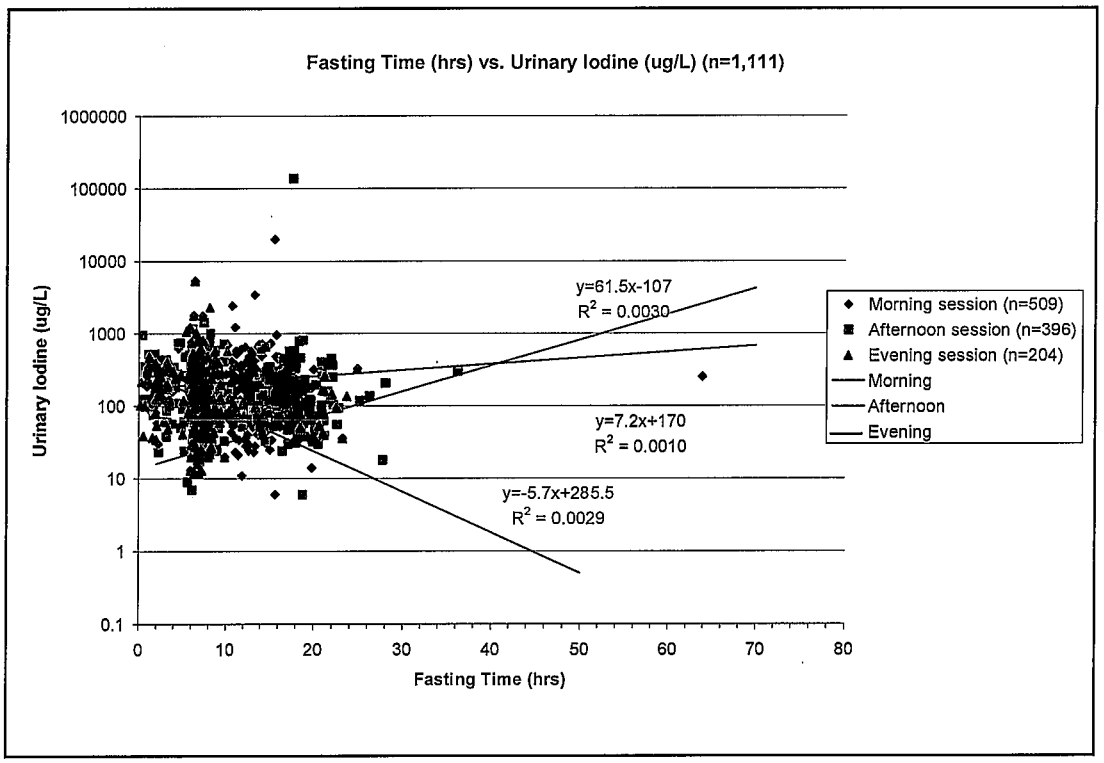
- 66% of study subjects reported regularly (five or more times per month) consuming dark leafy green vegetables. Among these, consumption amount varied widely (up to 91 times per month). 48% reported consuming cooked dried beans or peas five or more times per month. *Dark green vegetables from the Brassica family (e.g., broccoli, kale) are known to contain high concentrations of both thiocyanate and nitrate, two agents known to cause iodide uptake inhibition (IUI) by the thyroid in the same way as perchlorate. Legumes are also known to contain high concentrations of nitrates.*
- 46% of study subjects reported taking dietary supplements during the month preceding their examination interview. 2% (22 subjects) admitted taking a dietary supplement during the fasting period. *There is evidence that dietary supplements can affect thyroid function. For example, short-term dietary supplementation with kelp (high in iodine) significantly increases both basal and poststimulation TSH* (Clark et al., 2003).
- NHANES 2001-2002 does not report on consumption of soy or soy products. *Soy consumption has been associated with development of goiter and an increase in TSH levels in humans* (Doerge and Sheehan, 2002). *Soy products are heavily marketed to women as healthful, including such products as soy milk, tofu, energy bars and drinks, and meat substitutes. Soy isoflavones (e.g., genistein and daidzein) can also be measured in biological samples such as serum, to give an approximation of soy intake levels.*
- Several other important study variables, including location and time of year when samples were collected, were not reported due to privacy concerns. *Seasonally-related changes in thyroid hormone concentrations have been shown in adults, with higher T3 and T4 values seen in winter months, and a tendency to a greater TSH response to TSH-releasing hormone (TRH) was noted at this time* (Harrop et al., 1985). *These changes could reflect a centrally-mediated response of the hypothalamic-pituitary-thyroid axis to environmental temperature* (Harrop et al. 1985). *Significant annual, four-monthly and biannual rhythms were detected in serum TSH in adults, with the lowest detected in spring. A significant annual rhythm was detected in T3, with lower values in spring and summer than in the other seasons. The peak-trough differences in the yearly variation expressed as a percentage of the mean were 29.1% and 8.2% for TSH and T3, respectively* (Maes et al., 1997).

C. The "spot" urine and serum samples cannot be relied upon to establish chronic exposure levels or conditions. Spot samples are known to be inherently variable due to variations in urine volume and intake of exogenous compounds, and, in this population, fasting times varied widely between individuals and a substantial percentage of participants did not comply with study fasting requirements.

- For chemicals with a short biological half-life (e.g., perchlorate, iodine), concentrations in spot urine samples are known to be highly variable between samples, due to within- and between-day variations in urine volume and intake of exogenous compounds (Barr et al., 2005). Factors shown to influence concentrations include fasting time, time of day, nature of the last meal, sample dilution, collection method, preservation method, sample interferences, and analytical method (Rasmussen et al., 1999). Urinary iodine concentrations in 24-hour samples vary up to three fold from one day to another. This suggests that a single sample is insufficient to determine long-term iodine status (Rasmussen et al., 1999). Population iodine excretion estimates require 100 to 500 spot urine samples for each group or subgroup and fewer than 10 urine samples in an individual may be misleading (Anderson et al., 2008).
- Serum and urine samples were collected at either "morning" (46%), "afternoon" (36%), or "evening" (18%) examination sessions. Specific sample time of day is not given. *In humans, serum TSH concentrations are at their maximum at night, shortly before sleep, about 50-100% greater than the morning low* (Fisher, 1996). *Early morning values are greater than later morning values* (Surks et al., 2005). *TSH is secreted in pulses, with eight to fourteen pulses occurring in 24 hours. Sleep deprivation, strenuous exercise, or working during night or evening shifts accentuate the rhythms* (Surks et al., 2005).
- Fasting times were highly variable. Subjects appointed to a morning session were asked to fast for 9 hours while subjects appointed to an afternoon or evening session or a home exam session were asked to fast for 6 hours. However, the protocol states the "greater goal [is] completing as many components as possible within the time constraints of the session with phlebotomy as the highest priority component." Reported length of fast ranged from 0 to 63 hours, with a mean and 50th percentile of 10.3 hours and 5th and 95th percentiles of 1.7 hours and 19 hours, respectively. For the morning, afternoon, and evening sessions, 8%, 13% and 24%, respectively, did not meet the fasting requirement.
- About 2% (22/1111) admitted taking a dietary supplement during the pre-blood and urine collection fasting period. *There is evidence that dietary supplements can affect thyroid function. For example, short-term dietary supplementation with kelp (high in iodine) significantly increases both basal and poststimulation TSH* (Clark et al., 2003).
- Study participants were allowed to consume diet soda, black coffee or tea with saccharine or Equal since "these have no affect on study analytes." (Protocol p. 4-32). *It is relatively clear that sugar substitutes such as those used in diet sodas do not have a significant impact on thyroid function. However, the evidence for a lack of effect with caffeine is less clear. Studies in rats have shown that injection of caffeine results in a decrease in serum TSH followed by a subsequent decrease in serum T3 and T4* (Spindel et al., 1980). *However, no effect on thyroid parameters were found in men (TSH and T3) or Syrian hamsters (TSH, T4, and T3)* (Spindel et al., 1984; Bartsch et al., 1996).
- Figure 2A illustrates the variability in the fasting times. Though no direct correlation between urinary iodine (and perchlorate) measures and fasting times is seen, it is unclear whether fasting time, exclusive of all of the other variables

inherent in this dataset, could have affected urinary concentrations in a well-controlled dataset.

Figure 2A. Variability of Fasting Times from NHANES 2001-2002



D. The observed differences in perchlorate are that thyroid response between men and women are not explained, which could be due to another unexplained variable. Comparable differences have not been observed in previous studies of perchlorate exposure (e.g., Greer et al., 2002; Braverman et al., 2006; Crump et al., 2000). Missing database variables make it difficult to exclude other possible causes of the observed differences between men and women.

- Thyroid autoantibodies are not measured in NHANES 2001-2002. *Women are known to be about 2.7 times more likely to acquire an autoimmune disease than men* (Jacobson et al., 1997), *and to have a greater incidence of thyroid autoimmunity* (Chiovato et al., 1993). *Thyroid autoantibody levels have been shown to be positively correlated with TSH levels in humans* (Hollowell et al., 2002; O'Leary et al., 2006; Hoogendoorn et al.,2006). *In analysis of data from NHANES III, a significant association between female gender and elevated serum TSH levels disappeared when controlled for TPOAb* (Hollowell et al., 2002).

- The NHANES dataset does not include a variable directly characterizing time since last pregnancy; this value can be approximated based on reported "age at last live birth" and current age. Using this, about 7.5% of women not currently pregnant reported being pregnant within the previous 2 years. The possibility that postpartum thyroiditis afflicted some of these women cannot be excluded. *Postpartum thyroiditis, a syndrome of transient or permanent thyroid dysfunction caused by autoimmune inflammation of the thyroid, occurs frequently in the first year after delivery, with a prevalence of about 5-7%* (Muller et al., 2001). *In*

about 25-30% of these women, the condition progresses to permanent hypothyroidism (Lazarus, 2005b).

- Figures 3A and 4A depict the variability in the NHANES data set and the nominal changes noted in thyroid hormones.

Figure 3A. Variability of Perchlorate in NHANES Data Set

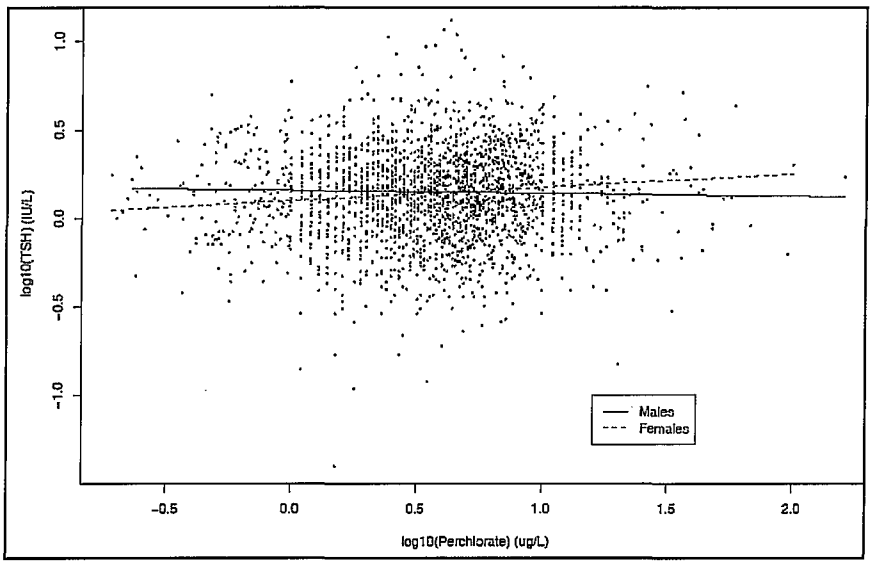


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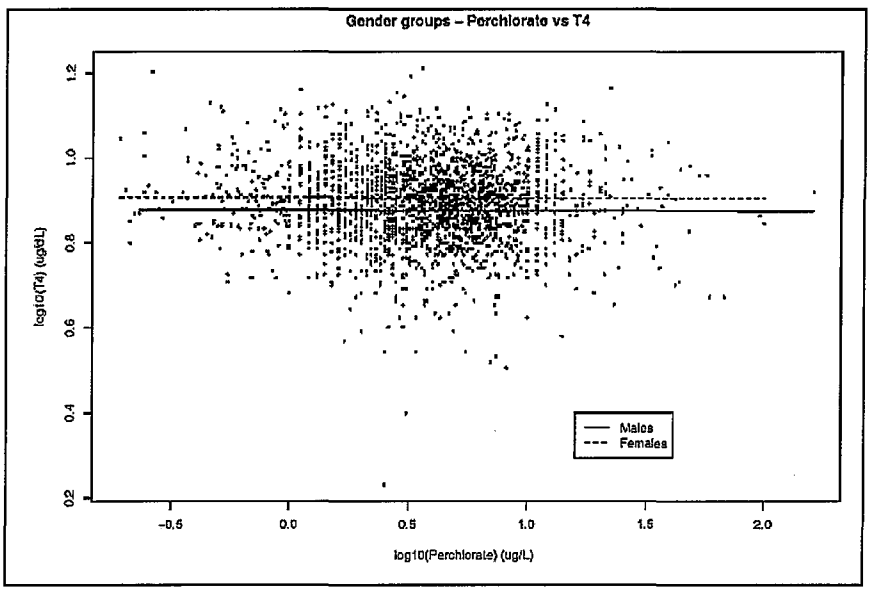
Figure 4A. Variability of Perchlorate and Changes in Thyroid Horomones



Charnley, *personal communication*

E. Spot urine concentrations of nitrate and thiocyanate, two other inhibitors of iodide uptake into the thyroid, were highly variable among study subjects. Since perchlorate, nitrate, and thiocyanate all act through the same mechanism of action (iodide uptake inhibition

(IUI) at the thyroid), evaluation of correlations between exposure to iodide uptake inhibitors and thyroid hormone or TSH concentrations should take into account combined exposures to all three anions.

- Perchlorate equivalent concentrations of nitrate, thiocyanate, and perchlorate in urine in the study population (calculated assuming that nitrate is 1/240 as potent and thiocyanate is 1/15 as potent as perchlorate; Tonacchera et al., 2004) ranged from 3.7 to 3329 µg/L. On average, for any given subject, perchlorate comprised about 1% of the total perchlorate equivalent concentration (geometric means are 2.8 and 270 µg/L for perchlorate and perchlorate equivalent concentration, respectively). One would expect to see thyroid effects of nitrate or thiocyanate before seeing perchlorate effects; however, Blount et al. (2006) report no reliable associations between urine levels of nitrate and thiocyanate and serum total T4 or TSH.
- No correlation between perchlorate equivalent concentration and serum total T4 or serum TSH is apparent. Perchlorate equivalent concentration was not a significant predictor of total T4 ($p=0.43$, coefficient -0.00011) or TSH ($p=0.25$, coefficient -0.00023). Conceivably, the same problems inherent in relying upon spot urine samples as indicators of longer term iodine intake would affect the reliability of spot urine samples for perchlorate, nitrate, and thiocyanate.

Missing Data

Some of the key data related to this study were not accessible in the time frame of this project. For instance, the analytical quality assurance/quality control data is available for review through the National Center for Health Statistics (NCHS), but NCHS requires reviewers to request an appointment and travel to their location to review the data. Other data would need to be obtained under the Freedom of Information Act (FOIA). Key data that could be obtained through these mechanisms might include exact times of blood samples and urine samples, the sequence of sample collection, QA/QC of the analyses, analytical logbooks, and other notes from the survey. These are critical pieces of information that could be useful to characterize the dataset and examine the significance of associations.

Summary

If one considered this as a pilot study which would be consistent with the stated intended use, then the appropriate and logical next step would be to address a number of these concerns with an enhanced experimental design. For example, adding critical thyroid function variables (e.g., free T4, anti-TPO), 24-hour urine collections, and better control of medications will provide a more appropriate study design.